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3 **Current and potential management strategies against**
4 ***Harmonia axyridis***

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8 **Abstract** This paper reviews the current and potential methods to control the harle-
9 quin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), an Asian
10 predatory beetle invasive in Europe and the Americas where it has become a human
11 nuisance, a grape and wine pest and a threat to native biodiversity. Current methods to
12 manage this invasive species include: techniques to mechanically prevent adult beetles
13 from entering buildings in autumn or to remove aggregates of beetles inside buildings,
14 e.g. using various trapping methods; the use of insecticides on buildings or in vineyards
15 to prevent aggregation in houses or on grapes; cultivation practices in vineyards to
16 lower the impact of the ladybird on grape production and wine quality; remedial
17 treatments for wine tainted by the ladybird. Other methods are presently being
18 developed or considered. Semiochemicals could be used as deterrents or as attractants
19 to develop more efficient trapping systems in buildings and open fields. Natural ene-
20 mies include pathogens, parasitoids, predators and a parasitic mite but few of them
21 show potential as biological control agents. While management methods presently used
22 or under development may eventually solve the problems caused by beetles aggregating
23 in buildings or vineyards, the issue of *H. axyridis* populations outcompeting native
24 species is much more challenging. Only the sudden adaptation of a native natural

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25 enemy or the importation of a natural enemy from the area of origin of the ladybird
26 may ultimately lower population densities. The problems linked to the importation of
27 an Asian natural enemy of *H. axyridis* are discussed.

28 **Keywords** *Harmonia axyridis* · Integrated pest management · Biological control ·
29 Mechanical control · Chemical control · Cultivation practices · Parasitoids ·
30 Entomopathogenic fungi · Parasitic mites

31 Introduction

32 *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), the harlequin ladybird, or
33 multicoloured Asian ladybeetle, is a polyphagous predatory coccinellid, native to
34 central and eastern Asia. It has been widely used as a biological control agent of aphids
35 in the Americas and Europe, in greenhouses as well as in outdoor crops (Koch 2003;
36 Pervez and Omkar 2006). In North America, since its establishment in the 1980s, it has
37 spread and increased dramatically so that it is now the dominant ladybird species in
38 much of the USA and Canada (Koch and Galvan 2008). Three categories of hazards
39 caused by this invasive ladybird are reported (Koch et al. 2006). First, it is considered
40 as a human nuisance because it aggregates in buildings when seeking overwintering
41 sites, causing cosmetic damage and, occasionally, biting humans and causing allergic
42 reactions (McCutcheon and Scott 2001; Huelsman and Kovach 2004; Kovach 2004;
43 Ray and Pence 2004). Second, *H. axyridis* has been reported to damage fruit crops in
44 late summer and to taint wine when harvested and crushed with grapes (Koch et al.
45 2004; Kovach 2004; Pickering et al. 2005). Finally, due to its predatory and competi-
46 tive abilities, *H. axyridis* may have strong negative effects on biodiversity, affecting
47 many non-target species, including native ladybirds and other aphidophagous insects
48 but also non-pest aphids or other herbivorous insects (see reviews by Majerus et al.
49 2006 and Koch and Galvan 2008).

50 In Europe, *H. axyridis* has been released in the wild since 1990 (Brown et al. 2008).
51 Since the first observations of feral populations were reported in 1999 in Germany and in
52 Belgium in 2001, numbers have increased exponentially and it is now regarded as
53 established in 12 European countries (Brown et al. 2008). In Belgium, recent surveys
54 showed that *H. axyridis* has become by far the most abundant ladybird on broadleaved
55 trees (Adriaens et al. 2008).

56 Since the establishment of *H. axyridis* in North America, this invasive ladybird has been
57 the target of numerous research programmes, which have been summarised in several
58 review articles (e.g. Adriaens et al. 2003; Koch 2003; Majerus and Roy 2005; Koch et al.
59 2006; Pervez and Omkar 2006). None of these reviews, however, cover the currently used
60 and potential management methods to control *H. axyridis*.

61 Several methods are presently implemented or being studied to control the ladybird in
62 North America. These methods differ according to the three main types of hazards caused
63 by the beetle (aggregation in buildings, damage to fruits and wine and competition with
64 the native fauna). Most management methods, proposed so far, concern preventive or curative
65 control against ladybird aggregation in houses or in vineyards. No method is presently
66 available to lower population densities in natural environments and to limit the impact of
67 the ladybird on native species.



68 Mechanical control and trapping methods

69 Numerous extension documents in North America describe methods to mechanically
70 prevent adult beetles from entering buildings in autumn or to remove aggregates of beetles
71 inside buildings (e.g. McCutcheon and Scott 2001; Jones and Boggs 2002; Baniecki et al.
72 2004; Potter and Townsend 2005). Recommendations to prevent the entrance of beetles in
73 buildings include: checking for all possible entry sites; sealing windows, cracks and small
74 holes throughout the house with weather stripping; placing insect screening over windows,
75 attic, exhaust vents, etc. To remove large aggregates of beetles in buildings, the use of a
76 broom and dust pan or, better, a vacuum cleaner is recommended (Jones and Boggs 2002;
77 Baniecki et al. 2004; Potter and Townsend 2005). Jones and Boggs (2002) describe how a
78 vacuum cleaner can be modified to capture ladybirds and avoid them entering the vacuum
79 by using a sweeper attachment with a knee-high nylon hose inserted in the end.

80 Various traps are recommended and commercially available to catch adult beetles in
81 buildings (Baniecki et al. 2004). Black light traps and other light traps can be used for
82 capturing beetles in dark confined places such as attics. Window traps may be effective
83 during daytime. Although it has been suggested that these traps could reduce beetle
84 populations by 50–70% (Jones and Boggs 2002; Baniecki et al. 2004), thorough testing is
85 required to ascertain whether they provide a solution for large infestations of *H. axyridis*
86 (Jones and Boggs 2002; Potter and Townsend 2005). Furthermore, it is highly unlikely that
87 light traps could efficiently control ladybirds in outdoor conditions, e.g. in vineyards or
88 orchards. The efficiency of trapping methods in buildings and open field could be greatly
89 enhanced by coupling with semiochemicals. There are many examples in the literature of
90 insects using olfactory communication for dispersal, migration, reproduction, aggregation
91 and alarm signalling (reviewed in a pest control context by Van Emden and Service 2004).
92 Over the last 30 years the behavioural manipulation of insects through semiochemical
93 techniques (for example: push–pull strategies) has been considered for the control of insect
94 populations, with varying success (Cook et al. 2007). However, our theoretical under-
95 standing of insect semiochemistry has increased enormously in recent years (reviewed by
96 Hassanali et al. 2005; Cook et al. 2007; Roy et al. 2008a) and applications exploiting this
97 knowledge, within pest control strategies, are being developed further. Pheromones have
98 been used within integrated pest management (IPM) strategies for: monitoring pest pop-
99 ulations, trapping out pest populations (“lure and kill”), confusing pests (saturating a crop
100 environment with synthetic pheromones to prevent males locating females), deterring
101 oviposition and manipulating or encouraging natural enemies.

102 Information on semiochemically-mediated behavioural mechanisms of coccinellids is
103 limited (Pettersson et al. 2005) but there are sufficient recent studies to suggest that, like
104 other insects, chemical communication is an important component of ladybird behaviour
105 (Burns et al. 1998; Hemptinne et al. 2004; Pettersson et al. 2005). The use of autumn
106 aggregation pheromones for the location of suitable overwintering sites perhaps has the
107 most significant potential for use in a control strategy. It has been widely speculated that
108 many species of ladybirds use species-specific aggregation chemicals in preparation for
109 dormancy (Majerus 1994). Hills (1969) suggested that *Adalia bipunctata* (L.) (Coleoptera:
110 Coccinellidae) used pheromonal volatiles from the excretal remains of individuals over-
111 wintering the previous year. Majerus (1994) demonstrated that *A. bipunctata* were less
112 likely to locate sites that had been washed between overwintering events, providing strong
113 circumstantial evidence of a long lasting, aggregation pheromone. In a more recent study,
114 Pettersson et al. (2005) demonstrated that *Coccinella septempunctata* L. (Coleoptera:
115 Coccinellidae) use an autumn aggregation pheromone to locate overwintering sites. There



116 is no current information on the chemical nature of these aggregation pheromones or
117 whether they are species specific or, indeed, seasonally specific (active only in autumn).
118 Nalepa et al. (2000, 2005) investigated factors influencing aggregation behaviour and
119 found little evidence for volatile aggregation pheromones. Their results suggest that beetles
120 are rather attracted by visual intensity contrast and by contact chemoreception with con-
121 specific or the faeces and residues that persist in aggregation sites from previous years.
122 However, if an aggregation semiochemical specific to *H. axyridis* could be produced, it
123 would be possible to utilise this in a “lure and kill” strategy for controlling this species in
124 sensitively determined areas. A recent paper suggests that two volatile sesquiterpenes, (E)-
125 β -farnesene (aphid alarm pheromone) and (–)- β -caryophyllene (isolated from the head-
126 space volatiles above *H. axyridis* aggregations in winter), are ecologically relevant to
127 *H. axyridis* (Verheggen et al. 2007). (E)- β -farnesene elicited electroantennogram (EAG)
128 and behavioural responses within an olfactometer in both male and female *H. axyridis*. In
129 contrast, (–)- β -caryophyllene resulted in EAG and behavioural activity (attraction) in male
130 *H. axyridis* but only EAG activity in female beetles (no associated behavioural response
131 within an olfactometer). However, interestingly Verheggen et al. (2007) demonstrated in a
132 bioassay that both male and female *H. axyridis* aggregated in the (–)- β -caryophyllene-
133 treated side of a ventilated plastic box as opposed to the untreated side. Perhaps the
134 presence of the male *H. axyridis* evokes the response of the females. The authors conclude
135 that (E)- β -farnesene and (–)- β -caryophyllene could have potential use within a push–pull
136 control strategy for *H. axyridis*, but it is possible that *H. axyridis* exhibits different
137 antennal sensitivity to (–)- β -caryophyllene in relation to season, responding more in
138 winter. The complexity of insect behavioural responses to semiochemicals should be
139 assessed thoroughly when considering manipulating insects as a component of an IPM
140 strategy.

141 Trapping methods based on aggregative pheromones are commonly used for the
142 monitoring and control of scolytid bark beetles (Byers 2004). A sap beetle, *Carpophilus*
143 *lugubris* Murray (Coleoptera: Nitidulidae) was successfully controlled at overwintering
144 locations, with minimal impacts on other insect species, using an autoinoculative device
145 baited with aggregation pheromone and the fungal pathogen *Beauveria bassiana* (Bal-
146 samo) Vuillemin (Ascomycota: Hypocreales) (Dowd and Vega 2003). A combination of
147 the fungal pathogen *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota: Hypocre-
148 ales) and an attractant (phenethyl propionate: eugenol: geraniol) has shown potential for
149 controlling Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Klein
150 and Lacey 1999). These systems could be appropriately designed for controlling *H. axy-*
151 *ridis*, however, recent studies have shown that *H. axyridis* is relatively resilient to fungal
152 (*B. bassiana*) mortality (Roy et al. 2008b). It is possible that a synthetic insecticide could
153 be used in an attractant trap which would provide a targeted method of control with limited
154 impacts on non-target insects and the environment.

155 Chemical control

156 Many insecticides have been tested against *H. axyridis*, but rather to test the non-target
157 impact of insecticides used against aphids and other pests than to assess their potential use
158 against the ladybird (see Koch 2003, for a review, and Galvan et al. 2005a, b, 2006d for the
159 most recent investigations). These studies showed that most insecticides commonly used in
160 agricultural environments are toxic to *H. axyridis*. Larvae were more sensitive than adults
161 and, in general, the toxicity of the insecticides was lower to the ladybird than to aphids.



162 Galvan et al. (2006a), Inclan et al. (2006) and Williams et al. (2006) recently carried out field
163 and laboratory evaluation of the efficacy and suitability of insecticides for management of
164 *H. axyridis* in vineyards. One of the limiting factors of using insecticides is that many of them,
165 e.g. most pyrethroids, have a pre-harvest interval of several weeks whereas, to be efficient,
166 treatments should be applied within a week before harvest (Galvan et al. 2006a). Among the
167 most efficient compounds, only carbaryl and imidacloprid were labelled for use on wine grape
168 within 7 days of harvest in USA at the time of the study (Galvan et al. 2006a). Insecticide
169 treatments against *H. axyridis* in vineyards should not be carried out preventively but should
170 rather follow decision protocols based on rigorous sampling plans and well defined action
171 thresholds (Galvan et al. 2006a, 2007). In addition, the potential effect of such treatments on
172 non-target beneficial organisms should be investigated.

173 In North America, it is sometimes advised to apply insecticides, as a preventive method,
174 to the exterior of buildings that are regularly infested year after year (McCutcheon and
175 Scott 2001; Jones and Boggs 2002; Baniecki et al. 2004; Potter and Townsend 2005).
176 Application of a residual formulation of synthetic pyrethroids (e.g. bifenthrin, cyfluthrin,
177 cypermethrin, deltamethrin, etc.) to walls and sidings, around eaves, attic vents, windows,
178 doors and other likely points of entry, in late-September–October, i.e. just before beetles
179 enter the structure is recommended. Insecticide use inside buildings is usually not advised
180 (McCutcheon and Scott 2001; Potter and Townsend 2005) and the application of chemicals
181 in other overwintering sites has never been considered.

182 Deterrents

183 Deterrents could be employed to reduce *H. axyridis* populations in locations where their
184 presence is undesired, for example, houses used as overwintering locations and vineyards.
185 *Harmonia axyridis* reduces rates of feeding and oviposition in the presence of conspecific
186 faeces but not with the faeces of *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae);
187 whereas *P. japonica* reduces feeding and oviposition in response to faeces of conspecifics
188 and *H. axyridis* (Agarwala et al. 2003). This is hypothesised to reduce intra-guild predation
189 (Agarwala et al. 2003). Further work could investigate the use of these pheromonal faecal
190 volatiles as deterrents for *H. axyridis*. However, it is possible that these could act as
191 deterrents to other ladybird species and so be detrimental by impacting on beneficial insects
192 in agricultural systems. Some plant compounds, such as camphor or menthol have proved to
193 be repellent to *H. axyridis* in field and laboratory tests and could be used to protect buildings
194 (Riddick et al. 2000). However, under field conditions, camphor was ineffective after 48 h
195 post-application (Riddick and Aldrich 2004). Laboratory bioassays also showed that
196 *H. axyridis* is repelled by DEET (*N,N*-diethyl-3-methylbenzamide), a widely used insect
197 repellent. A formulation of DEET plus paraffin had repellent activity for 23 days in the
198 laboratory, but has not been field-tested (Riddick et al. 2004).

199 Cultivation practices in fruit production and remedial treatment for tainted wine

200 *H. axyridis* is often reported as a pest of fruit production in North America (Koch 2003;
201 Kovach 2004). Nevertheless, the few studies on feeding activity suggest that it rarely
202 causes primary injury on fruits, but rather feeds on damaged fruits (Koch et al. 2004;
203 Kovach 2004; Galvan et al. 2006b). The main impact of the ladybird as a fruit pest is in
204 vineyards when it aggregates in clusters of grapes, in particular those containing



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205 damaged berries, and is processed with the grapes, resulting in tainted, unmarketable
206 wine or juice (Pickering et al. 2005). Several recommendations on cultivation practices
207 in vineyards have been suggested to lower the impact of the ladybird in regions where
208 *H. axyridis* causes recurrent problems to fruits, although these recommendations are not
209 always based on rigorous studies. Key components of an IPM strategy against *H. axy-*
210 *ridis* in vineyards include proper surveys for beetle densities before harvest and the
211 determination of a threshold density, to assist in management decisions. Galvan et al.
212 (2007) have described various sampling plans and assessed their usefulness. They found
213 binomial sampling plans to be more accurate methods to measure beetle densities than
214 enumerative plans. Kovach (2004) and Pickering et al. (2007) evaluated the threshold
215 density for wine contamination to be about 0.9 and 1.3–1.5 beetle per kg of grapes,
216 respectively, but the latter authors recommend a more conservative limit of 0.2–0.4
217 beetles per kg of grapes above which interventions in the field or in the winery should be
218 considered. Including berry injuries in the sampling procedures may also be useful since
219 ladybirds are primarily found on damaged fruits (Galvan et al. 2007). Such damage is
220 caused by a variety of mechanisms including by splitting, feeding by birds or other
221 insects, disease (rot) etc. (Galvan et al. 2007). Growers could reduce berry injury by
222 using irrigation to avoid long periods of drought and by avoiding injuring to berries
223 when pruning or spraying. Selecting varieties with higher resistance or tolerance to
224 splitting may also be envisaged, as a potential long-term measure, when vineyards are
225 replanted through the normal process of renewing stock.

226 Harvesting methods may have an impact on the density of beetles in harvested grapes.
227 Beetles may be more likely to leave the grapes during day harvesting rather than during
228 night harvesting (Anonymous 2005). Hand harvesting may be more favourable than
229 mechanical harvesting because aggregations of beetles in grape clusters can be monitored
230 during harvesting and infested grapes can be discarded. Beetles can be removed by shaking
231 clusters, by hand or by using shaking tables, and by floating clusters in water or vacuum
232 clusters (Galvan et al. 2006c). Shaker tables could also be used to monitor populations just
233 before harvest. However, these methods result in significant increase in time, labour and
234 costs of harvest (Galvan et al. 2006c). Managing the surrounding crops and vegetation
235 could also be critical because beetles found in vineyards and orchards in autumn probably
236 come from nearby aphid infestations. In North America, damage in vineyards is often
237 associated with the presence of soybean infested by the soybean aphid, *Aphis glycines*
238 Matsumura (Hemiptera: Aphididae), one of the preferred preys of *H. axyridis*, although the
239 exact relationship between the proximity of soybean, soybean aphids and *H. axyridis*
240 populations has not as yet been rigorously monitored.

241 Studies are being made on refining agents and other technologies to restore the quality
242 of wine tainted by *H. axyridis* (Pickering et al. 2006). The main compound responsible for
243 the taint is 2-isopropyl-3-methoxypyrazine (IPMP), a component of the reflex bleeding
244 fluid of *H. axyridis* (Pickering et al. 2005). IPMP concentrations in wine could be lowered
245 by activated charcoal in white wine and deodorized oak in red wine, although these
246 treatments do not translate into lower intensity of the sensory attributes associated with the
247 taint. In contrast, oak chips were successful in reducing the intensity of the taint charac-
248 teristics, probably through a masking effect (Pickering et al. 2006). However, this method
249 does not completely remove the taint from contaminated wine and, furthermore, it cannot
250 be used for all wine types, since many of them are not normally oaked. Thus, other
251 solutions that may be suitable for all wine types need to be sought. Pickering et al. (2006)
252 suggest searching for a technology to target and selectively bind IPMP, with subsequent
253 removal of the complex from the juice or wine.



254 **Biological control**

255 Natural enemies can be used in various ways to control an invasive insect such as
256 *H. axyridis* (Van Driesche and Bellows 1996). First, a species could be introduced from the
257 region of origin of the pest to the region of introduction for permanent establishment, a
258 method often called “classical biological control”. Second, in an “augmentative biological
259 control” programme, natural enemies can be increased through regular inundative or
260 inoculative releases. Finally, in a “conservation biological control” strategy, natural
261 enemies already present on site can be favoured, e.g. through various cultural practices, to
262 increase their impact on the target pest. All these methods could be developed and
263 employed for controlling *H. axyridis*, albeit not with the same agents.

264 Natural enemies reported, or tested against *H. axyridis* comprise pathogens, predators,
265 parasitoids, nematodes and a parasitic mite. There is little information available on the
266 importance of natural enemies in the population dynamics of *H. axyridis* in field condi-
267 tions, particularly in its region of origin. Research on mortality factors in field conditions
268 has been carried out in Japan (Osawa 1992, 1993) and South Korea (Park et al. 1996), but
269 natural enemies in the largest part of its distribution range, i.e. China and Russia, are
270 largely unknown. In Europe and North America, observations suggest that natural enemies
271 are of little importance in the population dynamics of the ladybird.

272 Pathogens

273 There have been a number of studies focussing on the impact of entomopathogens on
274 coccinellids. Undoubtedly, the literature on fungal entomopathogens dominates this field and
275 is mainly from the perspective of natural mortality in overwintering populations or, more
276 usually, the effect of biorational pesticides on non-target coccinellids. Entomopathogenic
277 fungi are common natural enemies of insects and other arthropods worldwide (Roy et al.
278 2006) and have considerable potential as biological control agents of pest insects.

279 Two Hypocrealean fungi have been found infecting ladybirds: *M. anisopliae* (Ginsberg
280 et al. 2002) and *B. bassiana* (James et al. 1995; Cottrell and Shapiro-Ilan 2003; Roy et al.
281 2008b). Most research on the direct interactions between fungi and ladybirds has concen-
282 trated on *B. bassiana* because of the potential non-target impact of this fungus when used as a
283 mycoinsecticide against various pest insects. Susceptibility of various ladybirds (Coleoptera:
284 Coccinellidae) to *B. bassiana* has been demonstrated: *Hippodamia convergens* Guérin-
285 Méneville (James et al. 1995), *A. bipunctata* (Roy et al. 2008b), *C. septempunctata* (Roy
286 et al. 2008b), *Coleomegilla maculata lengi* Timberlake (Todorova et al. 1996, 2000), *Ser-*
287 *angium parcesetosum* Sicard (Poprawski et al. 1998), *Olla v-nigrum* (Mulsant) (Cottrell and
288 Shapiro-Ilan 2003) and *Cryptolaemus montrouzieri* Mulsant (Smith and Krischik 2000). In
289 contrast, a number of studies have demonstrated the resilience of *H. axyridis* to *B. bassiana*
290 (Cottrell and Shapiro-Ilan 2003; Roy et al. 2008b). In North America one of the overwin-
291 tering habitats of *H. axyridis* is under bark in pecan orchards (Cottrell and Shapiro-Ilan 2003).
292 Adults of the native ladybird *O. v-nigrum* are also found in this overwintering location and are
293 commonly recorded infected with *B. bassiana* (Cottrell and Shapiro-Ilan 2003); whereas
294 *H. axyridis* are not. Cottrell and Shapiro-Ilan (2003) demonstrated in laboratory studies that
295 *H. axyridis* is extremely resistant to *B. bassiana* infection. Further studies using various
296 isolates of *B. bassiana* support this notion. Roy et al. (2008b) showed that *A. bipunctata* and
297 *C. septempunctata* are highly susceptible to doses of 10^7 spores ml^{-1} but even doses of
298 *B. bassiana* exceeding 10^9 spores ml^{-1} have limited impact on *H. axyridis*. However,



299 *B. bassiana* appears to have a sublethal effect on *H. axyridis* and even low doses
300 (10^5 spores ml^{-1}) reduce egg production significantly (Roy et al. 2008b).

301 There is one further fungal entomopathogen of coccinellids worthy of note: *Hesper-*
302 *omyces virescens* Thaxter (Ascomycota: Laboulbeniales). This intriguing fungus is an
303 obligate ectoparasite that infects many coccinellids worldwide (Nalepa and Weir 2007).
304 *Hesperomyces virescens* has been reported infecting *Chilocorus stigma* (Say) (Coleoptera:
305 Coccinellidae) (Thaxter 1891), *A. bipunctata* (Welch et al. 2001), *H. axyridis* (Garcés and
306 Williams 2004), *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae) (Kamburov et al.
307 1967; Applebaum et al. 1971), and *Coccinula crotchi* (Lewis) (Coleoptera: Coccinellidae)
308 and *Coccinula sinensis* Weise (Coleoptera: Coccinellidae) (M. Majerus and R. Ware
309 personal observation). This fungus is often reported on the ventroposterior of males and the
310 dorsoposterior of females; a sexual dimorphism that reflects the major transmission
311 mechanism which is thought to be via direct contact during mating (Weir and Beakes 1996;
312 Welch et al. 2001; Riddick and Schaefer 2005). Nalepa and Weir (2007) have studied the
313 interactions between *H. virescens* and *H. axyridis* in detail. In a field survey in North
314 Carolina they demonstrated infection levels of 22–38% at the beginning of winter
315 increasing to 62% by the end of winter. These overwintering ladybirds did not exhibit the
316 characteristic elytral distribution of *H. virescens* as hypothesised by the sexual transmis-
317 sion theory. In contrast, fungal thalli were distributed in accordance with direct contact
318 through aggregation and orientation behaviour, notably on the anterior part of the body. A
319 similar pattern was reported for *A. bipunctata* which, when collected from overwintering
320 aggregations, had fungal thalli distributed at the margins and front angles of the elytra
321 (Weir and Beakes 1996). Nalepa and Weir (2007) conclude that direct transmission
322 through contact with conspecifics is the most important mechanism for transmission of
323 *H. virescens* and sexual contact is of primary importance in the mating season but
324 aggregation in winter also plays a significant role.

325 Laboulbeniales characteristically do not penetrate the insect cuticle, however,
326 *H. virescens* is one of the few species, in this family, to exhibit rhizoidal penetration into
327 the host body by production of a circular appressorium, which attaches and penetrates the
328 host cuticle (Weir and Beakes 1996). Even though this fungus invades the host there are no
329 known deleterious impacts on the beetle (Nalepa and Weir 2007). However, *H. virescens* is
330 suspected to have a negative effect on *C. bipustulatus*, through increased mortality
331 (Applebaum et al. 1971; Kamburov et al. 1967). Heavy infections could also impede flight,
332 foraging and mating but this requires further investigation.

333 It is also of note that some Asian populations of *H. axyridis* are strongly female biased,
334 due to the presence of a male-killing bacterium of the genus *Spiroplasma* (Majerus et al.
335 1998). The bacterium is maternally inherited in the cytoplasm of eggs, but not sperm. As a
336 consequence, it kills male hosts early in embryogenesis. In doing so, the male-killer makes
337 the soma of these dead male eggs available as a food source to infected female sibling
338 neonate larvae, providing them with a significant fitness advantage (Majerus and Hurst
339 1997; Majerus 2006). Prevalence of this *Spiroplasma* in populations of *H. axyridis* in its
340 native range is variable, but can reach 80%. The male-killer has not been reported from
341 *H. axyridis* in its introduced range. Intuitively, it seems likely that introduction of male-
342 killer infected females into populations of *H. axyridis* in Europe and North America would
343 depress *H. axyridis*, because half the offspring (the males) of infected females would die in
344 the eggs. The male-killer would spread naturally through the population to an equilibrium
345 prevalence dictated by the vertical transmission efficiency of the male-killer (>0.99 for this
346 *Spiroplasma*), any direct fitness effects that the male-killer has on females and the level of
347 benefit gained by infected females as a result of the death and consumption of their male



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siblings (Hurst et al. 1997). The death of half the infected female's offspring would seem to be beneficial in reducing host population size, and consequently both intraguild competition and predation imposed by *H. axyridis*. However, this may not be the case in practice. As male *H. axyridis* can mate with many females, a bias in the sex ratio towards females is unlikely to lead to reduced fertility in females. Moreover, as *H. axyridis* larvae are highly cannibalistic, loss of a significant proportion of male progeny during embryogenesis may simply lead to a reduction in cannibalism of immatures, and not to a reduction in the adult population. Finally, as female ladybirds eat considerably more than males (Hodek 1996), the level of intraguild competition may actually be increased as a result of biasing the population sex ratio in favour of females. Due to these uncertainties, use of a male-killer in an attempt to reduce *H. axyridis* populations should not be undertaken until a full analysis of the consequences of such an action has been conducted.

Current research indicates that, under current knowledge, pathogens are unlikely to be suitable biological control agents for *H. axyridis*. The fungi examined so far (mainly *B. bassiana*) lack efficacy and specificity towards *H. axyridis* and could impact on non-target coccinellids and other insects. Nevertheless, the development of a novel strategy utilising pathogens as a component of a complex control strategy should not be ruled out. The sublethal effects of disease require further research and could provide a solution to controlling *H. axyridis*. Furthermore, *H. axyridis* should be screened throughout its native and non-native distribution to thoroughly assess the diversity of pathogens (fungi, protists, bacteria and viruses) and other "hitchhikers" (nematodes and other parasites) associated with it. One of these could be the key to control.

370 Predators

As other ladybirds, *H. axyridis* is considered to be largely protected from generalist predators thanks to its alkaloid-rich defence secretions and its aposematic colouration. Nevertheless, Nechayev and Kuznedsov (1973) mention eight species of birds feeding on *H. axyridis* in Russia. Although Kuznetsov (1997) reports that the two bird species *Picus canus* Gmelin (Piciformes: Picidae) and *Sitta europaea* L. (Passeriformes: Sittidae) will feed on *H. axyridis* in mass overwintering aggregations, he concludes that predation of coccinellids, including *H. axyridis*, by birds is generally rare. Eggs and larvae may also be preyed upon by other coccinellids (Pell et al. 2008; Ware and Majerus 2008) and by predatory bugs (De Clercq et al. 2003), although, in general, *H. axyridis* has better physical and chemical defences against predation than most ladybirds (see Pell et al. 2008, for review). It is also less attacked by ants and spiders than other ladybirds (Dutcher et al. 1999; Yasuda and Kimura 2001). It is very unlikely that any invertebrate or vertebrate predator will ever play a significant role in the natural control of *H. axyridis* in Europe or the Americas.

384 Parasitoids

Only two parasitoid species were found on *H. axyridis* in its introduction range and these have never been reported as being a significant source of mortality. The tachinid fly *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae) was found in adult beetles in North Carolina (Nalepa et al. 1996; Nalepa and Kidd 2002) and Oregon (Katsoyannos and Aliniaze 1998). All were reared from overwintering beetles. Parasitism was very variable between sites and from year to year but, on average, only 2–4% of the beetles were



391 parasitized. In Oregon, Katsoyannos and Aliniaze (1998) observed parasitism rates of
392 6.7–15.4% by dissection and 4.8% by rearing. *Strongygaster triangulifera* is a small
393 parasitic fly that is widely distributed in North America and very polyphagous. It is most
394 frequently reported as a parasitoid of adult Coleoptera and there are scattered reports of
395 parasitism within the Dermaptera, Hemiptera, Orthoptera and Lepidoptera (Reeves and
396 O'Hara 2004).

397 The braconid wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) is a
398 common endoparasitoid of ladybirds in Europe, Asia and the Americas. Females usually
399 parasitise adults, although larvae and pupae can also be attacked, in which case the
400 development continues in the adult beetle (Obrycki et al. 1985). *Dinocampus coccinellae*
401 is recorded from many ladybirds of the subfamily Coccinellinae but shows strong prefer-
402 ences for some large species. For example, in the UK it mainly attacks
403 *C. septempunctata*, *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) and *Har-*
404 *monia quadripunctata* Pontoppidan (Coleoptera: Coccinellidae) (Majerus 1997). In North
405 and South America it is most often recorded from *Coleomegilla maculata* (DeGeer) (e.g.
406 Obrycki et al. 1985; Berti Filho and Costa 1995; Hoogendoorn and Heimpel 2002). It is
407 occasionally observed on *H. axyridis* in North America (Hoogendoorn and Heimpel 2002;
408 Firlej et al. 2005), Europe (Koyama and Majerus 2008) and East Asia (Liu 1950; Maeta
409 1969; Park et al. 1996). In Europe and North America, *H. axyridis* is considered as a
410 marginal host for *D. coccinellae*. Hoogendoorn and Heimpel (2002) compared parasitism
411 in *H. axyridis* and the native ladybird *C. maculata* in the USA. They found similar para-
412 sitism rates by dissection of field-collected beetles, varying from 9% to 24%. But the
413 emergence rate was much higher in *C. maculata* than in *H. axyridis*, hardly any parasitoid
414 larvae being able to complete their development in *H. axyridis*. In laboratory assays,
415 female wasps attack the two hosts readily, but larvae were found in 90% of the attacked
416 *C. maculata* and in only 7% of the *H. axyridis*. Similar observations were made in Canada
417 (Firlej et al. 2005) and, in the UK (Koyama and Majerus 2008). Koyama and Majerus
418 (2008) have shown that while the attack rates of *D. coccinellae* on *H. axyridis* and
419 *C. septempunctata* are similar, successful development in *H. axyridis* is much lower than
420 in *C. septempunctata*. Firlej (2006) observed that, in *H. axyridis*, parasitism by *D. cocc-*
421 *inellae* was successful only when the ladybird larvae were parasitised, whereas adults were
422 attacked but no parasitoid developed in these adults. Firlej (2006) suggested that the poor
423 performance of *D. coccinellae* in *H. axyridis* may be at least partly due to the lower
424 number of teratocytes produced by the parasitoid in *H. axyridis* compared to suitable hosts.
425 In Asia, *D. coccinellae* is less commonly reared from *H. axyridis* than from, e.g. *Cocci-*
426 *nella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae) (Maeta 1969). However,
427 successful parasitism is higher in Asia compared to Europe (Liu 1950; Maeta 1969; Park
428 et al. 1996), suggesting either that Asian populations of *D. coccinellae* are better adapted
429 to *H. axyridis* than European populations, or that invasive populations of *H. axyridis* are
430 more resistant to *D. coccinellae*.

431 Other parasitoids of *H. axyridis* are known only from their area of origin, but details on
432 their impact, biology and ecology are scarce. The tachinid fly *Medina* (= *Degeeria*)
433 *luctuosa* (Meigen) (Diptera: Tachinidae) was found in adult beetles collected in October–
434 November in Korea (Park et al. 1996). Parasitism rates fluctuated between 0.7% and
435 21.1%. In laboratory rearing, parasitism by *M. luctuosa* shortened the life span and pre-
436 vented egg development and oviposition. The true identity of this parasitoid is questionable
437 because, in Europe, *M. luctuosa* is specific to adult chrysomelids of the genus *Haltica*,
438 whereas many Coccinellidae, but not yet *H. axyridis*, are parasitized by a closely-related
439 species, *Medina separata* (Meigen) (Diptera: Tachinidae), which was sometimes wrongly



440 identified as *M. luctuosa* (Tschorsnig and Herting 1994). A gregarious pupal parasitoid, the
441 phorid fly *Phalacrotophora philaxyridis* Disney (Diptera: Phoridae) was described from
442 Japan (Disney 1997). A congeneric species, *Phalacrotophora fasciata* (Fallén) (Diptera:
443 Phoridae) is reported from *H. axyridis* pupae in the Russian Far East by Kuznetsov (1997)
444 and Park et al. (1996) cite a *Phalacrotophora* sp. parasitizing 0.4–6.7% of the pupae in
445 Korea, 1–8 adults emerging from a single host pupa. The host range of *P. philaxyridis* is
446 unknown but, in Europe, *P. fasciata* and another species, *Phalacrotophora berolinensis*
447 Schmitz (Diptera: Phoridae), parasitize several coccinellid species (Hodek 1973; Majerus
448 and Kearns 1989).

449 From this survey, it appears that the potential of using parasitoids for the biological
450 control of *H. axyridis* in its area of introduction is limited. The parasitoids found on
451 *H. axyridis* in Europe and North America are of marginal importance and, in the case of
452 *D. coccinellae*, not well adapted to *H. axyridis*. Thus, there is little prospect for their use in
453 conservation or augmentative biological control programmes. The specificity of the par-
454 asitoids found only in Asia remains to be investigated but they belong to genera that are
455 known to comprise rather polyphagous species, at least within the family Coccinellidae. It
456 must be noted, however, that our knowledge on the parasitoid complex of *H. axyridis* in its
457 native range is based on a couple of studies in Japan and Korea, whereas the largest part of
458 the native range has never been investigated for parasitism. The parasitoid complex of
459 indigenous ladybirds often comprises more than three species. For example, at least seven
460 parasitoids are known from *C. septempunctata* in Europe (Klausnitzer and Klausnitzer
461 1997). Larval parasitism has never been recorded on *H. axyridis*, although several par-
462 asitoids of the genera *Aprostocetus*, *Oomyzus* and *Tetrastichus* (Hymenoptera: Eulophidae),
463 *Homalotylus* (Hymenoptera: Encyrtidae) and *Pachyneuron* (Hymenoptera: Pteromalidae)
464 are common larval parasitoids of Coccinellidae (Klausnitzer and Klausnitzer 1997; Noyes
465 2007). Larval parasitism by Encyrtidae or Eulophidae may kill up to 90–95% of ladybird
466 populations and severely affect their impact as aphid or coccid predators (Iperti 1964;
467 Hodek 1973). Thus, it would be worthwhile investigating parasitism in *H. axyridis* in
468 regions which have never or rarely been surveyed, such as China and Siberia. It remains to
469 be seen whether a parasitoid may be found that is totally specific to *H. axyridis*, as this
470 would be a pre-requirement for introduction into Europe or America. Most ladybird par-
471 asitoids are specific to Coccinellidae or to coccinellid sub-families, but often rather
472 polyphagous at family or sub-family level (Klausnitzer and Klausnitzer 1997). An
473 exception may be *Homalotylus platynaspidis* Hofer (Hymenoptera: Encyrtidae), which, in
474 Europe, may be specific to *Platynaspis luteorubra* (Goeze) (Coleoptera: Coccinellidae)
475 (Hodek 1973), although it has been recently reported from *Scymnus subvillosus* (Goeze)
476 (Coleoptera: Coccinellidae) in Russia (Noyes 2007).

477 Nematodes

478 To our knowledge, nematodes have never been recorded from wild populations of
479 *H. axyridis*. Parasitic nematodes of the families Allantonematidae and Mermitidae are able
480 to infest ladybird populations (Iperti 1964). For example, Iperti (1964) found a parasitic
481 nematode (later described as *Parasitylenchus coccinellinae* Iperti & Van Waerebeke
482 (Tylenchida: Allantonematidae) in Iperti and van Waerebeke 1968) parasitizing the
483 intestine of adults of several ladybirds, particularly *Propylea quatuordecimpunctata* (L.)
484 (Coleoptera: Coccinellidae) and *Oenopia conglobata* (L.) (Coleoptera: Coccinellidae).



485 Parasitism rates in *P. quatuordecimpunctata* reached up to 46% in early autumn. The
486 infection was not lethal, but strongly reduced egg maturation (Iperti 1964). Commercially
487 available entomopathogenic nematodes (*Heterorhabditis bacteriophora* Poinar (Nematoda:
488 Heterorhabditidae) and *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae)
489 were tested on *H. axyridis* and other ladybirds by Shapiro-Ilan and Cottrell (2005) in the
490 USA. They demonstrated that while *H. axyridis* and *C. septempunctata* (invasive alien
491 species in USA) are susceptible to nematodes they were both more resistant than two
492 native species (*C. maculata* and *O. v-nigrum*).

493 Parasitic mite

494 The mite *Coccipolipus hippodamiae* (McDaniel & Moril) (Acari: Podapolipidae) is a
495 sexually transmitted ectoparasite of adult coccinellids in Europe (McDaniel and Morrill
496 1969; Webberley et al. 2004). It lives on the ventral surface of the elytra. Larval *C. hip-*
497 *podamiae* pass between male and female hosts during copulation, in either direction (Hurst
498 et al. 1995). Once on a new host, larval mites embed their mouthparts into the ventral
499 surface of the host elytra and feed on haemolymph, metamorphosing into adults. Within a
500 few weeks, much of the ventral surface of the elytra is covered with mite adults, eggs and
501 larvae (Webberley et al. 2002). In *A. bipunctata*, the mite has strong negative effects on
502 male and female hosts, increasing overwintering mortality, particularly in males (Web-
503 berley et al. 2002) and reducing both fecundity and egg viability in females, the latter
504 eventually to zero (Hurst et al. 1995). Similar, but marginally less extreme, negative
505 effects of the mite have been found on female *Adalia decempunctata* (L.) (Coleoptera:
506 Coccinellidae) and *O. conglobata* (Webberley et al. 2004). *Coccipolipus hippodamiae* is
507 found on a variety of coccinellids, but shows highest prevalence on *A. bipunctata*, where
508 over 90% of some populations are infected by late summer (Webberley et al. 2004).
509 Comparative analysis of prevalence levels of the mite, on four European coccinellids, has
510 indicated that prevalence is positively correlated to promiscuity and voltinism (Webberley
511 et al. 2006).

512 Due to the high negative effects that *C. hippodamiae* imposes on its hosts, coupled to
513 the behaviour and ecology of *H. axyridis* in Europe, this mite is a possible candidate for
514 use as a biological control agent of *H. axyridis*. Importantly, *H. axyridis* has behavioural
515 characteristics (high promiscuity, no requirement for dormancy before reproducing, mul-
516 tivoltinism, over-lapping generations), to facilitate both horizontal transmission and
517 transmission down the generations of the mite, leading to high prevalence once the mite
518 has established. What is currently not known is whether *C. hippodamiae* can use
519 *H. axyridis* as a host, and, if it can, whether the strong negative effects that the mite
520 imposes on *A. bipunctata* will also be imposed on *H. axyridis*. Encouragingly, preliminary
521 tests in which *C. hippodamiae* have been artificially transferred from wild French *Har-*
522 *monia quadripunctata* to *H. axyridis* have shown that the mite can feed and survive on
523 *H. axyridis* (M. Majerus unpublished data). Moreover, transfer to *H. axyridis* might come
524 about naturally, for inter-specific hybrid matings between *H. axyridis* and *A. bipunctata*
525 has been recorded in the field in Europe (Pascoe et al. 2007), and Webberley et al. (2004)
526 report that mites can successfully transfer between species during interspecific hybrid
527 matings. If it is proven that *C. hippodamiae* is detrimental to *H. axyridis* and if the transfer
528 from European ladybirds does not occur naturally, artificially infested *H. axyridis* adults
529 could be inoculated to field populations for further propagation. *Coccipolipus hippodamiae*
530 also occurs in North America (McDaniel and Morrill 1969), and may thus have a role to



531 play there. However, given the wide host range of the mite, an appropriate risk assessment
532 would be required before integrating this biological control agent into management pro-
533 grammes. An introduction to continents from which *C. hippodamiae* has not been reported
534 (South America, Africa) should not be considered, although other congeneric mites with
535 similar ecologies that occur on these continents may be considered as alternatives.

536 The efficacy of sexually transmitted diseases (STD) such as *C. hippodamiae* and
537 *H. virescens* as controlling agents for *H. axyridis* might be improved in an IPM strategy
538 that also involves the male-killer of *H. axyridis*. In species in which both sexes are highly
539 promiscuous, such as *H. axyridis*, the rate of spread of an STD will be greater in popu-
540 lations in which sex ratios are female biased because males, due to their scarcity, will have
541 more mating partners and so more opportunity to both contract and to pass on the disease
542 (Majerus 2003). Thus, in a population of *H. axyridis* that is female biased due to the
543 presence of a male-killer, an STD, such as *C. hippodamiae*, would reach higher prevalence
544 than in a population with a 1:1 sex ratio.

545 Conclusions

546 Developing control methods against the harlequin ladybird remains a challenge, for several
547 reasons. It is the first time a predatory ladybird, or any other predatory beetle, requires
548 control. Thus, there is no previous experience on which to base new management strate-
549 gies, and research to date has been very limited. Furthermore, as most other ladybirds are
550 considered beneficial insects, control methods against *H. axyridis* will have to be highly
551 specific to avoid non-target effects. Suitable control methods will continue to be developed
552 to prevent or control aggregations in houses and fruit damage. Besides the use of chemical
553 insecticides, which are efficient but should preferably be avoided to prevent their negative
554 impact on human health and the environment, the most promising new strategies are the
555 development of new cultural practices in vineyards and, especially of new trapping
556 methods based on semiochemicals. Our current understanding of pheromonal and kairo-
557 monal communication by coccinellids, and specifically *H. axyridis*, is still limited.
558 However, there are many effective model systems using semiochemical methods for
559 controlling pest insects, which could prove to be informative in developing strategies for
560 controlling *H. axyridis*.

561 While control methods presently used or under development may eventually solve the
562 problems in buildings, orchards or vineyards, it is very unlikely that any of these methods
563 will ever limit the impact of outbreak *H. axyridis* populations on native biodiversity.
564 Unless a native European or America natural enemy (e.g. *C. hippodamiae*) adapts itself to
565 the new invader and provides control, the only method that may eventually lower invasive
566 population densities in a sustainable way is the importation of natural enemies from the
567 region of origin. However, *H. axyridis* is a difficult target for classical biological control,
568 partly because the invasion of *H. axyridis* is, in itself, most probably the result of bad
569 biological control practices. Thus convincing the public, authorities and a part of the
570 scientific community that a biological control agent needs to be introduced to control
571 another biological control agent that turned bad may be challenging. In addition, a can-
572 didate biological control agent should be specific to *H. axyridis*. The chance of finding a
573 specific pathogen or parasitoid in Asia is low but not negligible. As in all classical bio-
574 logical control programmes, the risk of introducing an exotic agent has to be weighted
575 against the risk of doing nothing or the risk related to traditional control measures. In the
576 case of *H. axyridis*, the introduction of a biological control agent should be considered



577 only if there are clear signs that native ladybirds, or other aphidophagous insects, are
578 seriously threatened over a significant part of their distribution range. At present, the
579 priority for research is to gather more reliable and quantitative data on the exact impact of
580 *H. axyridis* on the native fauna in the invaded regions. In the meantime, comparative
581 assessments of mortality factors affecting populations of *H. axyridis* in the native range
582 and the areas of introduction should be carried out, e.g. through life table analysis, to
583 understand which factors limit population densities in the native range and which do not
584 occur in the regions of introduction. These studies would also allow the identification of
585 potential classical biological control agents that could be considered for introduction,
586 should the situation in the invaded regions become dramatic for native biodiversity.

587 Finally, it must be noted that, in North America, there are various agricultural contexts
588 where *H. axyridis* is highly valued as a biological control agent, particularly in corn,
589 soybeans, citrus and apples (Koch 2003; Pervez and Omkar 2006). A control method that
590 would provide area wide reductions in *H. axyridis* populations might also result in reduced
591 levels of aphid biocontrol and, thus, create a conflict of interest between the agricultural
592 sector and conservationists.

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596

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