

# The effect of the coccinellid *Harmonia axyridis* (Coleoptera: Coccinellidae) on transmission of the fungal pathogen *Pandora neoaphidis* (Entomophthorales: Entomophthoraceae)

PATRICIA M. WELLS<sup>1,2</sup>, JASON BAVERSTOCK<sup>1</sup>, MICHAEL E.N. MAJERUS<sup>2</sup>, FRANCIS M. JIGGINS<sup>2</sup>, HELEN E. ROY<sup>3</sup> and JUDITH K. PELL<sup>1</sup>

<sup>1</sup>Department of Plant and Invertebrate Ecology, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; e-mail: trish.wells@bbsrc.ac.uk

<sup>2</sup>Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK

<sup>3</sup>Centre for Ecology & Hydrology, Wallingford, Oxfordshire, OX10 8BB, UK

**Key words.** Coccinellidae, *Harmonia axyridis*, *Coccinella septempunctata*, multicoloured Asian ladybeetle, harlequin ladybird, seven-spot ladybird, Entomophthorales, *Pandora neoaphidis*, enhanced transmission, vectoring

**Abstract.** The coccinellid *Harmonia axyridis* is a recent arrival in the UK and is an intraguild predator of the entomopathogenic fungus *Pandora neoaphidis*. *Harmonia axyridis* entirely consumes *P. neoaphidis*-sporulating cadavers and this may have a negative effect on the epizootic potential of *P. neoaphidis*. Here we assessed within plant transmission, and between plant vectoring, of *P. neoaphidis* in the presence of either *H. axyridis* or *Coccinella septempunctata*, a native coccinellid that only partially consumes fungal cadavers. Transmission was greater in the presence of coccinellids, with 21% of aphids becoming infected with the fungus whilst only 4% were infected in the control. However, there was no significant effect of coccinellid species or sex on fungal transmission. Between plant vectoring occurred infrequently in the presence of both species of coccinellid. The effect of *H. axyridis* on *P. neoaphidis* transmission is, therefore, likely to be similar to that of the native coccinellid *C. septempunctata*.

## INTRODUCTION

Aphid populations are regulated by predators, pathogens and parasitoids. Interactions between aphid enemies may have positive, neutral or negative effects on pest control (Rosenheim et al., 1995; Ferguson & Stiling, 1996; Straub et al., 2008). It is difficult to predict the outcome of intraguild interactions on pest suppression, especially when a non-native polyphagous predator enters the guild. *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), known as the multicoloured Asian lady beetle and the harlequin ladybird, is a polyphagous coccinellid species that is not native to Britain but established in 2004 and is predicted to have a negative impact on biodiversity (Majerus et al., 2006; Roy et al., 2006; Brown et al., 2008; Ware & Majerus, 2008). Although *H. axyridis* is a dominant competitor over many native coccinellid species, the interactions between *H. axyridis* and non-coccinellid aphid natural enemies are less well studied (Pell et al., 2008). Here we present data on the interactions between *H. axyridis* and an aphid-specific pathogen.

*Pandora neoaphidis* (Remaudière & Hennebert) Humber is an aphid-specific entomopathogenic fungus in the order Entomophthorales that can cause epizootics in field populations (Feng et al., 1992; Pell et al., 2001; Barta & Cagan, 2006). Transmission of *P. neoaphidis* occurs via conidia that are forcibly ejected from dead, infected aphid cadavers and can remain infective for up to 14 days (Brobyn et al., 1985). These conidia are either deposited on aphid hosts, on the substrate surrounding the *P. neoaphidis*-sporulating cadavers or may pass through

the boundary layer of the plant and passively disperse on wind currents to new host populations (Brobyn et al., 1985; Hemmati et al., 2001). The presence of foraging native predators, parasitoids and extraguild co-occurring arthropods all increase local transmission of *P. neoaphidis* by increasing aphid movement and, therefore, the encounter rate with infective conidia (Pell et al., 1997; Feuntes-Contreras et al., 1998; Roy et al., 1998, 2001; Baverstock et al., 2008). The native aphid predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) also vectors *P. neoaphidis* to previously uninfected aphid colonies on different plants, thereby enhancing dispersal (Roy et al., 2001).

*Coccinella septempunctata* is an intraguild predator of *P. neoaphidis*-sporulating cadavers, but rarely entirely consumes these cadavers in Petri dish experiments (Roy et al., 1998, 2008). Although conidia production from partially consumed *P. neoaphidis*-sporulating cadavers is reduced there is no significant reduction in transmission of the fungus (Roy et al., 1998). In contrast, *H. axyridis* completely consumes *P. neoaphidis*-sporulating cadavers in Petri dish arenas and does not discriminate between dead uninfected aphid prey and sporulating cadavers (Roy et al., 2008). The intraguild interactions between *P. neoaphidis* and *H. axyridis* may therefore be different to those with *C. septempunctata*. Here we compare the effect of *H. axyridis* and *C. septempunctata* on the transmission of *P. neoaphidis* within an *Acyrtosiphon pisum* (Harris) colony and investigate the potential of the coccinellid

TABLE 1. Percentage of aphids recovered from single plant arenas in the absence and presence of *H. axyridis*. Within the *H. axyridis* present treatment the percent aphids recovered in treatments containing male or female *H. axyridis* in the presence or absence of fungus are shown. Lower (LCI) and upper (UCI) confidence intervals at the 95% level are shown with sample size (n). Values are back-transformed from the logistic scale.

Treatment	Aphids	LCI	UCI	n
No enemy control	96	94	98	18
<i>H. axyridis</i> present	82	78	86	36
♂	87	80	93	9
♂ + fungus	80	71	87	9
♀	81	72	86	9
♀ + fungus	77	68	85	9

nellids to vector *P. neoaphidis* between *A. pisum* colonies.

## MATERIAL AND METHODS

Single plant arenas, each consisting of one sixteen day old *Vicia faba* plant (L. Cultivar: The Sutton) contained in a lamp glass (1.4 litre capacity) were used. Each plant was infested with twenty adult *A. pisum* aphids and maintained at 18°C (16L : 8D), as described by Roy et al. (1998). Eight treatments were prepared: (1) no enemy control, (2) ♂ *H. axyridis*, (3) ♀ *H. axyridis*, (4) *P. neoaphidis*, (5) ♂ *H. axyridis* + *P. neoaphidis*, (6) ♀ *H. axyridis* + *P. neoaphidis*, (7) ♂ *C. septempunctata* + *P. neoaphidis* and (8) ♀ *C. septempunctata* + *P. neoaphidis*. Where required, *P. neoaphidis* was added as rehydrated pairs of *P. neoaphidis*-sporulating cadavers on a water agar plug (isolate X4, from the Rothamsted Research collection, original host = *A. pisum*). One cadaver pair was placed on the adaxial surface of the four largest top leaves. The experiment was split into two consecutive parts, (a) within plant transmission and (b) between plant vectoring. (a) Where required, a single adult coccinellid (sex recorded) that had been starved for 24 h was added to arenas. Coccinellids were removed after 4 h followed by *P. neoaphidis* and the aphids. Aphids were counted and transferred to new single plant arenas previously free from aphids. (b) Coccinellids were transferred to new single plant arenas (1 per arena) containing twenty uninfected adult *A. pisum* and were allowed to forage for 16 h. All arenas were sealed with cling film for the first 24 h of the experiment to ensure a high relative humidity to allow the fungus to germinate, after this time the cling film was replaced with muslin. The numbers of uninfected and *P. neoaphidis*-infected aphids were assessed after a further five days. The experiment was set up as a completely randomized block design with three blocks of ten arenas, and was repeated on three occasions. Each coccinellid treatment was tested once in each block and the coccinellid absent treatments twice. In total, each coccinellid treatment was replicated nine times and each coccinellid-absent treatments 18 times. The proportions of aphids recovered after the initial 4 h foraging period in the presence or absence of *H. axyridis* (treatments 1–7) were analysed using logistic regression (generalized linear model with binomial error and logit link) in GenStat (Payne et al., 2009). For the fungus-present treatments, the proportions of *P. neoaphidis* infected aphids recovered at the end of the experiment were also analysed using logistic regression. The overall treatment effect was partitioned into contrasts representing a comparison between the coccinellid-absent treatment (treatment 4) and all coccinellid-present treatments combined (treatments

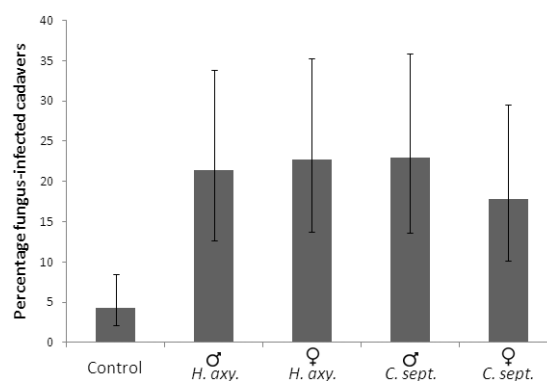


Fig. 1. Percentage of fungus-infected cadavers found in single plant arenas six days after initial exposure to inoculum. All treatments contained aphids and fungus plus: no predator (control) or a single ♂ *H. axyridis*, ♀ *H. axyridis*, ♂ *C. septempunctata* or ♀ *C. septempunctata*. Means and 95% confidence intervals shown are back-transformed from the logistic scale.

5–8), and the main effects of coccinellid species, coccinellid sex and their interaction within the coccinellid-present combinations. Over dispersion was accounted for by comparing ratios of deviances to the residual mean deviance against critical values of the F distribution. Means and 95% confidence intervals shown are back-transformed from the logistic scale.

## RESULTS AND DISCUSSION

Foraging by *H. axyridis* decreased the proportion of aphids recovered ( $\chi^2 = 47.44$ ,  $df = 1$ ,  $p < 0.001$ ) (Table 1). There was no difference in the proportion of aphids eaten by *H. axyridis* in the presence or absence of *P. neoaphidis* ( $\chi^2 = 1.08$ ,  $df = 1$ ,  $p = 0.345$ ) or by male and female *H. axyridis* ( $\chi^2 = 1.68$ ,  $df = 1$ ,  $p = 0.20$ ) (Table 1). There was no interaction between these treatments ( $\chi^2 = 0.55$ ,  $df = 1$ ,  $p = 0.463$ ). Significantly more aphids became infected with *P. neoaphidis* in treatments that contained a foraging coccinellid compared to those that did not ( $F_{1,41} = 33.42$ ,  $p < 0.001$ ) with infection levels at 21% (95% CI: 15–28%) and 4% (95% CI: 2–8%) respectively. However, transmission in the presence of *H. axyridis* and *C. septempunctata* was not significantly different ( $F_{1,41} = 0$ ,  $p = 0.997$ ) with a mean of 22% (95% CI: 7–51%) and 21% (95% CI: 7–49%) *P. neoaphidis*-infected aphids recovered in the presence of *H. axyridis* and *C. septempunctata* respectively (Fig. 1). There was no significant effect of coccinellid sex on transmission ( $F_{1,41} = 0$ ,  $p = 0.973$ ) nor was there an interaction between species and sex ( $F_{1,41} = 1.06$ ,  $p = 0.309$ ) (Fig. 1). The complete consumption of a *P. neoaphidis*-sporulating cadaver by a coccinellid was only observed on one occasion and was by a female *H. axyridis*. Partial consumption of cadavers may have occurred but this could not be determined after sporulation. Vectoring occurred on three occasions. Two *C. septempunctata* males vectored *P. neoaphidis*, with a single adult aphid becoming infected on each occasion. One female *H. axyridis* vectored *P. neoaphidis*, infecting three nymphs. There were no *P. neoaphidis*-infected aphids in treatments that did not contain *P. neoaphidis*.

Under natural conditions *H. axyridis*, *C. septempunctata* and *P. neoaphidis* co-occur on plants such as bean and common nettle (unpubl. data). The transmission of *P. neoaphidis* was similar in the presence of both coccinellid species, despite *H. axyridis* being a greater intraguild predator of *P. neoaphidis* in previous Petri dish studies (Roy et al., 2008). The positive effect on transmission in the presence of this non-native species is therefore similar to that found in the presence of native intra-guild and extra-guild species (Roy et al., 1998; Baverstock et al., 2008, 2009). Similar increases in *P. neoaphidis* transmission as a result of foraging by *C. septempunctata* were found by Roy et al. (1998) and Ekesi et al. (2005). Lower levels of transmission were found in this study, possibly because aphids were transferred to clean plants after being exposed to foraging coccinellids thereby preventing further transmission of conidia from the plant surface. Alternatively, the lower levels of transmission observed in this study may have been due to the age-dependent susceptibility of *A. pisum* to *P. neoaphidis* (Milner, 1982, 1985; Lizen et al., 1985). Indeed, Roy et al. (1998) assessed transmission of *P. neoaphidis* to 4<sup>th</sup> instar *A. pisum* which may have been more susceptible to *P. neoaphidis* than the adult aphids assessed here.

In the current study, no fungal cadavers were consumed by *C. septempunctata* and only one fungal cadaver was completely consumed by *H. axyridis* within four hours. This was surprising given that *H. axyridis* showed no discrimination between dead uninfected aphids and *P. neoaphidis*-sporulating cadavers in previous Petri dish experiments whereas *C. septempunctata* showed a preference for uninfected aphids (Roy et al., 2008). An increase in the proportion of uninfected prey will result in a reduced encounter rate between the coccinellid predator and fungal cadavers and, if the predator does not show a preference between prey types, this may decrease the predation of fungal cadavers. In addition, the presence of *P. neoaphidis* did not affect the predation of uninfected aphids by *H. axyridis*, therefore, *P. neoaphidis* may not disrupt aphid suppression by *H. axyridis* at the single plant scale.

Vectoring of conidia by non-host insects has been observed for entomopathogenic fungi in both the Hypocreales and the Entomophthorales and has been investigated in three collembolan species (Dromph, 2003), a black ant (Bird et al., 2004) and a bug (Down et al., 2009). In this study *H. axyridis* and *C. septempunctata* vectored *P. neoaphidis* at a similar rate. However, vectoring was very infrequent and experiments at a more realistic scale are required to determine the importance of vectoring for *P. neoaphidis*. Roy et al. (2001) showed that *C. septempunctata* could vector *P. neoaphidis* and that vectoring events are highly irregular, with no correlation between initial level of pathogen exposure and the number of aphids that became infected. In addition, *C. septempunctata* adults that had foraged on non-crop plants found in field margins such as nettle, knapweed or birds foot trefoil containing *P. neoaphidis* were able to vector the pathogen to aphids on bean plants (Ekesi et al.,

2005). In contrast to the passive dispersal of infective conidia on wind currents, the dispersal of *P. neoaphidis* by coccinellids is targeted as the pathogen is directly transported between aphid colonies. When *P. neoaphidis*-infected cadavers are low in number or the habitat is diverse, targeted dispersal by coccinellids could be the most important mode of dispersal (Roy et al., 2001; Baverstock et al., 2010). However, Roy et al. (2003) found that *C. septempunctata* inoculated with *P. neoaphidis* were only able to vector the pathogen within 4 h of inoculation and conidia vectored onto plants by foraging *C. septempunctata* were only infective up to 24 h post conidia dispersal (Roy et al., 2003). Nonetheless, vectoring is seen as an important form of dispersal and methods of manipulating this in augmentative and conservation biocontrol strategies are being investigated (Furlong et al., 1995; Bird et al., 2004; Ekesi et al., 2005; Down et al., 2009; Baverstock et al., 2010).

In conclusion, *H. axyridis* increases within plant transmission of *P. neoaphidis* and can vector the pathogen between aphid populations on different plants. The effect *H. axyridis* could have on *P. neoaphidis* epizootiology is, therefore, likely to be similar to that of the native coccinellid *C. septempunctata*. Experiments at larger temporal and spatial scales under more realistic conditions are needed to determine the effect *H. axyridis* will have on *P. neoaphidis* at the landscape level.

ACKNOWLEDGEMENTS. PMW is supported by the Biotechnology and Biological Sciences Research Council of the United Kingdom (BBSRC). JB is supported by the U.K. Department for Environment, Food and Rural Affairs (Defra). JKP is supported by Defra and BBSRC. Rothamsted Research is an Institute of the BBSRC. HER is in the Biological Records Centre (BRC) within the NERC Centre for Ecology & Hydrology. BRC is co-funded by the Joint Nature Conservation Committee. We thank Suzanne Clark for assistance with statistical methods.

## REFERENCES

- BARTA M. & CAGAN L. 2006: Observations on the occurrence of Entomophthorales infecting aphids (Aphidoidea) in Slovakia. *BioControl* **51**: 795–808.
- BAVERSTOCK J., BAVERSTOCK K.E., CLARK S.J. & PELL J.K. 2008: Transmission of *Pandora neoaphidis* in the presence of co-occurring arthropods. *J. Invert. Pathol.* **98**: 356–359.
- BAVERSTOCK J., CLARK S.J., ALDERSON P.G. & PELL J.K. 2009: Intraguild interactions between the entomopathogenic fungus *Pandora neoaphidis* and an aphid predator and parasitoid at the population scale. *J. Invert. Pathol.* **102**: 167–172.
- BAVERSTOCK J., ROY E.H. & PELL J. 2010: Entomopathogenic fungi and insect behaviour: from unsuspecting hosts to targeted vectors. *BioControl* **55**: 89–102.
- BIRD A.E., HESKETH H., CROSS J.V. & COPLAND M. 2004: The common black ant, *Lasius niger* (Hymenoptera: Formicidae), as a vector of the entomopathogen *Lecanicillium longisporum* to rosy apple aphid, *Dysaphis plantaginea* (Homoptera: Aphididae). *Biocontr. Sci. Techn.* **14**: 757–767.
- BROBYN P.J., WILDING N. & CLARK S.J. 1985: The persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis* on leaves in the field. *Ann. Appl. Biol.* **107**: 365–376.
- BROWN P.M.J., ROY H.E., ROTHERY P., ROY D.B., WARE R.L. & MAJERUS M.E.N. 2008: *Harmonia axyridis* in Great Britain:

- analysis of the spread and distribution of a non-native coccinellid. *BioControl* **53**: 55–67.
- DOWN R.E., CUTHBERTSON A.G.S., MATHERS J.J. & WALTERS K.F.A. 2009: Dissemination of the entomopathogenic fungi, *Lecanicillium longisporum* and *L. muscarium*, by the predatory bug, *Orius laevigatus*, to provide concurrent control of *Myzus persicae*, *Frankliniella occidentalis* and *Bemisia tabaci*. *Biol. Control* **50**: 172–178.
- DROMPH K.M. 2003: Collembolans as vectors of entomopathogenic fungi. *Pedobiologia* **47**: 245–256.
- EKESI S., SHAH P.A., CLARK S.J. & PELL J.K. 2005: Conservation biological control with the fungal pathogen *Pandora neoaphidis*: implications of aphid species, host plant and predator foraging. *Agr. Forest Entomol.* **7**: 21–30.
- FENG M.G., NOWIERSKI R.M., JOHNSON J.B. & POPRAWSKI T.J. 1992: Epizootics caused by entomophthorean fungi (Zygomycetes, Entomophthorales) in populations of cereal aphids (Hom., Aphididae) in irrigated small grains of southeastern Idaho, USA. *J. Appl. Entomol.* **113**: 376–390.
- FERGUSON K.I. & STILING P. 1996: Non-additive effects of multiple natural enemies on aphid populations. *Oecologia* **108**: 375–379.
- FEUNTES-CONTRERAS E., PELL J. & NIEMEYER H.M. 1998: Influence of plant resistance at the third trophic level: interactions between parasitoids and entomopathogenic fungi of cereal aphids. *Oecologia* **117**: 426–432.
- FURLONG M.J., PELL J.K., CHOO O.P. & RAHMAN S.A. 1995: Field and laboratory evaluation of a sex-pheromone trap for the autodissemination of the fungal entomopathogen *Zoopthora radicans* (Entomophthorales) by the Diamond-back moth *Plutella xylostella* (Lepidoptera, Yponomeutidae). *Bull. Entomol. Res.* **85**: 331–337.
- HEMMATI F., PELL J.K., MCCARTNEY H.A., CLARK S.J. & DEADMAN M.L. 2001: Conidial discharge in the aphid pathogen *Erynia neoaphidis*. *Mycol. Res.* **105**: 715–722.
- LIZEN E., LATTEUR G. & OGER R. 1985: Sensitivity to infection by the entomophthoraceous fungus *Erynia neoaphidis* Remaud. & Henn. of the aphid *Acyrtosiphon pisum* (Harris) according to its form, instar and age. *Parasitica* **41**: 163–170.
- MAJERUS M., STRAWSON V. & ROY H. 2006: The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. *Ecol. Entomol.* **31**: 207–215.
- MILNER R.J. 1982: On the occurrence of pea aphids, *Acyrtosiphon pisum*, resistant to isolates of the fungal pathogen *Erynia neoaphidis*. *Entomol. Exp. Appl.* **32**: 23–27.
- MILNER R.J. 1985: Distribution in time and space of resistance to the pathogenic fungus *Erynia neoaphidis* in the pea aphid *Acyrtosiphon pisum*. *Entomol. Exp. Appl.* **37**: 235–240.
- PAYNE R.W., HARDING S.A., MURRAY D.A., SOUTAR D.M., BAIRD D.B., GLASER A.I., CHANNING I.C., WELHAM S.J., GILMOUR A.R., THOMPSON R., WEBSTER R. 2009: *The Guide to GenStat Release 12, Part 2*. VSN International, Hemel Hempstead.
- PELL J.K., PLUKE R., CLARK S.J., KENWARD M.G. & ALDERSON P.G. 1997: Interactions between two aphid natural enemies, the entomopathogenic fungus *Erynia neoaphidis* Remaudiere & Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *J. Invert. Pathol.* **69**: 261–268.
- PELL J., EILENBERG J., HAJEK A. & STEINKRAUS D.C. 2001: Exploring the potential of entomophthorales in pest management. In Butt T.M., Jackson C. & Magan N. (eds): *Fungi as Biocontrol Agents: Progress, Problems and Potential*. CABI, Wallingford, pp. 71–153.
- PELL J.K., BAVERSTOCK J., ROY H.E., WARE R.L. & MAJERUS M.E.N. 2008: Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. *BioControl* **53**: 147–168.
- ROSENHEIM J.A., KAYA H.K., EHLER L.E., MAROIS J.J. & JAFFEE B.A. 1995: Intraguild predation among biological-control agents – Theory and evidence. *Biol. Control* **5**: 303–335.
- ROY H.E., PELL J.K., CLARK S.J. & ALDERSON P.G. 1998: Implications of predator foraging on aphid pathogen dynamics. *J. Invert. Pathol.* **71**: 236–247.
- 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*. *Biocontr. Sci. Technol.* **11**: 99–110.
- ROY H.E., ALDERSON P.G. & PELL J.K. 2003: Effect of spatial heterogeneity on the role of *Coccinella septempunctata* as an intraguild predator of the aphid pathogen *Pandora neoaphidis*. *J. Invert. Pathol.* **82**: 85–95.
- ROY H.E., BROWN P.M.J. & MAJERUS M.E.N. 2006: *Harmonia axyridis*: a successful biocontrol agent or an invasive threat? In Eilenberg J. & Hokkanen H. (eds): *An Ecological and Societal Approach to Biological Control*. Kluwer Academic Publishers, Dordrecht, pp. 295–309.
- ROY H.E., BAVERSTOCK J., WARE R.L., CLARK S.J., MAJERUS M.E.N., BAVERSTOCK K.E. & PELL J.K. 2008: Intraguild predation of the aphid pathogenic fungus *Pandora neoaphidis* by the invasive coccinellid *Harmonia axyridis*. *Ecol. Entomol.* **33**: 175–182.
- STRAUB C.S., FINKE D.L. & SNYDER W.E. 2008: Are the conservation of natural enemy biodiversity and biological control compatible goals? *Biol. Control* **45**: 225–237.
- WARE R.L. & MAJERUS M.E.N. 2008: Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. *BioControl* **53**: 169–188.

Received July 4, 2010; revised and accepted November 11, 2010