Overwintering Strategy of Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae): Cold-Free Space As a Factor of Invasive Success

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Ann. Entomol. Soc. Am. 101(5): 860–866 (2008)

ABSTRACT Successful overwintering of a coccinellid species is partially dependent on quality of hibernacula, for surviving cold temperatures, for drowning risk, and for avoiding parasitism or infection by pathogens. The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), an introduced species that has spread throughout North America since 1988, overwinters outside and inside houses or artificial structures. Five experiments were conducted during winter 2003–2004 to evaluate winter survival of the invasive *H. axyridis* compared with the indigenous *Coleomegilla maculata lengi* Timberlake outside and inside human houses, to assess its contribution to the invasive success of the multicolored Asian lady beetle. We formulated the hypothesis that *H. axyridis* will survive during winter inside human houses and that no survival would be observed outside in Quebec, Canada. Survival of *H. axyridis* ranged from 25 to 53% in different experiments inside houses, whereas no survival was recorded outside. *C. maculata* did not survive inside houses, but 12.5% of the individuals survived outside. The indigenous species *C. maculata* had higher proportion of lipids than the invasive species *H. axyridis*, suggesting that *H. axyridis* is not physiologically adapted to overwinter in Canada. Selection of human houses as overwintering sites by the multicolored Asian lady beetle may constitute a "cold-free space," which could explain its great invasive success in northern regions.

KEY WORDS overwintering survival, *Harmonia axyridis*, cold-free space, *Coleomegilla maculata lengi*

In temperate regions, overwintering strategies and cold survival may greatly influence the invasive success of a species. In many insects, for example, the level of winter survival is the primary determinant of their future abundance (Honěk 1989, Bale 1991). Choice of overwintering sites also could reduce natural enemies of these insects, such as entomopathogens or parasites that are commonly present on or in these sites (Honěk 1989). Entomogenous fungi may be present either on individuals that invade hibernacula or in soil of these sites, and they will infect individuals in spring, when humidity is very high (Honěk 1989, Nedved 1993, Ceryngier 2000, Riddick and Schaefer 2005, Harwood et al. 2006, Riddick 2006). Parasitoids in hibernacula may either enter diapause together with their hosts, or they may promptly develop inside the adult stage in the hosts, which are still not dormant (Hodek 1973, Ceryngier and Hodek 1996). For invasive insect species in temperate areas, overwintering strategies and survival should be determinant to reach great abundance and reinvade in high density the environment after each winter.

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The Palearctic multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), has spread rapidly through North America since 1988 (Chapin and Brou 1991, Tedders and Schaefer 1994, Coderre et al. 1995, Dreistadt et al. 1995, Colunga-Garcia and Gage 1998). This coccinellid species was introduced many times during the last century as a biological control agent of aphids and scales (McClure 1987, Tedders and Schaefer 1994). *H. axyridis* was first observed in Canada in 1994 (Coderre et al. 1995), and it is now widely distributed throughout Quebec Province (Lucas et al. 2002; E.L. et al., unpublished data), as far as 48′ latitude (G.L., unpublished data).

In Asia, *H. axyridis* overwinters in crevices in rocks or in concrete objects such as buildings, and sometimes in leaf litter (Obata 1986, Sakurai et al. 1993). The species shows a hypsotactic aggregation, i.e., it is connected with prominent isolated and contrasting objects visually present in relatively dry situations (Obata 1986, Obata et al. 1986, Sakurai et al. 1992, Nalepa et al. 2005), and hibernacula are found in the same place every year (Obata 1986). In Japan, the second or third generation of *H. axyridis* aggregates at overwintering sites in early November, and adults

0013-8746/08/0860–0866$04.00/0 © 2008 Entomological Society of America

enter reproductive diapause before winter, decrease their respiration rate, and accumulate glycogen and lipids (Sakurai et al. 1992). The adults emigrate to breeding sites in late March (Obata 1986, Osawa 2001). *H. axyridis* also accumulate myoinositol as a cryoprotectant, and it is considered as a freeze-intolerant species with a certain degree of chill tolerance that changes its supercooling ability seasonally (Somme 1982, Watanabe 2002, Koch et al. 2004). Experiments have shown that this species is paralyzed at −5°C, with an optimum overwintering temperature between 0 and −5°C, with considerable mortality occurring at −20°C (Watanabe 2002).

In North America, the most obvious overwintering sites of the multicolored Asian lady beetle are houses or other artificial structures, where they can constitute a nuisance for human beings (Kidd and Nalepa 1995; LaMana and Miller 1996; Nalepa et al. 1996; Hagley 1999; Yarbrough et al. 1999; Riddick et al. 2000, 2004; Huelsman et al. 2001; McCutcheon and Scott 2001; Schaefer 2003; Kovach 2004; Lucas et al. 2007). A study of caged lady beetles in Connecticut (McClure 1987) reported >90% overwintering mortality the first year and 100% mortality the next year, suggesting that low winter temperatures may be an important factor regulating populations of *H. axyridis* from year to year. Laboratory experiments have shown that the supercooling point of adult *H. axyridis* in the United States ranges from −12 to −16°C (Koch et al. 2004). Thus, this species cannot survive prolonged freezing below −16°C in the United States. The capacity of *H. axyridis* to survive winter conditions in northern locations may be more related to the availability of quality overwintering sites than to its capacity to increase cold hardiness (Koch et al. 2004).

The objective of this study was to evaluate the overwintering strategies of *H. axyridis* in northern North America as a contribution to its invasive success. We hypothesized that the multicolored Asian lady beetle increases its survival by overwintering in human houses. We chose to compare overwintering survival of *H. axyridis* with that of an ecologically similar indigenous species, *Coleomegilla maculata lengi* Timberlake. Observations of the invasive species at overwintering sites of this indigenous species have been reported in autumn (E.L., unpublished data).

Materials and Methods

Five experiments were conducted in southeastern Canada (Québec province) during winter 2003–2004 inside and outside houses to assess overwintering survival of *H. axyridis* and *C. maculata.* Specimens of *H. axyridis* were collected on exterior walls of houses (72° 42′ W, 45° 23′ N) when they aggregated in mass in October and November 2003. Specimens of *C. maculata* were collected beneath leaf litter in November 2003 on an overwintering site under willow, *Salix nigra* Marsh. (72° 56′ W, 45° 39′ N), where thousands of beetles hibernate year after year. *H. axyridis* individuals also were observed on these sites, on the bark of trees, or in the leaf litter (E.L., unpublished data). Both species were kept 1 wk in incubators set at 10°C with water until beginning the experiments.

Experiment 1: Winter Survival Outside. One hundred individuals of *H. axyridis* and *C. maculata* were marked with white paint on one elytra and deposited on three overwintering sites of *C. maculata* ($n = 300$) (72° 56′ W, 45° 39′ N; 72° 97′ W, 45° 28′ N; 72° 92′ W, 45° 35′ N) where individuals of *H. axyridis* were often seen in autumn. These sites were under big trees (willow, *S. nigra* Marsh.; poplar, *Populus* sp.; and maple, *Acer saccharum* Marsh.) and under snow cover during most of the winter. Individuals were placed in cavities beneath the leaf litter at the tree base. Temperature during release day in November 2003 was 5°C and 10°C in April 2004. Survival and gender were determined in laboratory and compared between species by likelihood ratio G-test (Sokal and Rohlf 1995).

Experiment 2: Winter Survival in Muslin Bags in Houses. Three closed muslin bags (10 by 10 cm) with sterile and dry wooden chips containing 20 coccinellids (*H. axyridis* or *C. maculata*) were installed in the basement of three houses ($n = 180$) (72° 42′ W, 45° 23′ N; 73° 45′ W, 45° 28′ N; 71° 55′ W, 45° 22′ N) in November 2003 and removed in April 2004. Muslin bags were installed on the floor in a corner of the basement, where they could be typically found (G.L., unpublished data). No food or water was available to the beetles. Temperature and humidity were recorded weekly by electronic thermometer. Survival was recorded in the laboratory as lady beetles walking normally after 24 h (Watanabe 2002), and gender of the individuals was determined. Differences in survival between species and gender were analyzed by likelihood ratio G-test (Sokal and Rohlf 1995).

Experiment 3: Winter Survival of *H. axyridis* in Quebec Houses. *H. axyridis* were collected in 15 houses in four regions of Quebec province, Canada (74° 10′ W to 70° 98′ W; 45° 08′ N to 46° 83′ N), during 1 d in March and April 2004. All apparent dead and live coccinellids were collected, and gender of each individual was determined. Temperature and relative humidity inside the house during the day of collection were recorded. Survival differences between genders were determined by chi-square analysis (Sokal and Rohlf 1995).

Experiment 4: Winter Survival in Controlled Chambers. Six petri of 10 *H. axyridis* and *C. maculata* with sterile and dry wooden chips were installed in controlled chambers at −5 and 10°C from the end of November 2003 to April 2004. Relative humidity was kept constant at 60%, which corresponded to the maximal relative humidity found in houses. Two petri dishes of each species and temperature treatment were removed each month from January to the end of March to assess survival and gender. Survival was compared between temperatures and month with likelihood ratio G-test.

Experiment 5: Proportion of Lipids. For experiments 1–4, proportion of lipids in the weight of coccinellids was determined for live individuals. For outside individuals, the measurement of proportion of lipids in *C. maculata* was conducted on 25 individuals captured on the overwintering site in November 2003 and at the beginning of April 2004. This measurement also was carried out on 25 *H. axyridis* captured outside houses in November and in April around houses. In muslin bags and controlled chambers, these measures were recorded on live individuals. In Quebec houses, the lipids were extracted for 20 individuals by site when available. Dry weight was measured with an analytical balance after 48 h in a dry oven at 60°C. Lipid weight was determined by the method of Huey (1966) and Zitzman and May (1989). Coccinellids were pierced two times on upper and underside of the abdomen with a fine needle and dried for 48 h. They were weighed (dry weight; DW) and put individually in 0.5-ml Eppendorf vials in a methanol/chloroform mixture (2:1) for 3 d. During this period, the solvents dissolved the fats diffusing from the insects. They were removed and dried outside during 24 h. They were dried in an oven during another 48 h and weighed again (lean weight; LW). The weight difference obtained determined the lipid weight (DW − LW). The proportion of lipids was calculated as the lipid weight on the lean weight ((DW − LW)/LW) for size standardization to eliminate dependency of fresh weight on water and fat (Nedved and Windsor 1994). The proportion of lipids was compared for each independent variable by analysis of variance (ANOVA) (Sokal and Rohlf 1995). Data on proportion of lipids were transformed by arcsine square root (Sokal and Rohlf 1995). All statistical analyses were performed with JMP 5.0 (SAS Institute 2002).

Results

Experiment 1: Winter Survival Outside. All 300 *H. axyridis* recaptured in April on the three overwintering sites were dead. There were 72 *C. maculata* recaptured in April, and nine of these beetles were alive, for a total 12.5 ± 5.53% survival. We did not attempt to evaluate survival with total individuals because they could have walked away, or died. There was no difference in survival between sites for *C. maculata* (G = 5.26, df = 72, $P = 0.072$), so data were pooled. Survival was significantly higher for *C. maculata* than for *H. axyridis* (G = 30.52, df = 372, $P < 0.0001$). There was no survival difference between genders for *C. maculata* (G = 0.51, df = 72, $P = 0.47$).

Experiment 2: Winter Survival in Muslin Bags in Houses. Mean relative humidity of houses was 30%, with a temperature fluctuating between 4 and 11°C. There was 24.81 ± 3.95% survival of *H. axyridis* in the muslin bags installed in three heated houses. Survival of *H. axyridis* was not different among houses, so data were pooled. No survival difference was observed between genders for *H. axyridis* ($\chi^2 = 2.18$, df = 2, $P = 0.34$). All 180 *C. maculata* in muslin bags inside the three houses were dead before the end of the experiment in April 2004. Survival was higher for *H. axyridis* than for *C. maculata* ($\chi^2 = 4.35$, df = 1, $P = 0.04$).

Experiment 3: Winter Survival of *H. axyridis* in Quebec Houses. During March and April 2004, 2,119 *H. axyridis* were collected in 15 houses. Mean survival of *H. axyridis* was 53.0 ± 6.18%. Survival of females was greater than males ($\chi^2 = 106.87$, df = 1, $P < 0.0001$) (Fig. 1). Temperature in the houses ranged from 7 to 31°C, and relative humidity ranged from 25 to 59%.

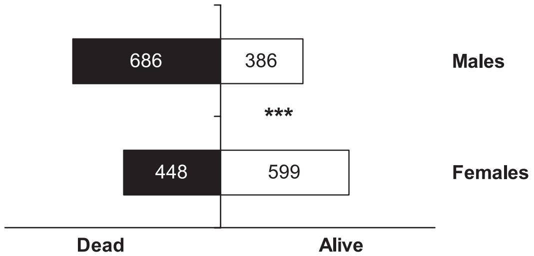

Fig. 1. Survival of females and males of *H. axyridis* collected in 15 houses in Quebec province of Canada during winter 2003–2004. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Experiment 4: Winter Survival in Controlled Chambers. A significant decrease in survival of *H. axyridis* between months was observed at −5°C (G = 9.96, df = 60, $P = 0.007$) and 10°C (G = 60.08, df = 58, $P = 0.0001$) (Fig. 2). At the end of winter, 45% of *H. axyridis* had survived at −5°C, whereas no survival at 10°C was reported. Survival was similar at the two temperatures during January (G = 2.77, df = 39, $P = 0.096$) and February (G = 2.18, df = 40, $P = 0.14$), but there was higher survival at −5°C in March than at 10°C (G = 14.61, df = 39, $P = 0.0001$) (Fig. 2). No survival difference between male and female *H. axyridis* was observed at −5°C (G = 0.02, df = 59, $P = 0.90$) or 10°C (G = 0.08, df = 58, $P = 0.78$) for all months. No individuals of *C. maculata* survived inside controlled chambers at the two temperatures from the beginning of January.

Experiment 5: Proportion of Lipids. *Outside Houses.* In November 2003 and April 2004, there was a greater proportion of lipids in *C. maculata* than in *H. axyridis* and in females than in males (Tables 1 and 2).

Inside Houses. In muslin bags in houses, the proportion of lipids in *H. axyridis* was not different between houses, so data were pooled for subsequent analysis. No proportion of lipid differences was ob-

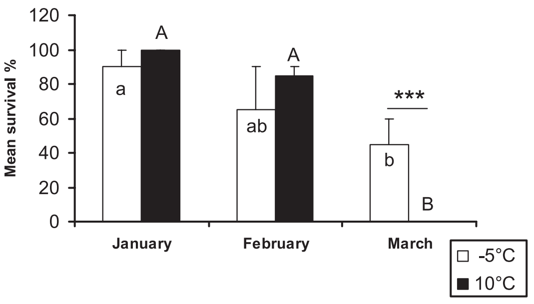

Fig. 2. Survival of *H. axyridis* inside controlled chambers at −5 and 10°C during winter 2003–2004. Different letters indicate significant differences ($P < 0.05$) between months at −5°C (lowercase letters) and 10°C (uppercase letters). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 1. Proportion of lipids of females (♀) and males (♂) of *H. axyridis* and *C. maculata* outside houses, inside houses, and in controlled chambers during winter 2003–2004

Exp	Variable	Species	Proportion of lipids (%) (mean ± SEM)	
			♀	♂
Outside	Nov.	*H. axyridis*	35.49 ± 3.30	26.41 ± 3.10
		C. maculata	73.18 ± 5.73	61.40 ± 5.05
Outside	April[a]	*H. axyridis*	23.95 ± 2.70	16.54 ± 2.08
		C. maculata	56.13 ± 3.18	45.31 ± 6.14
Inside	Muslin bags	*H. axyridis*	21.06 ± 1.35	22.02 ± 2.49
		C. maculata		
Inside	Quebec houses	*H. axyridis*	21.05 ± 1.10	17.65 ± 0.90
Controlled chambers	−5°C	*H. axyridis*	46.36 ± 2.58	40.26 ± 4.74
	−5°C	*C. maculata*		
	10°C	*H. axyridis*	32.73 ± 2.43	28.86 ± 2.75
	10°C	*C. maculata*		

[a] *H. axyridis* adults were collected outside, around houses.

served between genders ($F_{1, 41} = 0.06$; df = 1, 41; P = 0.81) (Table 1).

In Quebec houses, *H. axyridis* females in the 15 houses had a higher proportion of lipid (F = 4.86; df = 1, 221; P = 0.029) than did males (Table 1).

Controlled Chambers. No differences in proportion of lipids were found among the 3 mo at the two temperatures in incubators; therefore, data were pooled for subsequent analyses. The proportion of lipids in *H. axyridis* at −5°C was greater than at 10°C (Tables 1 and 2).

Discussion

We confirmed our hypothesis that *H. axyridis* was unable to survive outside human houses in southeastern Canada, whereas the indigenous species *C. maculata* had 12.5% survival. Conversely, *H. axyridis* survivorship inside houses ranged from 25 to 53% in different trials, whereas no *C. maculata* survived. These results suggest that the invasive success of *H. axyridis* may be linked to a cold-free space selection during winter.

Winter conditions in northeastern Canada can be detrimental to invasive species that are not morphologically, physiologically, or behaviorally adapted to these conditions. Temperatures during winter 2003–2004 ranged between 5 and −25°C, with 26 d below −15°C (normal conditions in this area; Environment Canada 2006). Air temperature above the snow line can be between −20 and −40°C during winter (Bale 1991). Fluctuating air temperature and snow cover are observed year after year, and they can generate high mortality in overwintering animals (Bale 1991). For example, mortality of *C. maculata* during winter can be variable among sites, ranging from 3 to 100% (Benton and Crump 1979, Wright and Laing 1982). The same trend in Europe is observed for *Coccinella septempunctata* L. where mortality ranged from 24 to 100% (Honěk 1997). No survival of *H. axyridis* outside on overwintering sites was observed in our experiments, whereas *C. maculata* survived at 12.5%. Experiments of Koch et al. (2004) demonstrated that 80% of the *H. axyridis* died when exposed to −15°C and 100% died at −20°C after 24 h. No winter survival also was observed by McClure (1987) in Connecticut on caged *H. axyridis* the second year of the experiment. In our experiment, no snow cover was present during some weeks in December and January, with very low temperatures, which may explain total mortality of the invasive species. Although observed on overwintering sites of *C. maculata, H. axyridis* should better survive in bark crevices or tops of hills (Obata 1986; Obata et al. 1986; Sakurai et al. 1992, 1993; Hodek and Honěk 1996). Additional observations of the multicolored Asian lady beetle overwintering on top of hills near Montreal (Quebec) (73° 09′ W, 45° 18′ N; 71° 82′ W, 45° 73′ N), showed that individuals observed were dead at the end of winter (E.L., personal communication).

Conversely, inside houses, temperature and relative humidity are much more stable during winter, and they may provide safe habitat during this period. Human houses could constitute a cold-free space for animal species able to exploit them during a part of their life cycle. The indigenous species *C. maculata* was unable to survive inside houses or in controlled chambers in our experiments. These results are in accordance with the experiments of Jean et al. (1990), who demonstrated no winter survival of this species at

Table 2. Analyses of proportion of lipids of *H. axyridis* and *C. maculata* outside houses in Nov. 2003 and April 2004 and in controlled chambers

Exp	Effect	df	F	P
Outside, Nov.	Whole model	3	22.83	<0.0001
	Species	1	65.32	<0.0001
	Gender	1	5.92	0.021
	Species × gender	1	0.02	0.89
	Error	31		
Outside, April	Whole model	3	27.47	<0.0001
	Species	1	74.18	<0.0001
	Gender	1	7.57	0.009
	Specie × gender	1	0.006	0.94
	Error	41		
Controlled chambers	Whole model	3	6.51	0.0006
	Temperature	1	13.93	0.0004
	Gender	1	3.14	0.081
	Temperature × gender	1	0.15	0.70
	Error	71		

temperatures >0°C. Relative humidity was also very low in our experiments (ranging from 30 to 60%), and *C. maculata* is known to be very susceptible to desiccation (Benton and Crump 1979, Nedved 1993) and needs 75–100% humidity to survive during winter (Jean et al. 1990). Alternatively, in our two experiments inside houses, we observed survival of *H. axyridis* between 25 and 53%. To our knowledge, only one other coccinellid species, *Adalia bipunctata* L., is known to overwinter in cracks in walls, in lofts, or behind windows and even in rooms of houses, however, without comparable use of human houses to *H. axyridis* (Hodek and Honěk 1996; Majerus 1994, 1997). However, a fair proportion of *A. bipunctata* apparently do not leave the habitats, orchards, parks, or forests, and they hibernate either in crevices of trees, bark, or in artificial objects (Hodek and Honěk 1996). Our experiments and observations demonstrated that *H. axyridis* was unable to survive outside, suggesting that this behavioral adaptation to overwinter inside houses could be the only way to survive for this species in northeastern Canada. One question that could be raised by our experiment and will need further investigation is whether the northern expansion of this species is linked to the presence of artificial structure in the landscape.

Evaluating the population of *H. axyridis* that is overwintering inside houses is not an easy task. In rural areas, many houses contained some coccinellids (in terms of dozens, hundreds, or thousands of individuals), with higher numbers in old houses that have more interstices to be invaded (Huelsman et al. 2001). Factors such as control by humans, incapacity to find the exit, or temperature inside house also could influence the number of individuals reinvading the environment at the end of winter. Our experiments in controlled chambers showed higher survival at the end of winter at −5°C than at 10°C. High mortality at 10°C at the end of winter indicated that *H. axyridis* cannot sustain this temperature more than 4 to 5 mo. Watanabe (2002) demonstrated that *H. axyridis* individuals survived much longer at 0°C than at 5°C, suggesting that the mortality at higher temperature could be caused by depletion of energy reserves or water rather than by chilling injury. Our evaluation of lipid proportion demonstrate clearly a higher depletion of lipids during winter at 10°C than at −5°C, suggesting that energy reserves are important for survival of *H. axyridis* in heated houses. No studies have attempted to evaluate the impact of these factors on the spring population of *H. axyridis,* and more experiments are needed.

Interestingly, female survival was higher than that of males in houses. Females also showed a higher proportion of lipids than males. In many coccinellid species, females have a higher absolute fat content than males (Hodek and Honěk 1996), and females are generally larger than males (Nalepa et al. 1996, Osawa 2001). In several insect species, body size is an important correlate of individual fitness (Honěk 1997), positively associated with fecundity (Dixon and Guo 1993), flight activity and endurance, resistance to starvation and desiccation as well as total longevity (Honěk 1997). Nalepa et al. (1996) had a higher proportion of female *H. axyridis* in their collection after winter. In our area, overwintering success of females could be due to higher fat content, which provides a food reserve in heated houses. Bazzochi et al. (2004) showed that female *H. axyridis* had more intense oviposition activity after winter than native *A. bipunctata* and *Propylea quatuordecimpunctata* Linné. Furthermore, they had the highest postoverwintering rate of increase, which will give a higher capacity to colonize the environment after winter. Temperature in a house in our experiments ranged between 7 and 30°C, with bigger females surviving better than males during hibernation. No such results have been found for the indigenous species *C. maculata* where females did not experience higher survival than males. Thus, postoverwintering rate of increase could be higher for *H. axyridis*, providing advantages to reinvade the environment after winter, to lay eggs in high numbers, and to compete for resources or by interference with other species.

Warm and dry sites also could provide conditions that prevent the spread of disease during the winter (Honěk 1989). Although many dead coccinellids were found inside houses, no entomopathogenic fungal mortality on *H. axyridis* was noted. A subsample of 50 *H. axyridis* installed in controlled chambers at 10°C until the end of May (30 d) did not show any effective parasitism or fungal pathogen infection (G.L., unpublished data). It is now crucial to study the survival during winter of parasitoids and fungal pathogens of *H. axyridis* inside houses. A differential susceptibility of *H. axyridis* compared with other species to such enemies should influence greatly its invasive success.

The potential northern extension of the distribution of an exotic organism, such as *H. axyridis,* is linked to the capacity of the organism to withstand unfavorable environmental conditions, such as temperature extremes (Tauber et al. 1986). Insects in cold regions require some degree of cold hardiness to protect them from low temperatures (Salt 1961, Bale 1987). As pointed by Koch et al. (2004), the cold hardiness of *H. axyridis* in Minnesota and Georgia seems to be a poor predictor of its northern distribution. In our experiment, we demonstrate that the survival of the exotic *H. axyridis* is linked to the selection of human houses by overwintering individuals. Human houses may be considered a cold-free space for this species, allowing its geographical extension into northern North America and contributing to its great invasive success. Our study confirms the opposite overwintering strategy of the invasive *H. axyridis* and the indigenous *C. maculata,* indicating niche differentiation of these two species, and consequently, potential coexistence.

Acknowledgments

We thank homeowners for providing houses and assistance with experiments. We also thank Janick Durocher for technical assistance. Denis Réale, Onofre Soares, and Tim Work provided suggestions on an earlier draft of the manuscript. This research was supported by a Natural Sciences and

Engineering Research Council of Canada (NSERC) scholarship to G.L. and NSERC grants to É.L. and D.C.

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Received 23 August 2006; accepted 16 April 2007.