Spanish Journal of Agricultural Research
13(2), e1006, 11 pages (2015)
eISSN: 2171-9292
http://dx.doi.org/10.5424/sjar/2015132-7054
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Characterization of a newly established aggregation of the invasive ladybeetle *Harmonia axyridis* and current status of the invader in Spain

Xavier Pons[1], Meritxell Roca[1], Belén Lumbierres[1] and Éric Lucas[2,3]

[1] *Universitat de Lleida, Department of Crop and Forest Sciences. Agrotecnio Center. Rovira Roure 191. 25198 Lleida, Spain.*
[2] *Institut de Recerca i Tecnologia Agroalimentària (IRTA), Unitat d'Entomologia. Ctra. de Cabrils, Km 2, 08348 Cabrils, Spain.*
[3] *Université du Québec à Montréal, Département des Sciences Biologiques. CP 8888, Succursale Centre-Ville, Montréal (Québec), H3C 3P8 Canada.*

Abstract

The multicoloured Asian ladybeetle, *Harmonia axyridis* (Pallas), an invasive biocontrol agent introduced in North and South America, as well as in Europe for aphid control, drastically affected assemblages of native coccinellid species, local communities and people. Although the insect is common in several European countries and it was released in Spain for aphid control in 1995, no evidence of population establishment has been reported until 2011. In the present paper, 1) we summarize the records of the invasive ladybeetle in Spain and provide numerous new mentions, and 2) we follow for the first time an overwintering aggregation from autumn to spring and characterize some individual features which allow us to determine its phenology of the establishment and spreading. The results establish clearly that: 1) *H. axyridis* is (until now) restricted to Catalonia (NE Spain) but the invading process is in progress and the insect is able to occupy different habitats; 2) the ladybeetle overwinters successfully in Spain without significant natural mortality or parasitism; 3) the form *succinea* represents 73-81% of the overwintering individuals, and 4) the invasion takes probably its origin from Southern France. The potential impact of the invasion by *H. axyridis* in Spain is discussed.

Additional key words: multicoloured Asian ladybeetle; overwintering; invasive species; coccinellids; European expansion; harlequin ladybird.

Citation: Pons, X.; Roca, M.; Lumbierres, B.; Lucas, E. (2015). Characterization of a newly established aggregation of the invasive ladybeetle *Harmonia axyridis* and current status of the invader in Spain. Spanish Journal of Agricultural Research, Volume 13, Issue 2, e1006, 11 pages. http://dx.doi.org/10.5424/sjar/2015132-7054.

Received: 06 Nov 2014. **Accepted**: 04 May 2015

Copyright © 2015 INIA. This is an open access article distributed under the Creative Commons Attribution License (CC by 3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Xavier Pons: xavier.pons@irta.cat

Introduction

The multicoloured Asian ladybeetle, *Harmonia axyridis* (Pallas), is an east-paleartic species (native from Japan, China, Korea, Mongolia and Siberia) introduced in North-and South-America, Asia and Europe for aphid control, which has generated unexpected impacts on native coccinellid populations, ecosystems and people (Koch, 2003; Roy & Wajnberg, 2008). In Europe, *H. axyridis* was introduced for the control of aphids in Ukraine and Byelorussia in the last sixties and in France in the last eighties, but was not commercialized until middle nineties (Brown *et al.*, 2008). Since its introduction in these countries, the expansion of *H. axyridis* through Europe has been continuous (Brown *et al.*, 2008) and its effects on crops, urban areas and ecosystems have been widely studied and described.

Nevertheless, data from Spain are scarce (Brown *et al.*, 2008, 2011a). The coccinellid was introduced in Almería (South East Spain) for aphid control in 1995 (Jacas *et al.*, 2006), some adults were observed in the Canary Islands in 2003 and 2004 (Machado, 2006) and one single specimen was found in a public garden in Bilbao (Northern Spain) in 2007 (Goldazarena & Calvo, 2007). In spite of these release and findings, no evidence of population establishment occurred (Brown *et al.*, 2008, 2011a). Beyond of these records, there were some years without public records until that Carbonell & Sesma (2013) reported the occurrence of isolated individuals in several localities of Catalonia (North-eastern Spain) and one overwintering aggregation, quite close to the France border. However, no description of the characteristics of any Spanish aggregation has been

reported and their ability to overwinter in Spain remains to be established.

We were alerted of several aggregations of coccinellids adults occurring in houses in four localities of Northern Catalonia: a bigger one in *Les Llosses* (Girona province) and two smaller ones in *Alpens* and *Borredà* (Barcelona province) in November 2013, and another one in *Capmany* (Girona province) in January 2014. We identified all aggregations as *H. axyridis* and started to monitor the overwintering population and to prospect for new occurrences of the ladybird, mainly in Catalonia.

The present report aims 1) to describe the current status of *H. axyridis* in Spain, and 2) to characterize and follow for the first time an overwintering aggregation of the invader in the Spanish context.

Material and methods

Status of *H. axyridis* in Spain

Data on the occurrence of *H. axyridis* in Spain were collected by a thorough review of the literature and also by direct mentions from authors and colleagues.

In addition to the monitoring of reported aggregations during autumn and winter, from the end of the overwintering period until August, periodically (every 2-3 weeks) a sampling was done in several locations of Catalonia and the Basque Country on different types of vegetation. Sampled locations in Catalonia were *La Seu d'Urgell* [Lleida province; alfalfa and maize crop fields and plants in field margins; sweep net and Vortis® suction sampler (Burkard Manufacturing Co. Ltd., Ricksmanworth, UK) in alfalfa and visual sampling in maize and field margins], *Alpens* and *Borredà* (Barcelona province; margin vegetation in roads and gravel roads; visual sampling), *Barcelona* city (urban parks, visual sampling and yellow sticky traps), *Lleida* city (botanical garden and urban parks; visual sampling and yellow sticky traps), Lleida's city surroundings and Gimenells (Lleida province; maize fields, visual sampling and yellow sticky traps), *Cabrils* (Barcelona province; margin vegetation in roads and in parks; visual sampling), and *Torroella de Montgrí* (Girona province; vegetation of the banks of river Ter; visual sampling). Scouting in the Basque Country was visually carried on in vegetable gardens and urban parks of *Zarautz* (Gipuzkoa province). Occasionally, some localities of northern Aragon (Jaca) and Navarra (Ochagavía, Elizondo, Pamplona) were also visually sampled by authors in forest and urban parks. The presence of the insect in Catalonia was also communicated by scientists and collaborators (colleagues from universities and research centres, urban landscape managers, farm advisers, etc.). Information to several colleagues and technical advisers in Central Spain, Valencia, Andalusia and Asturias was required for reporting current or existing observations of the recent presence of the ladybird in their geographical areas and for scouting.

Characterization of the aggregations

Firstly, we characterized the sites where aggregations occurred: geographic coordinates, altitude, surrounding landscape, building characteristics, etc. Among the four aggregation sites reported, we selected the population of *Les Llosses*, since it was the largest. Characterization of this aggregation included natural mortality, parasitism rate, and phenology of the overwintering beetles, elytra colour pattern, sex ratio, weight, size, and female ovariole developmental stage before and after the overwintering period.

To determine the overwintering phenology, the aggregation was periodically monitored from November to May and the changes in colonies settle in visible places recorded. Yellow sticky traps and pollen bait traps were also placed inside and outside the *Les Llosses* overwintering site at the end of February and weekly monitored to detect individuals leaving the site. Along the overwintering period, individuals were collected and brought to the laboratory in order to determine sex, weight, size, ovariole development and parasitism. The adults were maintained in a refrigerator at 5 °C for 1-2 days before being individually photographed under stereoscopic microscope (Leica MZ8) on a millimetre paper. Then, specimens were individually preserved in ethanol 70°. The body length and width was measured from photos using the software programme ImageJ (Rasband W.S., US National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/, 1997-2014). Alcohol preserved individuals were sexed under the microscope and then dissected in Beadle's solution in order to establish ovariole developmental stage and potential parasitism. Weight and size were compared at the beginning and after overwintering by an ANOVA. Sex ratio was compared to 1:1 proportion by chi-square analyses.

By the end of the overwintering period, beetles started to be more active. Their distribution within the house (level and orientation) was periodically recorded (from 14 March to 2 May).

Results

Status of *H. axyridis* in Spain

Table 1 and Fig. 1 show the historical records of *H. axyridis* in Spain, with the new mentions. In addition to the findings of the ladybird in Spain described in the

Table 1. Period and locality of the records of *Harmonia axyridis* in Spain. When the reference is "Authors", it means that *H. axyridis* was recorded by the authors of the present study or their collaborators

Year (Month)	Locality (Region)[1]	Insect developmental stage	No. of individuals	Site	Reference
1995	Almería (And)	—	Undetermined [2]	Greenhouses	SIFA (2004); Jacas *et al.* (2006)
2003-04 (Oct)	La Laguna (Tenerife, Can)	Adult	1 individual	Garden	Machado (2006)
2006	Tenerife (Can)	Adult	1 individual	Garden	Brown *et al.* (2008)
2007 (Jun)	Loiu (Biscay, Bas)	Adult	2 individuals	Urban park on *Tilia platyphyllos* Scop. (*Eucallipterus tiliae* L.)	Goldazarena & Calvo (2007)
2010 (Jul)	Sant Dalmai (Girona, Cat)	Adult	1 individual	Next to crops	Carbonell & Sesma (2013)
2010 (Nov)	Girona (Cat)	Larva	1 individual	Urban park	Carbonell & Sesma (2013)
2011 (Nov)	Girona (Cat)	Adult	1 individual	Garden	Carbonell & Sesma (2013)
2011 (Sep)	Beuda (Girona, Cat)	Adult	1 individual	House	Carbonell & Sesma (2013)
2011 (Oct)	St Llorenç de la Muga (Girona, Cat)	Adult	Overwintering aggregation (200 individuals)	House	Carbonell & Sesma (2013)
2011 (Nov)	Gualba (Barcelona, Cat)	Adult	1 individual	Village	Carbonell & Sesma (2013)
2012 (Jul)	Barcelona (Cat)	Adult	1 individual	Urban park	Carbonell & Sesma (2013)
2012 (Jul)	Santandria (Menorca, Bal)	Adult	1 individual	Garden	Carbonell & Sesma (2013)
2013 (Sep)	Zarautz (Bas)	Adult	1 individual	Beach	Lumbierres *et al.* (2014)
2013 (Nov)	Les Llosses (Girona, Cat)	Adult	Overwintering aggregation (>500 individuals)	House. Some individuals on weeds	Lumbierres *et al.* (2014)
2013 (Nov)	Borredà (Barcelona, Cat)	Adult	Overwintering aggregation (>200 individuals)	House	Authors
2014 (Jan)	Campmany (Girona, Cat)	Adult	Overwintering aggregation (100 individuals)	Farm hut	Authors
2014 (Feb-May)	Les Llosses (Girona, Cat)	Adult	Post overwintering dispersing individuals (>750 individuals, see text)	House	Authors
2014 (Mar)	Lleida (Cat)	Adult	1 individual	Urban (inside a car)	Authors
2014 (Mar)	Lluçà (Barcelona, Cat)	Adult	1 individual	House	Authors
2014 (April)	Alpens (Barcelona, Cat)	Adult	Post overwintering dispersing individuals (30 indiv.)	House	Authors
2014 (April)	Torroella de Montgrí (Girona, Cat)	Adult	Post overwintering (3 individuals)	Field margin on *Rumex* sp. (*Aphis fabae* Scopoli)	Authors
2014 (April)	Sant Dalmai (Girona, Cat)	Adult	Several individuals	Next to crops	Sesma (2015)
2014 (April)	Roses (Girona, Cat)	Adult	1 individual	Urban park	Sesma (2015)
2014 (April)	Vilassar de Mar (Barcelona, Cat)	Adults	1 individual	Urban	Sesma (2015)
2014 (May)	Torroella de Montgrí (Girona, Cat)	Adult + Egg	Mating adults + eggs (6 individuals)	Field margin on *Arundo donax* L. (*Melanaphis donacis* (Passerini))	Authors
2014 (Jun)	Cabrils (Barcelona, Cat)	Adult + Larva	2 adults + 1 larva	Gravel road on *A. donax* (*M. donacis*)	Authors
2014 (Jun)	Borredà (Barcelona, Cat)	Adult	1 individual	Road edge on *Urtica* sp. (*Aphis urticata* Gmelin)	Authors
2014 (Jun)	Sabadell (Barcelona, Cat)	Larva	1 individual	Urban park on *Liriodendron tulipifera* L. (*Illinoia liriodendri* (Monell)	Authors
2014 (Jun-Jul)	Barcelona (Barcelona, Cat)	Larva Adult	1 individual 1 individual	Urban park House	Authors
2014 (Jun)	Soria	Adult	1 individual	Urban park	Sesma (2015)
2014 (Jul)	La Jonquera-Cantallops (Girona, Cat)	Adult	1 individual	Meadow	Sesma (2015)
2014 (Jul)	Cal Riera (Barcelona, Cat)	Adult	1 individual	River bank	Sesma (2015)
2014 (Aug)	L'Estartit (Girona, Cat)	Adult	Several individuals	Beach	Sesma (2015)
2014 (Jun, Sep)	Sant Martí d'Albars (Barcelona, Cat)	Larva Adult	1 individual 1 individual	Next to crops	Sesma (2015)

[1] And: Andalusia; Can: Canary Islands; Bas: Basque Country; Cat: Catalonia; Bal: Balearic Islands. [2] Released for biological control.

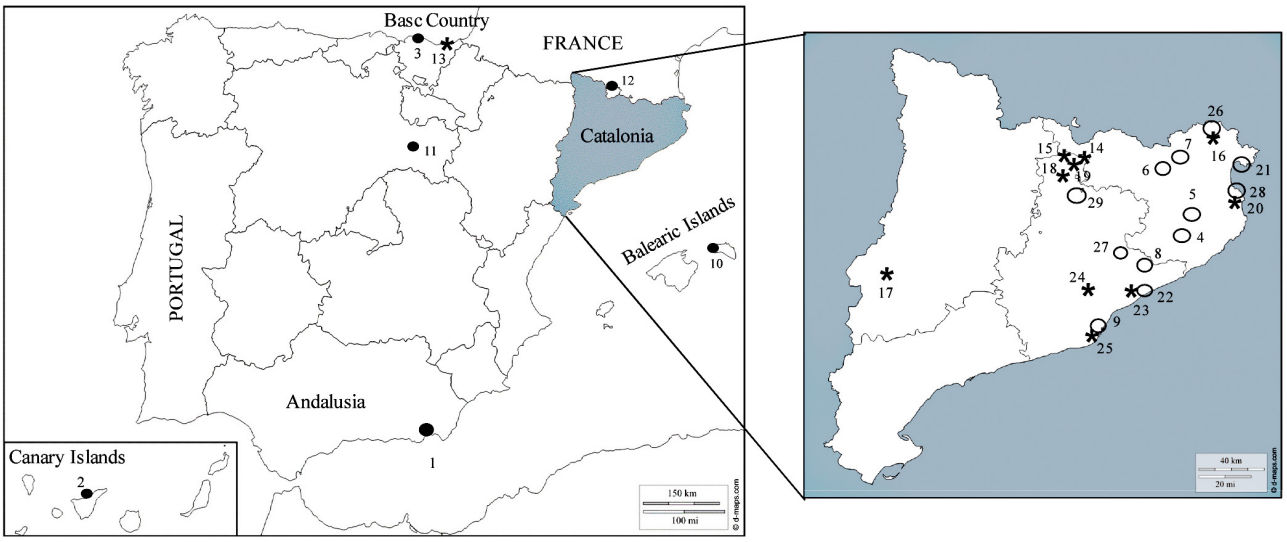

Figure 1. Geographic distribution of *Harmonia axyridis* in Spain (see also Table 1). ●: Records from other authors in Spain and Andorra: [1]Almeria; [2]Tenerife; [3]Loiu; [10]Menorca; [11]Soria; [12]Ordino (Andorra). ○: Records from other authors in Catalonia: [4]St Dalmai; [5]Girona; [6]Beuda; [7]St Llorenç de la Muga; [8]Gualba; [9]Barcelona; [21]Roses; [22]Vilassar de Mar; [26]La Jonquera-Cantallops; [27]Cal Riera; [28]L'Estartit; [29]St Martí d'Albars. *: Records from authors: [13]Zarautz (43°17'17.86"N; 2°10'01.34"W); [14]Les Llosses (42°08'37.28"N; 02°03'11.68E); [15]Borredà (42°08'10.24"N; 02°02'50.73"E); [16]Capmany (42°22'15.67"N; 02°55'22.92"E); [17]Lleida (41°37'50.45"N; 0°36'12.24"E); [18]Lluçà (42°05'00.61"N; 2°01'05.17" E); [19]Alpens (42°07'05.94N; 02°06'19.14"E); [20]Torroella de Montgrí (42°02'32.20"N; 3°07'40.45"E); [23]Cabrils (41°31'20.92N"; 2°22'18.95"E); [24]Sabadell (41°32'39"N; 2°05'28"E); [25]Barcelona (41°22/3.28"N; 2°9'22.65"E).

Introduction section, and those reported from Carbonell & Sesma (2013) and Sesma (2015) through photographic records in the website http://www.biodiversidadvirtual.org, we have recorded overwintering aggregations in several new sites of central and north Catalonia and the presence in spring and summer of individuals (adult, larvae or eggs) in some others close or far away localities from the overwintering sites. Most of the records are concentrated in Catalonia, the only region where overwintering aggregations have been reported.

In spring and summer, we found adults and larvae of *H. axyridis* in some herbaceous plants (*Rumex* sp., *Urtica* sp.), on giant reed (*Arundo donax* L.) and on some trees (*Liriodendron tulipifera* L.) infested by aphids (Table 1). Other coccinellid species were also collected in these sites: *Adalia decempunctata* L., *Coccinella septempunctata* L., *Hippodamia variegata* Goeze, *Propylea quatuordecimpunctata* L., and *Scymnus* sp.

Overwintering sites

The aggregation from *Les Llosses* occurred in a isolated three-storey house of 135 m^2 floor and 7.90 m height, at 795 m altitude in the south-east slope of a 915 m altitude forest hill dominated by pines (*Pinus sylvestris* L.) and oaks (*Quercus rubor* L.). The house is surrounded by a meadow field and a tiny garden of vegetables and with some pear, plum and quince trees. Fifty meters slope down of the building there is a water stream with bank river vegetation.

The aggregation from *Borredà* was observed within a room of the east side of a several attached buildings of an isolated four-storey farm house. The house was located on the SE slope of a hill of 970 m, at 800 m altitude. The building was surrounded by garden vegetation, meadows and cereal fields and separated from a pine and oak forest (around 100 m) and from a water stream with bank river vegetation (150 m).

The aggregation of *Alpens* was observed inside a room of the east side of a three storey farm-house, 150 m to the East from the end of the village at 870 m altitude. The house was located in an area dominated by pine and oak forest and uncultivated lands. Near the house there is a road and beyond it a cultivated area with fields of winter cereals and grasses which ends 400 m slope down at 810 m altitude.

The aggregation of *Capmany* occurred inside a tool-hut and in a wood pile besides that building. The site was in a plane area at 100 m altitude. The hut was surrounded by orchards with vegetables and some scattered fruit trees, an almond tree field (0.5 ha) and a vineyard of nearly 1 ha.

Characterization of the *Les Llosses* aggregation

Overwintering phenology. The house owner realized the presence of the coccinellids in middle October

when she observed that in several days a large number of a new "type" of ladybird stayed on the outer East and South walls of her house. More than 500 adults stayed on that walls for some days and later insects were punctually observed within the house. On 4 November 2014, we recorded more than 200 individuals in several quiet groups, mainly on the East inner wall of the house. Some few adults were also observed on herbaceous plants and grasses around the building. During winter, overwintering individuals were mostly hidden, but several clusters remained quiets in visible places. An adult of *Harmonia quadripunctata* Pontoppidan was also identified within the aggregation. On 5 March, we detected movement of individuals and from then to the end of April active adults in the house were recorded. These individuals abandoned progressively the house and dispersed. On 2 May, no adults remained inside the house. No captures of adults were obtained from yellow sticky traps or pollen bait traps.

The end of the overwintering period started at the same time for the populations from *Borredà* and *Alpens* but occurred a little bit earlier in *Capmany* (end of February).

Elytra colour pattern. Forms *succinea*, *conspicua* and *spectabilis* (according Osawa & Nishida, 1992; Mitchie *et al.*, 2010) were found over the sampling period. The form *succinea* was the prevalent over the whole period and represented between 73 to 81% of the individuals (Fig. 2). Among melanic forms, *spectabilis* was lightly more abundant than *conspicua*. There was no difference in the elytral colour pattern between sexes and between individuals collected before and after overwintering.

Sex. The sex ratio was not statistically different from 1:1 for any insect form and sampling date, although more females than males were recorded after overwintering (Table 2).

Weight and size. The weight of adult individuals was not affected by colour forms (F=1.93, p=0.1473; df=2, 271) but influenced by sex (F=4.18, p=0.0419, df=1, 271), females weighting more than males at each sampling date (Table 3). Also, individual weight changed according to the sampling date (F=4.52, p=0.0118, df=2, 271). Individuals collected before overwintering (4 November) being heavier (30.8±0.7 mg) than individuals collected after overwintering (14 and 28 March) (25.0±1.5 mg) (F=8.71; p=0.0034; df=1, 277). This difference was also significant according to beetle sexes (F=5.35; p=0.0214, df=1, 277) with a weight loss of 15% in females and of 28% in males.

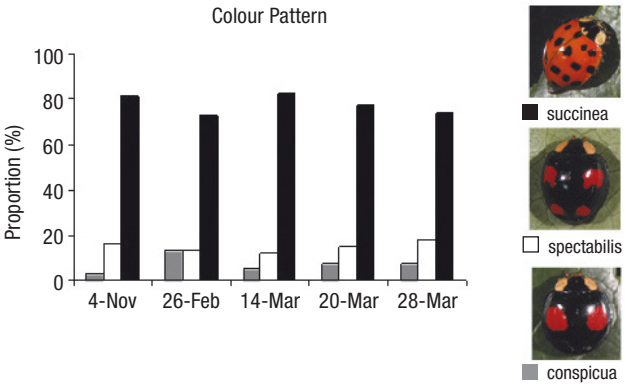

Figure 2. Proportion of colour forms of *Harmonia axyridis* from *Les Llosses* (Girona, Catalonia) at overwintering (November 4 and February 26) and after overwintering (March 14, 20 and 28).

Table 2. Sex ratio (%) of *Harmonia axyridis* individual from the population of Les Llosses before and after overwintering

Period	% of individuals		χ^2	p
	♀	♂		
Before overwintering	44.6	55.4	1.16	0.28
After overwintering	57.2	42.8	2.07	0.15

The length and width did not vary with the elytra pattern form, but again changes with sex. Body size of females was significantly greater than males at the three sampling dates (Table 3).

Ovariole development. Dissection of females showed that before overwintering the ovarioles were empty of eggs. However, after overwintering there was a progressive increase of the proportion of ovarioles with eggs, reaching more than 60% by the end of March (Table 4). No copulating individuals were observed.

Mortality and parasitism. No old dead corpse from previous years was found. Three hundred and fifty-eight adults (males and females) were dissected and no parasitized individuals were found. Neither ectoparasitic fungi, nor ectoparasite mites were recorded.

Table 3. Mean ± S.E. values of weight, length and width of females and males of *Harmonia axyridis* from *Les Llosses* (Girona, Catalonia) at the beginning of overwintering (4 November) and after overwintering (14 and 28 March)

Body measure	4 Nov		14 Mar		28 Mar	
	♀	♂	♀	♂	♀	♂
Weight (mg)	32.78 ± 1.22 a	19.22 ± 0.57 b	28.27 ± 1.26 a	21.98 ± 1.12 b	27.67 ± 3.04 a	20.73 ± 0.46 b
Length (mm)	6.91 ± 0.08 a	6.61 ± 0.16 a	7.20 ± 0.10 a	6.62 ± 0.08 b	7.16 ± 0.05 a	6.63 ± 0.05 b
Width (mm)	5.54 ± 0.07 a	5.37 ± 0.04 b	5.82 ± 0.09 a	5.56 ± 0.06 b	5.81 ± 0.04 a	5.59 ± 0.05 b

Table 4. Number of *Harmonia axyridis* females with ovarioles containing eggs at three dates of the overwintering process: before (November 4) and after (March 14 and 28) overwintering

Date	Female dissected number	No. of females	% of females
		with ovarioles containing eggs	
4 November	45	0	0
14 March	36	3	8.33
28 March	99	63	63.63

During the monitoring of the aggregation, no mortality was recorded.

Distribution of active ladybeetles in the overwintering house. A total of 761 observations of active individuals after overwintering were effectively done. Individuals were frequently found in groups, especially in the corners of the windows, or below window frames on the wall, or in the corners of ornamental devices on shelves on the wall. Most of the first active individuals were observed at the first floor, probably because it was the warmer level of the house. Later on the season, although active ladybeetles were observed in all house storeys, most of them were at the ground floor (Fig. 3A). In relation to the orientation, the insects preferred the East side of the house (Fig. 3B).

Discussion

The present results on the overwintering aggregations of the Asiatic ladybeetle and the numerous new mentions of the beetle reported establish clearly that the invasion process is in progress in Spain. *Harmonia axyridis* is present, reproduces, lays eggs, develops and overwinters successfully in Spain. The second conclusion is the fact that until now, the ladybeetle is restricted to the North-East of Spain (Catalonia). Records from other regions of Spain (Basque Country, Balearic and Canary Islands, Andalusia, and the latest in Soria (http://www.biodiversidadvirtual.org) suggest that the insect has not been established there yet.

What is the origin of the invasive population?

Two hypotheses can be formulated about the potential origin of the population: first, the Spanish origin (colonization derived from previously released ladybeetle in Almeria, south of Spain) and second, the European origin. Logically, the Almeria hypothesis should be discarded, since the release was considered unestablished (Jacas *et al.*, 2006; Brown *et al.*, 2011a), and no individual has been reported from the south of Spain since this time. Considering the European hypothesis, Lombaert *et al.* (2010, 2014) reported three routes of invasion of the European populations of the beetle, one coming from its native area released for biocontrol 1982 in France (named, European Biocontrol Strain), and another two, each one coming from eastern or western USA. In absence of genetic analyses, it is impossible to determine without any doubt the origin of the Spanish populations. However, the relative proportion of elytral color forms can be quite different among populations from different countries. For example, in USA and Eastern Canada, melanic forms are virtually absent (Koch, 2003; Lucas, *pers. obs.*). In the Spanish aggregation about 20% of the individuals have melanic forms and the relative proportion of elytral color forms (Fig. 2) is similar to other European populations (Adriaens *et al.*, 2008; Burgio *et al.*, 2008; Brown *et al.*, 2008; Stankovic *et al.*, 2011). Brown *et al.* (2008) suggest

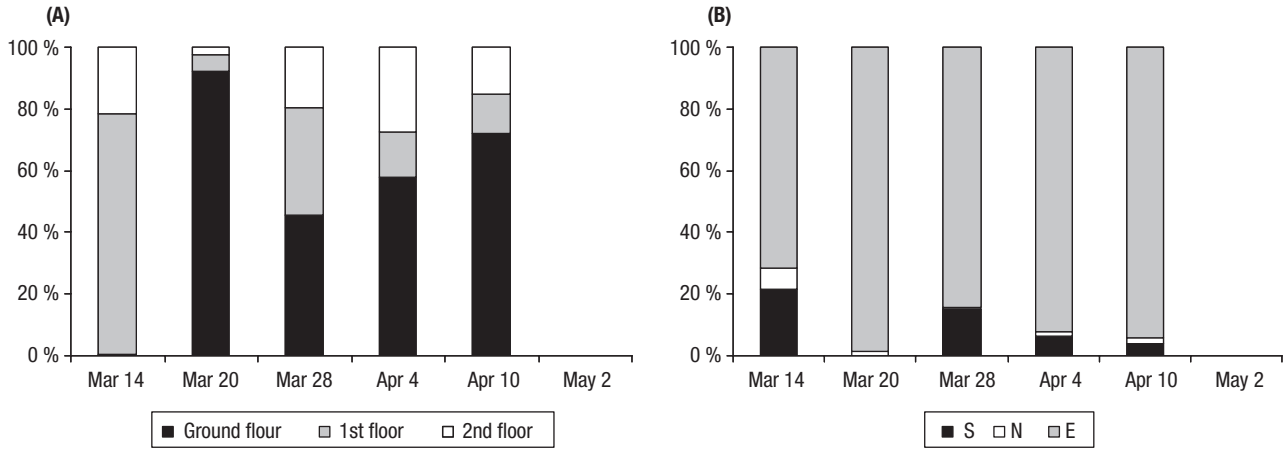

Figure 3. Proportion of active individuals recorded after overwintering in the three house-storeys (A) and on each orientation of the inner walls of the house (B). No ladybeetles were recorded on May 2 and on Western walls.

that the elytral color similarity in European populations is linked to limited points of origin in Europe, as Lombaert *et al.* (2014) has recently stated. Finally, all the reported mentions of *H. axyridis* in the present paper come from sites at less than 150 km from the French border. The entry may then have occurred from France trough the Pyrenees either by natural dispersion of the insect, or via human transport. The finding of the insect in Andorra (Sesma, 2015) supports this hypothesis.

What is the phenology of the establishment and spreading of the invasive aggregations?

Considering the timing of the invasion, the Spanish overwintering aggregation of *Les Llosses* is probably a new invasive population from 2013, since no cadavers were found from previous years and since the owner of the house who is living there had never seen the invader before. This aggregation successfully overwinters in 2013-14 and the phenology of the overwintering coincides with the described general pattern in the invaded countries of Europe and the Northern Hemisphere (Iperti & Bertrand, 2001; Koch, 2003; Raak-van den Berg *et al.*, 2012).

Overwintering started with the gathering of individuals in mid October, the hibernation by the end of October or the beginning of November, and the end of the overwintering occurred at the beginning of March. Adults preferred mainly the East side of the house for overwintering as is suggested by the records during the aggregation period and by the distribution of active insects within the house in March and April.

The presence of undeveloped ovaries in females and the weight decrease in male and females by loss of fat body content have been reported as signals of hibernating individuals (Sakurai *et al.*, 1992; Iperti & Bertand, 2001; Raak-van der Berg *et al.*, 2012). Females of the *Les Llosses*' aggregation had their ovaries empty of eggs until middle of March, when they started developing ovarioles and, by the end of March, nearly two of each three females had eggs in their ovarioles. Furthermore, males and females lose weight, although loss was higher in males than in females. The results on female ovariole development and adult weight suggest that the aggregation of *Les Llosses* has suffered a real hibernation. Ceryngier *et al.* (2004) showed that females of some coccinellid species may have inactive ovaries in early dormancy but sperm in their spermatheca, facilitating an early fertilization process in overwintering sites or nearby them. The lower weight loss in females than in males in the Les Llosses's aggregation may be an indicator that females started their reproductive processes. Several studies reports a short diapause followed by a quiescence period allowing insects to be active if weather conditions become favourable (Iperti & Bertrand, 2001; Raak-van den Berg *et al.*, 2012; Awad *et al.*, 2013). The population from *Campmany*, a northern locality close to France border, finished the overwintering period before that of *Les Llosses*. This may be due to a milder weather in *Capmany* (due to lower altitude) according to a closer proximity to the sea: 14.4 to 8.3 °C from October to March in *Les Llosses* and 18.3 to 11.9 °C in *Capmany*, with minimum 3.7 and 8.2 °C in *Les Llosses* and *Capmany*, respectively (Servei Meteorològic de Catalunya, http://www.meteocat.cat). Further studies should be conducted in order to precise overwintering features of the Spanish populations of *H. axyridis* according to temperature, altitude, latitude and distance from the sea.

Some Asiatic populations are female biased due to the presence of a male-killing bacterium from genus *Spiroplasma* sp. This male killer has not been reported from *H. axyridis* in its introduced range (Kenis *et al.*, 2008). In spite of that, Awad *et al.* (2013) found in Czech populations a lower survival rate during winter of males than females resulting in a low proportion of males in spring. We did not record significant differences to 1:1 sex ratio between sampling dates, although a lower proportion of males was recorded after overwintering.

After overwintering, insects from the aggregation of *Les Llosses* abandoned the house refugia and dispersed without evidence of their destination, although a few individuals were found in June relatively close to the different overwintering sites. The mentions of individuals in spring and summer far of the overwintering sites may be an evidence of the movement of the populations or could be due to the presence of other existing overwintering aggregations. *H. axyridis* is often considered as a good flyer (Hodek *et al.*, 1993) and a rate of spread up to 200 km per year in Europe has been evaluated (Brown *et al.*, 2011a), but spreading could also be favored by involuntary human transport (one mention reports an adult ladybeetle in Lleida within a car coming from northern Catalonia). It would be very important to follow the colonization of the Iberian Peninsula by systematic samplings of individuals during spring-summer and of overwintering populations during winter.

Beside the spreading capacity, individuals collected in the present study show that *H. axyridis* is able to occupy very different habitats (urban landscapes, reed beds, riparian or ruderal zones, etc.) on trees, shrubs and herbaceous plants and that it seems that there is an expansion from northern to central areas of Catalonia.

What will be the impact of the invasion by *H. axyridis* and what should we expect in the future?

The impacts of the invasion by the Asiatic ladybeetle have been thoroughly reported in the invaded regions, with positive but mainly negative effects related to its establishment [see Majerus *et al.* (2006) as a revision]. Since the establishment of the beetle in Spain is now demonstrated, impacts of *H. axyridis* should be expected, and negative ones should be similar to those described elsewhere. Firstly, domestic problems would occur with high densities of the insect invading human houses for overwintering, as it was the case in the present study.

Secondly, impact on native or already established ladybeetle species may also occur. The arrival of *H. axyridis* is associated to drastic changes in coccinellid assemblages all around the word (Colunga-Garcia & Gage, 1998; Michaud, 2002; Lucas *et al.*, 2007a,b; Pell *et al.*, 2008; Bélanger & Lucas, 2011; Roy *et al.*, 2012). Catalonia has a diverse agriculture dominated by winter cereals, maize, alfalfa, vegetables in open air and greenhouse, fruit orchards and vineyards. *H. axyridis* has been reported in all of these crops over Europe and America, as well as in urban landscapes. For example, in Belgium, Vandereycken *et al.* (2013) reported that maize is one of the crops most affected. Some of the most abundant coccinellids in Spanish arable crops (that is, *C. septempunctata*, *H. variegata* and *P. quatuordecimpunctata*) move between alfalfa, winter cereals and maize to follow aphid populations and demonstrate clear patterns of relative abundance (Pons & Eizaguirre, 2009; di Lascio *et al.*, unpublished data). In the present study, no *H. axyridis* has been recorded in maize and alfalfa (two crops where coccinellids are abundant) and this suggests that the invasion of the Asian ladybeetle is recent. However, the invasion of crop fields should be promptly observed, as has happened in other countries, and all these three native coccinellids species are showed to be in disadvantage intraguild predation experiments with *H. axyridis* (Ware & Majerus, 2008; Lucas, 2012; Katsanis *et al.*, 2013).

Spring and summer records from the present study show that *H. axyridis* has already colonized urban landscapes in Spain. In these environments, *Oenopia conglobata* L., *Adalia bipunctata* L., *A. decempunctata*, *H. variegata* and *Scymnus* sp. are the predominant ladybeetle species (Lumbierres *et al.*, 2005; Pons & Lumbierres, 2013). Since asymmetric intraguild predation in favor of the invader has been reported for all the native ladybeetle species (Ware & Majerus, 2008; Brown *et al.*, 2011b; Lucas, 2012; Katsanis *et al.*, 2013), negative impact on them should be expected also in urban landscapes. In Eastern Canada, *A. bipunctata* has disappeared from urban landscapes since the arrival of *H. axyridis* (Lucas, *pers. obs.*).

The Asian lady beetle would also probably invade forestal and more-open natural environments. For example, fifteen years after its arrival in Quebec (Canada), the multicoloured *H. axyridis* was clearly the dominant species in undisturbed wild meadow ecosystems demonstrating that these habitats will not constitute a natural refuge from the invader for native species (Bélanger & Lucas, 2011).

To improve their survival during winter, *H. axyridis* feeds on sugar-containing fruits like apples, pears or grapes in late autumn (Koch, 2003; Lucas *et al.*, 2007b; Galvan *et al.*, 2008). In the case of grapevines, insects could be harvested with the fruit and during the process beetles release haemolymph containing substances that can alter the smell and taste of wine (see Pickering *et al.*, 2004). The invasion by *H. axyridis* could then constitute a threat for the fruit and wine industry in Spain.

No parasitized individuals were recorded from the aggregation of *Les Llosses* in samples taken at the beginning and after overwintering. Furthermore, no parasitized individuals were collected in spring or summer. *Dinocampus coccinellae* Schrank (Hymenoptera: Ichneumonidae) and *Hesperomyces viriscens* Thaxt. (Ascomycota; Laboubeniales) are common parasites of coccinellids recorded in Catalonia on several coccinellid species as *C. septempunctata*, *H. variegata*, *O. conglobata* and *A. decempunctata* (Lumbierres, *pers. obs.*), so that *H. axyridis* is susceptible to be parasitized by these parasites. However, a recent study shows that natural enemies are still unable to control populations of *H. axyridis* in Europe (Raak-van den Berg *et al.*, 2014). In America also, despite the fact that *D. coccinellae* readily attacks *H. axyridis*, the success of the parasitism is very low and lower than on other coccinellid species, due to host behavioral defenses and unsuitability (Firlej *et al.*, 2005, 2010).

Van Lenteren *et al.* (2008) defined *H. axyridis* as a potentially risky species for Northwest Europe and Brown *et al.* (2008) predicted than the expansion of the invader in Europe would occur mainly to the northern and eastern countries and that the spread southward is uncertain (see also Kontodimas *et al.*, 2008; Soares *et al.*, 2008). However, the reports of Kulijer (2010) in Bosnia-Herzegovina, Stankovic *et al.* (2011) in Croatia and the present study, demonstrate the high potential of invasion in southern Mediterranean countries. The Asiatic ladybeetle has a huge phenotypical plasticity. The insect is euryphagous, eurytopic and

has successfully colonized drastically different countries and landscapes (Brown *et al.*, 2008). In spite of the different climatic conditions of Spain (oceanic in north and west, continental in center, and Mediterranean in northeast, east and south) we may expect the invasion process to continue until colonization of the complete country, even the sub-tropical area of southwest Almería, Granada, Málaga) may be colonized since *H. axyridis* was recently reported in Kenya (Nedved *et al.*, 2011). Furthermore, the relatively warm Spanish climate would probably allow a higher survival rate during winter, and would not act as a barrier in absence of human houses such as in Canada (Labrie *et al.*, 2008).

In conclusion, the results of this paper show that *H. axyridis* is already established in Spain and, although until now is restricted to Catalonia, the invading process is in progress.

Acknowledgements

We thank Carme Forcada and Montse Barniol for allowing us to monitor the overwintering aggregation in *Les Llosses*. We also thank Cristina Castañé, Matilde Eizaguirre, Ramon Albajes, Anaïs Lucas, Anna Lleixà, Joan Gual, Filipe Madeira, David Pons and Oscar Alomar for helping in sampling and the new records advising and all the other colleagues and technical advisers who scouted the ladybird for us in other regions of Spain.

References

Adriaens T, San Martin y Gomez G, Maes D, 2008. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. BioControl 53: 69-88. http://dx.doi.org/10.1007/s10526-007-9137-6

Awad M, Kalushkov P, Nedvedova T, Nedved O, 2013. Fecundity and fertility of ladybird beetle *Harmonia axyridis* after prolonged cold storage. BioControl 58: 657-666. http://dx.doi.org/10.1007/s10526-013-9512-4

Bélanger E, Lucas, E, 2011. Dominance of the multicoloured Asian lady beetle *Harmonia axyridis* in an undisturbed wild meadow ecosystem. Eur J Environ Sci 1: 1-14.

Brown PMJ, Adriaens T, Bathon H, Cuppen J, Goldazarena A, Hagg T, Kenis M, Klausnitzer BEM, Kovar I, Loomans AJM, *et al.*, 2008. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. BioControl 53: 55-67. http://dx.doi.org/10.1007/s10526-007-9124-y

Brown PMJ, Thomas CE, Lombaert E, Jeffries DL, Estoup A, Lawson Handley LJ, 2011a. The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. BioControl 56: 623-641. http://dx.doi.org/10.1007/s10526-011-9379-1

Brown PMJ, Frost R, Doberski J, Sparks T, Harrington R, Roy HE, 2011b. Decline in native ladybirds in response to the arrival of *Harmonia axyridis:* early evidence from England. Ecol Entomol 36: 231-240. http://dx.doi.org/10.1111/j.1365-2311.2011.01264.x

Burgio G, Santi F, Lanzoni A, Masetti A, De Luigi V, Melandri M, Reggiani A, Ricci C, Loomans AMJ, Maini S, 2008. *Harmonia axyridis* recordings in northern Italy. B Insectol 61: 361-364.

Carbonell R, Sesma JM, 2013. Confirmada la presencia de *Harmonia axyridis* (Pallas, 1773) en la Península Ibérica. Primeras citas para Cataluña e Islas Baleares (Coleoptera: Coccinellidae). BV news 16: 12-17.

Ceryngier PP, Havelka J. Hodek I, 2004. Mating and activity of gonads in pre-dormant and dormant ladybirds (Coleoptera: Coccinellidae). Invert Repr Dev 45: 127-135. http://dx.doi.org/10.1080/07924259.2004.9652582

Colunga-Garcia M, Gage SH, 1998. Arrival, establishment, and habitat use of the multicolored Asian ladybeetle (Coleoptera: Coccinellidae) in a Michigan landscape. Environ Entomol 27: 1574-1580. http://dx.doi.org/10.1093/ee/27.6.1574

Firlej A, Boivin G, Lucas E, Coderre D, 2005. First report of parasitism of *Harmonia axyridis* parasitism by *Dinocampus coccinellae* Schrank in Canada. Biol Invasions 7: 553-556. http://dx.doi.org/10.1007/s10530-004-5848-0

Firlej A, Lucas E, Coderre D, Boivin G, 2010. Impact of host behavioral defenses on parasitization efficacy of a larval and adult parasitoid. Biocontrol 55: 339-348. http://dx.doi.org/10.1007/s10526-009-9262-5

Galvan TL, Koch RL, Hutchinson WD, 2008. Impact of fruit feeding on overwintering survival of the multicolored Asian lady beetle, and the ability of this insect and paper wasps to injure wine grape berries. Entomol Exp Appl 12: 429-436. http://dx.doi.org/10.1111/j.1570-7458.2008.00731.x

Goldazarena A, Calvo D, 2007. First record of *Harmonia axyridis* (Coleoptera: Coccinellidae) from the Iberian Peninsula. Bol Soc Entomol Aragonesa 41: 437-439.

Hodek I, Iperti G, Hodkova M, 1993. Long-distance flights in Coccinellidae (Coleoptera). Eur J Entomol 90: 403-414.

Iperti G, Bertrand E, 2001. Hibernation of *Harmonia axyridis* (Coleoptera: Coccinellidae) in South-Eastern France. Acta Soc Zool Bohem 65: 207-210.

Jacas JA, Urbaneja A, Viñuela E, 2006. History and future of introduction of exotic arthropod biological control agents in Spain: a dilemma? BioControl 51: 1-30. http://dx.doi.org/10.1007/s10526-005-5808-3

Katsanis A, Barbendreier D, Nentwig W, Kenis M, 2013. Intraguild predation between the invasive ladybird *Harmonia axyridis* and non-target European coccinellid species. BioControl 58: 73-83. http://dx.doi.org/10.1007/s10526-012-9470-2

Kenis M, Roy HE, Zindel R, Majerus MEN, 2008. Current and potential management strategies against *Harmonia axyridis*. BioControl 53: 235-252. http://dx.doi.org/10.1007/s10526-007-9136-7

Koch RL, 2003. The multicolored Asian lady beetle, *Harmonia axyridis:* A review of its biology, uses in biological control, and non-target impacts. J Insect Sci 3: 1-16. http://dx.doi.org/10.1673/031.003.3201

Kontodimas DC, Stathas J, Martinou AF, 2008. The aphidophagous predator *Harmonia axyridis* (Coleoptera: Coccinellidae) in Greece, 1994-1999. Eur J Entomol 105: 541-544. http://dx.doi.org/10.14411/eje.2008.072

Kulijer D, 2010. First record of invasive species *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) in Bosnia and Herzegovina. Acta Entomol Serb 15: 141-143.

Labrie G, Coderre D, Lucas E, 2008. Overwintering strategy of multicolored Asian lady beetle (Coleoptera: Coccinellidae): cold-free space as a factor of invasive success. Ann Entomol Soc Am 101: 860-866. http://dx.doi.org/10.1093/aesa/101.5.860

Lombaert E, Guillemaud T, Cornuet JM, Malausa T, Facon B, Estoup A, 2010. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PloS ONE 5: e9743. http://dx.doi.org/10.1371/journal.pone.0009743

Lombaert E, Guillemaud T, Lundgren J, Koch R, Facon B, Grez A, Loomans A, Malausa T, Nedved O, Rhule E, Staverlokk A, Steenberg T, Estoup A, 2014. Complementarity of statistical treatments to reconstruct worldwide routes of invasion: the case of the Asian ladybird *Harmonia axyridis*. Mol Ecol 23: 5979-5997. http://dx.doi.org/10.1111/mec.12989

Lucas E, 2012. Intraguild interactions. In: Ecology and behaviour of the ladybird beetles (Coccinellidae) (Hodek I, van Emden HF, Honek A, eds.). Blackwell Publ Ltd, Chichester, UK, pp: 343-374.

Lucas E, Labrie G, Vincent C, Kovach J, 2007a. The multicoloured Asian ladybeetle: beneficial or nuisance organism? In: Biological control: a global perspective (Vincent C, Goettel M, Lazarovits G, eds.). CABI Int, UK, pp: 38-52. http://dx.doi.org/10.1079/9781845932657.0038

Lucas E, Vincent C, Labrie G, Chouinard G, Fournier F, Pelletier F, Bostanian N, Coderre D, Mignault MP, Lafontaine P, 2007b. The multicolored Asian ladybeetle *Harmonia axyridis* in Quebec agroecosystems ten years after its arrival. Eur J Entomol 104: 737-743. http://dx.doi.org/10.14411/eje.2007.094

Lumbierres B, Pons X, Starý P, 2005. Parasitoids and predators of aphids associated with public green areas of lleida (NE Iberian Peninsula). Adv Hort Sci 19: 69-75.

Lumbierres B, Roca M, Pons X, 2014. Las apariencias engañan: los peligros de la mariquita asiática *Harmonia axyridis*. Phytoma-España 258: 48-49.

Machado A, 2006. El sarantontón asiático *Harmonia axyridis* (Pallas) presente en Canarias (Coleoptera: Coccinellidae). Vieraea 34: 71-72.

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: 207-213. http://dx.doi.org/10.1111/j.1365-2311.2006.00734.x

Michaud JP, 2002. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. Environ Entomol 31: 827-835. http://dx.doi.org/10.1603/0046-225X-31.5.827

Mitchie LJ, Mallard F, Majerus MEN, Jiggins FM, 2010. Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. J Evol Biol 23: 1699-1707. http://dx.doi.org/10.1111/j.1420-9101.2010.02043.x

Nedved O, Hava J, Kulikova D, 2011. Record of the invasive alien ladybird *Harmonia axyridis* (Coleoptera, Coccinellidae) from Kenya. ZooKeys 106: 77-81. http://dx.doi.org/10.3897/zookeys.106.1242

Osawa N, Nishida T, 1992. Seasonal variation in elytral colour polymorphism in *Harmonia axyridis* (the ladybird beetle): the role of non-random mating. Heredity 69: 297-307. http://dx.doi.org/10.1038/hdy.1992.129

Pell JK, Baverstock J, Roy HE, Ware RL, Majerus MEN, 2008. Intraguild predation involving *Harmonia axyridis:* a review of current knowledge and future perspectives. BioControl 53: 147-168. http://dx.doi.org/10.1007/s10526-007-9125-x

Pickering G, Lin J, Riessen R, Reynolds A, Brindle I, Soleas G, 2004. Influence of *Harmonia axyridis* on the sensory propierties of white and red wine. Am J Enol Viticult 55: 153-159.

Pons X, Eizaguirre M, 2009. Cultivos extensivos en regadío: cereales de invierno, maíz y alfalfa. In: Control biológico de plagas agrícolas (Jacas JA, Urbaneja A, eds.). Phytoma-España, Valencia, pp: 384-398.

Pons X, Lumbierres B, 2013. Control integrado en espacios verdes urbanos. 12º Symposium de Sanidad Vegetal: Hacia la Gestión Integrada de Plagas. Servicio de Publicaciones, Junta de Andalucía, Consejería de Agricultura, Pesca y Medio Ambiente, Sevilla. pp: 145-164.

Raak-van den Berg CL, Hemerik L, de Jong PW, van Lenteren J, 2012. Mode of overwintering of invasive *Harmonia axyridis* in the Netherlands. BioControl 57: 71-84. http://dx.doi.org/10.1007/s10526-011-9394-2

Raak-van den Berg CL, van Wielink PS, de Jong PW, Gort G, Haelewaters D, Helder J, van Lenteren, JC, 2014. Invasive alien species under attack. Natural enemies of *Harmonia axyridis* in the Netherlands. BioControl 59: 220-240. http://dx.doi.org/10.1007/s10526-014-9561-3

Roy H, Wajnberg E, 2008. From biological control to invasion: the ladybird *Harmonia axyridis* as a model species. BioControl 53: 1-4. http://dx.doi.org/10.1007/s10526-007-9127-8

Roy HE, Adriaens T, Isaac NJB, Kenis M, Onkelinx T, San Martin G, Brown PMJ, Hautier L, Poland R, Roy DB *et al.*, 2012. Invasive alien predator causes rapid declines of native European ladybirds. Divers Distrib 18: 717-725. http://dx.doi.org/10.1111/j.1472-4642.2012.00883.x

Sakurai H, Hirano T, Takeda S, 1992. Physiological changes related to diapause of the lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). App Entomol Zool 27: 479-429.

Sesma JM, 2015. Primeras citas de *Harmonia axyridis* (Pallas, 1773) en Andorra, Castilla y León y su expansión en Cataluña (Coleoptera: Coccinellidae). BVnPC 4(43): 5-10.

SIFA, 2004. Organismos de control biológico. Servicio de Información Fitosanitaria de Almería, Spain.

Soares AO, Borges I, Borges PAV, Labrie G, Lucas E, 2008. *Harmonia axyridis:* What will stop the invader. BioControl 53: 127-145. http://dx.doi.org/10.1007/s10526-007-9141-x

Stankovic VM, Koren T, Stankovic I, 2011. The harlequin ladybird continues to invade southeastern Europe. Biol Invasions 13: 1711-1716. http://dx.doi.org/10.1007/s10530-010-9929-y

Van Lenteren JC, Loomans AJM, Brabendreier D, Bigler F, 2008. *Harmonia axyridis:* an environmental risk assessment for Northwest Europe. BioControl 53: 37-54. http://dx.doi.org/10.1007/s10526-007-9120-2

Vandereycken A, Brostaux Y, Joie E, Haubruge E, Verheggen FJ, 2013. Occurrence of *Harmonia axyridis* (Coleoptera: Coccinellidae) in field crops. Eur J Entomol 110: 285-292. http://dx.doi.org/10.14411/eje.2013.042

Ware RL, Majerus MEN, 2008. Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. BioControl 53: 169-168. http://dx.doi.org/10.1007/s10526-007-9135-8