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Life histories of an invasive and native ladybird under field experimental conditions in a temperate climate

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Abstract

Among characteristics that are thought to determine the success of invasive species, life-history traits feature prominently. However, in most cases, these have been determined under laboratory conditions. Here, we use a field set-up to determine immature development time and survival of invasive *Harmonia axyridis* (Pallas) and native *Adalia bipunctata* L. (both Coleoptera: Coccinellidae). On caged *Tilia × europaea* L. cv. Pallida trees (Malvaceae) with ample amounts of aphid food, we introduced freshly hatched larvae of a single or of both ladybird species and followed their development until emergence of adults. Under the condition of ample prey availability, both ladybird species apparently hardly interacted and intraguild predation did not cause significant mortality. Development time of both species is in line with data from laboratory tests under controlled conditions. Immature survival can reach high levels, but is considerably higher for *H. axyridis* (44–100%) than for *A. bipunctata* (11–77%), resulting in faster increase of *H. axyridis* populations, which is one of the factors that may explain its invasion success.

Introduction

Various causes have been suggested for the disproportionate success – in terms of population growth relative to that of native species – of invasive species (Kolar & Lodge, 2001). Among these are typical life-history characteristics such as high survival rate and fast development (Sakai et al., 2001). Especially important is how values of these traits in invasive species relate to those of native guild-members under natural circumstances. It is therefore important to perform comparative, empirical studies on life-history variables of invasive vs. native species under field conditions in order to understand the former's disproportionate success, because laboratory studies of life-

history variation might not necessarily have explanatory power in the study of invasion success. In this paper, we studied two key life-history traits (survival and development rate) of a currently recognised classical invasive species, the multicoloured Asian ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), under field experimental conditions, and compared these with the values found for a native species, *Adalia bipunctata* L. (Coleoptera: Coccinellidae). *Adalia bipunctata* is a common species which seems to be most endangered by *H. axyridis* (Adriaens et al., 2008; Brakefield & de Jong, 2011; Roy et al., 2012; Kenis et al., 2017). Both *H. axyridis* and *A. bipunctata* are primarily arboreal species (Honek & Rejmanek, 1982; Majerus, 1994; Nedved, 1999; Adriaens et al., 2008; Osawa, 2011; Vandereycken et al., 2012) with a large spatial-temporal co-occurrence (Adriaens et al., 2008; Vigišová et al., 2017).

Harmonia axyridis has been introduced in the USA, Europe, and some countries in South America and

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northern Africa as a biological control agent of aphids (Brown et al., 2011), but is now regarded as an invasive species (De Clercq & Bale, 2011). The establishment of *H. axyridis* has, among other negative side effects (van Lenteren et al., 2008), been associated with a decline in native ladybird populations (Alyokhin & Sewell, 2004; Roy et al., 2012). Many characteristics of *H. axyridis* are thought to contribute to its invasion success. The species is polyphagous, eurytopic, multivoltine (Hodek et al., 2012), has a good dispersal ability (Brown et al., 2011), a short generation time, high immature survival and high fecundity (Raak-van den Berg et al., 2017). All these characteristics contribute to rapid population growth. In addition, *H. axyridis* is a strong intraguild predator (Pell et al., 2008).

Most biological knowledge of *H. axyridis* is based on laboratory studies, whereas we are interested in its biology in the field, especially in comparison with native species that co-occur with *H. axyridis*. This study is the first to determine development and survival characteristics of ladybird species under field experimental conditions in northwestern Europe. Two field studies are known from other areas, i.e., France and Japan (Osawa, 1993; Kindlmann et al., 2000), but the experimental set-up and the type of food offered differed from the present study, making comparisons doubtful.

In order to contribute to an explanation of the invasion success of *H. axyridis*, we address the following questions in this paper: (1) what is the immature development time and immature survival of *H. axyridis* and *A. bipunctata* under field conditions in The Netherlands? And (2) does co-occurrence of the two species under conditions of ample prey availability result in differences in mortality or development time in one or both species? Assuming that immature development and survival indeed play an important role in the invasiveness of *H. axyridis*, we hypothesise that development is faster and/or immature survival is higher for *H. axyridis* than for *A. bipunctata*.

Material and methods

Insects and culturing

Laboratory populations of *H. axyridis* and *A. bipunctata* were initiated from field-collected specimens within a 100-km radius from Wageningen, The Netherlands (Raak-van den Berg et al., 2012b,c). Pairs of males and females from generations 1–3 of the laboratory population were put in Petri dishes (9 cm diameter) lined with filter paper in a climate room at 25 ± 1 °C (2009) or 24 ± 1 °C (2010), and L16:D8 photoperiod. The beetles received ad libitum *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) eggs,

bee pollen, and honey water, and they were fed pea aphids [*Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), reared on *Vicia faba* L. (Fabaceae)] 3–4× per week to provide eggs for the experiment. Aphids and *E. kuehniella* eggs were provided by Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands).

Infestation experiment in the field

The experiments were conducted on 12 young lime trees [*Tilia × europaea* L. cv. Pallida (Malvaceae); 12 cm stem circumference] in the orchard of the experimental farm Droevendaal, Wageningen University (Wageningen, The Netherlands). The trees were trimmed and trained in espalier form making the branches easily accessible for the observer. Branches were trained in a North–South direction. Trees were up to 3 m tall and planted in a 3 × 4 matrix with 3 m spacing. Each tree was enclosed by a cage (2.5 × 2 × 3.2 m) with a gauze cover (0.6 × 0.6 mm mesh). The soil was covered with natural vegetation of mainly grasses and weeds. Ladybirds, other insects interfering with aphids (e.g., ants, lacewings, hoverflies, caterpillars), and any natural enemies of ladybirds we saw (e.g., spiders) were removed before and during the experiments. Tape covered with olive oil around stems and supporting poles prevented ants from climbing into the trees.

Branches of *Tilia* spec. infested with aphids [*Eucalipterus tiliae* L. (Hemiptera: Aphididae)] were collected in the field and used to infest the experimental trees by putting the cut branches on those trees for 1 day. Once established, aphid populations grew rapidly, resulting in large aphid populations that were comparable with heavily infested trees in the wild, providing ad libitum food at the density in which ladybird larvae were introduced. Inside two cages, data loggers (MicrologPRO, www.fouriersystems.com) recorded the temperature every half hour.

To determine development and survival of *H. axyridis* and *A. bipunctata*, larvae were placed on the trees in three treatments: only *H. axyridis*, only *A. bipunctata*, or larvae of both *H. axyridis* and *A. bipunctata*. Three trees were used per treatment, whereas the three remaining trees served as controls and did not receive any larvae. Treatments were randomly assigned to the trees, with the restriction that each treatment contained one lush and one more sparsely leafed tree, as six trees were lush than the other six. The experiment was repeated 5×: in 2009, starting 22 May (I), 15 June (II), and 7 July (III), and in 2010, starting 8 June (IV) and 1 July (V). In period I, six larvae were placed on each tree. In the following periods, six larvae were placed on each of the six more sparsely leafed trees and 10 larvae on each of the six lush trees. In total 179 individuals of *H. axyridis* and 170 individuals of *A. bipunctata* were used.

The experiments were started with freshly hatched larvae that had not yet dispersed from their egg batch, except for period V, when 1-day-old larvae were used. Cannibalism of sibling eggs may have occurred, but has not been registered. To avoid family effect, larvae were taken from different parental pairs: three (for sparsely leafed trees) or five (for lush trees) parental pairs were used. Each larva was placed on a leaf of a separate branch in the vicinity of aphids. Every other day the developmental stage of the larvae was recorded and their position labelled. Skins that were shed after ecdysis were recorded as well, and removed. Developmental stage of larvae and skins was determined based on colour (Hodek, 1973; Koch, 2003; www.stippen.nl) and on reference individuals reared in the laboratory. Larvae and pupae were often difficult to find in this non-destructive experiment, and hence not all individuals were always found. Therefore, shed skins of earlier stages were also used to determine the number of individuals that successfully moulted into the next stage and the day on which that happened. To determine the number surviving to a next stage, the number of individuals in later stages was also taken into account. When larvae moulted into pupae, the size (length) of the pupa was measured with an electronic digital calliper (± 0.1 mm; ETC Tools, Malmö, Sweden) within 24 h of pupation, and a clip cage was placed over the pupa. After emergence, adults were weighed on a micro balance (± 0.1 mg, Sartorius, Göttingen, Germany, and Mettler Toledo, Tiel, The Netherlands). Two extremely small *H. axyridis* adults (outliers, period V) were removed from the analysis of pupal size and adult weight.

Statistical analysis

To estimate developmental time, data were pooled over trees per combination of period and species. The average durations of the larval stages and their standard errors were estimated with the non-parametric method of Pontius et al. (1989), performed by a programme for the analysis of single-cohort stage-frequency data written by Manly (1994). Data were pooled over trees per combination of period and species to have sufficient data to perform the analysis. Also in order to allow analysis, the development times of the fourth instar and the prepupal stage were summed and standard errors were appropriately corrected.

To determine survival, the data were pooled into three groups: the first three instars (L1+L2+L3; i.e., percentage of first instars that successfully moulted into the fourth larval stage), the fourth instar including prepupa (L4+PP; i.e., percentage of fourth instars that successfully moulted into the pupal stage), and the pupa (i.e., percentage of pupae that successfully emerged as adults). Overall immature survival was also determined.

Developmental response variables, survival proportions, and pupal and adult size measurements were analysed using mixed linear models (Proc MIXED of SAS/STAT software v.9.3; SAS Institute, Cary, NC, USA). The fixed part of the model contained main effects for species, competition, year, and, if applicable, sex, and interactions species*year and species*competition. The block factor tree type (lush vs. sparsely leafed) was included in the model, but removed if not significant ($P > 0.05$). Random effects for period within year, and, if applicable, tree within year and period-tree combination within year were included in the mixed models to follow the trial design as closely as possible. For developmental responses, data were pooled over trees, and random effects for period-tree pool combination within year were used. Degrees of freedom for F-tests of fixed effects were estimated using the method of Kenward & Roger (1997), which may result in non-integer numbers. To obtain approximate constant variance and normality, needed for mixed models, survival proportions were arcsine- \sqrt{x} transformed and weighted analyses were performed using the initial insect counts at the beginning of each period as weights. Attempts to analyse the survival proportions with more appropriate generalised linear mixed models failed, due to convergence problems of the algorithm for some variables. Developmental responses were weighted with $1/SE^2$, where the standard error was estimated simultaneously with the developmental time. The morphometric variables adult weight, pupal length, and pupal width were log-transformed before analysis to improve normality, homoscedasticity, and additivity of relationships. Relevant results are shown as estimate (\pm SE of the estimate in parentheses).

Results

In all relevant analyses, no block effect (lush vs. sparsely leafed trees) was found, and this factor was therefore removed from the models.

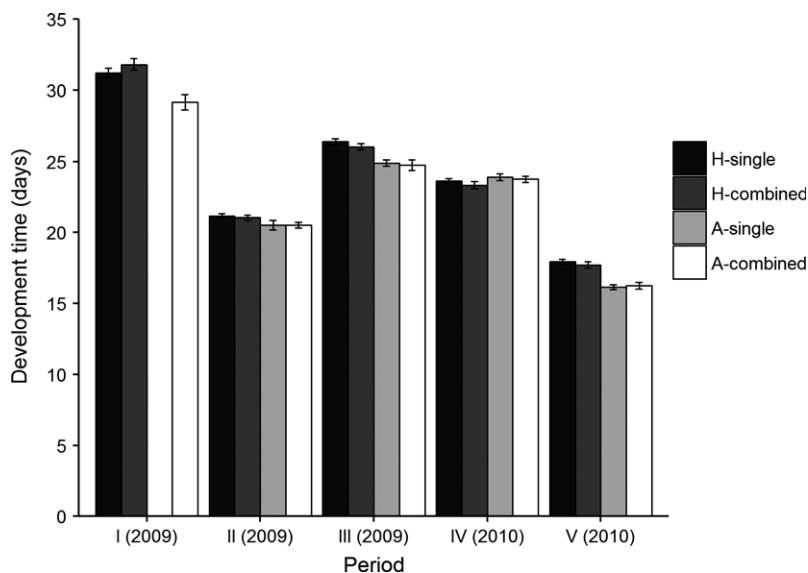
Development

The development time from L1 to adult of *H. axyridis* (23.4 days) was significantly longer than that of *A. bipunctata* (22.5 days), a difference of 0.9 (± 0.3) days ($P = 0.007$; Tables 1 and S1, Figure 1). Analysis of the individual stages indicates that this difference is also found for the fourth instar – *H. axyridis* development time is 0.7 (± 0.2) days longer ($P = 0.002$; Table 1) – whereas for the other stages no species effect was observed. Hence, differences in the fourth instar determined to a large extent the differences found for total development. No evidence for effects of year, competition, or of the interactions species*year and species*competition was found (Table 1). Inter-

Table 1 Test statistics for mixed models describing development time, survival, pupal size, and adult weight of *Harmonia axyridis* and *Adalia bipunctata*. The null hypotheses of 'no effect' were tested for fixed effects and variance components were estimated

		Year		Species		Sex	
		F	P	F	P	F	P
Development	L1	$F_{1,4.1} = 0.26$	0.64	$F_{1,10.0} = 0.80$	0.40	—	—
	L2	$F_{1,1.9} = 0.87$	0.45	$F_{1,10} = 2.34$	0.16	—	—
	L3	$F_{1,2.9} = 0.50$	0.53	$F_{1,10.0} < 0.01$	1.0	—	—
	L4PP	$F_{1,45.0} = 0.37$	0.57	$F_{1,10.4} = 17.5$	0.002	—	—
	Pupa	$F_{1,2.9} = 0.70$	0.47	$F_{1,9.9} = 0.21$	0.66	—	—
	L1toA	$F_{1,3.0} = 1.44$	0.32	$F_{1,10} = 11.4$	0.007	—	—
Survival	L1-L3	$F_{1,2.9} = 1.01$	0.39	$F_{1,32.1} = 25.1$	<0.0001	—	—
	L4PP	$F_{1,4.6} = 0.62$	0.47	$F_{1,40.5} = 0.01$	0.93	—	—
	Pupa	$F_{1,3.3} = 0.01$	0.92	$F_{1,46.7} = 0.25$	0.62	—	—
	L1toA	$F_{1,3.6} = 0.99$	0.38	$F_{1,42} = 13.9$	0.0006	—	—
Size and weight	Pupal length	$F_{1,3.3} = 2.23$	0.22	$F_{1,150} = 2072.9$	<0.0001	$F_{1,155} = 51.3$	<0.0001
	Pupal width	$F_{1,3.0} = 2.13$	0.24	$F_{1,82} = 2359.6$	<0.0001	$F_{1,154} = 63.9$	<0.0001
	Adult weight	$F_{1,3.0} = 2.22$	0.23	$F_{1,61.1} = 2761.2$	<0.0001	$F_{1,171} = 80.1$	<0.0001

L4PP = fourth instar including prepupa; L1toA = first instar until adult emergence; L1–L3 = first three instars (L1+L2+L3).

**Figure 1** Estimated mean (\pm SE, estimated with method of Pontius et al., 1989) development time (days) from first instar to adult emergence of *Harmonia axyridis* (H) and *Adalia bipunctata* (A) on lime trees in the field during three periods in 2009 – starting on 22 May (I), 15 June (II), and 7 July (III) – and two periods in 2010 – starting on 8 June (IV) and 1 July 2010 (V) – with the species separated (single) or combined.

period variance was large, except for the first instar. In the group with only *A. bipunctata* larvae in period I, the prepupal and pupal stages were not found, and, therefore, development time could not be estimated (Figure 1).

Survival

Under field experimental conditions, but with the exclusion of natural enemies, immature survival of *H. axyridis* ranged from 44 to 100%, whereas immature survival of *A. bipunctata* ranged from 11 to 77% (Table S2, Figure 2). Averaged over the 2 years, immature survival of *H. axyridis* (back-transformed estimated

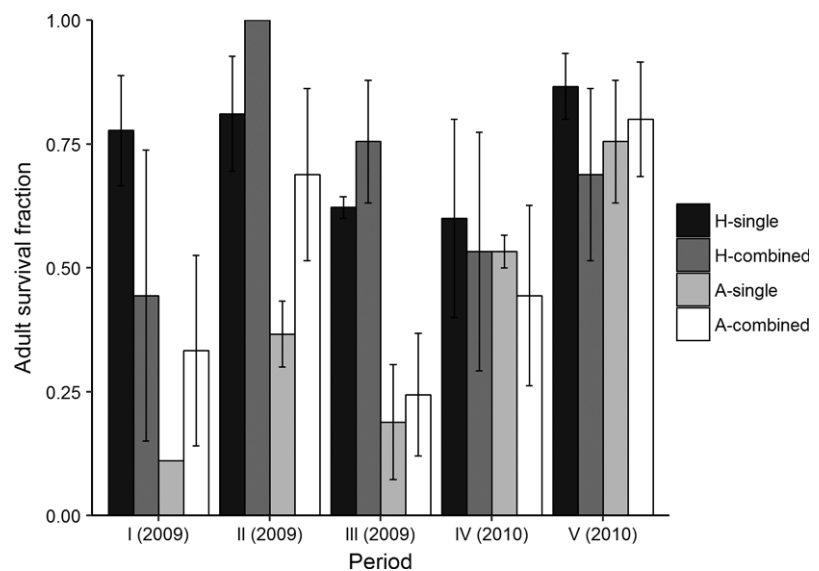
mean: 91%) was higher than that of *A. bipunctata* (53%) ($P = 0.0006$; Table 1), but the difference in survival rate was not constant over the years (species*year: $P = 0.003$): in 2009 a larger difference was found (94 vs. 26%) than in 2010 (88 vs. 82%). No effect of competition on survival was found: the difference in survival between the two species did not depend on competition (species*competition: $P = 0.43$; Table 1), nor did survival averaged over species depend on competition (main competition effect: $P = 0.41$; Table 1).

Statistical analysis of stage-specific survival proportion indicates that only survival of L1–L3 differed between

Table 1 Continued

Competition		Species*year		Species*sex		Species*competition	
F	P	F	P	F	P	F	P
$F_{1,12.6} = 0.08$	0.78	$F_{1,9.9} = 1.43$	0.26	—	—	$F_{1,9.7} = 1.41$	0.26
$F_{1,10.3} = 0.01$	0.91	$F_{1,9.9} = 0.16$	0.69	—	—	$F_{1,10.0} = 1.37$	0.27
$F_{1,10.1} = 4.19$	0.07	$F_{1,10.0} = 0.07$	0.80	—	—	$F_{1,10.1} = 1.43$	0.26
$F_{1,6.4} = 1.13$	0.33	$F_{1,9.2} = 0.48$	0.51	—	—	$F_{1,10.4} = 0.03$	0.86
$F_{1,9.9} = 0.06$	0.81	$F_{1,9.9} = 0.44$	0.52	—	—	$F_{1,9.9} = 0.49$	0.50
$F_{1,10} = 0.01$	0.93	$F_{1,10} = 0.32$	0.58	—	—	$F_{1,10} = 0.25$	0.63
$F_{1,30.2} = 3.37$	0.08	$F_{1,43.9} = 2.63$	0.11	—	—	$F_{1,31.5} < 0.01$	0.99
$F_{1,47.6} = 1.83$	0.18	$F_{1,42.2} = 3.5$	0.07	—	—	$F_{1,37.6} = 0.32$	0.57
$F_{1,46.4} = 0.49$	0.49	$F_{1,46.8} = 13.02$	0.0007	—	—	$F_{1,46.6} = 0.51$	0.48
$F_{1,50.5} = 0.68$	0.41	$F_{1,46.3} = 9.51$	0.003	—	—	$F_{1,42.3} = 0.64$	0.43
$F_{1,93.1} = 0.01$	0.90	$F_{1,146} = 0.54$	0.47	$F_{1,155} = 1.55$	0.22	$F_{1,154} = 0.04$	0.84
$F_{1,25.3} = 0.41$	0.53	$F_{1,78.97} = 14.4$	0.0003	$F_{1,155} = 0.02$	0.89	$F_{1,58.1} = 0.22$	0.64
$F_{1,19.2} = 0.02$	0.89	$F_{1,105} = 5.73$	0.018	$F_{1,170} = 0.21$	0.64	$F_{1,58.9} = 0.08$	0.77

Figure 2 Mean (\pm SE) immature survival proportions from first instar to adult emergence of *Harmonia axyridis* (H) and *Adalia bipunctata* (A) on lime trees in the field during three periods in 2009 – starting on 22 May (I), 15 June (II), and 7 July (III) – and two periods in 2010 – starting on 8 June (IV) and 1 July 2010 (V) – with the species separated (single) or combined.



species (100% *H. axyridis* vs. 67% *A. bipunctata*, $P < 0.0001$; Table 1), and that only pupal survival had a significant species*year interaction ($P = 0.0007$). In 2009 pupal survival of *H. axyridis* was higher than that of *A. bipunctata*, whereas in 2010 it was opposite. For none of the stages evidence was found for an effect of competition, or of species*competition interaction, which is similar to what was found for overall immature survival. For all stages as well as for overall immature survival, the variance component for period was consistently estimated to be positive, whereas variance components for tree and period*tree were bound at 0 in some cases. Variability

between periods within years was considerable for all stages.

Adult weight

Analysing adult weights indicated main effects of species and sex (both $P < 0.0001$; Tables 1 and S3; females were 1.2× heavier than males, Figure 3). The interaction species*sex was not significant ($P = 0.64$; Table 1), indicating that the female-to-male weight ratio was constant over the two species. Remarkably, the species effect was not constant over years (year*species: $P = 0.018$; Table 1): in 2009 *H. axyridis* adults were 2.9× heavier, in 2010 2.6×.

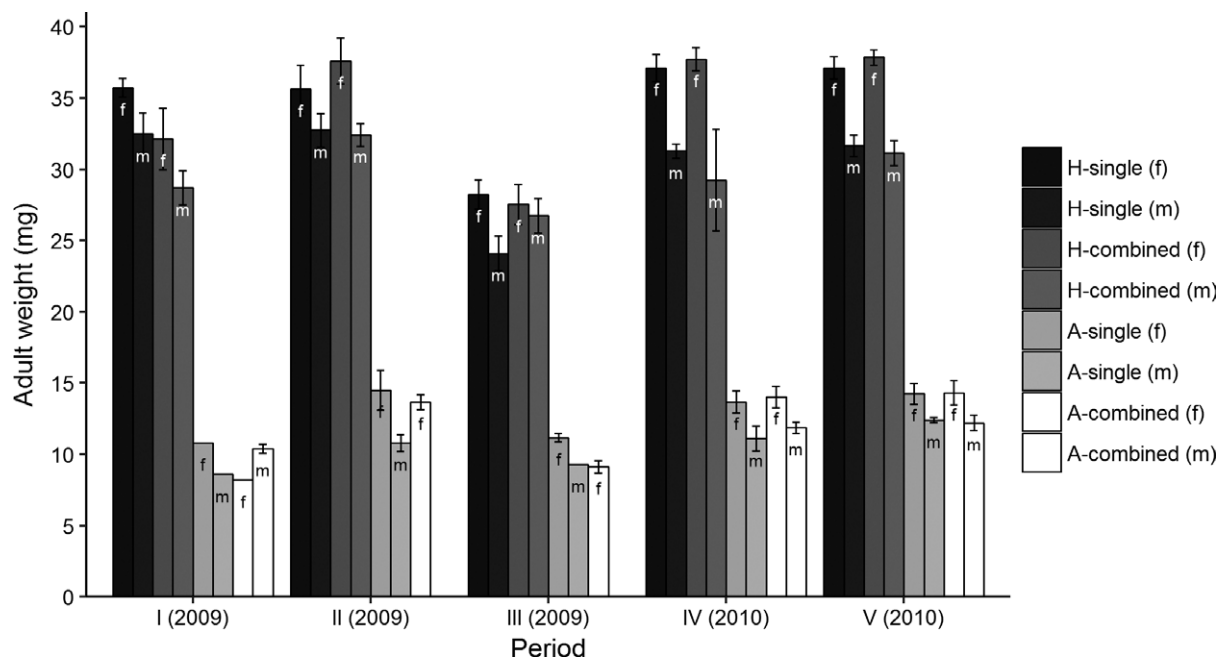


Figure 3 Mean (\pm SE) weight (mg) at adult emergence of female (f) and male (m) *Harmonia axyridis* (H) and *Adalia bipunctata* (A) on lime trees in the field during three periods in 2009 – starting on 22 May (I), 15 June (II), and 7 July (III) – and two periods in 2010 – starting on 8 June (IV) and 1 July 2010 (V) – with the species separated (single) or combined.

After removal of non-significant factors and averaging the data over the years, the back-transformed estimated means were: *H. axyridis*, females 34.7 mg, males 29.9 mg; *A. bipunctata*, females 12.6 mg, males 10.9 mg. For the analysis of pupal length and width, we refer to the supplementary information.

Temperature

The average temperature in the cages during the experiments ranged from 18.0 (I) to 23.8 °C (V), which is on average 2.6 °C higher than the temperature outside the cages (Table S4). Especially peak temperatures were high in the cages, with maxima ranging from 36.0 to 43.2 °C. Maximum temperatures in the cages were on average 8.4 °C higher than outside the cages.

Discussion

Development

The development times of *H. axyridis* found in our study fall within the range of available data on development times determined under laboratory conditions of unlimited food and long-day conditions. With a model based on those laboratory studies (Raak-van den Berg et al., 2017), estimates of development time at the average cage temperatures (Table S4) are made for each period (I: 26.4; II:

21.3; III: 22.8; IV: 22.6; V: 17.4 days). The development times observed in this study fall within this range of estimated development times. In Japan, *H. axyridis* and *Coccinella septempunctata* L. were observed on trees in a botanical garden from mid-May to mid-July (Kindlmann et al., 2000) and larval development was in both studies also estimated using the method of Manly (1997). The development times for *H. axyridis* found at comparable temperatures in this and one other study under field conditions were either much longer (18–37 days) (France; Ongagna et al., 1993) or considerably shorter (16.2–17.4 days) (Japan; Kindlmann et al., 2000) (Table S5); both studies were performed under shorter day length than our study and the type of available food was different. Differences in food quality and quantity, as well as extreme temperatures, may have caused these differences in development times (Reznik et al., 2015). The on average 2.6 °C higher temperature in our cages compared to outside suggests that development will be slower in the field, in spring in The Netherlands.

The observed development times from L1 to adult emergence for *A. bipunctata* are quite long compared to those found in previous studies (Table S6), but all literature data were determined in the laboratory with reared populations of *A. bipunctata*. In general, captivity rearing selects for shorter developmental times than found in the

field (Nunney, 2003; Tayeh et al., 2012), so this might explain the difference in the development time we found. When comparing literature data on reared populations for pupal development, we found that they are similar, whereas our larval development time is longer than that reported in the literature. Prolonged larval, but not pupal, development suggests that food availability limited the larval development. The few development times determined with offspring of individuals collected in the field for both larval and pupal stages were similar to the ones we found.

In general, *H. axyridis* develops more slowly than *A. bipunctata*. This is in accordance with the lower developmental threshold for *A. bipunctata* (8.5–10.1 °C) compared with that of *H. axyridis* (10.5–11.2 °C), whereas their thermal constants are comparable (*A. bipunctata*, 244.8–267.9 vs. *H. axyridis*, 231.3–258.3) (Obrycki & Tauber, 1981; Schanderl et al., 1985; LaMana & Miller, 1998; Jalali et al., 2010; Stathas et al., 2011). Analysis of the development time of the individual immature stages indicates that only the between-species difference in development of fourth larval stages including the prepupa determines the observed difference in overall development time. The slower development of *H. axyridis* is inconsistent with the hypothesis that a higher development rate in this species contributes to its invasive success.

Survival

Survival of *A. bipunctata* was significantly higher in 2010 than in 2009. This difference may be influenced by the fact that for period V in 2010 1-day-old first instars were used. These had the opportunity to feed on sibling eggs before being released and were therefore probably less fragile than freshly hatched larvae. Overall, immature survival of *H. axyridis* was higher than that of *A. bipunctata*. For individual stages, only L1–L3 shows differences in survival between species. This result is consistent with the hypothesis that high survival rates have contributed to the invasive success of *H. axyridis*.

When prey is limited, mortality of the larval stages is the key factor determining population dynamics of *H. axyridis* in the field (Osawa, 1993), in particular mortality of the fourth instar (Kindlmann et al., 2000). Osawa (1993) attributed mortality of younger stage larvae to their low prey-searching and capturing ability (Kawai, 1978) even when aphid density was high, and attributed fourth-instar mortality to absolute food shortage (Osawa, 1992) causing cannibalism and intraguild predation (Yasuda & Shinya, 1997). We did not observe high mortality of the fourth larval stage, possibly because we used high prey densities. Factors causing considerable larval mortality, other than cannibalism and intraguild predation, are unknown, but may include adverse weather conditions. In

this study visible natural enemies were excluded, but mortality caused by natural enemies is usually low (Osawa, 1993; Yasuda & Shinya, 1997), although some predation of eggs and younger larval stages by ants has been reported (Burgio et al., 2008).

In this experiment, larvae were placed on leaves individually, whereas both *H. axyridis* and *A. bipunctata* lay their eggs in clusters. Hemptinne et al. (2000) demonstrated that first instars are attracted to aphids eaten by conspecific larvae. These authors argued that this behaviour increased their chance of finding prey and surviving, because siblings are near each other, given that eggs are laid in clusters. This could indicate that our study underestimates first-instar survival under conditions of high prey density.

Reported survival rates for *H. axyridis* when determined in the laboratory at temperatures above 30 °C are variable: no survival at 30, 32 (Michaud, 2002), and 35 °C (Wang et al., 2009), reduced survival at 30 (22–56%; Lombaert et al., 2008), 33 (50%; Knapp & Nedved, 2013), and 34 °C (25%; LaMana & Miller, 1998), and full development at 30 (larvae and pupae; Kawauchi, 1979) and 38 °C (pupae; Knapp & Nedved, 2013), although only part of the pupae survived (O Nedved, pers. comm.). For *A. bipunctata*, Jalali et al. (2010) reported normal development at 30 °C and no development at 35 °C. In our field study, high temperatures around or above 35 °C were reached at some point during the experiments, still many larvae and pupae did survive these temperatures. In contrast to the laboratory conditions, temperatures in our field study fluctuated and larvae are apparently able to survive periods with high temperatures. Three laboratory studies confirm that most instars tolerate high temperatures for some time: 48 h at 33 °C (Knapp & Nedved, 2013; Knapp, 2014) and 2 h at 35 and 40 °C (Acar et al., 2004). Abiotic factors like shade and wind chill may allow for longer survival at high temperatures under field conditions.

Adult weight

Adult weight of female *A. bipunctata* is in line with previous studies under laboratory conditions at similar temperatures (10.7–14.1 mg; Table S7), whereas the weight of *A. bipunctata* males (9.5–10.5 mg; Table S7) is within the upper range of literature data on aphid-fed populations under laboratory conditions at similar temperatures, as is that of *H. axyridis* adults (females, 23.7–39.2 mg; males, 18.6–32.3 mg; Table S7). Food quality and quantity may have a big effect on adult size (Hodek & Honek, 1996). In this field study, food was not limited and, because of the high weight observed for *H. axyridis*, we may conclude that lime aphids apparently provide good food. Field surveys indicate that *H. axyridis* is found on lime trees in large numbers (Viglášová et al., 2017).

Effect of competition between species

Under conditions of low prey availability, intraguild predation is, in general, an important force structuring the guild of ladybirds (Yasuda & Shinya, 1997). High prey availability is generally known to decrease the incidence of intraguild predation (Obrycki et al., 1998; Schellhorn & Andow, 1999; Kajita et al., 2000; Burgio et al., 2002). However, even under conditions of high prey availability the presence of *H. axyridis* was found to slow down development of *A. bipunctata* in a study of Kajita et al. (2000). In our study no evidence was found for an effect of intraspecific competition, and we conclude that although intraguild predation under field conditions has been found (Gagnon et al., 2011; Hautier et al., 2011; Raak-van den Berg et al., 2012a; Thomas et al., 2013; Gagnon & Brodeur, 2014), it seems to be negligible at high prey densities.

Conclusion and implications

In conclusion, despite incomplete life tables, we were able to provide estimates of development time and survival of *H. axyridis* and *A. bipunctata* in the field. We also showed that with ample provision of prey (1) immature development in the field is in line with laboratory data for *H. axyridis*, but not for *A. bipunctata*, (2) survival is generally high, but higher for *H. axyridis* than for *A. bipunctata*, (3) *A. bipunctata* and *H. axyridis* do not negatively interfere, and (4) intraguild predation does not seem to occur under our experimental conditions. As for the native *A. bipunctata* the laboratory-obtained data on survival in the literature deviate from the survival we found in our semi-field experiment, it is crucial to measure life-history traits under field conditions if one aims to understand their contribution to the relative success of invasive species. We hypothesise that differences in survival of beetle species play a role in determining the numerical composition of ladybird guilds, as higher survival results in faster population growth. Finally, we speculate that the higher survival and, consequently, the faster population growth of *H. axyridis* may contribute to its invasive success, and that differences in development time are unlikely to play a direct role, as this was longer for *H. axyridis* than for the native *A. bipunctata*.

Our results indicate that comparison of life-history traits between competing native and invasive species may contribute to the understanding of the invasive character of the latter, but that it is important to compare the life histories under field conditions, as these are likely to differ from measurements taken in the laboratory. Ideally, however, studies of the role of life history in the invasive nature of a particular species also include comparisons of the life

histories of the invasive species in the invaded area with those in the area of origin, as was done for *H. axyridis* (Raak-van den Berg et al., 2017); life histories are not fixed and may respond to selection after invasion (Seiter et al., 2013). The invasive species itself might also impose selection on local native competitors in the invaded area, including on their life history (Stuart et al., 2014; Berthon, 2015). Thus, to evaluate to what extent certain characteristics of individuals of a species contribute to their invasive success, these should be studied under relevant conditions, i.e., under local abiotic circumstances and in local community context. Just as informative as the search for characteristics leading to invasive behaviour, is the analysis of factors that prevent an exotic species to become invasive. In Japan, where *H. axyridis* occurs naturally, *A. bipunctata* is a non-native species, but has not become invasive (Kajita et al., 2006), although it is expanding its distribution and prey range (Toda & Sakuratani, 2006). A comparison between characteristics of these two species, as well as local abiotic conditions and differences in community structure between Japan and western Europe is likely to single out key factors contributing to the invasion success of *H. axyridis* in western Europe.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Estimated mean (in parentheses SE, estimated with method of Pontius et al., 1989) immature development time (days) of *Harmonia axyridis* and *Adalia bipunctata* on lime trees in the field during three periods in 2009 – starting on 22 May (I), 15 June (II), and 7 July (III) – and two periods in 2010 – starting on 8 June (IV) and 1 July 2010 (V) – with the species separated (single) or combined.

Table S2. Immature survival (%) of *Harmonia axyridis* and *Adalia bipunctata* on lime trees in the field during three

periods in 2009 – starting on 22 May (I), 15 June (II), and 7 July (III) – and two periods in 2010 – starting on 8 June (IV) and 1 July 2010 (V) – with the species separated (single) or combined. Sample sizes are given in parentheses.

Table S3. Mean (and SD; n, sample size) pupal width and length (mm) and adult weight (mg) at emergence of female (f) and male (m) *Harmonia axyridis* and *Adalia bipunctata* on lime trees in the field during three periods in 2009 – starting on 22 May (I), 15 June (II), and 7 July (III) – and two periods in 2010 – starting on 8 June (IV) and 1 July 2010 (V) – with the species separated (single) or combined.

Table S4. Average, minimum, and maximum temperature (°C) inside (measured during the experiments in the cages) and in outside and minimum and maximum day length.

Table S5. Development of *Harmonia axyridis* under field conditions in France and Japan.

France: first instar to adult emergence (L1toA), small cages, excess of *Acyrtosiphon pisum* (Ongagna et al., 1993); Japan: larva (i.e., complete larval period: all four instars including prepupa), open field, declining numbers of *Aphis gossypii* Glover (Kindlmann et al., 2000).

Table S6. Literature data on development time (days) of *Adalia bipunctata* from first instar to adult emergence (L1toA; larva + pupa) and for larva (i.e., complete larval period: all four instars including prepupa) and pupa separately determined under laboratory conditions for reared and field-collected populations, with unlimited access to prey.

Table S7. Literature data on adult weight (mg, range in parentheses) at emergence of European, aphid-fed populations of *Harmonia axyridis* and *Adalia bipunctata* determined under laboratory conditions. Source populations are field collected within four generations ('wild') or laboratory reared ('reared').