

Alkaloids provide evidence of intraguild predation on native coccinellids by *Harmonia axyridis* in the field

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Abstract Over a period of less than 5 years, Belgium was thoroughly invaded by the multicolored Asian coccinellid, *Harmonia axyridis*. At the same time, a decline of some native coccinellid species was observed in tree habitats. One hypothesis about the cause of this decline was that it might

have been due to intraguild predation (IGP) by *H. axyridis*. In natural conditions, IGP between coccinellids can be tracked by using defensive compounds. Exogenous alkaloids in *H. axyridis* were therefore examined by gas chromatography-mass spectrometry (GC-MS), using individuals sampled from lime trees that were also occupied by other species of coccinellids. *Harmonia axyridis* was the dominant species at all life stages, in terms of both numbers of sites occupied and local abundance. The GC-MS analysis of *H. axyridis* larvae revealed traces of exogenous alkaloids from 19 of the 20 sites and, in nine of those 19 sites, more than 30% of the larvae analyzed contained exogenous alkaloids. Three alkaloids were detected: adaline from *Adalia* spp., calvine from *Calvia* spp. and propyleine from *Propylea quatuordecimpunctata*. Predation by *H. axyridis* on two different coccinellid species was also detected in the same larva, reinforcing the status of *H. axyridis* as a top predator. A generalized linear model indicated that IGP frequency was positively influenced by two variables: the abundance of extraguild and intraguild prey; and the interaction between these two variables. Our results therefore support the hypothesis that IGP by *H. axyridis* on native coccinellids in tree habitats has led to the decline of several of these species.

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Introduction

Invasive alien coccinellids, like other Coleoptera such as the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) and small hive beetle (*Aethina tumida* Murray), can have significant impacts. To date, only a few cases have been studied. The release in North America of the European seven-spot ladybird, *Coccinella septempunctata* L., as a classical biological control agent of pest aphids, led to dramatic changes in the composition of native coccinellid communities (Alyokhin and Sewell 2004; Elliott et al. 1996) with the decline in some native species due to competition (Evans 2004). In 2002, the harlequin ladybird, *Harmonia axyridis* Pallas, was first recognised in Belgium as an invasive species, having been introduced into the country in 1997 for aphid biological control (Adriaens et al. 2003). In less than 5 years it had colonized urban, agricultural and semi-natural habitats throughout the country, occupying niches of native species (Adriaens et al. 2008), and it subsequently invaded other European countries (Brown et al. 2008). In parallel with this invasion, a decline in native coccinellids, such as *Adalia bipunctata* (L.) and *Adalia decempunctata* (L.), was observed in Belgian tree habitats (Adriaens et al. 2010). In the USA, declines in native coccinellid species had been observed after *H. axyridis* colonisation (Colunga-Garcia and Gage 1998). The mechanisms involved in these declines are unclear, but competition or intraguild predation (IGP) could be implicated.

The intraguild interactions between two or more predator species can influence the structure, stability and diversity of animal communities (Polis et al. 1989). IGP is thought to occur mainly among arthropod generalist predators (Snyder and Evans 2006). Under laboratory conditions, *H. axyridis* has been shown to be an intraguild predator not only of other coccinellid species (Cottrell and Yeorgan 1998; Yasuda et al. 2001; Burgio et al. 2002; Ware and Majerus 2008), but also of other aphidophagous species (Phoofolo and Obrycki 1998; Gardiner and Landis 2007). It was even observed to feed upon an aphid pathogenic fungus (Roy et al. 2008). However, laboratory studies can be over-simplified and do not address the many parameters that affect IGP under field conditions (Pell et al. 2008; Weber and

Lundgren 2009), such as extraguild prey density (Rosenheim et al. 1995; Lucas et al. 1998; Hindayana et al. 2001) and habitat structure (Lucas and Brodeur 1999; Janssen et al. 2007), that can influence the magnitude and direction of IGP (Polis et al. 1989), the intraguild prey stage and the prey's ability to escape (Sato et al. 2005). The influence of these factors on IGP is difficult to predict but field surveys provide an opportunity to assess the prevalence of IGP. To date, however, IGP has been difficult to detect under field conditions (Lucas 2005).

Here we describe techniques for measuring the frequency, in the field, of *H. axyridis* IGP on native coccinellids by detecting exogenous alkaloids in *H. axyridis* larvae using gas chromatography-mass spectrometry (GC-MS) (Hautier et al. 2008; Sloggett et al. 2009). Many coccinellid species are protected from predation by alkaloids (Daloze et al. 1994; Glisan King and Meinwald 1996; Laurent et al. 2005) and these compounds can be used as predation tracers. The second purpose of this study was to examine parameters that could affect IGP in the field. Drawing on experimental results reported by Schellhorn and Andow (1999) and Kajita et al. (2000), and field observations reported by Hironori and Katsuhiko (1997), we assume that, in the field, IGP by *H. axyridis* on native coccinellids is influenced by several factors, including (1) native coccinellid larval densities (i.e. intraguild prey), (2) aphid densities (i.e. extraguild prey) and (3) total coccinellid densities at a given aphid abundance (reflecting competition for extraguild prey).

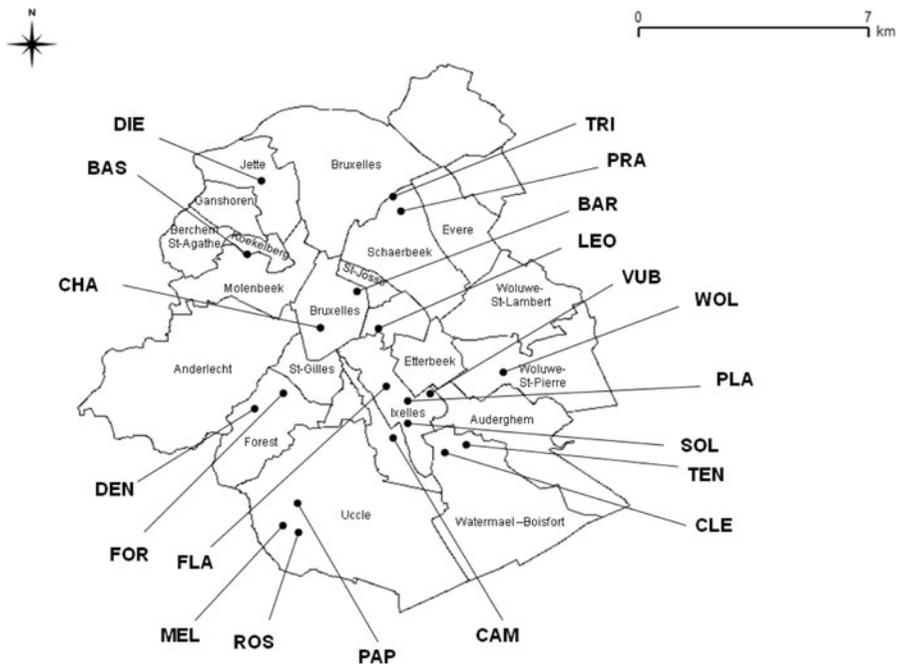
Materials and methods

The study was carried out in June and July 2008 on lime trees (*Tilia* spp.) in parks and along streets at 20 sites in Brussels (Fig. 1).

Coccinellid collection and aphid observations

There was one collection of coccinellids per site, conducted by beating branches with a stick above a collecting net (diameter 65 cm, depth 130 cm). Depending on the size of the site, 40–110 branches were sampled. All coccinellid adults were identified at species level following the identification key compiled by Baugnée and Branquart (2000). Where

Fig. 1 Sites sampled in Brussels. *LEO*: Parc Léopold; *BAR*: Place des Barricades; *CHA*: Place de la Chapelle; *PAP*: Rue du Papenkasteel; *PLA*: Campus de la Plaine; *VUB*: Boulevard Général Jacques; *SOL*: Campus du Solbosch; *CLE*: Église St Clément; *TEN*: Parc Ten Reuken; *FLA*: Abbaye de la Cambre; *BAS*: Basilique de Koekelberg; *DIE*: Parc de Dieleghem; *PRA*: Pont Van Praet; *TRI*: Chaussée de Vilvorde; *MEL*: Melkriek; *ROS*: La Roseraie; *CAM*: Bois de la Cambre; *WOL*: Parc de la Woluwe; *DEN*: Abbaye de St-Denis; *FOR*: Parc de Forest



possible, larvae were identified at species level, except for *Adalia* spp., which were identified at genus level because it was difficult to separate the species. Third and fourth instar *H. axyridis* larvae were placed individually in 1.5 ml Eppendorf microtubes and stored in a freezer at -20°C until alkaloid analysis. To estimate aphid density at each site, the number of aphids on 80 randomly selected leaves was counted.

Alkaloid analysis

The analysis of exogenous alkaloids in *H. axyridis* larvae was carried out using the method developed by Hautier et al. (2008). To increase accuracy in the interpretation of GC–MS analyses, the detection period of three exogenous alkaloids had been previously determined in *H. axyridis*. These alkaloids were selected because they are produced by three of the main native aphidophagous species found on lime trees: adaline from *Adalia* spp., calvine from *Calvia* spp. and propyleine from *Propylea quatuordecimpunctata* (L.). Starved fourth instar *H. axyridis* larvae were fed in Petri dishes (diameter 55 mm) with one first instar *A. bipunctata* or *Calvia quatuordecimguttata* or *C. septempunctata* larva. *Coccinella septempunctata* was used as a substitute for *P. quatuordecimpunctata* (L.), because the structure of its

alkaloid, coccinelline, is very close to that of propyleine, the alkaloid of the latter species (Tursch et al. 1972). Exogenous alkaloids were extracted as described here. Each Eppendorf microtube with a larva was filled with a 600 μl solution of bidistilled acetone. The larva was then crushed with a pestle. After 10 min of extraction by soaking, the mixture was filtrated in a Pasteur pipette containing a 1-cm thick layer of compressed cotton wool. To maximize alkaloid recovery, the pipette was rinsed with 200 μl of acetone. The filtrate was concentrated by evaporation. The residue was dissolved in 50 μl of acetone and vortexed until complete homogenization. Aliquots of 1 μl of the acetone extracts were then analysed by GC–MS using a Finnigan Polaris Q ion trap mass spectrometer linked to a Finnigan Trace GC equipped with a split/splitless injector and a DB-5MS column (30 m \times 0.25 mm internal diam. \times 0.25 μm film thickness) from J&W Scientific. The injection port and transfer line were set at 240 and 310 $^{\circ}\text{C}$, respectively. The splitless mode was used. Oven temperature was programmed from 50 $^{\circ}\text{C}$ (isothermal for 1 min) to 310 $^{\circ}\text{C}$ at 15 $^{\circ}\text{C}/\text{min}$, then isothermal for 1 min, using helium as the carrier gas (1.2 ml/min). The alkaloids were identified by an analysis of their mass spectra produced by electron impact (ion source operating at 250 $^{\circ}\text{C}$ with an ionization energy of 70 eV, scan range m/z 20–400)

and by comparing their GC retention times with those of the reference compounds.

Data analysis

The correlation between *H. axyridis* and native coccinellid densities (individuals per 100 beatings), and between densities of aphids (aphids per 80 leaves) and of coccinellids, were analyzed using Pearson correlation coefficients.

Generalized linear models with a binomial distribution and logit link function (Dobson 2002) were used to examine the relationship between IGP frequency and three variables measured in the field: aphid density (shared prey, extraguild prey), native coccinellid larval density (intraguild prey) and total coccinellid density, including all native and exotic larvae and adults (reflecting the competitors for extraguild prey). Explanatory variables were mean-centered and divided by their standard deviation to allow a direct comparison of model coefficients to be made (Gelman and Hill 2007). Model selection was based on using the information criterion approach described by Burnham and Anderson (2002). A set of biologically relevant models was fitted and compared using the Akaike Information Criterion (AIC), corrected for small sample size and over-dispersion, QAICc. Model selection uncertainty, indicated by the weight of each model, was estimated by calculating the probability of a given model being selected as the best model. Model averaged coefficients were estimated as the mean of each parameter from each model, weighted by the model weight estimated for each model. For model averaging, we considered only a confidence set of models whose QAICc weight amounted to 0.95. A similar approach was used to estimate coefficient standard errors, taking model selection uncertainty into account, and was not restricted to one model.

All statistical analyses were performed using R 2.10 (R Development Core Team 2009).

Results

Coccinellids

At the 20 sites sampled, 13 species, 1,000 coccinellid adults and 787 larvae were collected after 1,540

beatings. Among the coccinellid adults, *H. axyridis* was the most abundant species (mean density of 47.44 ± 8.66 individuals/100 beatings/site) and was dominant at all sites apart from two (Table 1). Adults of four native species were observed: three aphidophagous tree-dwelling species (*A. decempunctata*, *Calvia decemguttata* and *C. quatuordecimguttata*) and one mycetophagous species (*Halyzia sedecimguttata*); although 15 times less abundant than *H. axyridis*, these species were present at 15, 11, 12 and 11 sites, respectively. Two generalist species (*A. bipunctata* and *P. quatuordecimpunctata*) and two other tree-dwelling species (*Exochomus quadripustulatus* and *Oenopia conglobata*) were caught. Three conifer-dwelling coccinellids were also observed (*Anatis ocellata*, *Myrrha octodecimguttata* and *Aphidecta oblitterata*). Among the larvae, the most abundant species was *H. axyridis*, with a mean density of 55.11 ± 7.30 individuals/100 beatings/site; it was the dominant species at all sites. Four genera of native coccinellid larvae were collected (*C. quatuordecimguttata*, *Adalia* spp., *C. decemguttata* and *P. quatuordecimpunctata*). Species determination was not possible for 17 larvae. At 10 sites, no native larvae were found. The density of *H. axyridis* adults was positively correlated with the density of indigenous coccinellid adults ($r = 0.55$, $t_{1,18} = 2.80$, $P = 0.012$), but negatively correlated with the density of *H. axyridis* larvae ($r = -0.60$, $t_{1,18} = -3.18$, $P = 0.0052$).

Aphid populations

The density of extraguild prey was highly variable across sites, from 0.18 aphids/leaf to 6.25 aphids/leaf, with a mean of 2.24 ± 1.40 aphids/leaf. No significant correlations were observed between the aphid population and the density of adult and larval native species ($r = -0.029$, $t_{1,18} = -0.12$, $P = 0.93$ and $r = -0.26$, $t_{1,18} = -1.13$, $P = 0.27$), or between the aphid population and the density of adult and larval *H. axyridis* ($r = -0.077$, $t_{1,18} = -0.33$, $P = 0.75$ and $r = 0.013$, $t_{1,18} = 0.055$, $P = 0.96$).

Exogenous alkaloid content in *H. axyridis*

Exogenous alkaloids were detected in *H. axyridis* larvae and identified on the basis of their retention time and mass spectra (Table 2). These alkaloids can

Table 1 Densities of coccinellid larvae and adults per site and number of sites where the species is present (No. sites)

Species	Larvae					Adults				
	Mean	SEM	Min	Max	No. sites	Mean	SEM	Min	Max	No. sites
<i>Adalia</i> spp.	0.53	0.28	0	5	5	3.99	0.86	0	15	16
<i>A. bipunctata</i> (L.)						0.59	0.22	0	3.3	7
<i>A. decempunctata</i> (L.)						3.40	0.70	0	12	15
<i>Aphidecta oblitterata</i> (L.)						0.10	0.07	0	1	2
<i>Anatis ocellata</i> (L.)						0.10	0.10	0	2	1
<i>Calvia</i> spp.	1.39	0.52	0	9	8	5.36	1.13	0	21	17
<i>C. decemguttata</i> (L.)	1.14	0.50	0	9	7	3.00	0.96	0	17	11
<i>C. quatuordecimguttata</i> (L.)	0.25	0.18	0	3.3	2	2.37	0.56	0	8.3	12
<i>Exochomus quadripustulatus</i> (L.)						0.10	0.07	0	1	2
<i>Halyzia sedecimguttata</i> (L.)						3.28	1.11	0	18	11
<i>Harmonia axyridis</i> Pallas	55.11	7.30	10	135	20	47.44	8.66	0	126	18
<i>Myrrha octodecimguttata</i> (L.)						0.05	0.05	0	1	1
<i>Oenopia conglobata</i> (L.)						0.08	0.08	0	1.7	1
<i>Propylea quatuordecimpunctata</i> (L.)	0.05	0.05	0	1	1	0.10	0.07	0	1	2
<i>Psyllobora vigintiduopunctata</i> (L.)						0.08	0.08	0	1.7	1
Unidentified	0.93	0.32	0	4.5	8					
Native coccinellid	2.01	0.74	0	12	10	13.25	2.08	0	40	19
Exotic coccinellid	55.11	7.30	10	135	20	47.44	8.66	0	126	18
Total coccinellid	59.44	7.37	14	135	20	60.68	9.96	7.5	153	20

Mean: mean density per site (No. individuals/100 beatings/site), SEM: standard error of mean, Min and Max: minimum and maximum density per site (No. individuals/100 beatings/site)

be accurately detected because of their distinct retention times, resulting in no peak overlap. Adaline, the alkaloid from *A. bipunctata* and *A. decempunctata*, could be detected 96 h after ingestion. Calvine from *C. quatuordecimguttata* and precoccinelline from *C. septempunctata* were not sequestered and were detected only during the first hour, or the first 24 h after ingestion, respectively.

Out of the 590 *H. axyridis* larvae analysed (third to fourth instar), 121 showed exogenous alkaloids from native coccinellid larvae (Table 3). Positive *H. axyridis* larvae (i.e. containing exogenous alkaloids) were detected at all sites apart from one (LEO) where no exogenous alkaloid was detected in the 52 larvae analysed. At nine sites (BAR, CLE, MEL, PLA, PRA, ROS, SOL, TEN, VUB), more than 30% of the *H. axyridis* larvae analysed were positive. The highest proportion of positive larvae was 50% (MEL). Three exogenous alkaloids were detected in the positive larvae: adaline, propyleine and calvine. Adaline was the most commonly detected one and was found in 104 larvae, whereas propyleine and

calvine were detected in only seven and three larvae, respectively. In 6% of the positive larvae, two alkaloids were detected in the same larvae from four sites (CLE, PAP, SOL, VUB). All possible combinations were recorded—adaline and calvine, adaline and propyleine, and calvine and propyleine—and were detected in four, two and one positive larvae, respectively.

Factors influencing IGP under field conditions

We fitted eight models describing the relationship between IGP frequency and three variables observed at the sites: aphid density as extraguild prey, native coccinellid larval density as intraguild prey, and total coccinellid density (native and *H. axyridis*) reflecting competitors (Table 4). According to cumulative sum of model weights, there was a 0.85 probability that the best model would be among the first two models and a 0.97 probability that it would be among the first three. The other models were clearly less well supported by the data. The first three models included

Table 2 Detection period of exogenous alkaloids in 4th instar larvae of *H. axyridis*

Intraguild prey (1st instar larva)	Alkaloid detected	Retention time (min)	Time after ingestion				
			1 h	24 h	48 h	72 h	96 h
<i>Adalia bipunctata</i>	Adaline	10.85–10.93	+ (10/10)	+ (10/10)	+ (10/10)	+ (10/10)	+ (9/9)
<i>Adalia decempunctata</i>	Adaline	10.79–10.88	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
<i>Calvia quatuordecimguttata</i>	Calvine	12.50–12.51	+ (5/5)	– (0/5)	– (0/5)	– (0/5)	
<i>Coccinella septempunctata</i>	Precoccinelline	8.82–8.92	+ (5/5)	+ (5/5)	– (0/5)	– (0/5)	– (0/5)

“+”: presence of alkaloid peak; “–”: absence of alkaloid peak. In brackets: number of positive larvae/number of larvae analysed

aphid density and native coccinellid larvae, total coccinellid density and the interaction between aphids and native coccinellids as explanatory variables. Aphids and native coccinellids were clearly the most important variables, with similar weights (0.99) (Table 5). Both these variables were present in the three best models. The aphids * native coccinellids interaction had a weight of 0.322 and was present in only the second of the three best models. The total coccinellid density had a weak weight (0.135) and was present in only the third of the three best models. In addition, the averaged coefficient of this last variable was estimated with poor precision ($\beta = -0.166$, $SE = 0.199$).

From the three best models, it was possible to describe how IGP frequency varies when fixing either aphid density or native ladybird larval density (Fig. 2). When aphid density was fixed, IGP frequency increased slowly with native ladybird larval density at a low aphid density. It was independent of intraguild prey at medium aphid density and, at a higher aphid density, decreased slightly when intraguild prey increased. When native larval coccinellid density was fixed, IGP frequency was independent of aphid density at high native ladybird larval density, but increased slightly with aphid density when native ladybird larval densities had lower values.

Discussion

At all the sites sampled, *H. axyridis* was the most numerous coccinellid species at both the larval and adult stages. It appeared to have replaced the native

aphidophagous species commonly observed on European trees before the invasion, such as *A. decempunctata*, *A. bipunctata* and *C. quatuordecimguttata* (Wratten 1973; Iablokoff-Khnzorian 1982; Honěk 1985; Majerus 1994). At the adult stage, no significant difference in site preference was observed between native species and *H. axyridis*, as indicated by the significant correlations between relative densities at each site. At the larval stages, however, the native species were rare and were observed at only half the sites, whereas *H. axyridis* was present at all sites. This absence of native larvae could have resulted from reduced fecundity or increased predation when *H. axyridis* was present and fed on aphids and other coccinellids (Soares and Serpa 2007; Dixon 2000).

Our analytical technique for detecting the exogenous alkaloids of native coccinellids in *H. axyridis* larvae highlighted the existence of IGP on lime trees. IGP was not a rare event; it was apparent at all sites except one. Three exogenous alkaloids were detected: adaline, propyleine and calvine. These alkaloids are naturally produced by *Adalia* spp., *P. quatuordecimpunctata* and *Calvia* spp., respectively (Laurent et al. 2005). Laboratory experiments by Kajita et al. (2000), Burgio et al. (2002) and Ware and Majerus (2008) have shown that these species could be prey for *H. axyridis*, but here we show that, under field conditions, these native species are effectively consumed by *H. axyridis*. The different alkaloid retention-detection periods and the different densities of native larvae make it difficult to suggest the prey preferences of *H. axyridis*. The long persistence of adaline in the predators increased the likelihood of

Table 3 Numbers of *H. axyridis* larvae analysed and positive (i.e. with alkaloids), and alkaloids detected at the 20 sampled sites

	Sites																			Total No. sites			
	BAR	BAS	CAM	CHA	CLE	DEN	DIE	FLA	FOR	LEO	MEL	PAP	PLA	PRA	ROS	SOL	TEN	TRI	VUB		WOL		
No. larvae analysed	20	19	29	39	23	31	4	50	31	52	4	38	13	24	85	38	5	26	26	33	590	20	
No. positive larvae	7	1	2	8	8	3	1	2	4	0	2	9	4	10	28	15	2	3	9	3	3	121	19
No. positive larvae with:																							
Adaline	7	1	2	8	7	2	1	2	4	0	2	6	4	10	25	14	2	3	3	3	1	104	19
Calvine	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	3	2
Propyleine	0	0	0	0	0	1	0	0	0	0	0	1	0	0	3	0	0	0	0	0	2	7	4
Adaline + calvine	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	4	3
Adaline + propyleine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1
Calvine + propyleine	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1

detecting IGP of *Adalia* spp., compared with the other native coccinellid species. However, predation on *Adalia* spp. was detected at 19 sites, including 14 sites where no *Adalia* larvae were collected, whereas *Calvia* spp. larvae were observed at 5 of these 14 sites. In Petri dishes, *C. quatuordecimguttata* was attacked less often than *A. bipunctata* or *P. quatuordecimpunctata* (Ware and Majerus 2008). In addition, *C. quatuordecimguttata* eggs are chemically protected from predation by *H. axyridis* (Ware et al. 2008), unlike *A. bipunctata* eggs, which are completely consumed (Burgio et al. 2002). *Harmonia axyridis* could have initially attacked and consumed *Adalia* spp. eggs and larvae present at the site before switching to attack the well-defended *Calvia* spp. In view of these results, IGP on *Adalia* spp. could at least partly explain the observed decline of these species in tree habitats in Belgium (Adriaens et al., 2010).

In 6% of the *H. axyridis* larvae analysed, two different alkaloids were detected simultaneously. This was probably the result of the predation of two different coccinellid species or (but less likely in view of the short alkaloid persistence) of the consumption of a coccinellid that was itself an intraguild predator (secondary predation). The observed consumption of several coccinellid species reinforces the status of *H. axyridis* as a top predator and confirms its polyphagous food habits. Several traits of *H. axyridis* seem to make it a top predator, as suggested by Dixon (2000). Larval development in *H. axyridis* is not affected by the consumption of heterospecific coccinellid larvae (Yasuda and Ohnuma 1999; Sato et al. 2008), and *H. axyridis* tolerates the coccinellid chemical defence because it is able to modify prey alkaloids rapidly (Sloggett and Davis 2010). This ability to prey on other coccinellids is further reinforced by defences against predation by other coccinellids, such as chemical defences (Sato et al. 2009) and the physical protection provided by strong dorsal spines (Hautier et al., in prep.). The phenological patterns of the different coccinellids also suggest that *H. axyridis* is a top predator. In *Hibiscus* spp. trees, alfalfa and potato fields, *H. axyridis* arrives after the other coccinellids (Takahashi 1989; Hironori and Katsuhiko 1997; Jansen and Hautier 2008) and its young larvae are able to develop on resources already exploited by other predators and to complete their development even while these resources are declining.

Table 4 QAICc statistics for generalized linear binomial models describing the relationships between IGP frequency and three variables: aphid density (aphids), native coccinellid larval density (Lnative) and total coccinellid density (ldbtot)

Model	<i>k</i>	<i>n</i>	Loglik	QAICc	Delta	Weight	Sum.w
Aphids+ Lnative	3	20	-46.25511	57.534	0	0.531	0.531
Aphids+ Lnative+ aphids*Lnative	4	20	-43.70211	58.566	1.032	0.317	0.849
Aphids+ Lnative+ ldbtot	4	20	-45.55034	60.439	2.905	0.124	0.973
Lnative	2	20	-57.34482	65.604	8.07	0.009	0.982
Aphids	2	20	-57.80379	66.069	8.535	0.007	0.99
Aphids+ Lnative+ ldbtot+ aphids*Lnative+aphids*ldbtot	6	20	-42.8398	66.74	9.206	0.005	0.995
Aphids+ ldbtot+ aphids*ldbtot	4	20	-52.7022	67.685	10.151	0.003	0.998
Aphids+ ldbtot	3	20	-57.72667	69.157	11.623	0.002	1
(intercept)	1	20	-70.40693	76.045	18.511	0	1

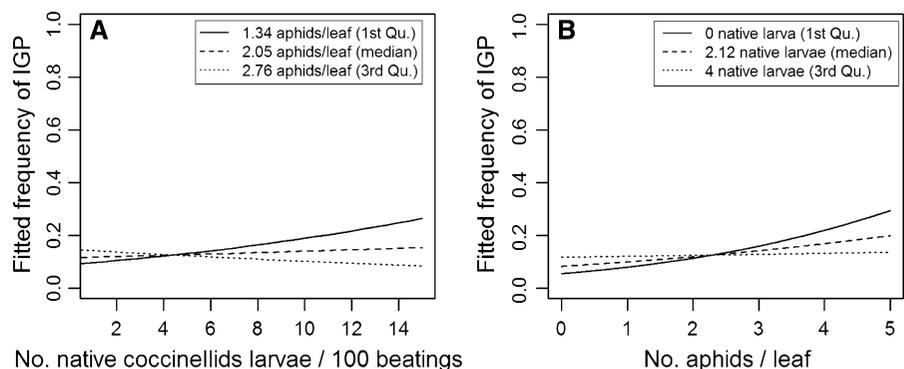
k: number of parameters in the model; *n*: number of observations; Loglik: Log Likelihood; QAICc: Akaike Information Criterion corrected for small sample size and overdispersion; Delta: QAICc – QAICc_{minimum}; Weight: model weight based on QAICc; Sum.w: cumulative sum of model weights. All models include an intercept (not shown)

Table 5 Averaged coefficients and standard errors in the model

Variables	Frequency	Weight	Coefficient	SE
(intercept)	1	1	-1.552	0.169
Aphids	0.778	0.991	0.434	0.144
Lnative	0.556	0.988	0.480	0.141
Aphids*Lnative	0.222	0.322	-0.428	0.268
Ldbtot	0.444	0.135	-0.166	0.199
Aphids*ldbtot	0.222	0.009	NA	NA

The variables are expressed in standard deviation units. Frequency: frequency of the variable in the models; Weight: weight of the variable in the models; Coefficient: corresponding to a logit scale (log of odd ratio), SE: standard error of the coefficient. NA: parameter not present in the 95% confidence set of models

Our field data provided evidence of the correlation between IGP and intra- and extra-guild prey density. Two main factors affecting the frequency of *H. axyridis* IGP on native species were identified:

Fig. 2 Change of IGP frequency depending on the number of coccinellid larvae (A) or the number of aphids (B). Models based on averaged coefficients and mean value of total ladybird density

aphids (i.e. extraguild prey) and native larvae (i.e. intraguild prey). They both had a positive influence on IGP frequency, with a similar magnitude. Their interaction, however, negatively influenced IGP frequency. It should be noted that there are some caveats regarding these conclusions, because they cannot be interpreted unequivocally and because many factors could not be controlled during the field observations. The explanatory variables that we identified might not be entirely causal factors, but also consequential. The measured densities of intraguild prey could be partly or entirely the result of IGP, instead of the trigger for it, and the same could be said of extraguild prey density which, in addition, was measured once and not at the instant of IGP. Surveys were made on various dates and at different sites, and therefore the age structure of aphid colonies (growth or decline) differed because aphid population structure is linked to the quality of food, predators and parasites action, intraspecific effects and weather (Dixon 1971, 1998).

The age structure of aphid colonies could also have a strong influence on IGP level. When an aphid colony is growing, it constitutes a rich source of food for predators, thus reducing competition among these predators, whereas a declining colony would induce a high level of competition. In addition, the variables that we identified could have masked other factors. Competitors other than coccinellids (syrphid and lacewing larvae) were present at the sites and could have influenced IGP between coccinellids. Cannibalism could not be assessed, although we know that this behaviour can regulate predator density in terms of food resources (Polis 1981), notably in coccinellids (Mills 1982; Osawa 1993).

In conclusion, in the field, IGP by *H. axyridis* on native coccinellids is frequent and widespread. These results support the hypothesis that IGP by *H. axyridis* is responsible for the observed decline of several species in arboreal habitats.

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