

Invasive alien species under attack: natural enemies of *Harmonia axyridis* in the Netherlands

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Abstract The aphid predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an invasive alien species in Europe and North America with negative effects on non-target species (including a decline of native ladybird populations), as well as fruit production, and human health. It is, therefore, important to find out which natural enemies could be used to reduce their numbers. Knowledge of *H. axyridis*' natural enemies is summarised and data collected from the Netherlands over the past ten years are presented. Beetles were sampled from winter aggregations and from spring through to autumn with illuminated

screens at night. Natural enemies were not found in samples of *H. axyridis* from 2003–2007. From 2008 onward *H. axyridis* adults were infested by: *Hesperomyces virescens* Thaxt. fungi (summer and winter), *Parasitylenchus bifurcatus* Poinar and Steenberg nematodes (winter), *Coccipolipus hippodamiae* (McDaniel and Morrill) mites (winter), and *Dinocampus coccinellae* (Schrank) parasitoids (summer and winter). Our results indicate that these natural enemies are starting to use *H. axyridis* as a host, but are as yet not sufficiently abundant to control the population.

Keywords *Hesperomyces virescens* ·
Parasitylenchus bifurcatus · *Coccipolipus*
hippodamiae · *Dinocampus coccinellae* ·
Parasitylenchus coccinellinae

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Introduction

The multicoloured Asian ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an invasive alien species in Europe and North America with negative effects on non-target insect species, fruit production, and human health (Koch and Galvan 2008; van Lenteren et al. 2008). Moreover, a decline in native ladybird populations is associated with its establishment and rapid spread (Roy et al. 2012). *Harmonia axyridis*, an efficient predator of aphids, is native to northeast Asia (Brown et al. 2011). It was first introduced into North America as a biological control agent in 1916, followed by numerous releases at many locations through the 1980s (Gordon 1985), but first became an established species in 1988 in the state of Louisiana, USA (Krafsur et al. 1997). In northwest Europe *H. axyridis* was first introduced in 1982, it was used for aphid control in greenhouse crops from 1990, and it was released in the Netherlands in outdoor crops in 1996. In the Netherlands the first specimen (a pupa) was reported from the wild in October 2002 (Cuppen et al. 2004), and the population of *H. axyridis* has rapidly increased in size and range since then.

Increased understanding of the invasion process and knowledge of mechanisms that promote alien species in becoming established and invasive is needed to limit the effect of these species on native communities (Roy et al. 2011a). In addition to life history traits that promote invasion, an alien species introduced to a new range may experience reduced impacts from natural enemies because it invaded without its native natural enemies, and/or because natural enemies in the invaded area are not (yet) effective in attacking the invader (Enemy Release Hypothesis, Roy et al. 2011a). Many parasitoids, parasites, and pathogens are reported to attack ladybirds, and under certain circumstances these may cause important ladybird mortality (Ceryngier et al. 2012).

Knowledge about natural enemies of *H. axyridis* in the native and invaded range is currently very limited and more detailed studies are needed to draw conclusions on, and predict, the effect of natural enemies on population dynamics of *H. axyridis*. Roy et al. (2011b) provided an overview of natural enemies of coccinellids and discussed potential novel interactions between *H. axyridis* and natural enemies. In the supporting materials for the current paper, we provide

an extensive survey of the current knowledge of natural enemies attacking adult *H. axyridis*: biology, ecology, and prevalence of entomopathogenic fungi *Hesperomyces virescens* Thaxt. (Ascomycota: Laboulbeniomyces: Laboulbeniales), nematodes *Parasitylenchus bifurcatus* Poinar and Steenberg (Tylenchida, Hexatylinea: Iontonchioidea, Parasitylenchidae), ectoparasitic mites *Coccipolipus hippodamiae* (McDaniel and Morrill) (Acarina: Podapolipidae), and insect parasitoids *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae).

Here, we document natural enemies that attack *H. axyridis* in the Netherlands, describe the infection rates over the past decade, and discuss the potential effects of these natural enemies on *H. axyridis* populations. This study serves as a baseline for monitoring the prevalence of natural enemies of *H. axyridis* over time in the Netherlands and surrounding areas. It may also be used to evaluate strategies to control this invasive organism.

Materials and methods

Sampling *Harmonia axyridis*

Winter sampling

Over the course of five successive winters (2006–2010), aggregations of hibernating *H. axyridis* were collected ($n = 378, 878, 13521, 19085,$ and 3105 respectively) at various locations and on various dates in the Netherlands. Beetles were also sampled during their migratory flights [2004, ‘De Kaaistoep’ ($n = 333$) and 2010, Wageningen ($n = 185$)]. For details see supporting materials (Supporting Material S1).

Ladybirds sampled in 2008, 2009, and 2010 were transported at ambient temperature to Wageningen. The beetles were allowed to continue overwintering in outdoor cages behind wooden shutters on the wall of an experimental farm of Wageningen University (N51°59′32″, E5°39′43″) as described earlier (Raak-van den Berg et al. 2013). Over the course of the winter, samples of beetles were taken from the overwintering cages and then either directly frozen or first used in experiments and frozen afterwards (Raak-van den Berg et al. 2012a, 2013). Beetles sampled in 2004, 2006, and 2007 were immediately stored in ethanol (70 %).

Summer sampling

Since 1995 insects have been monitored regularly, at night, from March to November (hereafter referred to as summer) each year, in 'De Kaaistoep', near Tilburg (N51°32'25", E5°0'53"). Insects, attracted to a vertically-placed, white, illuminated, polyester screen (3.5 m wide, 1.9 m high), were sampled with an aspirator after they had landed on the screen, as described in van Wielink and Spijkers (2013). Observations started at sunset and continued for 4 h.

Sampling efforts were not constant over the years. In mid-July 2003, the first *H. axyridis* adult was caught. As a result of this observation and to monitor the arrival of *H. axyridis*, sampling effort was intensified in the years 2004–2006. In the period from 2003 to 2011 insects were observed during 361 nights and *H. axyridis* was caught during 208 nights ($n = 6216$ specimens). No *H. axyridis* were caught in the months March and November in any year. All *H. axyridis* individuals were collected, killed with ethyl-acetate and stored in ethanol (70 %).

Prevalence of natural enemies

Laboulbeniales

All ladybirds collected during summer 2003–2011, during migratory flight in 2004 and 2010, during winter 2006, 2007, and 2010 and a subset of beetles collected during winter 2008 and 2009 were checked for visible infection with *Laboulbeniales*. During winter 2010 beetles were still alive during examination. Ladybirds without visible infection were used in experiments for two to four weeks at warm experimental conditions (25 °C) until shortly after first oviposition and were subsequently frozen. Beetle couples that reproduced late, or not at all, were kept longer at warm conditions until death or late oviposition. Voucher specimens were identified by D. Haelewaters and are deposited at Farlow Herbarium, Harvard University, USA. DNA was extracted (Weir and Blackwell 2001) from seven thalli from one host (# D. Haelew. 113, female from Tilburg, winter 2010) and the sequence of small subunit ribosomal DNA (SSU rDNA) was established (methods in Hansen et al. 2005) with primer set NS1/NS4 (White et al. 1990) (GenBank KC833471).

Parasitoids, mites, and nematodes

To determine infection with mites, nematodes and parasitoids, the frozen or ethanol-stored beetles were inspected under the elytra for mites, and the abdomen was dissected to detect nematodes and parasitoids. Beetle sex and colour were recorded. Female ladybirds collected during summer (2003–2011) as well as (sub) samples of beetles (both sexes) collected during winter (2006–2010) were examined (Supporting Material S1).

Nematodes from the different locations were examined by G. Karssen with light microscopy and compared with Danish *P. bifurcatus* specimens provided by G.O. Poinar (Poinar and Steenberg 2012). DNA was extracted from five nematode specimens, collected in Alkmaar, Deelen-K, Tilburg, and Winssen (winter 2010). After amplification of nearly full length SSU rDNA, fragments were cloned and sequenced as described in Holterman et al. (2006) (GenBank KC875397, KC875398, KC875399, KC875400, KC875401). Following alignment of these new sequences in a phylum-wide framework harbouring about 2700 SSU rDNA sequences (Vervoort et al. 2012), a local phylogenetic tree was constructed, including members of the suborder Hexatyliina only.

When mites were found, the elytrae with the mites were stored in 70 % ethanol, and specimens were sent to R. W. Husband for identification.

Parasitoid larvae found during dissection and parasitoid larvae emerging from adult ladybirds during the experiments of 2008 and 2010 were collected. For identification, the CO1 gene of two parasitoids, collected in Tilburg and Kootwijk (winter 2010), was sequenced (Folmer et al. 1994) (GenBank KF021264) and compared with that of *D. coccinellae* specimens that had earlier been identified morphologically by C. van Achterberg.

Statistical analysis

Statistical analyses were performed separately for winter and summer data, as data collection strategies were different. During winter, aggregations of hibernating *H. axyridis* were collected at different locations in the Netherlands, but not all locations were visited each year. During summer, *H. axyridis* was collected at a single location over the years 2003–2011.

Table 1 Overview tested relationships, datasets, response and explanatory variables. *H. vir* codes for *H. virescens*

Part	Dataset	Response variable	GLM specification	Explanatory variables	Period
1	All	Fraction <i>H. vir</i> infected	Binomial ($n > 1$), logit link	Location, sex	2010
2a	Dissected	<i>H. vir</i> infected (y,n)	Binomial ($n = 1$), logit link	Location, sex, colour, nematode	2010
2b		Nematode infected (y,n)	Binomial ($n = 1$), logit link	Year, location, sex, colour, <i>H. vir</i>	2008–2010
2c	Experiment ^a	Female non-fertile (y,n)	Binomial ($n = 1$), logit link	Location, colour, nematode, <i>H. vir</i>	2010
3	Summer	Fraction <i>H. vir</i> infected	Binomial ($n > 1$), logit link ^b	Year, sex, season (Jun–Sep)	2008–2011

^a Excluding six females: (non-)fertility could not be determined, (Raak-van den Berg et al. 2013)

^b Excluding individuals with unknown sex

Sampling efforts were neither constant over years nor months.

Statistical analysis consisted of fitting generalised linear models (GLM., McCullagh and Nelder 1989) [procedure GENMOD of SAS/STAT® software, version 9.2 (SAS Institute Inc., 2008)], see Table 1. We allowed for interactions between explanatory variables, if possible, but removed these from the final models if not significant ($P > 0.05$). Regression coefficients (rc) in the logistic regression models were interpreted in terms of odds ratios (OR, where OR is reported as $\exp(\text{rc})$). Likelihood ratio test statistics χ^2 were used, unless mentioned otherwise.

Results

No fungi, nematodes, mites or wasps were found attacking *H. axyridis* in 2003–2007. In 2008, 2009, and 2010, several natural enemies were found. Results are presented below for each natural enemy.

Hesperomyces virescens

Before 2010 no *H. virescens* infection was found in winter samples described in the present study. In another study (Raak-van den Berg et al. 2012b), however, five (out of 753) beetles sampled in winter 2009 were infected with Laboulbeniales but these were not identified to species level. During the winter of 2010, a remarkably larger number of infected beetles was found. Figure 1 shows the counts, with infection rate ranging from 0 % (Deelen-K and Winssen) to 55.6 % (Winterswijk). When analysing the 2010 infection rates (Table 1, part 1), we found significant differences between locations ($P < 0.0001$, $\chi^2 = 671.6$, $\text{df} = 11$) and sex ($P < 0.0001$, $\chi^2 = 38.6$, $\text{df} = 1$). Male ladybirds had

1.73 [= $\exp(\text{rc}) = \exp(0.55)$, $\text{se}(\text{rc}) = 0.09$] greater odds of infection than female ladybirds.

From six locations, all beetles (or a representative sample) were dissected individually (Supporting Material S1, $n = 1472$): 348 beetles were infected with *H. virescens*, 89 with nematodes, and 23 with both. Statistical analysis (Table 1, part 2a) showed significant differences in *H. virescens*-infection rates between the locations ($P < 0.0001$, $\chi^2 = 289.5$, $\text{df} = 5$). Tilburg had the highest infection rate of those six locations. Again, males had higher infection rates than females [$P < 0.0001$, $\chi^2 = 20.4$, $\text{df} = 1$; OR = 1.87 (= $\exp(0.63)$, $\text{se}(\text{rc}) = 0.14$)]. An association of fungal infection with nematode infection was found for location Tilburg ($P = 0.0009$, $\chi^2 = 11.0$, $\text{df} = 1$), where nematode-infected beetles had 4.9 [= $\exp(1.60)$, $\text{se}(\text{rc}) = 0.48$] times greater odds (for infection with *H. virescens*) than non-nematode-infected beetles. In other locations, *H. virescens*-infection rates were low and were not significantly associated with nematode infection ($P = 0.67$, $\chi^2 = 0.18$, $\text{df} = 1$). No relationship of *H. virescens* infection with ladybird colour was found ($P = 0.94$, $\chi^2 = 0.12$, $\text{df} = 2$). A partial SSU rDNA sequence (728 bp) was obtained, which perfectly matches two known SSU rDNA sequences of *H. virescens* (GenBank AF298233, JQ941711).

In the summer samples the first infection with *H. virescens* was recorded in July 2008. During the years 2008–2011, *H. axyridis* beetles were collected from 56 of 111 observation nights. On 18 nights ladybirds were infected with *H. virescens* ($n = 65$, Table 2), 63 of which could be used in the analysis, as sex was unknown for two adults (July 2008 and July 2009). Analysis of the infection rate (Table 1, part 3) showed significant differences between years ($P < 0.0001$, $\chi^2 = 55.7$, $\text{df} = 3$). No significant association with

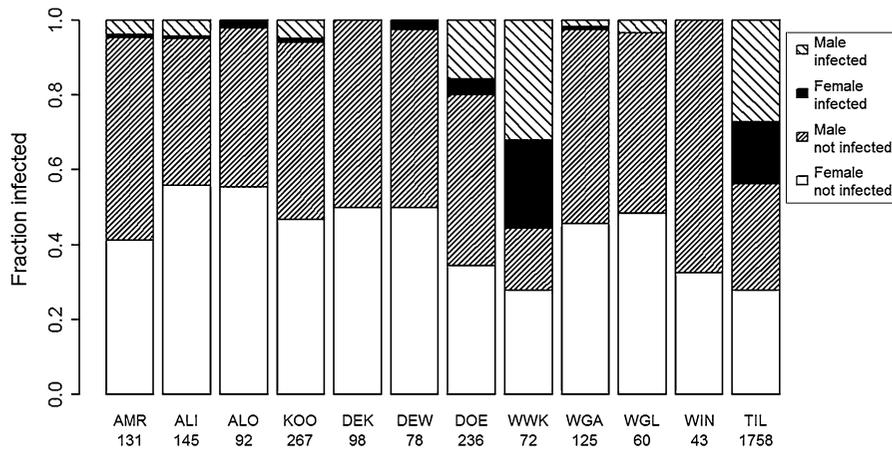


Fig. 1 Prevalence of *H. virescens* on *H. axyridis* adults, sampled from winter aggregations in 2010. The horizontal axis shows the sampling locations and number of beetles collected.

Table 2 Prevalence of *H. virescens* on *H. axyridis* adults, collected with an illuminated screen in ‘De Kaaistoep’ during spring–autumn in the years 2008–2011

Year ^a	Season ^b	Observation nights (N)	Total collected (N)	<i>H. virescens</i> infected [N (%)]
2008	Spring	21	42	0 (0)
	Summer	14	1,088	5 (0.5)
	Autumn	0		–
2009	Spring	14	1	0 (0)
	Summer	20	699	27 (3.9)
	Autumn	2	0	0 (0)
2010	Spring	6	0	0
	Summer	13	242	18 (7.4)
	Autumn	2	7	0 (0)
2011	Spring	5	5	1 (20)
	Summer	10	339	14 (4.1)
	Autumn	4	8	0 (0)

^a See also Haelewaters et al. (2012) (period 2008–2010)

^b Spring: March–May, summer: June–September, autumn: October–November

season ($P = 0.81$, $\chi^2 = 0.057$, $df = 1$) or sex ($P = 0.44$, $\chi^2 = 0.58$, $df = 1$) was found.

Coccipolipus hippodamiae

Ten ladybirds were found to be infected with *Podapolipus* mites. Mites on three specimens were positively identified as *C. hippodamiae* by R. W. Husband.

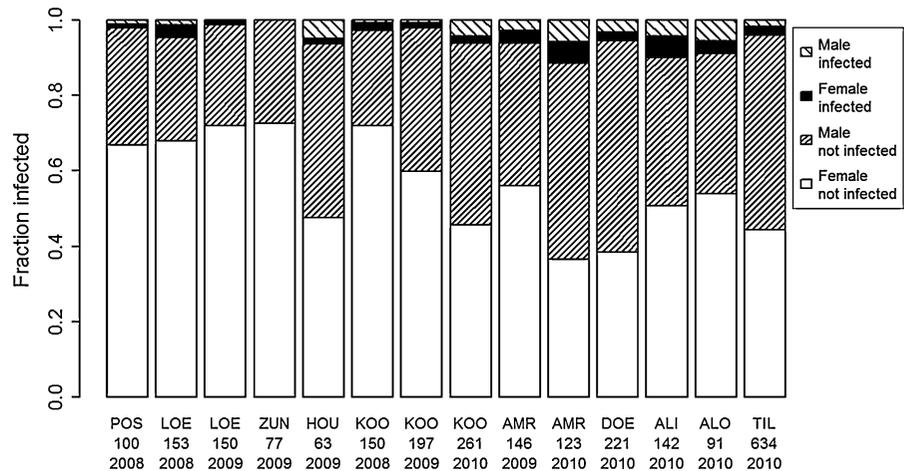
Prevalence was recorded by means of visual inspection. Abbreviations for locations are given in Supporting Material S1

One infected voucher specimen has been deposited at the Museum of Zoology of the University of Michigan, USA. The mites were found on ladybirds sampled during the winters of 2009 [Kootwijk (1) and Zundert (2)] and 2010 [Alkmaar (2), Alphen-O (1), Kootwijk (1), Tilburg (3)]. Prevalence ranged from 0.38 % (Kootwijk 2010) to 2.6 % (Zundert). Both male and female ladybirds were infected. Two male ladybirds from Tilburg were infected with both mites and *H. virescens*. No co-infection with nematodes was observed. No mites were found in the summer samples.

Parasitylenchus bifurcatus

In the winter samples from 2008, 2009, and 2010, nematode infection was found in ladybirds from all locations studied, except Zundert. Additionally, in 2010, incomplete samples from six other locations were dissected (Supporting Material S1), namely only the few beetles that died during the experiment. In five cases at least one of the dissected beetles was nematode infected (Deelen-K, Deelen-W, Winssen, Wageningen-A, and Wageningen-L), indicating that the prevalence was at least 5 %. In 2008, beetles from four locations (Alphen-O, Deelen-K, Deelen-W, Loenen-O, $n = 2097$) overwintered together in one cage: four of the 52 dissected beetles were found infected. Thus, nematode infection has been reported in almost all populations. Both juveniles, males and adult

Fig. 2 Prevalence of *P. bifurcatus* in *H. axyridis* adults collected from winter aggregations in 2008, 2009, and 2010. The horizontal axis shows the sampling year, sampling location, and the number of beetles dissected. Abbreviations for locations are given in Supporting Material S1



females, were found in one ladybird. Determination based on morphological characteristics showed that these nematodes were identical to the nematodes found in Denmark and, therefore, belong to the species *P. bifurcatus* (Poinar and Steenberg 2012).

The nearly complete SSU rDNA sequences (approx. 1700 bp) of the nematodes originating from the different locations were identical and did not match any known SSU rDNA sequence. The sequences represented a well-supported separate group within the suborder Hexatyulina constituting a part of a cluster which also includes *Fergusobia* and *Deladenus*. Hence, ribosomal DNA sequence data show that the species found belongs to a separate genus, *Parasitylenchus*, which is in line with the morphological identification of this species.

Analysis (Table 1, part 2b) showed that infection rates differed between years ($P = 0.010$, $\chi^2 = 9.2$, $df = 2$) and locations ($P = 0.00026$, $\chi^2 = 32.1$, $df = 9$) (Fig. 2). Neither sex ($P = 0.94$, $\chi^2 = 0.0062$, $df = 1$) nor colour ($P = 0.51$, $\chi^2 = 1.33$, $df = 2$) was significantly linked to nematode infection rate. In Tilburg, nematode infection showed a positive relationship with *H. virescens* infection [$P = 0.002$, $\chi^2 = 9.68$, $df = 1$; OR = 4.40 = exp(1.48), se(rc) = 0.48]. This relationship was not found at the other locations ($P = 0.71$, $\chi^2 = 0.14$, $df = 1$).

During winter experiments in 2010, 5.4 % of the females (from the locations where all ladybirds were dissected, $n = 478$) failed to reproduce. Interestingly, 65.4 % of the non-reproducing females were infected with nematodes, compared to 2.7 % of the reproducing females. Analysis (Table 1, part 2c) showed that

failure to reproduce was neither significantly related to location ($P = 0.60$, $\chi^2 = 3.63$, $df = 5$), nor to colour ($P = 0.12$, $\chi^2 = 4.17$, $df = 2$). The association with nematode infection was very strong [$P < 0.0001$, $\chi^2 = 76.7$, $df = 1$; OR = 121 (= exp(4.80), se(rc) = 0.64)]. Failure to reproduce was also associated with *H. virescens* infection ($P = 0.012$, $\chi^2 = 6.36$, $df = 1$). No nematodes were found in the summer samples.

Dinocampus coccinellae

During the winter of 2009, one braconid larva was found by dissection in a ladybird from Zundert representing 0.2 % of all dissected beetles in 2009 and 1.3 % of dissected beetles from Zundert. In winter 2010, two parasitoids were found: one braconid larva by dissection in a ladybird from Tilburg (0.1 %), and one braconid larva emerged from a ladybird from Kootwijk and spun a cocoon (0.004 %). No other larvae emerged from the ladybirds during experiments in 2008 and 2010. The sequence of the CO1 gene of both wasps confirmed that the two larvae from 2010 (Tilburg and Kootwijk) belonged to the species *D. coccinellae*. Unfortunately, the larva from Zundert was lost during processing.

Of the summer samples, two parasitoids were found: one braconid larva—probably *D. coccinellae* by dissection (0.4 % of 245 dissected females in 2009)—and one *D. coccinellae* larva emerged from a ladybird (0.3 % of 352 caught adults in 2011). This specimen was identified by R. Comont and H. Disney. Both male and female ladybirds were infected.

Discussion

Six years after the first record of *H. axyridis* in the Netherlands in 2002 (Cuppen et al. 2004), we first observed attacks by natural enemies (fungi and nematodes). Here we show that, although the immune system of *H. axyridis* protects against most fungi, nematodes, bacteria, and yeast (Gross et al. 2010), three species, *H. virescens*, *P. bifurcatus*, and *C. hippodamiae*, are able to overcome the immune system of *H. axyridis*.

Hesperomyces virescens

Hesperomyces virescens was found parasitising *H. axyridis* for the first time during the summer of 2008 in samples collected from ‘De Kaaistoep’. In overwintering aggregations, the first *H. axyridis* found infected with Laboulbeniales was recorded in Zundert, which is close to ‘De Kaaistoep’. The variation in prevalence which we observed between years as well as between locations is also reflected in other studies (see S2, S3 in Supporting Material, and Haelewaters et al. 2012). For comparison, *H. virescens* infection rates on *H. axyridis* in North America varied both over season (Riddick 2006) and between sampling sites (Nalepa and Weir 2007). Successful establishment of *H. virescens* requires both a suitable host and favourable environmental conditions, as experimentally shown for Carabidae (De Kesel 1996), which may explain the variability in infection rates. Moreover, apparent absence of infection (e.g. on *A. bipunctata* in France and Switzerland, Supporting Material S3) can be attributed to a combination of low sampling intensity and (very) low parasite prevalence (Welch et al. 2001).

In our study, host sex was significantly linked to infection rate in winter populations (more males than females were infected) but not in summer populations. In other studies the relationship between sex and infection is not consistent: some observe differences in infection rate (summer, Garcés and Williams 2004; winter, Nalepa and Weir 2007) while others do not (fall or winter, Riddick and Schaefer 2005; summer, Riddick 2006). Host sex is also associated with thallus distribution over the body (e.g. Riddick 2006; Nalepa and Weir 2007), and with thallus density (Riddick and Cottrell 2010). Differences between sexes in infection rate, thallus distribution and density are suggested to

be caused by different (mating) activity (Riddick and Schaefer 2005; De Kesel 2011), as active beetles have a higher chance of coming into contact with fungal spores than inactive beetles.

Infection rates in Doesburg, Tilburg, Winterswijk, and those observed in Germany in 2009 (Herz and Kleespies 2012) are almost as high as the infection rates in overwintering populations in the USA (Supporting Material S2). Infection rates in Belgium have increased from 2007 to 2011 (De Kesel 2011) in line with summer infection rates reported here. This may imply that *H. virescens* is spreading through the *H. axyridis* population in Europe, resulting in higher infection rates, confirming the suggestion by Roy et al. (2011b).

Winter prevalence of *H. virescens* in Tilburg, 2010 (which is near ‘De Kaaistoep’) is much higher than in the summer (2010 and 2011). Several factors influence infection levels: Firstly ‘dilution’ of the proportion of infected beetles following the emergence of a new, uninfected cohort of adults during the summer could explain this difference (Welch et al. 2001). Secondly, a substantial part (20 %; range 0–65 %) of the beetles caught in summer had soft elytra, indicating that these beetles had only recently emerged and are therefore uninfected, as it takes three to four days for the exoskeleton to sclerotise and darken (Obata 1988). Finally, summer observation nights were not equally distributed over the months. This is partly due to the fact that *H. axyridis* only flies when the following requirements are met: (a) temperatures not far below 14 °C, (b) no rain, and (c) wind not too strong (pers. obs. P. van Wielink), i.e. conditions that are not easily met in spring and autumn.

The observed negative effect of *H. virescens* infection on reproduction in our study can be explained by the fact that beetles that did not reproduce were kept at warm conditions in the experiment for much longer than the other beetles. Hence, spores had time to develop into thalli that were detectable during visual inspection.

Based on the available data we are limited to speculating about the origin of the *H. virescens* infection in Europe. The recent discovery of two *H. virescens*-infected *H. axyridis* individuals from the native range, China, sampled in the 1930s (Haelewaters et al. 2014) suggests that *H. virescens* is a historically global species and that at least one isolate was able to attack *H. axyridis* before it started

spreading globally. However, we do not expect that infected beetles from Asia were the source of infection in Europe and the USA. Firstly, *H. virescens* has been reported in both Europe (Santamaría et al. 1991) and North America (Thaxter 1891), long before the arrival of *H. axyridis*. Secondly, *H. axyridis* populations have been in quarantine before introduction as biological control agents (Krafsur et al. 1997; van Lenteren et al. 2008). Thirdly all populations introduced in the USA until 1980 originated from Japan or the USSR (Gordon 1985), where until now no *H. virescens* has been observed.

Instead we suggest that local strains of *H. virescens* have adapted to *H. axyridis* in both Europe and North America. Cottrell and Riddick (2012) showed with their laboratory experiment of forced confinement of healthy and infected beetles, that it is possible that different isolates of *H. virescens* exist and that these isolates may have a high degree of host specificity. We hypothesise that many isolates of *H. virescens* exist. The record from China shows that *H. virescens* is able to attack *H. axyridis*, but in Europe and the USA no isolate adapted to *H. axyridis* existed. Therefore, local isolates of *H. virescens*, infecting other hosts, had to adapt to *H. axyridis*. This can explain the time lag observed between *H. axyridis* introduction and first observation of *H. virescens* in both Europe (six years) and North America (ten years), where during a survey on museum collections of *H. axyridis* (from 1992 onwards) the first *H. virescens* infected specimens were observed only in 2002 (Supporting Material S2, S.Y. Zhao pers. comm.). Other possibilities are firstly that recent, undocumented, introductions from China are the source of the *H. virescens* infection in North America and/or Europe. Secondly, that infected beetles from North America have been introduced in Europe, although one would expect a shorter time lag in this case, as the possible *H. virescens* isolate was already adapted to *H. axyridis*, unless this had occurred in 2007 or 2008 and it took a few years only to spread and be detected. Further studies comparing invasion routes of *H. axyridis* with *H. virescens* distribution can shed light on the invasion process of *H. axyridis* and the interaction with *H. virescens*.

Based on our results we suggest that *H. virescens* appears to have established on *H. axyridis* in the Netherlands. How *H. virescens* will affect population densities of *H. axyridis* is unknown, as the effect of this fungus on other coccinellids is negligible (Riddick

et al. 2009). However, *H. axyridis* infected with laboulbeniales, had a slightly lower winter survival than uninfected beetles (Riddick 2010).

Coccipolipus hippodamiae

The first *H. axyridis* infected with *C. hippodamiae* in the Netherlands was found in winter 2009. The observed infection rates were low. Because mite infection negatively influences fitness (Rhule et al. 2010), the impact on beetle mortality may increase when mite infection rates approach levels that are reported for native *A. bipunctata* (Supporting Material S4).

Coccipolipus hippodamiae mites were reported in Europe before the arrival of *H. axyridis* (Supporting Material S4), hence we assume that they are beginning to use the exotic ladybird as a novel host. Webberley et al. (2006) did not detect mite infection on *A. bipunctata* in the Netherlands, nor in other populations from northwestern Europe, while moderate to high prevalence was observed in eastern and southern European populations. Mite infection of *H. axyridis* is also low in the Netherlands (this study) compared to eastern Europe (Supporting Material S2). This study might indicate the recent arrival of this parasite in Northwest Europe. It seems likely that the Netherlands constitute the current border of the geographical distribution of the mites.

Parasitylenchus bifurcatus

The first observation of *P. bifurcatus* nematodes was in the winter samples of *H. axyridis* in 2008, which is earlier than the first report of *Parasitylenchus* sp. from Denmark in 2009 (Harding et al. 2011) (Supporting Material S2) and concurrent with the first report of ‘Allantonematidae’ from Germany in October 2008 (Herz and Kleespies 2012, A. Herz pers. comm.). The infection rates are comparable with infection rates found in Germany (Herz and Kleespies 2012) and Denmark in 2009 (Harding et al. 2011) (Supporting Material S2). In our study, infection rates differed significantly between years and locations (but not across sexes). Infection rates also differed significantly between locations in studies in Germany [Pearsons exact χ^2 , $P = 0.0216$ (Herz and Kleespies 2012)] and Denmark [Pearsons χ^2 , $P = 0.0005$ (Harding et al. 2011)].

The specimens we found are morphologically similar to *P. bifurcatus* as described by Poinar and

Steenberg (2012). It is unknown how *H. axyridis* acquired this nematode infection. The species *P. bifurcatus* was not reported before 2012, and unfortunately, we cannot rule out that this species is similar to *P. coccinellae*, as the descriptions by both Iperiti and Waerebeke (1968) and by Poinar and Steenberg (2012) were not complete. Moreover, no DNA data are available for *P. coccinellinae* specimens to make a comparison with our DNA data.

The original source of infection in Europe could be parasitised individuals introduced from Asia or from North America. No records of nematodes infecting ladybirds are known from Asia, while from North America, infection of *H. axyridis* with ‘Allantonematidae’ (species unknown) has been reported (Roy et al. 2011b). Poinar and Steenberg (2012) suggest that infection could also be obtained from native European ladybirds. The species *P. coccinellinae* attacks several multivoltine coccinellid species: *Propylea quatuordecimpunctata* (L.), *Oenopia conglobata* (L.), *A. bipunctata*, *Hippodamia variegata* (Goeze), and *Harmonia quadripunctata* (Pontopiddian) (Iperiti and Waerebeke 1968).

Despite low infection rates, nematode infection has been observed in three successive years and rates were highest in the final year (2010). We therefore conclude that the nematodes have established on *H. axyridis* in the Netherlands. Harding et al. (2011) also report increasing infection rates. Infection can reach moderate levels in natural *H. axyridis* populations, up to 33.3 % in Denmark (Harding et al. 2011). The highly significant relationship between non-fertility of female ladybirds and nematode infection supports the hypothesis that *Parasitylenchus* sp. affects the reproduction of the ladybirds (Harding et al. 2011). The combination of moderate infection rates and reduction of reproductive ability of the host signals the potential effect of *P. bifurcatus* on populations of *H. axyridis* and suggests the possible use of *P. bifurcatus* as biological control agent of *H. axyridis*.

Dinocampus coccinellae

Infection of *H. axyridis* with *D. coccinellae* in the Netherlands was observed for the first time in 2009. Prevalence in the Netherlands is very low compared with prevalence in other ladybird species (Supporting Material S5) and is even low in contrast with prevalence among *H. axyridis* in other European

countries and the USA (Supporting Material S6). Parasitism by *D. coccinellae* currently has only a marginal impact on the population dynamics of *H. axyridis* (Berkvens et al. 2010). With similar attack rates for both *H. axyridis* and native species and a lower emergence from *H. axyridis*, the latter may act as an egg-sink for *D. coccinellae* (as hypothesized by Hoogendoorn and Heimpel 2002). Such an egg-sink may lead to a decline in *D. coccinellae* populations and result in a decline of infection rates in native coccinellid species (Berkvens et al. 2010).

Simultaneous infection with two natural enemies

Some ladybirds were infected with both *H. virescens* and mites (0.14 %) or nematodes (1.6 %). Co-infection has been observed earlier for mites in Austria (Christian 2002) and the USA (Riddick 2010), and for nematodes in Germany (Herz and Kleespies 2012).

Little is known about the effect of co-infection on ladybird fitness. Infection with mites and *H. virescens* results in a lower winter survival than infection with mites or fungus alone (Riddick 2010). We found that in Tilburg nematode and *H. virescens* infection were positively associated but, interestingly, not in other locations. If we assume that nematode-infected beetles are more sensitive to *H. virescens* infection, then a high *H. virescens* pressure as observed in Tilburg should result in a high percentage of infection with *H. virescens* in those beetles that are already infected with nematodes. The reduced total number of living beetles with nematodes in Tilburg indicates that co-infection of nematodes and *H. virescens* might also result in a lower survival. Further experiments are needed to study the effect of co-infection of *H. virescens* and mites or nematodes. If survival is indeed reduced, it might open options for biological control of the invasive ladybird.

Mechanisms of transfer

We have suggested that European populations of *H. virescens*, *C. hippodamiae* and nematodes, are switching from native coccinellids and are starting to use *H. axyridis* as a new host. All three natural enemies are reported in more than one coccinellid host, indicating that they have a more generalist nature. The biological control literature shows that generalist natural enemies make host switches easier than specialist species.

Examples for natural enemies of leaf miners are given in Minkenbergh and van Lenteren (1986). Host shifts have been reported for nematodes (McFrederick and Taylor 2013) and Laboulbeniales (Rossi 2011).

In winter, transfer between species could occur during overwintering. Although overwintering ladybirds usually form monospecific clusters, sometimes a few adults of other species are present in the aggregations (Hodek 2012). We found *A. bipunctata* and *O. conglobata* adults during sampling of overwintering populations of *H. axyridis* at very low numbers (< 0.2 %, Raak-van den Berg et al. 2012a, b, 2013). Both species are reported as hosts of *C. hippodamiae* and *P. coccinellinae*, and *A. bipunctata* is also reported as a host of *H. virescens* (see S3, S4 in Supporting Material, Iperiti and Waerebeke 1968). Therefore these ladybird species could be a possible source of infection of *H. axyridis* populations.

In summer, transfer between species could occur during interspecific mating. Reproductive mature males will mount almost any beetle they find—whether male or female, dead or alive, conspecific or heterospecific—to copulate (Obata 1987; Majerus 1994). Interspecific mating in the field has been observed between *H. axyridis* and *Asiocesta* sp. and in a treesome of *H. axyridis* with *C. septempunctata* and *A. bipunctata* (A.J.M. Loomans pers. obs.). Female rejection behaviour reduces the actual number of hybrid matings (Majerus 1994), but mounting alone can already facilitate transfer of natural enemies between individuals.

In this study we report that various types of natural enemies are starting to use *H. axyridis* as a host. We suggest that these enemies have transferred from native coccinellid hosts during overwintering or interspecific mating. Nevertheless, as yet, they are insufficiently effective to control this invasive alien species.

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References

- Berkvens N, Moens J, Berkvens D, Samih MA, Tirry L, De Clercq P (2010) *Dinocampus coccinellae* as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe. *Biol Control* 53:92–99
- Brown PMJ, Thomas CE, Lombaert E, Jeffries DL, Estoup A, Lawson Handley L-J (2011) The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl* 56:623–641
- Ceryngier P, Roy DB, Poland RL (2012) Natural enemies of ladybird beetles. In: Hodek I, van Emden HF, Honek A (eds) *Ecology and behaviour of the ladybird beetles (Coccinellidae)*. Blackwell Publishing Ltd, Chichester, UK, pp 375–443
- Christian E (2002) On the distribution and bionomics of the ladybird parasite *Coccipolipus hippodamiae* (McDaniel & Morrill, 1969) (Acari, Podapolipidae). *Abh Ber Naturkundemus Gorlitz* 74:9–14
- Cottrell TE, Riddick EW (2012) Limited transmission of the ectoparasitic fungus *Hesperomyces virescens* between lady beetles. *Psyche* 2012:7
- Cuppen J, Heijerman T, van Wielink PS, Loomans AJM (2004) Het Lieveheersbeestje *Harmonia axyridis* in Nederland: een aanwinst voor onze fauna of een ongewenste indringer (Coleoptera: Coccinellidae)? *Ned Faun Med* 20:1–12
- De Kesel A (1996) Host specificity and habitat preference of *Laboulbenia slackensis*. *Mycologia* 88:565–573
- De Kesel A (2011) *Hesperomyces* (Laboulbeniales) and coccinellid hosts. *Sterbeeckia* 30:32–37
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294–299
- Garcés S, Williams R (2004) First record of *Hesperomyces virescens* Thaxter (Laboulbeniales: Ascomycetes) on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *J Kans Entomol Soc* 77:156–158
- Gordon RD (1985) The Coleoptera (Coccinellidae) of America north of Mexico. *J N Y Entomol Soc* 93:1–912

- Gross J, Eben A, Muller I, Wensing A (2010) A well protected intruder: the effective antimicrobial defense of the invasive ladybird *Harmonia axyridis*. *J Chem Ecol* 36:1180–1188
- Haelewaters D, van Wielink P, van Zuijlen JW, Verbeken A, De Kesel A (2012) New records of *Laboulbeniales* (Fungi, *Ascomycota*) for the Netherlands. *Entomol Ber (Amst)* 72:26–33
- Haelewaters D, Comont RF, Zhao S, Pfister DH (2014) *Hesperomyces virescens* (Fungi, *Ascomycota*, *Laboulbeniales*) attacking *Harmonia axyridis* (Coleoptera, *Coccinellidae*) in its native range. *Chin Sci Bull* 59. doi:10.1007/s11434-013-0060-1
- Hansen K, LoBuglio KF, Pfister DH (2005) Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: RPB2, beta-tubulin, and LSU rDNA. *Mol Phylogen Evol* 36:1–23
- Harding S, Poinar GO, Dimitrova DV, Steenberg T (2011) *Parasitylenchus* sp (Tylenchomorpha: Allantonematidae) parasitizing field populations of *Harmonia axyridis* (Coleoptera: *Coccinellidae*). *Eur J Entomol* 108:487–488
- Herz A, Kleespies RG (2012) Occurrence of natural enemies in different populations of the invasive ladybird *Harmonia axyridis* (Pallas, 1771) (Coleoptera, *Coccinellidae*) in Germany. *Mitt Dtsch Ges Allg Angew Ent* 18:201–206
- Hodek I (2012) Diapause/dormancy. In: Hodek I, van Emden HF, Honek A (eds) *Ecology and behaviour of the ladybird beetles (Coccinellidae)*. Blackwell Publishing Ltd., Chichester, UK, pp 275–342
- Holterman M, van der Wurff A, van den Elsen S, van Megen H, Bongers T, Holovachov O, Bakker J, Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol Biol Evol* 23:1792–1800
- Hoogendoorn M, Heimpel GE (2002) Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. *Biol Control* 25:224–230
- Iperti G, Waerebeke DV (1968) Description, biologie et importance d'une nouvelle espèce d'Allantonematidae (nematode) parasite des coccinelles aphidiphages: *Parasitylenchus coccinellinae*, n. sp. *Entomophaga* 13:107–119
- Koch RL, Galvan TL (2008) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. *BioControl* 53:23–35
- Krafsur ES, Kring TJ, Miller JC, Nariboli P, Obrycki JJ, Ruberson JR, Schaefer PW (1997) Gene flow in the exotic colonizing ladybeetle *Harmonia axyridis* in North America. *Biol Control* 8:207–214
- Majerus MEN (1994) *Ladybirds*. Harper Collins Publishers, London, UK
- McCullagh P, Nelder JA (1989) *Generalized linear models*, 2nd edn. Chapman and Hall, London, UK
- McFrederick QS, Taylor DR (2013) Evolutionary history of nematodes associated with sweat bees. *Mol Phylogen Evol* 66:847–956
- Minkenbergh OPJM, van Lenteren JC (1986) The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. *Wageningen Agric Univ Pap*. 86:1–50
- Nalepa CA, Weir A (2007) Infection of *Harmonia axyridis* (Coleoptera: *Coccinellidae*) by *Hesperomyces virescens* (Ascomycetes: *Laboulbeniales*): role of mating status and aggregation behavior. *J Invertebr Pathol* 94:196–203
- Obata S (1987) Mating behavior and sperm transfer in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: *Coccinellidae*). *Appl Entomol Zool* 22:434–442
- Obata S (1988) Mating refusal and its significance in females of the ladybird beetle, *Harmonia axyridis*. *Physiol Entomol* 13:193–199
- Poinar GO, Steenberg T Jr (2012) *Parasitylenchus bifurcatus* n. sp. (Tylenchida: Allantonematidae) parasitizing *Harmonia axyridis* (Coleoptera: *Coccinellidae*). *Parasit Vectors* 5:218
- Raak-van den Berg CL, Hemerik L, de Jong PW, van Lenteren JC (2012a) Mode of overwintering of invasive *Harmonia axyridis* in the Netherlands. *BioControl* 57:71–84
- Raak-van den Berg CL, Stam JM, de Jong PW, Hemerik L, van Lenteren JC (2012b) Winter survival of *Harmonia axyridis* in the Netherlands. *Biol Control* 60:68–76
- Raak-van den Berg CL, de Jong PW, Hemerik L, van Lenteren JC (2013) Diapause and postdiapause quiescence demonstrated in overwintering of *Harmonia axyridis* (Coleoptera: *Coccinellidae*) in northwestern Europe. *Eur J Entomol* 110:585–591
- Rhule EL, Majerus MEN, Jiggins FM, Ware RL (2010) Potential role of the sexually transmitted mite *Coccipolipus hippodamiae* in controlling populations of the invasive ladybird *Harmonia axyridis*. *Biol Control* 53:243–247
- Riddick EW (2006) Influence of host gender on infection rate, density and distribution of the parasitic fungus, *Hesperomyces virescens*, on the multicolored Asian lady beetle, *Harmonia axyridis*. *J Insect Sci* 6:1–15
- Riddick EW (2010) Ectoparasitic mite and fungus on an invasive lady beetle: parasite coexistence and influence on host survival. *Bull Insectol* 63:13–20
- Riddick EW, Cottrell TE (2010) Is the prevalence and intensity of the ectoparasitic fungus *Hesperomyces virescens* related to the abundance of entomophagous coccinellids? *Bull Insectol* 63:71–78
- Riddick E-W, Schaefer P-W (2005) Occurrence, density, and distribution of parasitic fungus *Hesperomyces virescens* (*Laboulbeniales*: *Laboulbeniaceae*) on multicolored Asian lady beetle (Coleoptera: *Coccinellidae*). *Ann Entomol Soc Am* 98:615–624
- Riddick EW, Cottrell TE, Kidd KA (2009) Natural enemies of the *Coccinellidae*: parasites, pathogens, and parasitoids. *Biol Control* 51:306–312
- Rossi W (2011) New species of *Laboulbenia* from Ecuador, with evidence for host switch in the *Laboulbeniales*. *Mycologia* 103:184–194
- Roy HE, Lawson Handley L-J, Schonrogge K, Poland RL, Purse BV (2011a) Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? *BioControl* 56:451–468
- Roy HE, Rhule E, Harding S, Lawson Handley L-J, Poland RL, Riddick EW, Steenberg T (2011b) Living with the enemy: parasites and pathogens of the ladybird *Harmonia axyridis*. *BioControl* 56:663–679
- Roy HE, Adriaens T, Isaac NJB, Kenis M, Onkelinx T, San Martin G, Brown PMJ, Hautier L, Poland R, Roy DB, Comont R, Eschen R, Frost R, Zindel R, van Vlaenderen J, Nedved O, Ravn HP, Gregoire JC, de Biseau JC, Maes D (2012) Invasive alien predator causes rapid declines of native European ladybirds. *Divers Distrib* 18:717–725

- Santamaría S, Balazuc J, Tavares II (1991) Distribution of the European *Laboulbeniales* (fungi, *Ascomycotina*). An annotated list of species. *Treb Inst Bot Barc* 14:1–123
- Thaxter R (1891) Supplementary note on North American *Laboulbeniaceae*. *Proc Am Acad Arts Sci* 25:261–270
- van Lenteren JC, Loomans AJM, Babendreier D, Bigler F (2008) *Harmonia axyridis*: an environmental risk assessment for Northwest Europe. *BioControl* 53:37–54
- van Wielink P, Spijkers H (2013) Insects nightly attracted to light at a single site in De Kaaistoep, The Netherlands. Orders, families and species identified in 1995–2011. *Entomol Ber (Amst)* 73:200–214
- Vervoort MTW, Vonk JA, Mooijman PJW, van den Elsen SJJ, van Megen HHB, Veenhuizen P, Landeweert R, Bakker J, Mulder C, Helder J (2012) SSU ribosomal DNA-based monitoring of nematode assemblages reveals distinct seasonal fluctuations within evolutionary heterogeneous feeding guilds. *PLoS ONE* 7:e47555
- Webberley KM, Tinsley MC, Sloggett JJ, Majerus MEN, Hurst GDD (2006) Spatial variation in the incidence of a sexually transmitted parasite of the ladybird beetle *Adalia bipunctata* (Coleoptera: Coccinellidae). *Eur J Entomol* 103:793–797
- Weir A, Blackwell M (2001) Extraction and PCR amplification of DNA from minute ectoparasitic fungi. *Mycologia* 93:802–806
- Welch VL, Sloggett JJ, Webberley KM, Hurst GDD (2001) Short-range clinal variation in the prevalence of a sexually transmitted fungus associated with urbanisation. *Ecol Entomol* 26:547–550
- White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, USA, pp 315–322
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