

Ectoparasitic mite and fungus on an invasive lady beetle: parasite coexistence and influence on host survival

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Abstract

This study documents the interaction of two coccinellid-specific ectoparasites, a podapolipid mite *Coccipolipus hippodamiae* (McDaniel et Morrill) and a laboulbeniellan fungus *Hesperomyces virescens* Thaxter with the lady beetle *Harmonia axyridis* (Pallas). The study objectives were to (1) determine the prevalence and intensity of both parasites and (2) predict the influence of parasitism on host survival during the winter season. The prevalence of mite and fungus in host aggregations ranged from 3.2 to 17.4% and 3.6 to 43.5%, respectively, in winter aggregations in five different locations in Mississippi, southeastern USA, from 2007-2009. The mite colonized the subelytral space of male and female *H. axyridis*. Mite intensity was greatest on fungus-infected beetles, especially females. Fungus intensity was greatest on the abdomen of *H. axyridis* males that harbored mites than those that did not. *H. axyridis* adults (particularly males) infected with the mite and fungus or the fungus alone had lower survival rates, under simulated winter conditions, than those not infected with any parasites. This research documents the parasitic mite *C. hippodamiae* infecting *H. axyridis* under natural field conditions for the first time anywhere in the world.

Key words: *Coccipolipus hippodamiae*, *Hesperomyces virescens*, horizontal transmission, *Harmonia axyridis*, multicolored Asian lady beetle, harlequin ladybird, natural enemies, symbionts.

Introduction

Ectoparasitic mites (Acarina Podapolipidae) and ectoparasitic fungi (Order Laboulbeniales, Family Laboulbeniaceae) occur on lady beetles throughout the world (Riddick *et al.*, 2009). Approximately 14 podapolipids (all confined to the genus *Coccipolipus*) attack entomophagous and phytophagous lady beetles (Schroder, 1982; Husband, 1984; Cantwell *et al.*, 1985; Hochmuth *et al.*, 1987; Husband, 1989; Ceryngier and Hodek, 1996; Hajiqanbar *et al.*, 2007). Horizontal transmission occurs during bodily contact between mating (Knell and Webberley, 2004) or overwintering lady beetles (Webberley and Hurst, 2002). Very little information is available on the biology and ecology of mites in this family and their impact on coccinellid hosts. One species *Coccipolipus hippodamiae* (McDaniel et Morrill) reduces the fecundity and egg viability of several entomophagous coccinellids in Europe (Hurst *et al.*, 1995; Webberley *et al.*, 2004). Mite intensity (number of life stages present on the same host) can affect potential fecundity of the two-spotted lady beetle *Adalia bipunctata* (L.); high mite intensity causes a decline in host fecundity (Ryder *et al.*, 2007). This mite is also known to cause mortality of overwintering *A. bipunctata* adults, particularly males (Webberley and Hurst, 2002; Webberley *et al.*, 2006 a) and increase the mortality of food-deprived, non-overwintering *A. bipunctata* adults (Ryder *et al.*, 2007). Kenis *et al.* (2008) propose that *C. hippodamiae* can help reduce populations of *Harmonia axyridis* (Pallas) in some localities within an integrated pest management program.

Four Laboulbeniales species in the genus *Hesperomyces* attack entomophagous lady beetles (Thaxter, 1931; Riddick *et al.*, 2009). Very little information is available on the biology and ecology of laboulbeniellan fungi and their interactions with hosts. As far as is known, all spe-

cies require a living host for survival and reproduction. Typically, they cause little harm to their coccinellid hosts (Tavares, 1979; Weir and Beakes, 1995). However, one species *Hesperomyces virescens* Thaxter caused premature mortality of a predatory coccinellid *Chilocorus bipustulatus* L. under field conditions (Kamburov *et al.*, 1967). *H. virescens* has attacked several other predatory lady beetles (Tavares, 1985; Weir and Beakes, 1996; Christian, 2001), including *H. axyridis* (Garcés and Williams, 2004; Riddick and Schaefer, 2005; Harwood *et al.*, 2006) without having any pronounced negative effects on survival of its hosts (Riddick *et al.*, 2009).

H. virescens is transmitted horizontally between adult coccinellids as they attempt to mate at feeding sites or as they form aggregations at overwintering sites (Welch *et al.*, 2001; Riddick and Schaefer, 2005; Harwood *et al.*, 2006; Riddick, 2006; Nalepa and Weir, 2007). Images of the fungus *H. virescens* on the lady beetle *H. axyridis* have been presented in previous works (Riddick, 2006; Nalepa and Weir, 2007; Harwood *et al.*, 2007). The prevalence of this parasite in the host population can vary considerably from one season to the next (Riddick, 2006). The intensity of *H. virescens* on male and female hosts (*H. axyridis*) is often dependent on the extent of bodily contact between conspecifics rather than on any preference for a specific body part by the fungus (Riddick and Schaefer, 2005; Riddick, 2006; Nalepa and Weir, 2007).

H. axyridis is an entomophagous lady beetle with origins in central and eastern Asia. Through years of purposeful or accidental introductions dating back to 1916 (Gordon, 1985), *H. axyridis* became established in North America in the late 1980's (Chapin and Brou, 1991; Tedders and Schaefer, 1994). Over the last several years, wild *H. axyridis* populations have established in Europe, South America, and Africa (Koch *et al.*,

2006; Majerus *et al.*, 2006; Stals and Prinsloo, 2007; Adriaens *et al.*, 2008; Burgio *et al.*, 2008). *H. axyridis* is an important predator of aphids and other soft-bodied insects in soybean fields and pecan, apple, and citrus orchards (Tedders and Schaefer, 1994; LaRock and Ellington, 1996; Brown, 2004; Rutledge *et al.*, 2004; Costamagna and Landis, 2006; Mignault *et al.*, 2006). Unfortunately, *H. axyridis* feeds on damaged fruit in vineyards, taints wine, out-competes native coccinellids, feeds on non-target species, becomes a nuisance in households during the fall and winter seasons, and causes allergic reactions in humans (Koch and Galvan, 2008). There is a need to identify effective methods of managing this insect to curb its negative impacts in some urban and agricultural landscapes worldwide (Kenis *et al.*, 2008) and fostering its positive impact as a predator of crop pests.

Lack of effective natural enemies to suppress *H. axyridis* populations could be one of the reasons why outbreaks of this insect occur. Nevertheless, several non-adapted natural enemies attack *H. axyridis*. These include tachinid flies (Nalepa and Kidd, 2002), braconid wasps (Koyama and Majerus, 2008), pathogenic bacteria (Majerus *et al.*, 1998), pathogenic fungi (Roy *et al.*, 2008) and nematodes (Shapiro-Ilan and Cottrell, 2005). Unfortunately, none of these species, alone, can regulate the population dynamics of *H. axyridis*. Maybe a suite of natural enemies operating together could significantly affect the population dynamics of *H. axyridis*. This study documents the interaction of two coccinellid-specific ectoparasites, a podapolipid mite *C. hippodamiae* and a laboulbenian fungus *H. virescens* with the lady beetle *H. axyridis*. The study objectives were to (1) determine the prevalence and intensity of both parasites and (2) predict the influence of parasitism on host survival during the winter season. This study should increase our understanding of the interactions between parasitic mites and fungi and their beetle hosts.

Materials and methods

Parasite prevalence and intensity

H. axyridis adults were collected from five fall/winter aggregations in western Mississippi (USA) in December 2007, December 2008, and January 2009 (table 1). Dis-

tance between aggregation (collection) sites was a minimum of 9.7 km between sites 1 and 3 and a maximum of 134.1 km between sites 4 and 5. Beetles were transported back to the laboratory on the same day or the next day, in plastic vials or cardboard ice cream containers with lids. Because the ambient temperature was cool and the beetles were in a state of reduced activity at the time of capture, there was no need to transport beetles in a cooler (ice chest).

Beetles were placed in a refrigerator, in the same containers that they were captured in, upon arrival in the laboratory. All of the beetles were examined under an Olympus™ SZX16 Zoom Stereo microscope for the presence of parasitic mites (Podapolipidae) and parasitic fungi (Laboulbeniaceae). Parasite prevalence was the percentage of the sample populations (aggregations) infected with the mite or fungus. Infection rate was the percentage of male and female lady beetles harboring the mite or fungus. Fungus intensity was the number of mature or nearly mature thalli on body parts of the lady beetle. Mite intensity was the number of life stages (egg, larval female, adult female, and larviform male) underneath the elytra of the lady beetle.

The percent prevalence of both parasites was determined in the five *H. axyridis* populations (aggregation sites). The influence of host gender, parasite status (presence or absence), and the interaction between both factors on fungus and mite intensity was determined in this study. Sample size for fungus intensity was 50 fungus-infected males (40 without the mite, 10 with the mite) and 46 fungus-infected females (37 without the mite, 9 with the mite) for a total of 96 observations. Sample size for mite intensity was 13 mite-infected males (3 without the fungus, 10 with the fungus) and 21 mite-infected females (12 without the fungus, 9 with the fungus) for a total of 34 observations. Sample size reflects data combined from the five distinct *H. axyridis* aggregations.

Voucher specimens of the mite and fungus are currently stored in vials of 70-80% ethanol, along with the host, at the USDA-ARS, National Biological Control Laboratory, in Stoneville, Mississippi, USA. In addition, vouchers of the mite are currently maintained on slides in the mite collection at Adrian College, Adrian, Michigan, USA and the USDA-ARS, Stoneville, Mississippi.

Table 1. Location of collection sites and number of live *H. axyridis* collected in each overwintering aggregation.

Collection Date	Site	Location	<i>H. axyridis</i>
17 Dec. 2007	1	Leland, MS (Washington Co.) 33°24.30'N 90°59.34'W	146 ♂, 134 ♀
4 Dec. 2008	2	Grenada, MS (Grenada Co.) 33°46.79'N 89°46.38'W	9 ♂, 14 ♀
17 Dec. 2008	3	Stoneville, MS (Washington Co.) 33°27.32'N 90°55.46'W	48 ♂, 47 ♀
20 Dec. 2008	4	Benoit, MS (Bolivar Co.) 33°59.78'N 91°07.76'W	92 ♂, 75 ♀
28 Jan. 2009	5	Delta National Forest, MS (Sharkey Co.) 32°48.82'N 90°48.58'W	66 ♂, 76 ♀

Parasitism and winter survival of hosts

Winter survival of *H. axyridis* males and females was determined under simulated conditions in the laboratory for 18 consecutive weeks (4 January 2008 to 9 May 2008), using lady beetles collected from site 1 (see table 1). Each beetle was randomly assigned to a 50-ml plastic centrifuge tube (with screened lid for air circulation) to prevent bodily contact between beetles, which may or may not influence winter survival. A lab-top refrigerator set at 8 °C and 58-60% RH simulated the winter conditions. Each week, survivorship was assessed across treatment groups, which included beetles with mite only (0 males, 7 females), with mite and fungus (5 males, 4 females), with fungus only (23 males, 17 females), and without any parasite (51 males, 51 females). Dead individuals were removed and frozen (-20 °C) for post-winter examination of mite intensity. Repeated measurements of the proportion of beetles in each treatment group surviving the winter resulted in 72 observations in this experiment.

Statistical analysis

Pearson product moment correlation measured the strength of association between percent prevalence of the parasitic mite and the fungus across five host populations (aggregations) and the strength of association between the percentage of host males and females infected with the mite or fungus. Following a completely randomized design, a factorial analysis of variance (ANOVA) using the general linear model (GLM) procedure compared the influence of host gender and parasite status (presence or absence), and the interaction of these two factors on mite intensity and fungus intensity. A Kaplan-Meier survival analysis (KMSA), followed by a Log-Rank test of equality, estimated the difference between the survival curves of beetles in the different treatment groups. Following a completely randomized design, a repeated measures analysis of variance (RM ANOVA) compared the influence of parasitism on proportion of hosts surviving the winter. Absolute data were square root transformed prior to ANOVA and proportional data were arcsine transformed prior to the RM ANOVA. A Tukey test separated means after ANOVA and RM ANOVA. Means were considered significantly different when $p \leq 0.05$. SigmaStat 3.0.1 (Systat Software Inc., Richmond CA, USA) and SAS 9.1.3 (SAS Institute Inc., Cary NC, USA) software assisted with analysis of data. All data given in this manuscript represent non-transformed values.

Results

Parasite prevalence

Two ectoparasites, *C. hippodamiae* and *H. virescens* occurred in five *H. axyridis* fall/winter populations (aggregation sites) in western Mississippi, southeastern USA, from 2007-2009. Mite stages were underneath the elytra and visible through worn-out areas in the elytra, as seen via a dorsal view of *H. axyridis* under a zoom stereo microscope (figure 1). Worn-out or de-pigmented sections in the elytra result from female mites feeding

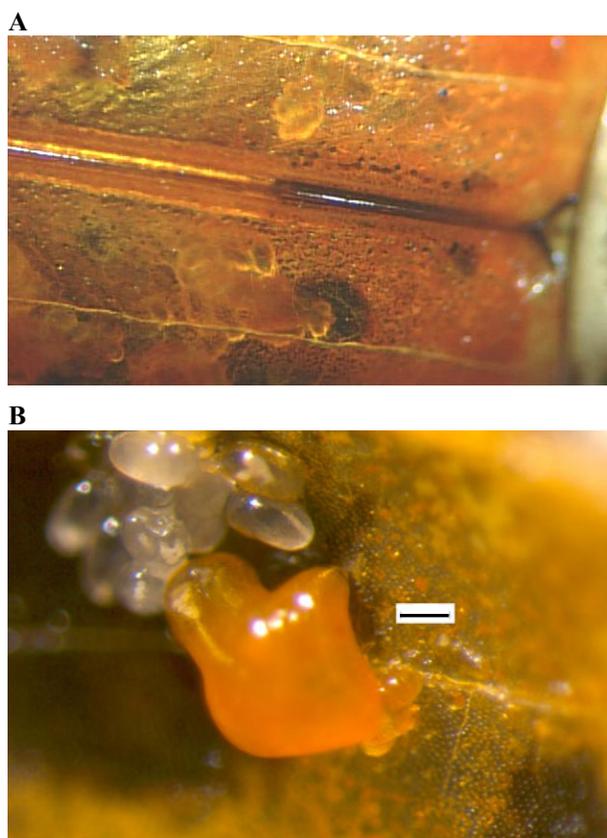


Figure 1. Dorsal microscopic view of worn-out (de-pigmented) areas of the elytra of lady beetle *H. axyridis* male showing mite *C. hippodamiae* female with eggs (A), and underside of elytra showing close-up view of mite female with eggs (B). Scale bar represents 400 μm in A and 100 μm in B. (In colour at www.bulletinofinsectology.org)

along the underside of the elytra (McDaniel and Morrill, 1969). Parasite prevalence ranged from 3.6 to 43.5% and 1.8 to 17.4% for the fungus and mite, respectively, across all aggregation sites (figure 2A). Prevalence of the mite and fungus correlated positively across the five aggregation sites ($r = 0.98$, $P = 0.004$, $n = 5$). The percentage of *H. axyridis* infected with the fungus and mite was variable (figure 2B). Infection of males by the fungus and mite ranged from 3.3 to 33.3% and 0 to 33.3%, respectively. Infection of females by the fungus and mite ranged from 4.0 to 19.15% and 2.7 to 8.95%, respectively. Infection rate correlated positively between males infected with the fungus versus males infected with the mite ($r = 0.88$; $P = 0.046$, $n = 5$), and between females infected with the fungus versus males infected with the mite ($r = 0.91$, $P = 0.03$, $n = 5$).

Parasite intensity

The parasitic fungus *H. virescens* was more often on the abdomen, mesothorax, metathorax, legs, and dorsal side of the elytra of the host, *H. axyridis* (figure 3). The interaction of host gender and mite status (presence or absence) had a significant effect on fungus thallus intensity on the abdomen (table 2). Fungus intensity was greater on the abdomen of males with the mite than males

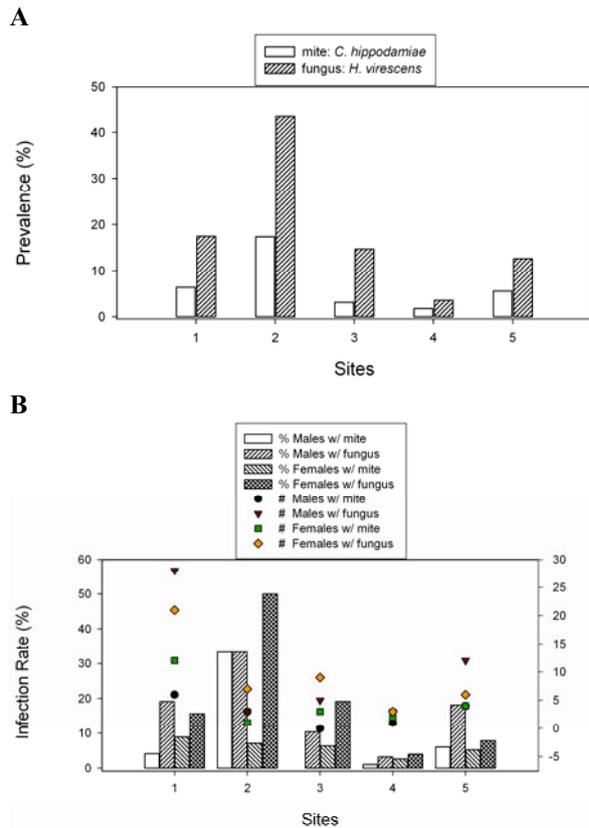


Figure 2. Percent prevalence of parasitic mite and fungus on *H. axyridis* collected in fall/winter aggregations (A) and percent infection rate of both parasites on male and female hosts (B). Sample size for parasite prevalence was five observations (aggregations). Sample size for infection rate was five observations for each of males and females harbouring mite or fungus.

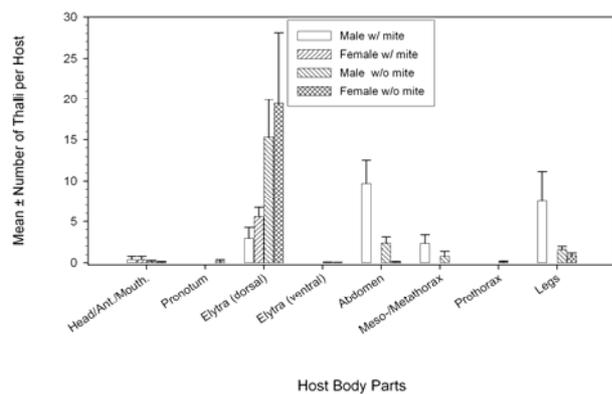


Figure 3. Mean \pm SEM number of mature or nearly mature *H. virescens* thalli (i.e., intensity) on body parts of the beetle *H. axyridis*. Sample size was 50 fungus-infected males (40 without the mite, 10 with the mite) and 46 fungus-infected females (37 without the mite, 9 with the mite) for a total of 96 observations.

without the mite or females with or without the mite. Host gender and mite status, and the interaction between the two factors, affected the number of thalli on the meso- and metathorax. Fungus intensity was greater on the meso- and metathorax of males with the mite than of

males without the mite or females with or without the mite. Host gender and the interaction between the two factors affected the intensity of the fungus on the legs. Fungus intensity was greater on the legs of males with the mite than on males without the mite and females with or without the mite. Fungus intensity was unaffected by host gender, mite status, or the interaction of the two factors on other body parts, including head, antennae, and mouthparts combined, pronotum, elytra, and prothorax (table 2).

The parasitic mite *C. hippodamiae* was usually in egg, larval (immature) female or adult female stages of development underneath the elytra of *H. axyridis*. Larviform (but sexually mature) males were rare (figure 4). The intensity of mite eggs was unaffected by host gender, fungus status or the interaction of the two factors (table 3). There was a significant interaction between both factors on the intensity of larval females; fewer were on *H. axyridis* males without fungus than males with fungus or females with or without fungus. Intensity of adult mite females was less on male rather than female hosts and less under the elytra of hosts without fungus. The intensity of larviform males was unaffected by host gender, fungus status, or the interaction of the two factors.

Parasitism and winter survival of hosts

Most *H. axyridis* males and females survived for 18 weeks (4.5 months) at a temperature of 8 °C in the laboratory. The proportion of adults surviving to the end of the experiment ranged from 0.75 to 1.0, depending on treatment (figure 5). Survival curves differed significantly between some of the treatments (KMSA; Log-Rank test, $X^2 = 30.4$, $df = 3$, $p < 0.0001$). Weekly measures of survival differed between treatment groups (RM ANOVA, $F = 52.9$; $df = 3, 51$; $p < 0.001$). Survival was greatest for *H. axyridis* adults in the mite only and the no parasite treatments rather than the mite and fungus or the fungus only treatment group. Adult survival was also greater in the fungus only than the mite and fungus treatment group. A few infected males expired within the first several weeks of the experiment; the others expired within the final weeks (table 4). There was no discernible pattern in parasite intensity between the adults that failed to survive. Pathogenic fungi, parasitoid wasps, or parasitoid flies did not develop on or emerge from any of the individuals in the treatment groups.

Discussion

Parasite prevalence and infection of lady beetles

This study documents the discovery of the mite *C. hippodamiae* as a parasite of the lady beetle *H. axyridis* under natural field conditions. The mite was first discovered on the convergent lady beetle *Hippodamia convergens* Guérin-Méneville in South Dakota, USA (McDaniel and Morrill, 1969) and later on the two-spot ladybird beetle *A. bipunctata* in New Jersey, USA (Husband, 1981). The fact that the prevalence of *C. hippodamiae* never exceeded 20% in any of the *H. axyridis* aggregations in this study suggests that this mite is not

Table 2. ANOVA statistics for the influence of host gender, mite status, and the interaction of both factors on intensity of the fungus *H. virescens*.

Source	Statistic					
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>p</i>
		Abdomen			Meso-/Metathorax	
Host gender	34.19	1, 92	< 0.001	12.28	1, 92	< 0.001
Mite status	9.15	1, 92	0.003	5.26	1, 92	0.024
Interaction	10.64	1, 92	0.002	5.26	1, 92	0.024
		Legs			Head/Ant./Mouthparts	
Host gender	17.33	1, 92	< 0.001	0.001	1, 92	0.97
Mite status	1.38	1, 92	0.24	2.17	1, 92	0.14
Interaction	9.53	1, 92	0.003	0.03	1, 92	0.86
		Pronotum			Elytra (dorsal)	
Host gender	0.26	1, 92	0.61	1.15	1, 92	0.28
Mite status	0.26	1, 92	0.61	1.96	1, 92	0.16
Interaction	0.26	1, 92	0.61	0.21	1, 92	0.65
		Elytra (ventral)			Prothorax	
Host gender	0.008	1, 92	0.93	0.22	1, 92	0.64
Mite status	0.46	1, 92	0.50	0.22	1, 92	0.64
Interaction	0.008	1, 92	0.93	0.22	1, 92	0.64

Table 3. ANOVA statistics for influence of host gender, fungus status, and the interaction of both factors on the intensity of the mite *C. hippodamiae*.

Source	Statistic					
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>p</i>
		Egg			Larval female	
Host gender	3.23	1, 30	0.08	7.67	1, 30	0.01
Fungus status	2.49	1, 30	0.125	9.61	1, 30	0.004
Interaction	0.145	1, 30	0.71	4.28	1, 30	0.047
		Adult female			Larviform male	
Host gender	7.41	1, 30	0.01	2.55	1, 30	0.12
Fungus status	5.62	1, 30	0.02	0.06	1, 30	0.81
Interaction	0.89	1, 30	0.35	2.16	1, 30	0.15

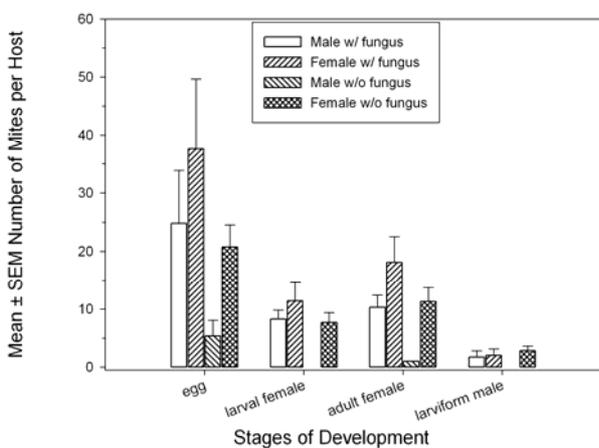


Figure 4. Mean \pm SEM number of *C. hippodamiae* life stages found underneath fungus-free and fungus-laden males and females. Sample size was 13 mite-infected males (3 without the fungus, 10 with the fungus) and 21 mite-infected females (12 without the fungus, 9 with the fungus) for a total of 34 observations.

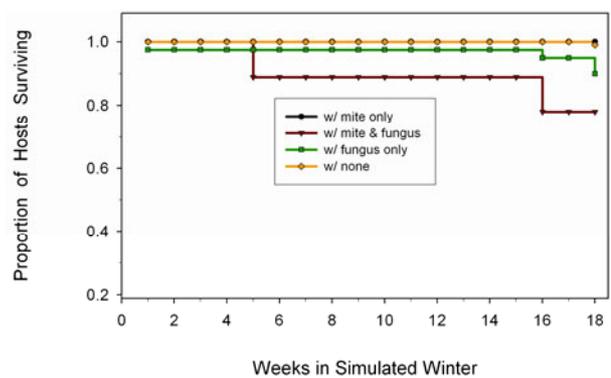


Figure 5. Proportion of *H. axyridis* males and females surviving winter storage in relation to parasite infection. Sample size was 18 repeated measurements of beetles in four treatment groups for a total of 72 observations. Treatment groups included beetles with mite only (0 males, 7 females), mite and fungus (5 males, 4 females), fungus only (23 males, 17 females), and no parasites (51 males, 51 females).

Table 4. Intensity of parasitic fungus and mite on lady beetle *H. axyridis* adults expiring in designated treatment groups during the simulated winter experiment.

Treatment Group	Dead <i>H. axyridis</i>	Fungus Intensity	Mite Intensity	Week Expired
Fungus only	♂	30	----	1
Mite and fungus	♂	24	63	5
Mite and fungus	♂	22	50	16
Fungus only	♂	44	----	16
Fungus only	♂	168	----	18
Fungus only	♀	2	----	18
None	♀	----	----	18

Fungus intensity represented mature (and nearly mature) *H. virescens* thalli found on the host body and mite intensity represented life stages of *C. hippodamiae* found underneath the host elytra.

very abundant, at least not in western Mississippi. The prevalence of *C. hippodamiae* in *A. bipunctata* populations can exceed 50% in some years in certain localities in Europe (Webberley, 2000), but it can decline during the winter, maybe due to parasite-induced mortality of infected beetles and low temperature that restricts movement from infected to non-infected hosts (Webberley *et al.*, 2006a).

The parasitic fungus *H. virescens* can have a prevalence that reaches 80% in summer (Harwood *et al.*, 2006) and 62% in late winter (Nalepa and Weir, 2007) in *H. axyridis* populations in the United States. *H. virescens* prevalence in *A. bipunctata* populations during the winter was 24% in Europe (Weir and Beakes, 1996) and 29% in Austria (Christian, 2001). Upon arrival at an overwintering site in the fall season, less than 14% of *H. axyridis* harbored the fungus (Riddick and Schaefer, 2005). In light of prior research, *H. virescens* prevalence of up to 43% in a *H. axyridis* aggregation in early winter, in this study, was moderate.

The presence of both parasites in the same *H. axyridis* aggregations and occasionally on the same host individuals is previously unreported. Apparently, co-existence of both the fungus and mite on the same host is possible because of ample nutritional resources provided by the host. The observation of a strong association (positive correlation) between the prevalence of *H. virescens* and *C. hippodamiae* in aggregations of the lady beetle *H. axyridis* was not expected. Co-existence of *C. hippodamiae* and *H. virescens* on other lady beetles has been reported only once before. Christian (2001, 2002) found both parasites on the lady beetle *A. bipunctata* in Vienna, Austria. In contrast, Webberley *et al.* (2006b) did not find both parasites in the same *A. bipunctata* populations or on the same host individuals across multiple locations in Europe.

Parasite intensity on hosts

The observation that *C. hippodamiae* immature and adult females outnumbered males approximately 5 to 1 on *H. axyridis* was lower than expected when considering densities reported in previous studies. For example, *C. hippodamiae* immature females outnumbered adult females and males by a ratio of approximately 100 to 1 and 1000 to 1, respectively, on the convergent lady beetle *H. convergens* (McDaniel and Morrill, 1969).

The observation that mite intensity was less on *H. axyridis* males devoid of fungus than males with fungus or females with or without fungus was not expected. The simplest explanation for this observation is that many of the males in this study were young (new generation) and unmated and had little or no contact with mite-infected conspecifics. Mite transmission often occurs as lady beetles are mating (Hurst *et al.*, 1995). Therefore, these males were at less risk of infection and the parasites did not have ample time to proliferate on them. An alternative explanation that fungus-free males are less suitable hosts for colonization by the mite is untenable.

The observation of greater fungus intensity on the abdomen, mesothorax, metathorax, and legs of *H. axyridis* males with the mite, than without the mite, under the elytra was not expected. Typically, *H. virescens* is often on the dorsum (elytra) and ventrum (abdomen, mesothorax, and metathorax) of mite-free *H. axyridis* males and on the dorsum (elytra) of mite-free *H. axyridis* females (Riddick, 2006; Nalepa and Weir, 2007). The greater intensity of *H. virescens* on the ventrum of mite-laden males could suggest a physiological interaction between the mite and fungus. When the mite is feeding on hemolymph, via penetrating through the cuticle of the underside of the host elytra, development of *H. virescens* on the outer surface of the same host elytra could be restricted. In contrast, development of the fungus on the ventrum of a shared host would not be restricted, since the mite only feeds from the underside of the elytra of its host. No comparable studies on the position and intensity of ectoparasitic fungi and mites on their shared coccinellid hosts occur in the literature.

Parasitism and winter survival of hosts

The observation of slight mortality of *H. axyridis* adults, particularly males, in the mite and fungus and fungus only treatment groups under simulated winter conditions suggests that ectoparasites have the potential to alter the survival of this lady beetle under natural field conditions. The observation that one beetle in the fungus only and one in the mite and fungus treatment groups expired within the first and fifth week of this experiment, respectively, suggests that old age and perhaps a weakened immune system due to parasitism, rather than starvation, might have been responsible for

their demise. *H. axyridis* adults can live more than two years (Savoïskaya, 1970 in Nalepa *et al.*, 1996), so it is conceivable that old generation adults overwinter with new generation adults.

Interestingly, no beetles (all females) in the mite only treatment group died during the course of this experiment. This result can only suggest that the mite does not reduce the winter survival of *H. axyridis* females. In southern USA (specifically in Mississippi), *H. axyridis* adults are usually inactive from mid- to late November until late February or early March. In this study, beetles remained in simulated winter conditions until early May, approximately eight weeks beyond normal conditions in the field. Webberley and Hurst (2002) found that *C. hippodamiae* caused a decline in winter survival of *A. bipunctata*, especially of males, in cold conditions from September until March in the United Kingdom. The winter season is a few months longer in the United Kingdom than in southern USA.

Conclusion

This study documents the invasive lady beetle *H. axyridis* as a new host for the parasitic mite *C. hippodamiae*. The mite *C. hippodamiae* and the fungus *H. virescens* can co-exist in the same *H. axyridis* populations (aggregations) and on some of the same host individuals. The mite has the potential of altering the position and intensity of the fungus on shared hosts, but further research is necessary to confirm this observation. Parasitism by the mite and fungus combined and the fungus alone might reduce winter survival of *H. axyridis*, especially males. Follow-up experiments are necessary to clarify the interaction of *H. axyridis* age and gender on the influence of parasitism on winter survival. Defining the role of native parasites on the life history of *H. axyridis* is a logical step in identifying a suite of natural enemies that can help curb populations of this invasive lady beetle in some localities.

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References

- ADRIAENS T., SAN MARTIN y GOMEZ G., MAES D., 2008.- Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium.- *BioControl*, 53: 69-88.
- BROWN M. W., 2004.- Role of aphid predator guild in controlling spirea aphid populations on apple in West Virginia, USA.- *Biological Control*, 29: 189-198.
- BURGIO G., SANTI F., LANZONI A., MASETTI A., DE LUIGI V., MELANDRI M., REGGIANI A., RICCI C., LOOMANS A. J. M., MAINI S., 2008.- *Harmonia axyridis* recordings in northern Italy.- *Bulletin of Insectology*, 61: 361-364.
- CANTWELL G. E., CANTELO W. W., CANTWELL M. A., 1985.- Effect of a parasitic mite, *Coccipolipus epilachnae*, on fecundity, food consumption and longevity of the Mexican bean beetle.- *Journal of Entomological Science*, 20: 199-203.
- CERYNGIER P., HODEK I., 1996.- Enemies of the Coccinellidae, pp. 319-350.- In: *Ecology of Coccinellidae* (HODEK I., HONEK A., Eds).- Kluwer Academic Publishers, Dordrecht, The Netherlands.
- CHAPIN J. B., BROU V. A., 1991.- *Harmonia axyridis* (Pallas), the third species of the genus to be found in the United States.- *Proceedings of the Entomological Society of Washington*, 93: 630-635.
- CHRISTIAN E., 2001.- The coccinellid parasite *Hesperomyces virescens* and further species of the order Laboulbeniales (Ascomycotina) new to Austria.- *Annalen des Naturhistorischen Museums in Wien*, 103 B: 599-603.
- CHRISTIAN E., 2002.- Zur Verbreitung und Lebensweise des Marienkäfer-Parasiten *Coccipolipus hippodamiae* (McDaniel & Morrill, 1969) (Acari, Podapolipidae).- *Abhandlungen und Berichte des Naturkundemuseums Görlitz*, 74: 9-14.
- COSTAMAGNA A. C., LANDIS D. A., 2006.- Predators exert top-down control of soybean aphid across a gradient of agricultural management systems.- *Ecological Applications*, 16: 1619-1628.
- GARCÉS S., WILLIAMS R., 2004.- First record of *Hesperomyces virescens* Thaxter (Laboulbeniales: Ascomycetes) on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae).- *Journal of the Kansas Entomological Society*, 77: 156-158.
- GORDON R. D., 1985.- The Coccinellidae (Coleoptera) of America north of Mexico.- *Journal of the New York Entomological Society*, 93: 1-912.
- HAIJQANBAR H., HUSBAND R. W., KAMALI K., SABOORI A., KAMALI H., 2007.- *Ovacarus longisetosus* n. sp. (Acari: Podapolipidae) from *Amara* (Paracelia) *saxicola* Zimm. (Coleoptera: Carabidae) and new records of *Coccipolipus*, *Dorsipes*, *Eutarsopolipus* and *Tarsopolipus* from Iran.- *International Journal of Acarology*, 33: 241-244.
- HARWOOD J. D., RICCI C., ROMANI R., PITZ K. M., WEIR A., OBRZYCKI J. J., 2006.- Prevalence and association of the laboulbenialean fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on coccinellid hosts (Coleoptera: Coccinellidae) in Kentucky, USA.- *European Journal of Entomology*, 103: 799-804.
- HOCHMUTH R. C., HELLMAN J. L., DIVELY G., SCHRODER R. F. W., 1987.- Effect of parasitic mite *Coccipolipus epilachnae* (Acari: Podapolipidae) on feeding, fecundity, and longevity of soybean-fed adult Mexican bean beetles (Coleoptera: Coccinellidae) at different temperatures.- *Journal of Economic Entomology*, 80: 612-616.
- HURST G. D. D., SHARPE R. G., BROOMFIELD A. H., WALKER L. E., MAJERUS T. M. O., ZAKHAROV I. A., MAJERUS M. E. N., 1995.- Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*.- *Ecological Entomology*, 20: 230-236.
- HUSBAND R. W., 1981.- The African species of *Coccipolipus* with a description of all stages of *Coccipolipus solanophilae* (Acarina: Podapolipidae).- *Revue de Zoologie Africaine*, 95: 283-299.

- HUSBAND R. W., 1984.- The taxonomic position of *Coccipolipus* (Acarina: Podapolipidae), a genus of mites which are parasites of ladybird beetles (Coccinellidae), pp. 328-336. In: *Acarology*, vol. 1 (GRIFFITHS D. A., BOWMAN C. E., Eds).- Ellis-Harwood Ltd., Chichester, UK.
- HUSBAND R. W., 1989.- Two new species of *Coccipolipus* (Acari: Podapolipidae) parasites of *Chilocorus* spp. (Coccinellidae) from Vera Cruz and Morelos, Mexico and Florida and Wisconsin, USA.- *Proceedings of the Entomological Society of Washington*, 91: 429-435.
- KAMBUROV S. S., NADEL D. J., KENNETH R., 1967.- Observations on *Hesperomyces virescens* Thaxter (Laboulbeniales), a fungus associated with premature mortality of *Chilocorus bipustulatus* L. in Israel.- *Israel Journal of Agricultural Research*, 17: 131-134.
- KENIS M., ROY H. E., ZINDEL R., MAJERUS M. E. N., 2008.- Current and potential management strategies against *Harmonia axyridis*.- *BioControl*, 53: 235-252.
- KNELL R. J., WEBBERLEY K. M., 2004.- Sexually transmitted diseases of insects: distribution, evolution, ecology and host behaviour.- *Biological Reviews*, 79: 557-581.
- KOCH R. L., GALVAN T. L., 2008.- Bad side of a good beetle: the North American experience with *Harmonia axyridis*.- *BioControl*, 53: 23-35.
- KOCH R. L., VENETTE R. C., HUTCHISON W. D., 2006.- Invasions by *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the western hemisphere: implications for South America.- *Neotropical Entomology*, 35: 421-434.
- KOYAMA S., MAJERUS M. E. N., 2008.- Interactions between the parasitoid wasp *Dinocampus coccinellae* and two species of coccinellid from Japan and Britain.- *BioControl*, 53: 253-264.
- LAROCK D. R., ELLINGTON J. J., 1996.- An integrated pest management approach, emphasizing biological control, for pecan aphids.- *Southwestern Entomologist*, 21: 153-167.
- MAJERUS T. M. O., MAJERUS M. E. N., KNOWLES B., WHEELER J., BERTRAND D., KUZNETZOV V. N., UENO H., HURST G. D. D. 1998.- Extreme variation in the prevalence of inherited male-killing microorganisms between three populations of *Harmonia axyridis* (Coleoptera: Coccinellidae).- *Heredity*, 81: 683-691.
- MAJERUS M., STRAWSON V., ROY H., 2006.- The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain.- *Ecological Entomology*, 31: 207-215.
- MCDANIEL B., MORRILL W., 1969.- A new species of *Tetrapolipus* from *Hippodamia convergens* from South Dakota (Acarina: Podapolipidae).- *Annals of the Entomological Society of America*, 62: 1465-1468.
- MIGNAULT M.-P., ROY M., BRODEUR J., 2006.- Soybean aphid predators in Quebec and the suitability of *Aphis glycines* as prey for three Coccinellidae.- *BioControl*, 51: 89-106.
- NALEPA C. A., KIDD K. A., 2002.- Parasitism of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) by *Strongygaster triangulifera* (Diptera: Tachinidae) in North Carolina.- *Journal of Entomological Science*, 37: 124-127.
- NALEPA C. A., WEIR A., 2007.- Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae) by *Hesperomyces virescens* (Ascomycetes: Laboulbeniales): role of mating status and aggregation behavior.- *Journal of Invertebrate Pathology*, 94: 196-203.
- NALEPA C. A., KIDD K. A., AHLSTROM K. R., 1996.- Biology of *Harmonia axyridis* (Coleoptera: Coccinellidae) in winter aggregations.- *Annals of the Entomological Society of America*, 89: 681-685.
- RIDDICK E. W., 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*.- *Journal of Insect Science*, 6 (42): 1-15. [online] URL: <http://insectscience.org/6.42/>
- RIDDICK E. W., SCHAEFER P., 2005.- Occurrence, density, and distribution of parasitic fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on multicolored Asian lady beetle (Coleoptera: Coccinellidae).- *Annals of the Entomological Society of America*, 98: 615-624.
- RIDDICK E. W., COTTRELL T. E., KIDD K. A., 2009.- Natural enemies of the Coccinellidae: Parasites, pathogens, and parasitoids.- *Biological Control*, 51: 306-312. [online] DOI 10.1016/j.biocontrol.2009.05.008.
- ROY H. E., BROWN P. M. J., ROTHERY P., WARE R. L., MAJERUS M. E. N., 2008.- Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*.- *BioControl*, 53: 265-276.
- RUTLEDGE C. E., O'NEIL R. J., FOX T. B., LANDIS D. A., 2004.- Soybean aphid predators and their use in integrated pest management.- *Annals of the Entomological Society of America*, 97: 240-248.
- RYDER J. J., HATHWAY J., KNELL R. J., 2007.- Constraints on parasite fecundity and transmission in an insect-STD system.- *Oikos*, 116: 578-584.
- SCHRODER R. F. W., 1982.- Effect of infestation with *Coccipolipus epilachnae* Smiley (Acarina: Podapolipidae) on fecundity and longevity of the Mexican bean beetle.- *International Journal of Acarology*, 8: 81-84.
- SHAPIRO-ILAN D. I., COTTRELL T. E., 2005.- Susceptibility of lady beetles (Coleoptera: Coccinellidae) to entomopathogenic nematodes.- *Journal of Invertebrate Pathology*, 89: 150-156.
- STALS R., PRINSLOO G., 2007.- Discovery of an alien invasive, predatory insect in South Africa: the multicolored Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae).- *South African Journal of Science*, 103: 123-126.
- TAVARES I. I., 1979.- The Laboulbeniales and their arthropod hosts, pp. 229-258. In: *Insect-fungus symbiosis: nutrition, mutualism, and commensalism* (Batra L. R., Ed.).- John Wiley and Sons, New York, USA.
- TAVARES I. I., 1985.- Laboulbeniales (Fungi, Ascomycetes).- *Mycologia Memoir*, 9: 1-627.
- TEDDERS W. L., SCHAEFER P. W., 1994.- Release and establishment of *Harmonia axyridis* (Coleoptera: Coccinellidae) in the southeastern United States.- *Entomological News*, 105: 228-243.
- THAXTER R., 1931.- Contribution towards a monograph of the Laboulbeniaceae. V.- *Memoirs of the American Academy of Arts and Sciences*, 16: 1-435.
- WEBBERLEY K. M., 2000.- Study of the distribution and effects of a sexually transmitted parasitic mite on coccinellid beetles. *Ph.D. Thesis*. University of Cambridge, Cambridge, UK.
- WEBBERLEY K. M., HURST G. D. D., 2002.- The effect of aggregative overwintering on an insect sexually transmitted parasite system.- *Journal of Parasitology*, 88: 707-712.
- WEBBERLEY K. M., HURST G. D. D., HUSBAND R. W., SCHELENBURG J. H. G. V. D., SLOGGETT J. J., ISHAM V., BUSZKO J., MAJERUS M. E. N., 2004.- Host reproduction and a sexually transmitted disease: causes and consequences of *Coccipolipus hippodamiae* distribution on coccinellid beetles.- *Journal of Animal Ecology*, 73: 1-10.
- WEBBERLEY K. M., BUSZKO J., ISHAM V., HURST G. D. D., 2006a.- Sexually transmitted disease epidemics in a natural insect population.- *Journal of Animal Ecology*, 75: 33-43.
- WEBBERLEY K. M., TINSLEY M. C., SLOGGETT J. J., MAJERUS M. E. N., HURST G. D. D., 2006b.- Spatial variation in the incidence of a sexually transmitted parasite of the ladybird beetle *Adalia bipunctata* (Coleoptera: Coccinellidae).- *European Journal of Entomology*, 103: 793-797.
- WEIR A., BEAKES G. W., 1995.- An introduction to the Laboulbeniales: a fascinating group of entomogenous fungi.- *Mycologist*, 9: 6-10.
- WEIR A., BEAKES G. W., 1996.- Correlative light- and scanning electron microscope studies on the developmental morphology of *Hesperomyces virescens*.- *Mycologia*, 88: 677-693.
- WELCH V. L., SLOGGETT J. J., WEBBERLEY K. M., HURST G. D. D., 2001.- Short-range clinal variation in the prevalence of a sexually transmitted fungus associated with urbanisation.- *Ecological Entomology*, 26: 547-550.

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