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Is the prevalence and intensity of the ectoparasitic fungus *Hesperomyces virescens* related to the abundance of entomophagous coccinellids?

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Abstract

Hesperomyces virescens Thaxter is an obligate ectoparasitic fungus that parasitizes coccinellids in several countries. It transmits horizontally between coccinellid adults via social contact. The relative abundance of coccinellids in an agroecosystem may affect the transmission dynamics of *H. virescens*. We predicted that the prevalence and intensity of *H. virescens* would be greatest on the more abundant coccinellid species. We collected lady beetles from plant foliage in a 480 ha agroecosystem in Byron, Georgia, USA, from April through October 2007. The prevalence and intensity of the parasite was greatest on *Harmonia axyridis* (Pallas), which was the most abundant coccinellid collected in early spring and summer. There was a positive relationship between parasite infection of the exotic *H. axyridis* and the native *Olla v-nigrum* Mulsant; parasite infection increased as relative abundance of both species increased. The parasite was seldom on one of its original hosts, *Hippodamia convergens* Guérin-Méneville, and never on the exotic *Coccinella septempunctata* L. even though the latter species was the second most abundant coccinellid in the agroecosystem. Lack of infection of an abundant coccinellid such as *C. septempunctata* could result from low encounter rates and not just low susceptibility to infection. *H. virescens* transmission may vary depending on frequency of contact between infected and uninfected coccinellids in shared habitats.

Key words: Laboulbeniaceae, Coccinellidae, horizontal transmission, ectoparasite, symbionts.

Introduction

The order Laboulbeniales contains almost 2,000 described species of ascomycetous fungi that parasitize arthropods, 80% of which are beetles (Coleoptera) (Weir and Hammond, 1997; Santamaria, 2001; Weir and Blackwell, 2004). The obligate ectoparasitic fungus *Hesperomyces virescens* Thaxter (Family Laboulbeniaceae) is known to infect 11 entomophagous and two mycophagous species of Coccinellidae (Coleoptera), reviewed in Riddick *et al.* (2009). A better understanding of the transmission dynamics of *H. virescens* is critical to assessing its biological impact on hosts. Most species of Laboulbeniales do not harm their hosts (Tavares, 1979; Weir and Beakes, 1995). However, Kamburov *et al.* (1967) reported that *H. virescens* caused mortality of 95% of the adults of *Chilocorus bipustulatus* L. in citrus groves in Israel. Sublethal effects on life history parameters of coccinellids may result from infection by *H. virescens*. Nalepa and Weir (2007) indicated that concentration of *H. virescens* thalli around mouthparts, head or antennae of *Harmonia axyridis* (Pallas) could hamper the detection of food, mates, or predators.

Transmission of *H. virescens* can depend on host behavior. Social (bodily) contact between infected and non-infected conspecific males and females facilitates the transmission of fungus onto *Adalia bipunctata* (L.) and *H. axyridis* (Welch *et al.*, 2001; Riddick and Schaefer, 2005). Physical contact of adults in moderate to large aggregations during the winter season exacerbates the spread of the fungus (Nalepa and Weir, 2007). Overlap of host generations during the spring and summer seasons and availability of susceptible life stages allow for persistence of *H. virescens* in some host populations (Riddick, 2006). The effect of host abundance or density on transmission of *H. virescens* is not clear. Few studies have monitored the prevalence or intensity of this parasite over an extended time (Riddick, 2006) and none examined this parasite in an assemblage of coccinellid species. If the parasite depends heavily on host behavior to facilitate transmission, parasite infection should relate positively to the relative abundance of its host. Seasonally abundant species that have moderate to high encounter rates with conspecifics might experience greater levels of infection by *H. virescens*. Encounters can occur within winter aggregations or on plant foliage, as adults search for mates or forage for food. *H. virescens* prevalence and intensity should be greatest on the more abundant species. Parasite prevalence could also be greater on multivoltine than on univoltine species. In addition, parasite prevalence could be greater on species that do not undergo summer diapause. The goal of this study was to determine the transmission potential of the parasitic fungus *H. virescens* in an assemblage of native and exotic lady beetles. We predicted that the prevalence and intensity of *H. virescens* would be greatest on the more abundant species. This study contributes to our knowledge of the transmission dynamics of laboulbenialean parasites on their coccinellid hosts.

Materials and methods

The coccinellid assemblage in non-orchard and orchard habitats (table 1) on the premises of a 480 ha, USDA facility (Southeastern Fruit & Tree Nut Research Labo-

Table 1. List of sampling dates and the habitats (coded*) in which coccinellids were sampled for the presence of ectoparasite *H. virescens* in Byron, Georgia, USA during spring, summer, and fall 2007. X, represents one sample taken per date; 2X, represents two samples taken per date.

Date (2007)	Mu	We	Cl	Pea	Ve	Pec	So	Pl	Cr	Jo	Br
April 9	X	X	X	X	X						
April 13	X	X	X	X	X						
April 19	X	X	X	X	X						
April 26	X	X	X	X	X						
May 3	X	X	X	X	X	X					
May 10				X	X	X					
May 17				X	X	X					
May 22				X	X	X					
May 29				2X	X	X					
June 5				2X							
June 14		X		2X		X				X	
June 20				X	X		X				
June 26				X	X		X				
July 5				X	X		X				
July 12				2X			X			X	
July 19				X			X			X	
July 25				X			X				X
August 2				X			X	2X			
August 9				X		X	X	X			
August 17				X		X	X	X			
August 23				X		X	X	X			
August 29				X		X	X	X			
September 6				X		X	X				
September 14				X		X					
September 20				X		X	X				
September 25		2X		X		X	X		X		
October 5				X		X	X				
October 10				X		X	X				
October 23				X		X	X				
October 30				X		X	X				

*Codes for habitats: Mu (mustard), We (weeds, nonspecific), Cl (crimson clover), Pea (peach trees), Ve (vetch), Pec (pecan trees), So (sorghum), Pl (plum trees), Cr (crape myrtle trees), Jo (Johnson grass), Br (briars, nonspecific).

ratory, SEFTNRL) in Byron, Georgia, USA, was sampled along line transects. Distances between habitats varied but at least 100 m separated those that were sampled concurrently. Vetch and Johnson grass habitats were adjacent, but sampling was separated temporally (i.e., neither were sampled on the same date). Unidentified weed and Johnson grass (*Sorghum halepense* (L.) Pers.) habitats were periodically mowed and thus not always available. Mustard (*Sinapis arvensis* L.), clover (*Trifolium incarnatum* L.) and vetch (*Vicia* spp.) were only available for sampling during early season. Briars (*Smilax* spp.) were sampled once, when other non-orchard habitats were not available. Orchard habitats were generally available for sampling throughout the study. Daily temperature and rainfall readings were available from a weather station on the premises of the SEFTNRL.

We used a standard insect sweep net to collect coccinellid adults on plant foliage, along line transects, in the non-orchard and orchard habitats. In order to standardize the sampling procedure, 100 sweeps were taken in each non-orchard habitat, along the line transect within 15 min anytime between 0800 and 1200 hrs on each sampling date. Using a left to right sweeping motion with the hand-held sweep net, sampling was completed after 100 sweeps or the habitat edge was reached and then sweeps were made back across an undisturbed transect of the habitat until 100 sweeps were completed. Length of non-orchard habitats ranged from 30 to 130 m and the length of orchard habitats ranged from 80 to 200 m. Total transect distance within a habitat was approximately 90 m. In the orchard habitats, 100 tree terminals were selected at random from trees along a line transect (tree row) and sampled by placing the sweep net over a limb terminal and shaking to dislodge any beetles. An orchard was sampled within 60 min typically anytime between 0800 – 1200 hrs. We transferred the captives into plastic bags, which were stored in a cooler, and later returned to a research laboratory at SEFTNRL.

In the laboratory, coccinellids were examined under a dissecting microscope for the presence of ectoparasites, specifically parasitic Laboulbeniales fungus. Coccinellids with Laboulbeniales thalli growing on any body part were stored in a 1-dram glass vial containing ethanol (70%). All infected beetles were shipped to the National Biological Control Laboratory (NBCL), USDA,

Table 2. Prevalence and presence (+) or absence (---) of parasitic fungus *H. virescens* on coccinellids in Byron, Georgia, USA.

Coccinellids	Sample size	Prevalence (%)	Presence/Absence ♂	♀
Harmonia axyridis	646	50.1	+	+
Coccinella septempunctata	502	0	---	---
Hippodamia convergens	297	1.3	+	---
Olla v-nigrum	142	33.1	+	+
Coleomegilla maculata	6	0	---	---
Scymnus loewii and *Scymnus socer*	45	0	---	---
Cycloneda munda	4	0	---	---

in Stoneville, Mississippi for closer examination. The gender of coccinellid adults was determined by examining the shape of the abdominal sternites under a zoom stereo microscope (Olympus™ SZX16). To determine the relative abundance of infected vs uninfected coccinellids for one season, the number of infected beetles (of each species) was compared to the total number collected each date throughout the season.

At the NBCL, fungus prevalence and intensity were determined in the laboratory as infected coccinellids were examined under an Olympus™ SZX16 zoom stereo microscope. Prevalence was defined as the percentage of coccinellids (of each species) in the entire seasonal sample infected with Laboulbeniales fungus. Infected coccinellids were those that had mature (or nearly mature) thalli anywhere on their body. Intensity was defined as the number of mature (or nearly mature) fungal thalli on the body of each coccinellid beetle.

Statistical analysis

Linear regression predicted the dependence of parasite infection on relative abundance of two common species (*H. axyridis*, *Olla v-nigrum* Mulsant). Pearson Product-Moment Correlation measured the strength of an association between the abundance of infected males versus infected females (*H. axyridis*, *O. v-nigrum*). The Student's *t*-test determined the significance of host sex on abundance of infected adults and the intensity of parasite infection. Data subjected to the *t*-test were square root transformed prior to analysis (Zar, 1999). SigmaStat 3.0.1 (Systat Software Inc., Richmond, CA) statistical software generated the analyses. Means were considered significantly different when $p \leq 0.05$. This manuscript presents only untransformed data.

Results

When combining data from all habitats, eight entomophagous coccinellid species were on plant foliage in the study area (table 2). The ectoparasitic fungus *H. virescens* infected only three of the species, *H. axyridis*, *O. v-nigrum*, and *Hippodamia convergens* Guérin-Méneville. Season-long fungus prevalence ranged from a high of 50% (for *H. axyridis*) to a low of 1% (for *H. convergens*). The fungus infected both sexes of *H. axyridis* and *O. v-nigrum*, but only infected males of *H. convergens.* Native species that were not infected included *Coleomegilla maculata* DeGeer, *Scymnus loewii* Mulsant, *Scymnus socer* LeConte, and *Cycloneda munda* (Say). One exotic species, *Coccinella septempunctata* L. was devoid of the fungus even though it was one of the most abundant species (table 2).

When the number of infected and non-infected individuals of the four most abundant species (*H. axyridis*, *H. convergens*, *O. v-nigrum*, *C. septempunctata)* were pooled across habitats, a trend of greater abundance for *C. septempunctata* and *H. axyridis* adults early in the growing season (April and May) was apparent (figure 1) Most *C. septempunctata* and many *H. axyridis* adults were relatively abundant during April and May. The minimum and maximum temperature during the season (spring and summer) was typical for the region, but there was no rainfall at the location from mid-April to early June 2007. Early season peaks in abundance were not evident for *O. v-nigrum* or *H. convergens*. In late August to early September, *H. convergens* was abundant in comparison to the other species.

The number of *H. axyridis* and *O. v-nigrum* infected with the parasite was related to the number captured per date over the season (*H. axyridis*, $r^2 = 0.74$, $p < 0.001$, n = 30, figure 2A; *O. v-nigrum*, $r^2 = 0.72$, $p < 0.001$, n = 30, figure 2B). The number of infected males and females per sample date correlated very well for *H. axyridis* ($r = 0.98$, $p < 0.0001$, n = 30) but not for *O. v-nigrum*

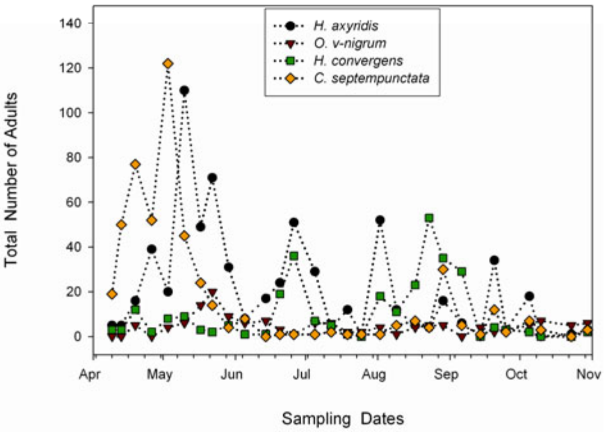

Figure 1. Total number of dominant coccinellids trapped in all habitats combined. Sample size of *H. axyridis*, *C. septempunctata*, *O. v-nigrum*, and *H. convergens* pooled over the season was 646, 502, 142, and 297, respectively.

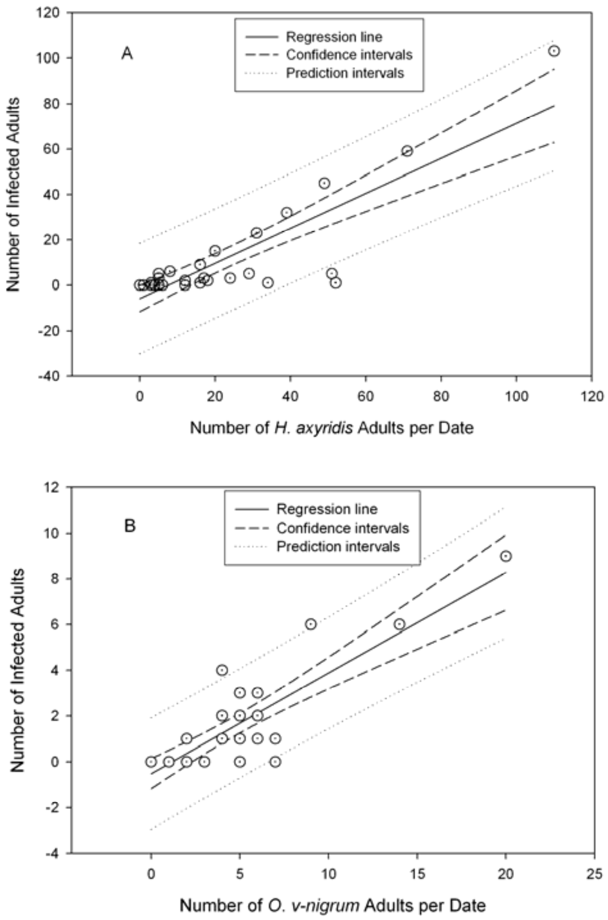

Figure 2. Relationship between the number of *H. axyridis* (A) and *O. v-nigrum* (B) infected with the parasitic fungus and the total number of adults captured per date. For *H. axyridis*, the regression line defines the relationship between infected (y) versus all adults (x); simple linear regression; $y = a + bx$; a, -5.83; b, 0.77; $r^2 = 0.74$; n, 30 replicate collection dates. For *O. v-nigrum*, the regression line defines the relationship between infected (y) versus all adults (x); simple linear regression; $y = a + bx$; a, -0.51; b, 0.44; $r^2 = 0.72$; n, 30 replicate collection dates. The solid line, dashed line, and dotted line represent the regression line, 95% confidence intervals and 95% prediction intervals, respectively.

($r = 0.24$, $p = 0.21$, n = 30). Infected *H. axyridis* males appeared slightly more abundant than infected females early in the season (figure 3A), however, data pooled across habitats for the season indicated no significant difference between sexes ($t = 1.0$, $df = 58$, $p = 0.31$). The abundance of infected *O. v-nigrum* males and females was not markedly different early in the season (figure 3B) or over the entire season ($t = 1.0$, $df = 58$, $p = 0.32$). The mean ± SEM number of infected *H. axyridis* adults per date over the season was 7.1 ± 2.7 and 3.7 ± 1.4 males and females, respectively. The mean ± SEM number of infected *O. v-nigrum* adults per date over the season was 0.97 ± 0.31 and 0.60 ± 0.18 males and females, respectively.

The total number of *H. virescens* thalli on the integument of *H. axyridis* and *O. v-nigrum* males and females was variable. Several *H. axyridis* males had over 300 mature thalli on their body and one male had nearly 600 mature thalli on its body (figure 4A). *H. axyridis* males harbored significantly more parasite thalli than females ($t = 5.7$, $df = 322$, $p < 0.001$); the mean ± SEM number of parasite thalli was 87.5 ± 5.5 and 45.7 ± 4.8 on males and females, respectively. One *O. v-nigrum* male had nearly 200 mature thalli on its body and another one had over 300 mature thalli on its body (figure 4B). Male and female *O. v-nigrum* harbored similar total numbers of parasite thalli ($t = 0.16$; $df = 45$; $p = 0.87$). The mean ± SEM number of parasite thalli was 49.2 ± 12.9 and 41.0 ± 8.9 on *O. v-nigrum* males and females, respectively. One *H. convergens* male had a total of 40 mature thalli on its body. The mean ± SEM number of parasite thalli was 17.2 ± 9.3 on *H. convergens* males (n = four infected males, no infected females).

When considering the distribution of the parasite on separate body parts, *H. axyridis* males and females had similar numbers of mature (or nearly mature) thalli on the dorsal elytra ($t = 1.2$, $df = 322$, $p = 0.22$), ventral elytra ($t = 0.25$; $df = 322$, $p = 0.8$), and pronotum ($t = 1.2$, $df = 322$, $p = 0.2$) as indicated in table 3. Males had significantly more thalli on the meso- and metathorax ($t = 8.2$, $df = 322$, $p < 0.001$), abdomen ($t = 7.25$, $df = 322$, $p < 0.001$), prothorax ($t = 3.4$; $df = 322$, $p < 0.001$), legs ($t = 7.0$; $df = 322$, $p < 0.001$) and head ($t = 4.0$; $df = 322$, $p < 0.001$). *O. v-nigrum* males had significantly more mature thalli on the prothorax ($t = 2.25$; $df = 45$, $p = 0.03$), and meso- and metathorax ($t = 2.6$, $df = 45$, $p = 0.01$) and females had more thalli on the dorsal elytra ($t = 2.2$; $df = 45$, $p = 0.03$) as indicated in table 3. *Hippodamia convergens* males had *H. virescens* thalli on the abdomen (7.7 ± 4.3, mean ± SEM), legs (6.2 ± 6.2, mean ± SEM) and meso- and metathorax (3.2 ± 2.0, mean ± SEM), and none on the elytra or any other body part (n = four infected males, no infected females).

Discussion

Few studies have documented the influence of host relative abundance on the transmission potential of parasitic fungi. Zerm and Adis (2004) reported that *Laboulbenia phaeoxanthae* W. Rossi et E. Arndt infection rate was greatest when the abundance of its carabid host was lowest and vice versa in the field. In another field study, Riddick (2006) determined that *H. virescens* infection rate decreased as the abundance of *H. axyridis* males increased in one season but not in another. In both of these prior studies, high abundance of uninfected adults was often due to emergence of new generation adults, which did not have significant encounters with old (overwintered) generation adults harboring the parasite. The observation that parasitic *H. virescens* infection increased along with the abundance of two host species, *H. axyridis* and *O. v-nigrum*, in this study suggests that host abundance can in fact have a positive effect on parasite transmission.

The behavior of both coccinellids (*H. axyridis*, *O. v-nigrum*) during the winter and early spring could have increased the spread of fungus in this study. Both spe-

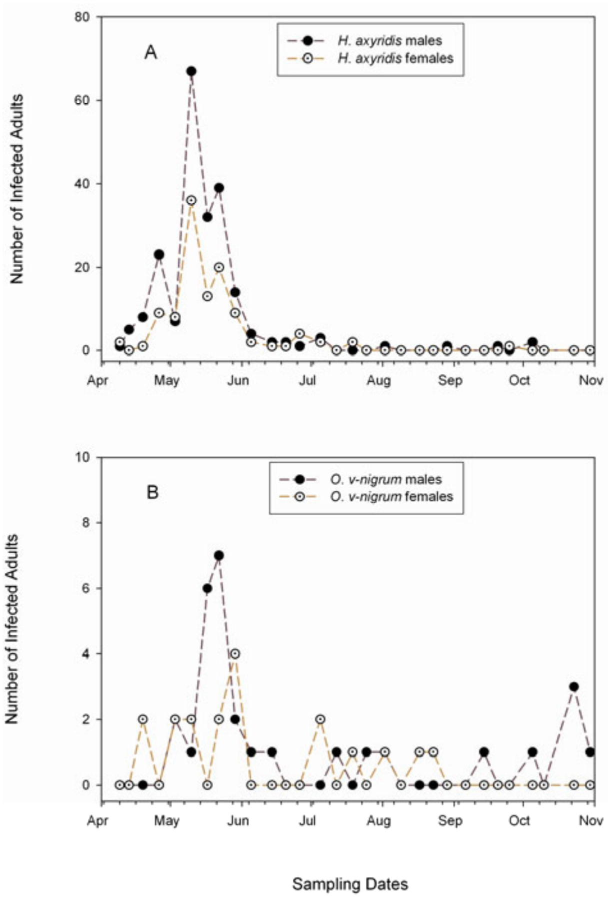

Figure 3. Number of infected *H. axyridis* males and females (A) and *O. v-nigrum* males and females (B) captured throughout the season. For *H. axyridis*, sample size was 213 infected males and 111 infected females. For *O. v-nigrum*, sample size was 29 infected males and 18 infected females.

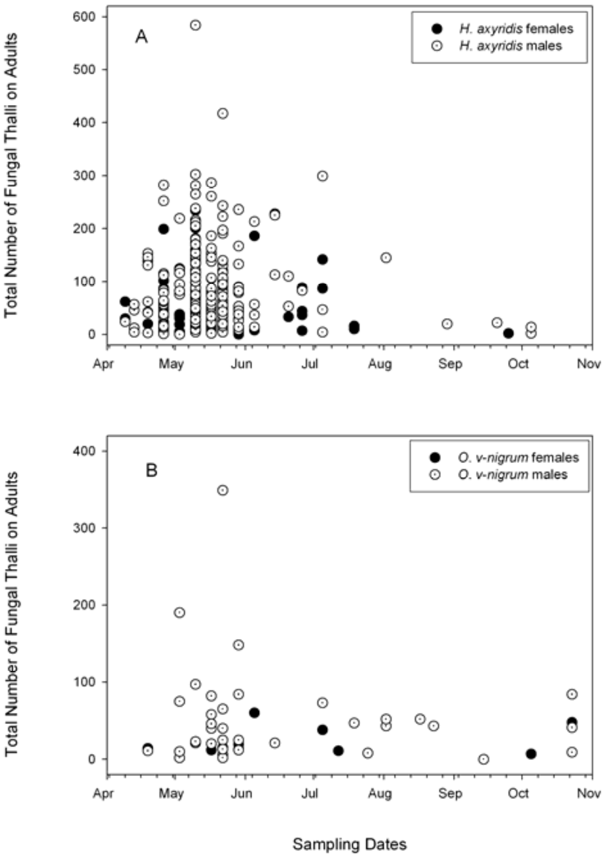

Figure 4. Total number of *H. virescens* mature (or nearly mature) thalli on *H. axyridis* males and females (A) and on *O. v-nigrum* males and females throughout the season (B). Dotted circle and blackened circle represent male and female hosts, respectively. Sample size was 213 infected males and 111 infected females for *H. axyridis*, and 29 infected males and 18 infected females for *O. v-nigrum*.

Table 3. Mean ± SEM number of *H. virescens* mature thalli on body parts of male (♂) and female (♀) *H. axyridis* and *O. v-nigrum*.

Host Body Part	*H. axyridis* ♂ ♀	*O. v-nigrum* ♂ ♀
Elytra (dorsal)	26.4 ± 2.3a 20.3 ± 2.5a	16.9 ± 4.6b 29.0 ± 6.1a
Elytra (underside)	4.0 ± 0.7a 4.3 ± 1.1a	0.8 ± 0.4a 1.3 ± 0.7a
Mesothorax and Metathorax	10.2 ± 1.0a 1.9 ± 0.47b	12.2 ± 5.3a 1.2 ± 1.1b
Abdomen (ventral)	19.5 ± 2.1a 6.0 ± 1.3b	4.2 ± 1.5a 1.6 ± 1.1a
Prothorax	1.7 ± 0.2a 0.5 ± 0.2b	2.6 ± 1.0a 0.2 ± 0.1a
Legs	19.4 ± 1.4a 8.3 ± 1.3b	10.4 ± 4.1a 5.9 ± 4.0a
Pronotum	4.7 ± 0.5a 3.7 ± 0.8a	1.8 ± 0.8a 1.5 ± 0.6a
Head (incl. mouthparts, antennae)	1.9 ± 0.4a 0.4 ± 0.1b	0.3 ± 0.1a 0.3 ± 0.2a

Means followed by a different letter in a column (for each body part) are significantly different. Sample size: infected *H. axyridis* (213 males, 111 females); *O. v-nigrum* (29 males, 18 females).

cies overwinter in aggregations of mixed sexes. In southeastern USA, *O. v-nigrum* adults overwinter beneath loose bark of live pecan trees (Mizell and Schiffhauer, 1987; Cottrell and Shapiro-Ilan, 2003) and *H. axyridis* adults overwinter in wall voids, ceiling/wall corners, and other secluded places in man-made structures (Nalepa *et al*., 1996, 2005; Schaefer, 2003). The physical contact with conspecifics in aggregations may facilitate transmission especially near the end of winter as temperatures rise and beetles mate prior to dispersing from overwintering sites. Consequently, early season infection of *H. axyridis* and *O. v-nigrum* was possible even before estimates of abundance were taken, beginning in April. Both coccinellid species were more abundant early in the season, despite the drought conditions that prevailed during April and May 2007.

Is it possible that the fungus transferred from *H. axyridis* to *O. v-nigrum* in some habitats (pecan and peach orchards) in this study? Both species are arboreal with a proclivity for searching for mates and food on tree foliage during spring and summer. There are no published records of the parasitic fungus *H. virescens* attacking *O. v-nigrum* anywhere in North America. Nevertheless, *H. virescens* has been observed on both *O. v-nigrum* and *H. axyridis* in Georgia, USA, since summer 2003 (TEC, unpublished observations). The potential for this species to spread between heterospecific adults as promiscuous *H. axyridis* males search for mates is unknown. Reproductively mature male coccinellids will mount conspecific as well as heterospecific females and males in their propensity to mate (Majerus, 1994). Bodily contact between related species, during mating or mating attempts, could provide a viable means of transmission of Laboulbeniales between coccinellid populations in shared habitats. Pascoe *et al.* (2007) observed mating between *H. axyridis* and the two-spotted lady beetle *A. bipunctata* under field conditions in the United Kingdom. Therefore, fungus transmission between *H. axyridis* and *O. v-nigrum* could occur during mating attempts.

The parasite *H. virescens* was rarely present on the convergent lady beetle *H. convergens* and was not present on the seven-spotted lady beetle *C. septempunctata* in this study. Since there was only one season of sampling, it is difficult to ascertain the reason for the low or no prevalence of the parasite on these two coccinellids. Some individuals in *H. convergens* and *C. septempunctata* populations might have undergone summer diapause in this study, therefore, limiting their potential for exposure to infection. The number of generations produced per year (voltinism) can vary from one to as many as five in *H. convergens* (Michaud and Qureshi, 2006) and from one to four generations in *C. septempunctata* populations (Hodek and Michaud, 2008). In contrast, *H. axyridis* does not undergo summer diapause and can produce two to four generations per year (Bazzocchi *et al*., 2004) depending on climate. Lack of infection of *C. septempunctata* in this study could indicate low encounter rates with infected cohorts and not just low susceptibility to infection. Both the rate at which coccinellid adults encounter conspecifics and the density of infected individuals in a population can influence the spread of a sexually transmitted parasite (Ryder *et al*., 2005, 2007). Interestingly, this parasitic fungus infected *H. convergens* in the early 1900's in Alabama, USA (Thaxter, 1931) and recently infected *C. septempunctata*, for the first time anywhere in the world, in Kentucky, USA at a prevalence of 1-2% (Harwood *et al*., 2006a). Maybe there is a time-lag before the parasite becomes fully adapted to *C. septempunctata*. Establishment of *C. septempunctata* in several eastern USA states, including Georgia, occurred in the 1970's (Angalet *et al*., 1979), prior to the establishment of *H. axyridis*, in the late 1980's to early 1990's (Tedders and Schaefer, 1994).

The observation that *H. axyridis* males had significantly more parasite thalli on their integument suggests that they are more active than female conspecifics. Riddick (2006) surmised that *H. axyridis* males encounter more conspecific males and females due to increased activity, which increased the chances of spreading the parasitic fungus. Riddick and Schaefer (2005) found that overwintering *H. axyridis* males harbored more parasite thalli than females, suggesting that males encounter more conspecifics and therefore increase opportunities for spread of infection. Therefore, differences in behavior of male and female beetles, rather than any gender preference on behalf of the parasite, can account for the unequal distribution of *H. virescens* on its host, *H. axyridis*. Interestingly, *O. v-nigrum* males did not harbor significantly more parasite thalli on their integument than female conspecifics in this study, which could suggest that males are not likely to encounter more individuals than their female cohorts. The low number of parasite thalli on the head and mouthparts of males and females of both *H. axyridis* and *O. v-nigrum* suggests that parasitism does not impede the detection of food, mates, or predators.

The manner in which Laboulbeniales fungi position themselves on male and female arthropod hosts has intrigued researchers for decades (Benjamin and Shanor, 1952; Weir and Blackwell, 2004). Physical contact may initiate spore discharge; therefore, the position of thalli on the body may reflect the type of contact that occurred (Weir and Beakes, 1996). Welch *et al*. (2001) proposed a sexual transmission hypothesis to explain the apparent distribution of *H. virescens* thalli on coccinellids (the two-spotted lady beetle, *A. bipunctata*). According to this hypothesis, the positioning of thalli on the dorsum of females and the ventrum of males should mirror the position of a male on top of a female conspecific while mating. Garcés and Williams (2004) were the first to report that *H. virescens* thalli were concentrated on the dorsoposterior of females and the ventroposterior of males of its host, *H. axyridis*. In contrast, others have reported that this parasite was concentrated on the dorsum and ventrum of *H. axyridis* males (Riddick, 2006; Harwood *et al*., 2006b; Nalepa and Weir, 2007). Since the parasite was also concentrated on the dorsum, ventrum, and legs of *H. axyridis* males in this study, the sexual transmission hypothesis cannot consistently account for the distribution of *H. virescens* on male hosts. The promiscuous behavior of *H. axyridis* males, including their propensity for mounting male as well as female conspecifics, in their search for mating partners, could result in the transfer of the parasite to the dorsum of other males as well as females. In contrast, the distribution of the parasite on *O. v-nigrum*, in this study, tends to fit a sexual transmission hypothesis; parasite thalli were generally more concentrated on the dorsum (elytra) and ventrum (meso- and metathorax) of females and males, respectively.

In conclusion, the obligate ectoparasitic fungus *H. virescens* is polyphagous on entomophagous coccinellids and transmission is not solely dependent on the abundance of potential hosts. Our prediction that the prevalence and intensity of *H. virescens* would be greatest on the more abundant coccinellid species was only partially true. Although infection of the most abundant coccinellid (*H. axyridis*) was evident, no infection of the second most abundant coccinellid (*C. septempunctata*) indicates that other factors, such as the rate at which infected and uninfected adults contact one another (for mating, overwintering, or summer aestivating), can affect parasite prevalence and intensity. Another possibility is that *C. septempunctata* is less susceptible to infection than *H. axyridis* and *O. v-nigrum*. Maybe *H. virescens* prevalence will increase in *C. septempunctata* populations as it becomes more adapted to this novel host over time. The coexistence of several related coccinellids in the same habitats and utilizing some of the same food sources may provide opportunities for transfer of *H. virescens* between heterospecific coccinellids. Future research should determine the frequency of transfer of *H. virescens* between heterospecific coccinellids across multiple habitats.

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References

ANGALET G. W., TROPP J. M., EGGERT A. N., 1979.- *Coccinella septempunctata* in the United States: recolonizations and notes on its ecology.- *Environmental Entomology*, 8: 896-901.

BAZZOCCHI G. G., LANZONI A., ACCINELLI G., BURGIO G., 2004.- Overwintering, phenology and fecundity of *Harmonia axyridis* in comparison with native coccinellid species in Italy.- *BioControl*, 49: 245-260.

BENJAMIN R. K., SHANOR L., 1952.- Sex of host specificity and position specificity of certain species of *Laboulbenia* on *Bembidion picipes*.- *American Journal of Botany*, 39: 125-131.

COTTRELL T. E., SHAPIRO-ILAN, D. I., 2003.- Susceptibility of a native and an exotic lady beetle (Coleoptera: Coccinellidae) to *Beauveria bassiana*.- *Journal of Invertebrate Pathology*, 84: 137-144.

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.

HARWOOD J. D., RICCI C., ROMANI R., OBRYCKI J. J., 2006a.- Historic prevalence of a laboulbenialean fungus infecting introduced coccinellids in the United States.- *Antenna*, 30: 74-79.

HARWOOD J. D., RICCI C., ROMANI R., PITZ K. M., WEIR A., OBRYCKI J. J., 2006b.- 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.

HODEK I., MICHAUD J. P., 2008.- Why is *Coccinella septempunctata* so successful? (A point of view).- *European Journal of Entomology*, 105: 1-12.

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.

MAJERUS M. E. N., 1994.- *Ladybirds*.- The New Naturalist. Harper Collins Publishers, London, UK.

MICHAUD J. P., QURESHI J. A., 2006.- Reproductive diapause in *Hippodamia convergens* (Coleoptera: Coccinellidae) and its life history consequences.- *Biological Control*, 39: 193-200.

MIZELL R. F. III, SCHIFFHAUER D. E., 1987.- Trunk traps and overwintering predators in pecan orchards: survey of species and emergence times.- *Florida Entomologist*, 70: 238-244.

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.

NALEPA C. A., KENNEDY G. C., BROWNIE C., 2005.- Role of visual contrast in the alighting behavior of *Harmonia axyridis* (Coleoptera: Coccinellidae) at overwintering sites.- *Environmental Entomology*, 34: 425-431.

PASCOE S., BROWN P., MAJERUS M., 2007.- Hybrid mating between a harlequin ladybird, *Harmonia axyridis*, and a 2-spot ladybird, *Adalia bipunctata*.- *Bulletin of the Amateur Entomologist's Society*, 66: 156-159.

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. W., 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.

RYDER J. J., WEBBERLEY K. M., BOOTS M., KNELL R. J., 2005.- Measuring the transmission dynamics of a sexually transmitted disease.- *Proceedings of the National Academy of Sciences*, 102: 15140-15143.

RYDER J. J., MILLER M. R., WHITE A., KNELL R. J., BOOTS M., 2007.- Host-parasite population dynamics under combined frequency- and density-dependent transmission.- *Oikos*, 116: 2017-2026.

SANTAMARIA S., 2001.- Los Laboulbeniales, un grupo enigmatico de hongos parasitos de insectos.- *Lazaroa*, 22: 3-19.

SCHAEFER P. W., 2003.- Winter aggregation of *Harmonia axyridis* (Coleoptera: Coccinellidae) in a concrete observation tower.- *Entomological News*, 114: 23-28.

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.

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.

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.

WEIR A., HAMMOND P. M., 1997.- Laboulbeniales on beetles: host utilization patterns and species richness of the parasites.- *Biodiversity and Conservation*, 6: 701-719.

WEIR A., BLACKWELL M., 2004.- Fungal biotrophic parasites of insects and other arthropods, pp. 119-145. In: *Insect-fungal associations: ecology and evolution* (VEGA F. E., BLACKWELL M., Eds).- Oxford Press, New York, USA.

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.

ZAR J. H., 1999.- *Biostatistical Analysis*. 4th edition.- Prentice Hall, New Jersey, USA.

ZERM M., ADIS J., 2004.- Seasonality of *Laboulbenia phaeoxanthae* (Ascomycota, Laboulbeniales) and its host *Phaeoxantha aequinoctialis* (Coleoptera, Carabidae) at a central Amazonian blackwater floodplain.- *Mycological Research*, 108: 590-594.

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