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Prevalence and association of the laboulbenialean fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on coccinellid hosts (Coleoptera: Coccinellidae) in Kentucky, USA

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Abstract. The laboulbenialean fungi occur throughout the world and are closely associated with a range of arthropods, including many coleopteran hosts. Throughout the summer of 2004, coccinellids were collected from a Bluegrass savanna woodland ecosystem, dominated by blue ash *Fraxinus quadrangulata* and Chinkapin Oak *Quercus muehlenbergii*, and the adults were examined for the presence of *Hesperomyces virescens* using binocular and scanning electron microscopy. Over 80% of adult *Harmonia axyridis*, a species previously reported as having a persistent association with the fungus, were infected. No significant differences were observed in incidence on male and female hosts, however, the distribution of fungus differed between sexes. Female *H. axyridis* had a greater percentage of infection on their elytron compared to other parts of their body whilst male infection was concentrated around their elytra, legs and abdomen. Although infection rates were significantly lower, we report, for the first time, the presence of this fungus on the hosts *Cycloneda munda*, *Brachiacantha quadripunctata* and *Psyllobora vigintimaculata*. This is the first study documenting the incidence of this insect-associated fungus with these native coccinellids of North America. In the samples collected from the Bluegrass savanna, two species (*Coleomegilla maculata* and *Hyperaspis signata*) were not infected by this fungus.

INTRODUCTION

The association of ectoparasitic laboulbenialean fungi with Coleoptera is widely reported (e.g., Weir, 1993, 1994; Weir & Hammond, 1997; Rossi & Santamaria, 2000; Garcés & Williams, 2004; Riddick & Schaefer, 2005). These obligate, ectoparasitic fungi are estimated to number between 10,000 and 50,000 species worldwide on Coleoptera (Weir & Hammond, 1997), with smaller numbers associated with other host species in the Acari, Blattodea, Dermaptera, Diplopoda, Diptera, Heteroptera, Hymenoptera, Isoptera, Orthoptera and Thysanoptera. Not only do these Laboulbeniales parasitize many different species, but they occur from the tropics (Rossi & Santamaria, 2000; Weir & Rossi, 2001; Zerm & Adis, 2004) to the sub-Antarctic (Hughes et al., 2004), and on both terrestrial (e.g., Rossi & Santamaria, 2000, Weir & Rossi, 2001) and aquatic (Santamaria, 2000, 2004) hosts.

Although some Coccinellidae are reported as hosts of Laboulbeniales (Weir & Hammond, 1997), relatively few studies document the incidence of their infection on these hosts; for example, they infect *Adalia bipunctata* (L.) (Weir & Beakes, 1996, Welch et al., 2001), *Olla v-nigrum* Casey (Weir & Beakes, 1996), and *Harmonia axyridis* (Pallas) (Garcés & Williams, 2004; Riddick & Schaefer, 2005). Despite the relatively few reports of insect-host associations between coccinellids and laboulbenialean fungi, the consequence of such interactions, and those with other generalist predators which are known to be important in biological control (Meijer, 1975), could be profound given that some generalist predator communities feed extensively on pest populations in agroecosystems. The Coccinellidae, including *H. axyridis*, are important in the control of many pest species (Obrycki & Kring, 1998; Koch, 2003; Pervez & Omkar, 2006) and, as part of an assemblage of natural enemies (Sunderland et al., 1997), are capable of restricting pest population growth. Whilst most laboulbenialean fungi have few detrimental effects on their hosts (Whisler, 1968; Weir & Beakes, 1995), some negative fitness consequences from these associations are documented (Bro Larsen, 1952; Strandberg & Tucker, 1974; Gemeno et al., 2004), and have been attributed to causing declines in the summer populations of *Chilocorus bipustulatus* L. in Israel (Kamburov et al., 1967).

The laboulbenialean fungus, *Hesperomyces virescens* Thaxter (Laboulbeniales: Laboulbeniaceae) was first reported on *Chilocorus stigma* (Say) (Thaxter, 1931) and has subsequently been reported on other coccinellids including *Hippodamia convergens* Guerin-Meneville (Thaxter, 1931), *Cycloneda sanguinea* (L.) (Tavares, 1979), *Adalia bipunctata* L. (Weir, 1996), *Psyllobora* spp. (Tavares, 1985), *Eriopis connexa* Germar (Thaxter, 1931) and *Olla v-nigrum* (Mulsant) (Weir & Beakes, 1996). However, given that historic specimens of exotic coccinellids in the United States contained no individuals with laboulbenialean infection (Harwood et al., 2006), the aim of this study was to examine present-day associations

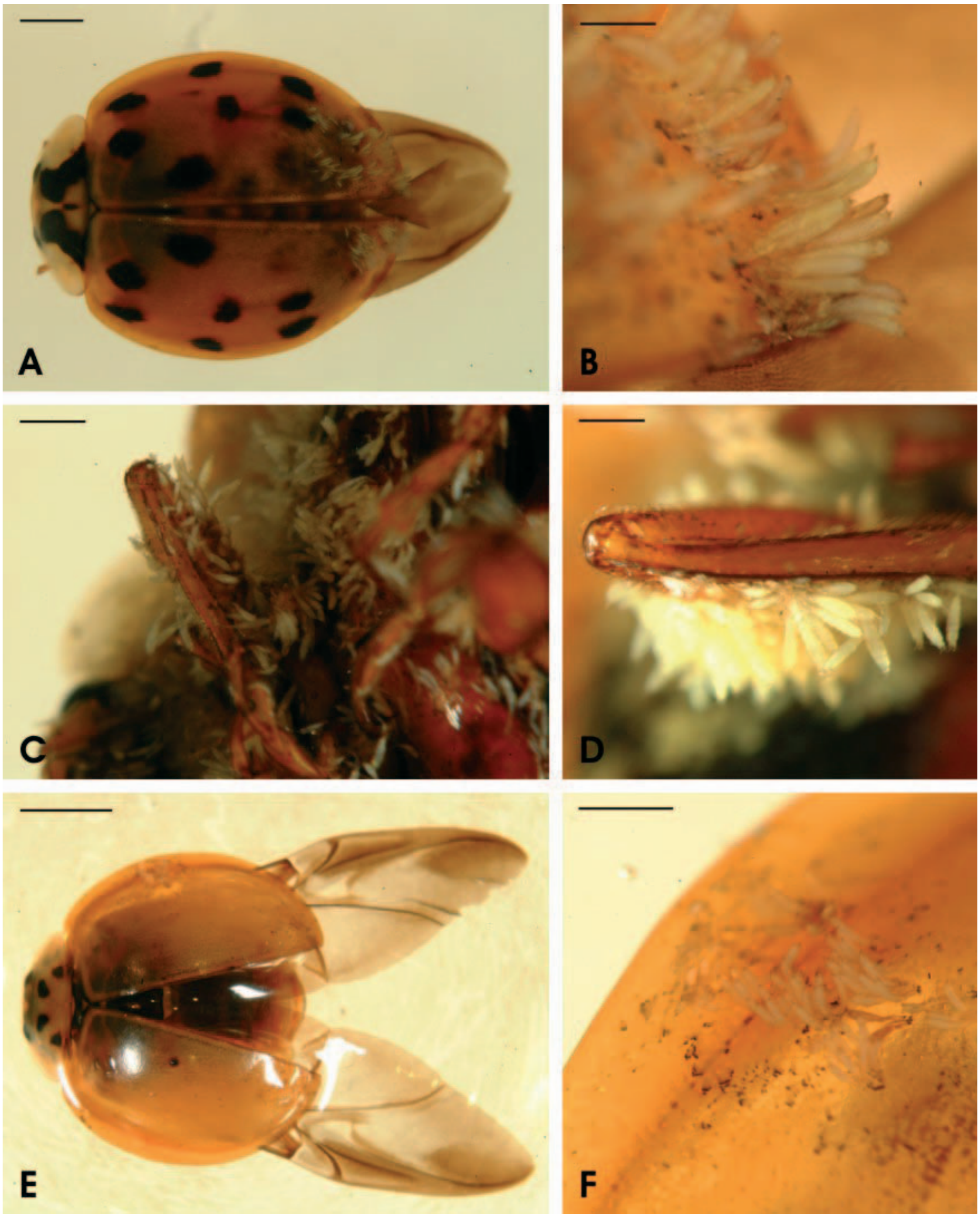

Fig. 1. *Harmonia axyridis* females infected with *Hesperomyces virescens*: A – dorsal view of the whole insect with both elytra distally infected; B – high magnification image of A; C – ventral view of *H. axyridis* female heavily infected with *H. virescens*; D – leg of *H. axyridis* heavily infected with *H. virescens*; E – dorsal view of the whole insect with one elytra infected; F – high magnification image of E. Scale bars: A – 1 mm, B – 200 µm, C – 500 µm, D – 250 µm, E – 1 mm, F – 500 µm.

between Coccinellidae and Laboulbeniales in non-agricultural habitats. The intention was to quantify these associations and identify new fungal-host interactions between ectoparasitic fungi and both exotic and native coccinellids in the field.

MATERIAL AND METHODS

Coccinellid adults were collected by trapping in numerous randomly located Townes style (Townes, 1972) Malaise Traps (Sante Traps, Lexington, KY, USA) at discrete locations within the woodland at Silver Lake Farm, Harrison County, Kentucky, USA. Samples were collected weekly from May until August 2004. The field site is located in the Bluegrass Region of central/northern Kentucky, ranging between approximately 160 and 320 m above sea level. The habitat is characterized by remnants of ancient Bluegrass savanna, dominated by the blue ash *Fraxinus quadrangulata* Michx. (Scrophulariales: Oleaceae) and Chinkapin Oak *Quercus muehlenbergii* Engelm. (Fagales: Fagaceae) with a grass (*Festuca* sp.) understory. The site also

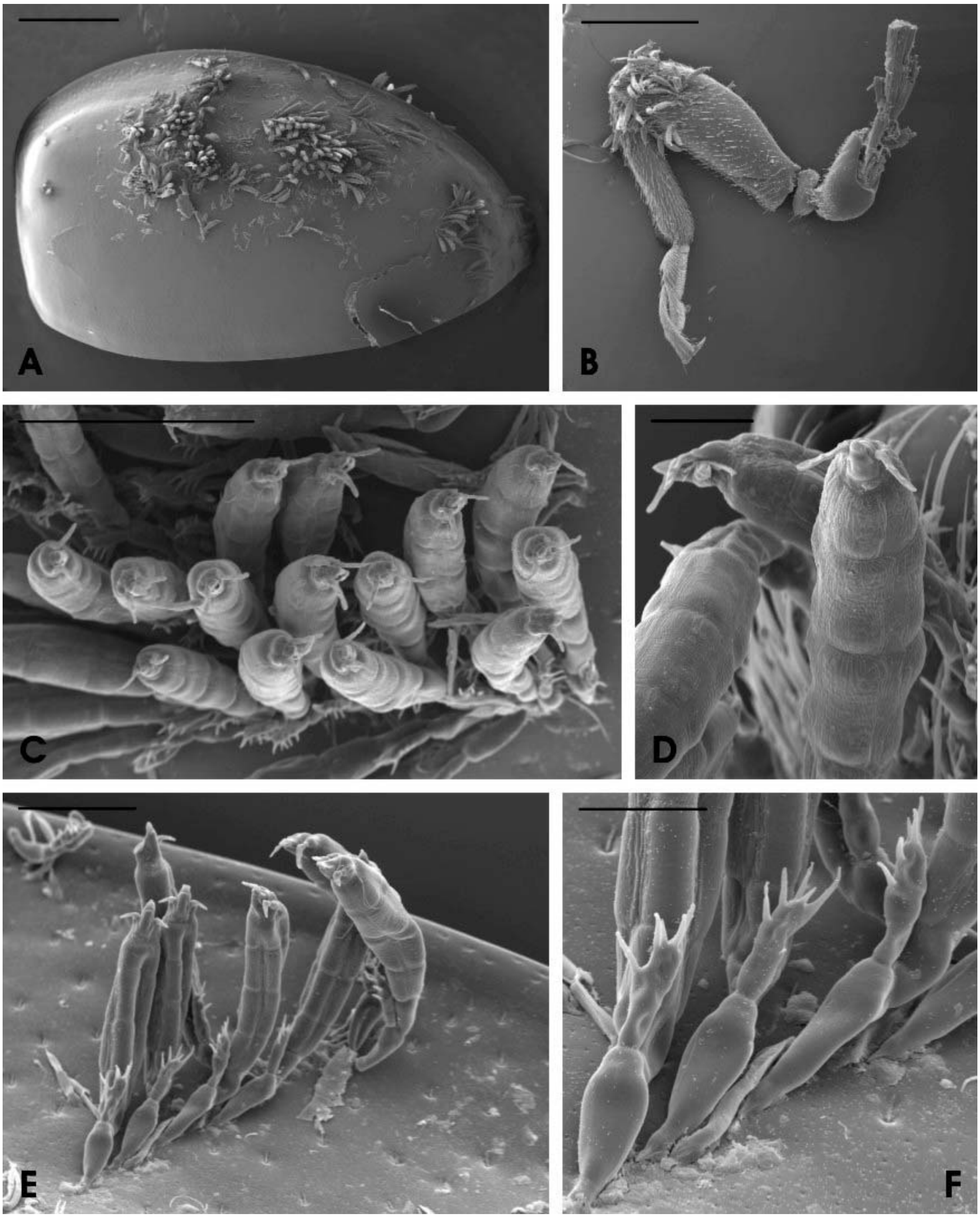

Fig. 2. Scanning electron micrographs (SEM) of *Harmonia axyridis* females infected with *Hesperomyces virescens*. A – dorsal view of the right elytron with diffuse fungal infection; B – fungal infection of the right leg; C – mycosis of mature *H. virescens* attached to coccinellid elytra showing close spatial arrangement of the fungi; D – mature *H. virescens* showing the upper portion of the perithecium, including apical outgrowths that may function as "trigger organs" in ascospore discharge; E – mature and developing thalli of *H. virescens* on the elytra; F – basal portion of developing *H. virescens* showing the antheridial appendage and receptacle. Scale bars: A – 1 mm, B – 1 mm, C – 200 micron, D – 50 micron, E – 100 micron, F – 50 micron.

contains closed-canopy woodlands with an understory consisting of forbes and rye.

Following collection, all species were transferred into 95% ethanol for subsequent examination for, and identification of, fungal parasites. The presence and distribution of laboulbenialean fungi was examined by binocular microscopy and species-level identification of *Hesperomyces virescens* confirmed due to the morphology of the perithecial apex and wall cells of mature thalli using light- and scanning electron microscopy (SEM) (after Weir & Beakes, 1996). SEM preparation on five male and female *H. axyridis* and two *Cycloneda munda* (Say) was undertaken for photographic presentation by initially preserving individuals in alcohol and subsequently completely dehydrating with successively higher concentrations of ethanol (70%, 80%, 90%, 95% and 99%). After dehydration, whole insect bodies or parts (i.e. elytra and legs) were critical point dried in a Balzers

TABLE 1. Number of adult coccinellids collected from a Bluegrass savanna ecosystem, and number infected with the laboulbenialean fungus *Hesperomyces virescens*.

Species	Number collected	Number infected	% infected
Harmonia axyridis	147	121	82.3
Psyllobora vigintimaculata	170	8	4.7
Brachiacantha quadripunctata	48	2	4.2
Cycloneda munda	81	2	2.5
Coleomegilla maculata	59	0	0
Hyperaspis signata	23	0	0

Union® Critical Point Dryer 020 unit (Balzers Union Ltd., Balzers, Liechtenstein), gold coated in a Balzers Union® SCD040 unit (Balzers Union Ltd., Balzers, Liechtenstein), and examined using a Philips® XL 30 scanning electron microscope.

The differences in infection rates between male and female *H. axyridis* were analyzed using a χ^2 test (Zar, 1999) by compiling results from individuals into a single sample unit of unreplicated data. The location of infection on *H. axyridis* was also analyzed using a χ^2 test on absolute count data of numbers infected followed by a Goodman's post hoc procedure (Marascuilo & Serlin, 1988) for discerning levels of significance between infection locations.

RESULTS

The examination of 528 coccinellids under binocular microscope revealed extensive formations of mycosis on *Harmonia axyridis* (Figs. 1A–F). These mycoses were later identified as thalli of the laboulbenialean fungus *H. virescens* using both light- and scanning electron microscopy (Fig. 2). The fungal infections were predominantly present in a clumped distribution around the distal part of the elytra, but were also recorded on the legs, sternites, thorax and head of infected individuals.

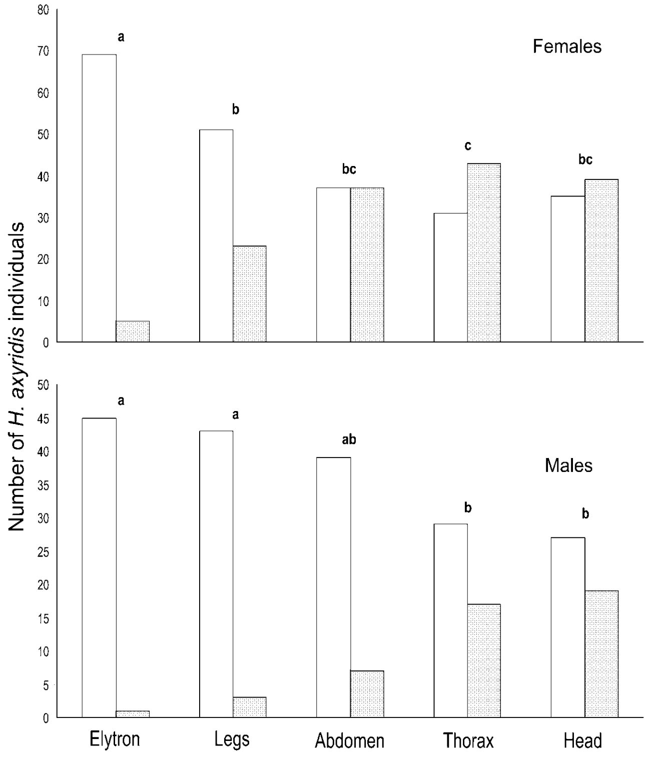

Fig. 3. Distribution of *Hesperomyces virescens* on different body parts in females and males of *Harmonia axyridis*. Empty bars represent number of infected individuals; grey bars represent number of uninfected individuals. Columns with the same letter are not different at $P < 0.001$.

Although *H. axyridis* were heavily infected with these fungal ectoparasites (82.3% infected, Table 1), three other coccinellids were collected (*C. munda*, *Psyllobora vigintimaculata* (Say) and *Brachiacantha quadripunctata* Melsheimer) in which infection rates were much lower (Table 1). Two species, *Hyperaspis signata* (Olivier) and *Coleomegilla maculata* (De Geer), were not observed to be hosts for this fungus (Table 1). Interestingly, despite the high prevalence of *H. virescens* on *H. axyridis* hosts, there was no significant difference in infection rates between males (78% infected) and females (85% infected) ($\chi^2 = 1.279$, $df = 1$, $P = 0.258$). No other invertebrates captured in Malaise traps at this field site were identified as hosts of *H. virescens*.

Analysis of fungal infection rates indicated non-random distribution of *H. virescens* on *H. axyridis* (females: $\chi^2 = 35.73$, $df = 4$, $P < 0.001$; males: $\chi^2 = 54.81$, $df = 4$, $P < 0.001$) with greater fungal infection on the elytron of females compared to all other body parts (Fig. 3a). The distribution of fungal infection differed in males, with a high prevalence of *H. virescens* on the legs, abdomen and elytron (Fig. 3b).

DISCUSSION

The high levels of infection of *Hesperomyces virescens* on their coccinellid hosts indicated the potential for detrimental fitness consequences resulting from such associations if this laboulbenialean fungus negatively impacts its hosts. *Harmonia axyridis*, a species previously reported as being susceptible to infection by this laboulbenialean fungus in host populations in Ohio (Garcés & Williams, 2004) and Pennsylvania (Riddick & Schaefer, 2005), was also highly parasitized in this study. However, infection rates of male and female *H. axyridis* did not vary despite considerable between-gender differences reported in these previous studies. Clear differences in the distribution of infection existed between males and females. These differences, with a greater incidence of *H. virescens* on the dorsal surface of the elytron of females and high incidence on the ventral surface (legs and abdomen) of males suggest sexual transmission may be operating in these hosts. Riddick & Schaefer (2005) also reported the likelihood for non-sexual transmission of laboulbenialean fungi between hosts. Similar distribution patterns are documented here whereby the high infection rates on the dorsal surface of males, and the incidence on the ventral surface of females, suggested that other non-sexual forms of contact may be operating in dispersing this fungus.

This high prevalence of *H. virescens* on *H. axyridis* could therefore be due to the large overwintering aggregations (Nalepa et al., 1996; Schaefer, 2003) thus increasing levels of host-to-host contact, given that transmission of this ectoparasitic fungus is believed to occur primarily by direct contact between an infected and uninfected individual (De Kesel, 1995). If such aggregations facilitate an increased spread of the fungus between hosts, which could ultimately affect the density and growth of coleopteran populations (De Kesel, 1993), changes in population structure may occur. However, without the long-term monitoring of coccinellid populations and laboratory evaluation of fitness consequences resulting from these infections, it is difficult to confirm this hypothesis. Interestingly, *Coleomegilla maculata,* another coccinellid which overwinters in large aggregated communities (Benton & Crump, 1979) below ground or under leaf litter, thus avoiding direct contact with *H. axyridis*, showed no incidence of fungal infection suggesting that *H. virescens* is not entirely generalist in terms of host susceptibility. However, despite relatively low infection rates, the first association with a number of other coccinellids (*Cycloneda munda*, *Brachiacantha quadripunctata* and *Psyllobora vigintimaculata*) is reported here. Further experiments are therefore required to ascertain the consequence of exposure to this ectoparasitic fungus and determine whether infection influences the long-term fitness of these hosts, given the common assumption that no negative fitness consequences result from associations with laboulbenialean fungi.

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