Infection of *Harmonia axyridis* by *Hesperomyces virescens:* Role of Mating Status and Aggregation Behavior

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Introduction *Hesperomyces virescens* is an ectoparasitic fungus infecting a variety of Coccinellidae (**Fig.1**); it was first reported on *Harmonia axyridis* by Garces & Williams (2003; J. Kans. Ent Soc. 77: 156-158). Although the transmission process has not been studied, the fungus is thought to be spread primarily between beetles via inter-individual contact. If so, we would expect that the pattern of fungal thalli on the body would differ between the sexes during the feeding/reproductive season. At that time, physical contact with conspecifics is largely limited to copulation, with the venter of the male in contact with the dorsal abdominal tip of the female. Moreover, if the fungus is transmitted by mating behavior, virgin females should be free of infection. Finally, the pattern of infection on individuals at the end of the feeding/reproductive season should differ from the pattern on beetles emerging from an overwintering aggregation, as these beetles have just spent four or more months in physical contact with thousands of other tightly packed individuals.

Fig. 1. SEM of a single mature fungal thallus of *Hesperomyces virescens* Thaxter (Ascomycetes: Laboulbeniales) on the elytron of *Harmonia axyridis*.The two elongated appendages at the tip of the perithecium are thought to act as triggers, initiating spore discharge in response to physical contact.

Goal Because the transmission process is critical in the evolution and ecology of the host-disease interaction, our goal was to determine if the presence and location of fungal thalli of *Hesperomyces virescens* on *Harmonia axyridis* could be correlated with host behavior. We therefore compared the infections of males and females collected from two distant sites at fall flight, at the conclusion of the feeding/breeding season, and within one site from fall flight until the end of the winter aggregation period. The role of female mating status was also examined.

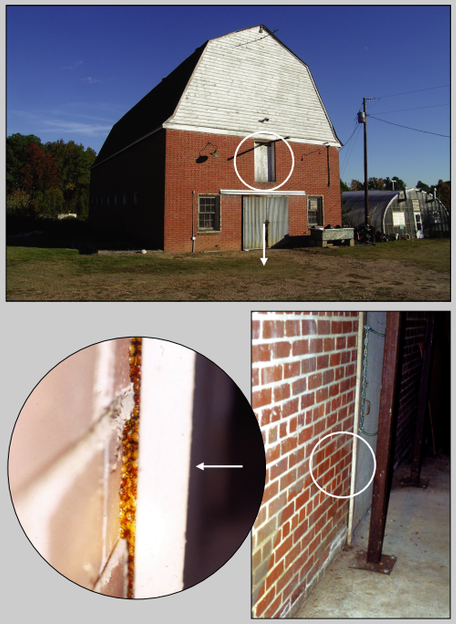


Fig. 2. Site 2, Piedmont. Beetles alight on the outside of the barn during fall flight, then aggregate behind a loose interior board framing a hay loft door; this aggregation is accessible to subsampling during winter.

Methods Four samples of *Harmonia axyridis* were collected and analyzed. Sample 1): Beetles (n = 300) collected on the 2nd day of autumn flight (13 Oct 2003) from the exterior of a home in the mountains of North Carolina (= Site 1). Sample 2): Beetles (n = 300) collected on the first day of flight (30 Oct 2003) from the exterior of a barn in the piedmont of North Carolina (Site 2; **Fig. 2**). Sample 3): Beetles (n = 100) collected at mid-winter (30 Dec 2003) from an interior aggregation in the barn at Site 2. Sample 4): Beetles (n = 100) collected from the same Site 2 aggregation at the end of winter (2 March 04).

The beetles were refrigerated until processed (within 10 days of collection).The insects were sexed, then examined under a binocular microscope for the presence of fungal thalli. The thalli were counted and their location on the insect mapped on a worksheet designed for that purpose. Females were dissected and their spermathecae examined for the presence of sperm.

The location of fungal thalli on beetles was analyzed by dividing the body into 6 zones: (1) pronotum and head, including antennae and palps; (2) anterior half of the dorsal surface of the elytra; (3) posterior half of the dorsal surface of the elytra; (4) legs; (5) anterior half of the ventral surface; (6) posterior half of the ventral surface. Only beetles with low level infections (< 15 fungal thalli) were included in the analysis of fungal location. These "young" infections are the result of recent interindividual contact with a diseased individual and give the most precise depiction of where on the body the infection was initiated.

Table 1. Male and Female Infection Status: Comparison in Space and Time[1]

		% Infected			
Sample[2]	n	Overall	Males	Females	P[3]
Site 1: At flight	300	38	33.1	39.0	0.29
Site 2: At flight	300	22	29.4	17.8	0.02
Site 2: End of winter	100	62	73.7	56.4	0.08

[1] Infection status = yes / no, without regard to number or location of thalli

[2] Site 1 = Mountains; Site 2 = Piedmont

[3] Males vs. females

Results Infection levels varied between sites, varied significantly between the sexes in one site but not the other, and increased by ~40% over the course of winter.

Table 2. Dynamics of Mating Status of Infected and Uninfected Females during the Aggregation "Season"[1]

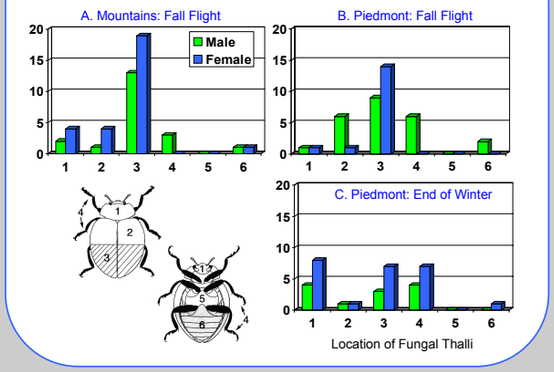
	% Females with Sperm in Spermatheca[2]		
Sample	Overall	Infected	Uninfected
At flight	40.0	66.7[a]	34.4[a]
Mid-winter	51.0	80.9[a]	30.0[a]
End of winter	66.0	69.0[a]	63.0[b]

[1] All from Site 2: Piedmont

[2] Within a column, values followed by the same letter are not significantly different.

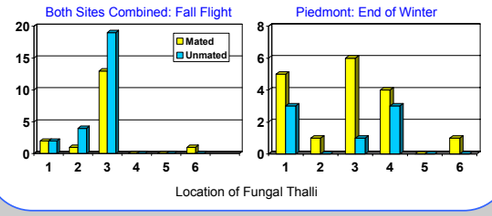
Results At flight, 1/3 of females sporting fungal thalli were virgins. The presence of infected virgins at the end of the feeding/breeding season suggests transmission via a mechanism other than successful copulation. Overall, the proportion of mated females in an aggregation increased over the course of the winter, but this increase was due to the higher number of uninfected females with sperm in their spermatheca. The proportion of "clean" females that were mated increased significantly, while the proportion of infected females did not. At the end of winter prior to spring flight, uninfected females were preferred as mating partners.

Fig. 3. Location of New Infections (≤ 15 thalli) on Bodies of Males and Females



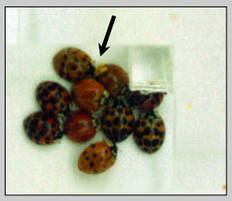
Results At fall flight, males and females exhibit similar patterns of infection: most fungal thalli are located on the posterior half of the dorsal surface of the elytra. Some males, however, had fungal thalli on legs, while females did not. At the end of the aggregation period, the pattern of initial infection shifts to one that it more evenly distributed over the body in both sexes. Male vs. female: (A) p = 0.27; (B) p < 0.01; (C) p = 0.97 (2 x 5 contingency table; p computed using Fisher's Exact Test).

Fig. 4. Location of New Infections (≤ 15 thalli) on Bodies of Mated vs. Unmated Females



Results At fall flight, the pattern of infection in females is unrelated to the presence of sperm in the spermatheca. In both mated and unmated females, fungal thalli were found most often on the dorsal abdominal tip. At the end of winter, thalli are distributed more evenly over the body in both mated and unmated females, though in mated females the thalli were more often located on the dorsal abdominal tip.

Conclusions



During the feeding/breeding season *Hesperomyces virescens* is primarily a sexually transmitted disease. Indiscriminate mounting by males of any dome shaped object of appropriate size probably accounts for the occurrence and location of young fungal infections on the posterior dorsal elytra not only of mated females, but also of males and of virgin females, which are unreceptive for 7-10 days after emergence.

During the winter *Hesperomyces virescens* is primarily a socially transmitted disease. The more even distribution of the fungal thalli over the body, including increased occurrence on the head and pronotum, may be attributed to the intimate physical contact typical of aggregations and to positional polarity within the group. Beetles in aggregations are typically oriented with their anterior end in contact with the body of a conspecific. Arrow points to the head of a beetle in contact with a tuft of fungal thalli on the posterior elytra of another beetle.The increased mating activity at the end of winter with uninfected females preferred as mating partners, suggests that many "clean" females depart the aggregation with incipient infections.