Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae) by *Hesperomyces virescens* (Ascomycetes: Laboulbeniales): Role of mating status and aggregation behavior

Christine A. Nalepa [a,*], Alexander Weir [b]

[a] *Beneficial Insects Laboratory, North Carolina Department of Agriculture and Consumer Services, Plant Industry Division, 1060 Mail Service Center, Raleigh, NC 27699-1060, USA*
[b] *Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210, USA*

Abstract

The ectoparasitic fungus *Hesperomyces virescens* was studied on *Harmonia axyridis* in North Carolina, in the southeastern United States. A primary goal was to investigate transmission of the disease by examining the correlation between the pattern of fungal infection and seasonal change in host behavior. Beetles were collected as they arrived at their winter quarters at two sites; in one site they were also subsampled at mid- and late winter. Insects were sexed and weighed, fungal thalli were counted, and their location on the host body mapped; spermathecae of females were examined for sperm. Infection levels varied between sites, differed significantly between the sexes in one site but not the other, and increased by ∼40% during winter. The distribution of thalli on the body changed seasonally, in concert with behavioral changes in the host. At fall flight, thalli were found most often on the posterior elytra of mated females, virgin females, and males. This is suggestive that the disease had been spread among both sexes via successful and failed copulation attempts; however, the relatively low incidence of infection on the male venter does not fit the sexual transmission scenario. During winter, thallus location shifts in concert with beetle aggregation behavior, with infections more often located on the head and legs. Fresh weight of beetles decreased by approx. 20% during winter, but was not affected by disease status. Prior to spring flight, uninfected females were preferred as mating partners, but the probable relationship between female age and infection status complicates interpretation of the data.
© 2006 Elsevier Inc. All rights reserved.

Keywords: *Harmonia axyridis*; Lady beetle; Coccinellidae; *Hesperomyces virescens*; Laboulbeniales; Spore transmission; Mate choice; Pathogenicity; Sexually transmitted disease; Socially transmitted disease

1. Introduction

Hesperomyces virescens Thaxter is a globally distributed, obligate, ectoparasitic fungus that infects a variety of beetles in the family Coccinellidae. It is a member of the Laboulbeniales, minute ascomycete fungi that complete their entire life cycle on a single arthropod host (Huldén, 1983; Tavares, 1985). The species was first noted in the western United States on *Chilocorus bivulnerus* Muls. (now *Chilocorus stigma* Say—Chagnon and Robert, 1962) collected in California by Thaxter (1891), and was more recently reported on the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) in Ohio (Garcés and Williams, 2004), and Pennsylvania (Riddick and Schaefer, 2005). The former authors noted that the infection was concentrated on the ventroposterior of males and dorsoposterior of females; the latter, however, found that the fruiting bodies of *H. virescens* on *H. axyridis* were found predominantly on the dorsal elytra of both sexes. These discrepancies in the reported bodily location of the fungus on *Harmonia* are significant, because the position of the fungus on the host is thought to reflect the mechanism by which the parasite is spread.

---

[*] Corresponding author. Fax: +1 919 233 8394.
E-mail address: christine.nalepa@ncmail.net (C.A. Nalepa).

For most species of Laboulbeniales the mode of transmission is still poorly understood, but it is likely that they are spread via direct contact between host individuals (De Kesel, 1993; Weir and Beakes, 1995). Two elongate appendages at the tip of the perithecium (fruiting body) of the fungus are thought to act as triggers, initiating spore discharge in response to physical contact (Weir and Beakes, 1996). When a healthy host touches a mature perithecium on a diseased host, sticky spores are mechanically liberated and adhere at the point of contact. The position of the fungus on the new host therefore reflects where the intraspecific contact occurred and can be indicative of behavioral patterns in the insect. A prime example is that foci of infection are often sexually dimorphic as a result of the male superior position during mating. Spores from thalli growing on the elytra of the female are transferred to the ventral surface of the male and vice versa, reinforcing the pattern (Benjamin and Shanor, 1952; De Kesel, 1993, 1995; Hedström, 1994; Scheloske, 1976). This type of localized infection has been reported for *H. virescens* on the coccinellids *Chilocorus bipustulatus* (L.) (Applebaum et al., 1971) and *Adalia bipunctata* (L.) (Welch et al., 2001), and supports the sexual transmission hypothesis.

Little is known regarding indirect transmission of Laboulbeniales, i.e., transmission via spores deposited on the substrate. It is thought to be of minor importance because of the typically low output of spores into the environment, their short life span (less than a week in the case of *Laboulbenia slackensis* Cépède and Picard), and the variable efficiency of this mode of transmission (Huldén, 1983; De Kesel, 1993, 1995). In some host-parasite systems, however, contact with infected substrate may be more important than contact with infected hosts (Lindroth, 1948).

The primary objective in the present study was to determine if the presence and location of fruiting bodies of *H. virescens* on *H. axyridis* could be correlated with patterns of physical contact among hosts. If direct transmission is the primary mode of disseminating spores, it is expected 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 during mating, 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 five or so months in intimate physical contact with thousands of other tightly packed individuals. A secondary objective of the study was to examine one aspect of pathogenicity in the fungus; we determined patterns of weight loss in infected and uninfected beetles over the course of the winter aggregation period.

2. Materials and methods

Infected beetles were first noted in North Carolina in 2003 during the autumn flight to overwintering aggregations. Four samples of *H. axyridis* were analyzed. Sample 1: beetles ($n = 300$) collected on the second day of autumn flight (13 October 2003) in the mountains of North Carolina (Site 1). Beetles were collected as they were alighting on a house and storage building situated next to a stream in a valley of the Appalachian Mountains in North Carolina near Weaverville (35.71°N, 82.45°W, 664 m in elevation). Sample 2: beetles ($n = 300$) collected on the first day of flight (30 October 2003) in the piedmont of North Carolina (Site 2). The insects were collected as they landed on a brick barn on the grounds of the NCDA & CS Beneficial Insects Laboratory in Cary (35.79°N, 78.73°W, 146 m elevation). Site 2 is approximately 340 km east of Site 1, but at comparable latitude. 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. Sample 3: beetles ($n = 100$) collected at mid-winter (30 December 03) from an overwintering aggregation inside the Site 2 barn. Sample 4: beetles ($n = 100$) collected from the same Site 2 aggregation at the end of winter (2 March 04). These four samples allowed us to make comparisons between beetles collected from two different locations in North Carolina at the time autumn flights were initiated (Samples 1 and 2), and to track the progression of the disease over the course of winter in one population (Samples 2, 3, and 4). The aggregation sampled inside the barn was a relatively small one, estimated to be about 400–500 individuals. Beetles typically begin dispersing from winter aggregations about the second week of March in central North Carolina.

Beetles were transported from collection sites in insulated boxes containing artificial ice packs, then refrigerated in glass jars with crumpled paper towels for shelter and a small square of wet sponge for a water source. They were held at ~7 °C for ≤10 days until processed. The fresh weight of individuals was measured to the nearest 0.1 mg on an Ohaus Explorer microbalance. The entire insect was examined for the presence of all developmental stages of fungal thalli; if present, the fruiting bodies were counted and their location mapped on an outline drawing of the dorsal and ventral surfaces of an adult coccinellid. Infection levels were categorized as low (≤15 thalli), medium (16–50 thalli) or high (>50 thalli). The insects were sexed using the shape of the 5th abdominal sternite, and the mating status of females was determined by dissection. The spermatheca was removed, crushed on a slide, and examined microscopically under phase contrast illumination for the presence of sperm.

Analysis of variance (Proc GLM; SAS Institute, 2001) was used to determine the relationships among beetle weight, length of the right elytron, infection level (uninfected, low, medium, and high), site, and collection date within Site 2 (at flight, mid-winter, and early spring). None of the interactions were significant and were eliminated from the analysis. Logistic regression (Proc GENMOD) was used to evaluate the relationship of sex and disease status.

The location of fungal thalli on beetles was analyzed by dividing the body into six zones: (1) pronotum and head, including antennae and palps; (2) the anterior half of the dorsal surface of the elytra; (3) the posterior half of the dorsal surface of the elytra; (4) legs; (5) the anterior half of the ventral surface, including the metasternum, mesosternum, prosternum, and anterior epipleuron; and (6) the posterior half of the ventral surface, including all abdominal sternites and the posterior half of the epipleuron.

After initial contact with fungal spores, secondary spread from the primary locus of infection is common. Established thalli mature and release spores, and these spread to additional locations on the host's body (autoinfection) (Whisler, 1968), possibly aided by autogrooming. Therefore, only beetles classified as having a low-level infection ($\leqslant 15$ thalli) were included in the analysis of fungal location. These "young" infections ("primary" infections of Whisler, 1968) are the result of recent contact with a diseased individual or infectious substrate and give the most precise depiction of where on the body the infection was initiated. The location of these low-level infections allows us to make inferences about the behaviors that may have resulted in the spread of the fungus. Even at low infection levels, however, thalli were often (22%) found in more than one location on the body. In these cases, the zone with the majority of thalli was used in the analysis. Body zone 5 was eliminated from statistical analysis, as none of the beetles with a low-level infection had thalli in this body region. Results were analyzed using a 2×5 contingency table (sex and location); probabilities were computed using Fisher's Exact Test.

To analyze the relationship of female mating status to fungal presence and body location over time (comparison of samples 2, 3, and 4), infected and uninfected females were analyzed separately using mating status as the dependent variable and using logistic regression to contrast samples (Proc GENMOD in SAS).

3. Results

3.1. Infection levels

Infection of *H. axyridis* by *H. virescens* varied over space and time, with slightly different trends for the two sexes. At the initiation of fall flight at the end of the active field season, overall infection levels were 38% in Sample 1 (mountains), and 22% in Sample 2 (piedmont). Infection levels increased from this Sample 2 baseline during the course of the winter aggregation period. At mid-winter (Sample 3) 39% of the beetles were infected, and 62% bore fungal thalli by the end of winter (Sample 4).

There was a significant interaction of sex and sample ($P = 0.03$) in beetle infection levels. When arriving at the aggregation site in autumn, the two sexes exhibited no differences in infection levels in Site 1, but at a comparable time in Site 2, significantly more males carried fungal thalli

Table 1
Male and female infection status: comparison in space and time (infected = yes or no, without regard to number or location of fungal thalli)

Sample	n	% Infected		P
		Males	Females	
#1 (Site 1, at flight)	300	33.1	39.0	0.29
#2 (Site 2, at flight)	300	29.4	17.8	0.02
#3 (Site 2, mid-winter)	100	36.7	41.2	0.65
#4 (Site 2, end of winter)	100	73.7	56.4	0.08

(Table 1). By mid-winter, infection levels of both sexes in Site 2 increased and converged. By the end of winter, when insects in the aggregation were in the pre-dispersal mating period, levels of infection in males again increased to near significance over that of females (Table 1). Overall, however, sex was not a significant predictor of health status ($P = 0.17$).

3.2. Location of infection on body

In both males and females with low-level infections, thalli could be found in any body location except the anterior half of the ventral body surface (location 5). At fall flight there was no significant difference in location of fungal thalli between the sexes at Site 1 (Fig. 1A; $P = 0.27$); in both sexes, thalli were found most often in location 3, on the posterior half of the elytra. At a comparable time in Site 2, however, the location of fungal thalli was significantly different between the sexes ($P < 0.01$). As in Site 1, thalli were most often found on the posterior elytra of both sexes, but in males they were also frequently found on the anterior half of the elytra and on the legs (Fig. 1B). Intrasexual comparison of thallus location on beetles collected at autumn flight in Sites 1 and 2 indicate no significant difference between the males ($P = 0.22$) or females ($P = 0.77$) of the two collection sites.

If the fungus is indeed transmitted during copulation, then the relative lack of low-level infections on the venter of males at fall flight is unexpected. We therefore examined the pattern of infection in males at all disease levels, i.e., low, medium, and high, pooled. Overall, 44% of diseased males had fungal thalli on their ventral abdomen (location 6) and/or legs (location 4), but in half of these (22% of total) fungal thalli were also found in other body locations. It is notable that as the level of infection intensifies the probability that some thalli will be found on the ventral abdomen or legs increases. Of the males with low level infections, 39% bore thalli in one or both of these locations. In males with medium and high-level infections, 50 and 57%, respectively, carried thalli in these areas. In Site 2, the pattern of fungal infection on the body shifted over the course of winter (compare Fig. 1B and C). Although at this site the low-level infection pattern of the sexes differed at fall flight, by the end of winter, there was no significant difference between them in fungal location ($P = 0.97$). This shift is significant, how-
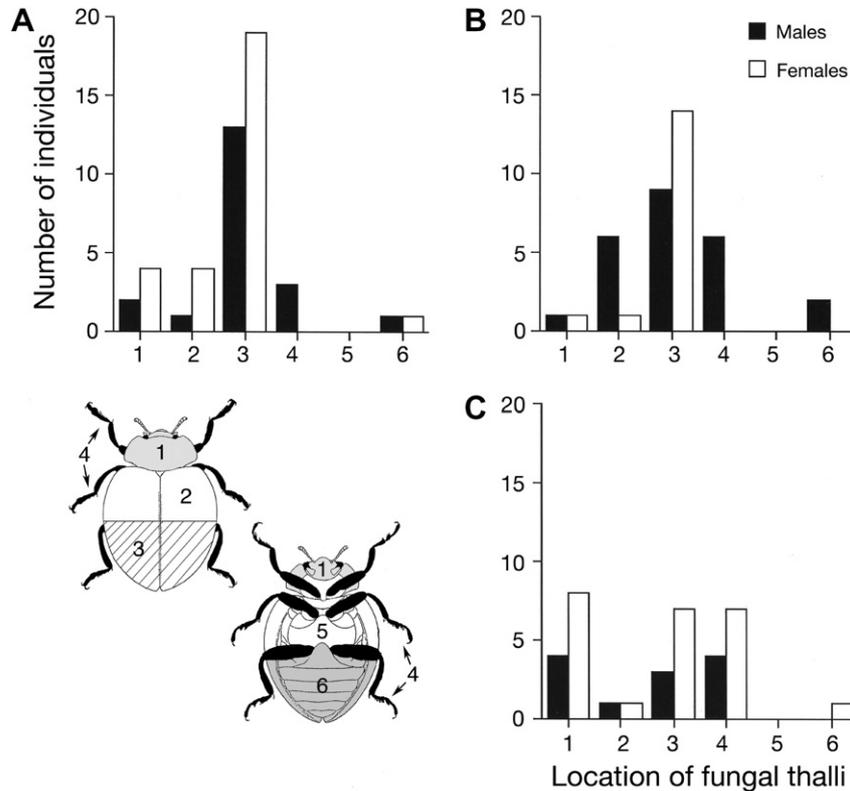
Fig. 1. Location of fungal thalli of *Hesperomyces virescens* on the bodies of adult male and female *Harmonia axyridis* with low-level, recently initiated infections ($\leqslant 15$ fruiting bodies). (A) Site 1, at fall flight; (B) Site 2, at fall flight; (C) Site 2, at end of winter aggregation period.

ever, only in the females ($P < 0.001$, Fisher's Exact Test). At fall flight most thalli on females were located on the posterior half of the elytra; by the conclusion of the winter aggregation period, incipient infections were almost equally located in the head region, on the legs, and on the posterior elytra.

In both sexes, the number of thalli notably increased in the head region over the course of winter. In beetles collected on the first day of fall flight at Site 2, just 3% of all infected individuals had thalli on the head, antennae or palps, and in none of these was the fungus located exclusively on these body parts. By the end of winter, however, 45% of all infected individuals had fungal thalli on the head, antennae or palps, and in 23% of the individuals with low-level infections, these were the only body locations where thalli were found.

3.3. Mating status of infected vs. uninfected females

At fall flight, 29.4% of all females from Site 1 were mated. When analyzed by infection status, it was found that 37.7% of infected females were mated, while 24.2% of uninfected females were mated. A similar pattern was seen at Site 2. Overall, 40% of females were mated at fall flight in this location. Of the infected females at this site, 66.7% were mated, while 34.4% of uninfected females were mated. Although in both sites a higher proportion of infected than uninfected females were mated, it is notable

that so many virgins were diseased (62.3 and 33.3% of infected females in Sites 1 and 2, respectively). Moreover, the pattern of fungal location on infected virgins was similar to that of infected females that had mated. In both, thalli were located most often on the dorsal abdominal tip (posterior elytra) (Fig. 2A). In Site 2, the overall proportion of mated females increased from 40 to 66% over the course of the winter. This increase, however, was due almost entirely to significantly increased matedness among uninfected females. The mating status of infected females did not change significantly from fall of 2003 to the following spring (Table 2).

3.4. Weight loss in relation to infection status

As expected, beetle wet weight was positively related to elytron length (Table 3). The wet weight of beetles in the Site 2 aggregation decreased significantly over the course of the winter (Table 3). Most weight loss occurred at the end of winter, as the mid-winter collection was similar to that of the baseline insects (data not shown). Males and females lost a similar proportion of body weight over winter (20.3 and 20.7%, respectively). In none of the collections was the weight of beetles infected with *H. virescens* significantly different from that of uninfected beetles (Table 3), nor did infection level (low, medium, and high) have an influence on the weight of infected beetles ($F = 1.20$, $P = 0.31$).
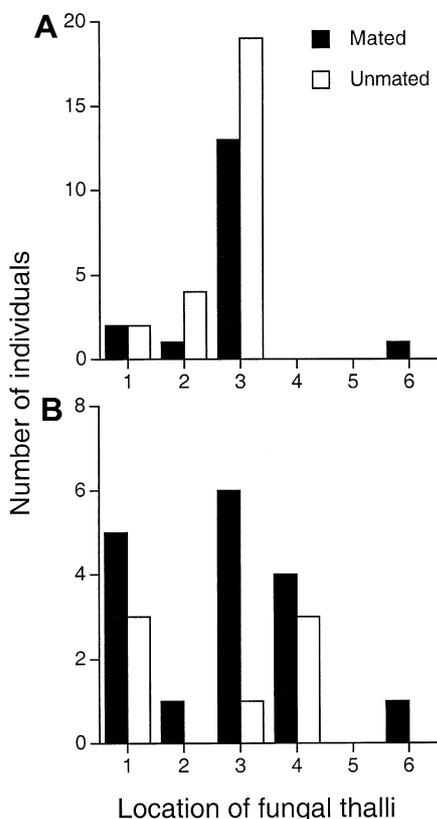
Fig. 2. Location of fungal thalli of *Hesperomyces virescens* on the bodies of mated and unmated female *Harmonia axyridis* with low-level, recently initiated infections ($\leqslant 15$ fruiting bodies). (A) Sites 1 and 2 combined, at fall flight; (B) Site 2 at end of winter aggregation period. Note that the *y*-axes are on different scales. See key to body locations in Fig. 1.

Table 2
Mating status of infected and uninfected females in Site 2 over the course of winter

Sample	% Females mated		
	Overall	Infected	Uninfected
#2 (Site 2, at flight)	40.0	66.7a	34.4a
#3 (Site 2, mid-winter)	51.0	80.9a	30.0a
#4 (Site 2, end of winter)	66.0	69.0a	63.0b

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

Table 3
Relationship between beetle weight (mg) and length of right elytron, infection status (yes or no), collection site, and date of collection in Site 2

Source	DF	MS	*F*	*P*
Length of elytron	1	12389.53	1403.23	<0.001
Infection status	1	9.11	1.03	0.31
Site	1	729.48	82.62	<0.001
Date (Site 2)	2	1740.32	197.11	<0.001

4. Discussion

The probability of being infected at the conclusion of the feeding/breeding season is related at least in part to popula-

tion dynamics, particularly the period of time that has elapsed since an individual's adult emergence. Appearance of the last generation of the year varies in different parts of the state and from year to year in response to a variety of environmental variables, including prevailing temperatures and the quantity and quality of food available to the beetles. The timing of the fall flight is also annually and geographically variable; autumn migration flights typically occur earlier in the mountains (Site 1) than in the piedmont (Site 2) of North Carolina (Kidd et al., 1995). Consequently there is substantial year to year difference in the proportion of females that have had physical contact with a potentially diseased male prior to arriving at aggregation sites in autumn. In North Carolina, an average of about 12% of dissected females collected as they flew into aggregations in 1993 had sperm in their spermatheca, ranging from 0 to 25% among nine sites. In 1994, a larger number of females had mated prior to winter—an average of 41%, with a range of 0–70%. Variation between years was also recorded within a site. In one Wake County location no females were mated in 1993 ($n = 41$) but 70% were mated in 1994 ($n = 59$) (Nalepa et al., 1996).

4.1. Location of fungal infection: fall flight

Host mating systems have a complex relationship with disease transmission (e.g., Kokko et al., 2002). Female *H. axyridis* are promiscuous (Obata, 1988b; Osawa, 1994), mating an average of 3.8 times in natural populations (Ueno, 1996). This is likely an underestimate, however, since sperm displacement apparently occurs (Ueno, 1994). Sexual behavior, then, can explain the prevalence of fungal thalli on the dorsal abdominal tip of mated females. Our data, however, indicate that in females collected at fall flight, infection with *H. virescens* is not tightly correlated with the presence of sperm in the spermatheca; furthermore, the pattern of primary fungal infection in virgin females is similar to that of mated females. It is likely that virgin females are infected via unsuccessful copulatory attempts; males mount newly emerged females but are rejected prior to genitalic engagement or sperm transfer. Female virgins of *H. axyridis* typically refuse to mate until they are sexually mature, which takes 7–10 days from when they emerge (Obata, 1988b; Obata and Johki, 1991; Obata et al., 1986). Refusal is of two types: precopulatory refusal, which occurs within 15 s of the male mounting the female, and interrupted genital contact, which occurs within 2 min (Osawa, 1994). Unreceptive females run away, shake off the male, or bend the abdomen upwards (Obata, 1988b). Fungal transmission that occurs during either attempted or completed copulation is known in other insect species (e.g., Hedström and Monge-Najéra, 1998).

Homosexual mounting may account in part for the position of infections on male dorsal elytra. Males seeking sexual partners are not overly choosy in what they climb onto, and they do so quickly, with few preliminaries. Visual factors such as body size and shape are

involved in short range mate recognition by males, and, according to Obata (1987), males may jump to mount. One third of tested male *H. axyridis* will mount a freshly dead male (Obata, 1988a). Inappropriate encounters in coccinellids are typically broken via rejection behavior of the mounted insect (Majerus, 1997).

Regardless of the sex of the mounted insect or the successful completion of copulation, a significant problem with the "transmission during mating" scenario is that incipient fungal infections were not typically located on the ventral surface of males collected during fall flight. Fungal thalli were most often found on the posterior half of the dorsal elytra (location 3); the venter of males typically becomes an infection site only as the disease progresses. This suggests that insects infected on the dorsal elytra may not successfully transmit the infection to the venter of mounting males; concomitantly, mounted insects may be acquiring their infections from somewhere other than the infected sternites of their suitors. One possibility is that spores are transferred to the venter of mounting males but have difficulty germinating in that body location; subsequently these spores are transferred to the elytra of the next beetle the male mounts. Thus, there may be two modes of direct transmission. Males may serve as passive vectors of spores acquired from an infected insect, and also transmit spores ejected from mature fungal thalli on their own bodies. Factors that may hinder spore germination on sternites include regional differences in cuticular chemistry, and the presence of setae, which could offset the direct contact of spores with the cuticle. Like other Coccinellinae, the dorsal surface of *H. axyridis* is glabrous (Gordon, 1985, p. 679), but the legs and sternites are finely pubescent (Nalepa, pers. obs.). With the exception of some Laboulbeniales that appear adapted for growing solely on setae, few fungal species in this group are consistently associated with insects cloaked in thick pubescence (Weir, pers. obs.); bristles and hairs appear to be a deterrent to spore attachment and/or successful germination.

4.2. Location of fungal infection: winter aggregation

Both the increased levels of infection and the shift in the body location of fungal thalli over the course of the winter suggest that *H. virescens* is spread in *H. axyridis* by the non-sexual physical contact typical of winter aggregations. Gregarious behavior in many coccinellids is known to favor the rapid spread of fungal epizootics. *Beauveria bassiana*, for example, is considered the main biotic factor responsible for winter mortality in several species, and the risk of mycosis greatly increases if the aggregation period is prolonged (Hodek and Honek, 1996; Iperti, 1999). *Harmonia axyridis* typically overwinters in multi-layered piles, frequently filling the crevice they have chosen as winter harborage. Positional polarity is obvious in the single layered aggregations typical of small groups at less severe temperatures and more open spaces (Fig. 3). Each beetle is oriented with its front end
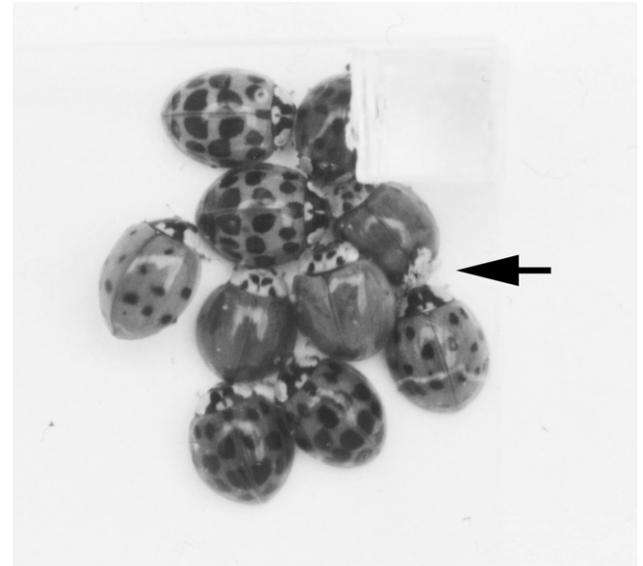

Fig. 3. Small aggregation of *Harmonia axyridis*. The anterior of each individual is in contact with the body of an adjacent beetle. Note that the head of one is touching a tuft of fungal thalli on the posterior elytra of a conspecific (arrow).

touching the body of another beetle (Anderson and Richards, 1977; Hagen, 1962). The increased frequency of fungal thalli on the anterior part of the body at the end of winter no doubt reflects this orientation behavior; the pattern has been documented in other gregarious species. *Adalia bipunctata* collected from aggregations develop fungal thalli most often at the margins and front angles of the elytra (Weir and Beakes, 1996).

The number of mated females rose from 40% at fall flight to 66% at the end of winter in Site 2. Four females from the final sample were carrying spermatophores, corroborating the suggestion that there is increased mating activity prior to spring dispersal. Our final sample was collected on 2 March, about two weeks prior to the initiation of dispersal from North Carolina aggregation sites. It is likely that high levels of sexual activity continue until spring exodus from the site, and that most females leave with sperm in their spermatheca. It is of interest that some mating apparently occurs throughout the winter (Table 2), probably whenever the temperature is warm enough to allow activity. Beetles have been noted walking near aggregation sites and taking short local flights during balmy days in January (Nalepa, unpubl. obs.).

Direct transmission via contact with conspecifics, then, appears to be the most important mechanism for transmitting *H. virescens* among *H. axyridis*. Sexual contact plays a primary role during the feeding/mating season, but both sexual and social contact are responsible for spreading *H. virescens* during aggregation in winter. Transmission via substrate borne spores also may play a role during winter. Indirect transmission is dependent on a large host population and a great number of spores (Huldén, 1983), both of which exist in *Harmonia* aggregations. In addition, the higher humidity levels typically surrounding grouped

insects (e.g., Yoder et al., 2002) may provide favorable conditions for not only fungal establishment and growth (e.g., Hedström, 1994; Arndt and Desender, 2002) but also for the survival of spores on the substrate. Spores of Laboulbeniales fungi usually perish quickly if they are not attached to a suitable host (De Kesel, 1997). Favorable micrometeorological conditions in an aggregation, however, may allow spores to survive longer on the substrate, and high concentrations of beetles in a limited space also increase the odds of a spore quickly contacting a fresh host. Increased number of incipient infections on beetle legs during winter (Fig. 1C) may reflect indirect transmission.

4.3. Pathogenicity

Hesperomyces virescens attaches to host cuticle via a melanized sucker-like organ ("foot"), beneath which is a small, circular penetration hole (Weir and Beakes, 1996). It is one of the few Laboulbeniales where rhizoidal penetration into the host body has been demonstrated (Kamburov et al., 1967; Weir and Beakes, 1996). Nonetheless, there is little evidence to suggest that the fungus has a deleterious effect on beetles (Weir and Beakes, 1995), and this study provides no new evidence of pathogenicity. The weight of infected *Harmonia* in relation to body size did not decrease more than that of healthy individuals during winter; any potential host effect, then, did not involve weight loss. The fungus may, however, reduce the vigor of its host in more subtle ways, undetected by the mechanisms employed so far. Minor changes in beetle physiology may occur, such as alteration in levels of lipids or cryoprotectants. Heavy loads of fruiting bodies (>100) on the elytra of *H. axyridis* found during this study and others (Riddick and Schaefer, 2005) suggest that the fungus may mechanically impair flight in badly diseased beetles, or increase flight costs. Heavy infections also may interfere with the sensory detection of food, mates, or predators, particularly in spring when fungal thalli are common on the head, antennae, and mouthparts. One beetle in Sample 4 had 27 thalli on the antennae and clypeus. A few individuals were noted as missing a maxillary palp, and in one case there were fungal thalli on the palp that remained. Broken antennae, palpi and cerci have been noted in insects infected with other species of Laboulbeniales fungi (Bro Larson, 1951; Gemeno et al., 2004).

4.4. Mate choice and disease status

Among the diseases known to be sexually transmitted in insects, attempts to demonstrate mate choice for uninfected partners have so far failed (Knell and Webberley, 2004). In the current study, however, the proportion of healthy mated females rose significantly at the end of winter, while the proportion of mated, diseased females remained static (Table 2). We consider this indirect evidence that in winter aggregations of *Harmonia*, infected females may be either less desirable sexual partners, or more reluctant to mate. A complication is that older females have had greater oppor-

tunity to acquire infections, and males may prefer younger mating partners regardless of their disease status. Nonetheless, at the end of winter all females were at least five months old and would have had similar probabilities of infection via social transmission. Regardless of the operant choice criteria, the end result is that prior to spring dispersal, healthy females were sperm recipients significantly more often than diseased females. Male mate choice would be unusual, given the typically indiscriminate nature of male coccinellids (e.g., Majerus, 1997). *Harmonia axyridis* males do not choose females on the basis of mating status (Obata, 1988b), but males of the summer generation in Japan apparently have a preference for melanic females (Osawa and Nishida, 1992). The conditions and stimuli that influence choice of sexual partners during the active season may be quite different from those operating in winter aggregations. The presence of hundreds to tens of thousands of potential mates in a limited space relatively safe from predators allows for interesting possibilities in terms of mate choice, particularly as beetles prepare to depart from their overwintering quarters in spring. The concept of winter aggregations as huge mate choice arenas is unexplored.

References

Anderson, J.M.E., Richards, A.M., 1977. First records of reproductive diapause and aggregation in Australian Coccinellidae (Coleoptera). Proc. Linn. Soc. N.S.W. 102, 13–17.

Applebaum, S.W., Kfir, R., Gerson, U., Tadmor, U., 1971. Studies on the summer decline of *Chilocorus bipustulatus* in citrus groves of Israel. Entomophaga 16, 433–444.

Arndt, E., Desender, K., 2002. Laboulbeniales (Ascomycota) on Carabidae (Insecta: Coleoptera) from the Galápagos Archipelago. Belg. J. Zool. 132, 155–164.

Benjamin, R.K., Shanor, L., 1952. Sex of host specificity and position specificity of certain species of *Laboulbenia* on *Bembidion picipes*. Am. J. Bot. 39, 125–131.

Bro Larson, E., 1951. On subsocial beetles from the salt marsh, their care of progeny and adaptation to salt and tide. In: Transactions of the IX International Congress of Entomology, Amsterdam, pp. 502–506.

Chagnon, G., Robert, A., 1962. Principaux Coleopteres de la Province de Quebec, Second ed. Université de Montreal Press, Montreal. 440 p.

De Kesel, A., 1993. Relations between host population density and spore transmission of *Laboulbenia slackensis* (Ascomycetes, Laboulbeniales) from *Pogonus chalceus* (Coleoptera, Carabidae). Belg. J. Bot. 126, 155–163.

De Kesel, A., 1995. Relative importance of direct and indirect infection in the transmission of *Laboulbenia slackensis* (Ascomycetes, Laboulbeniales). Belg. J. Bot. 128, 124–130.

De Kesel, A., 1997. Contribution towards the study of the specificity of Laboulbeniales (Fungi, Ascomycetes), with particular reference to the transmission, habitat preference, and host-range of *Laboulbenia slackensis*. Ph.D. thesis, Universiteit Antwerpen, Antwerpen, Belgium, pp. 1–132.

Garcés, S., Williams, R., 2004. First record of *Hesperomyces virescens* Thaxter (Laboulbeniales: Ascomycetes) on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). J. Kans. Entomol. Soc. 77, 156–158.

Gemeno, C., Zurek, L., Schal, C., 2004. Control of *Herpomyces* spp. (Ascomycetes: Laboulbeniales) infection in the wood cockroach, *Parcoblatta lata* (Blattodea: Blattellidae), with benomyl. J. Invertebr. Pathol. 85, 132–135.

Gordon, R.D., 1985. The Coccinellidae (Coleoptera) of North America North of Mexico. J. N.Y. Entomol. Soc. 93, 1–912.

Hagen, K.S., 1962. Biology and ecology of predaceous Coccinellidae. Ann. Rev. Entomol. 7, 289–326.

Hedström, I., 1994. *Stigmatomyces* species on guava fruit flies in seasonal and non-seasonal environments. Mycol. Res. 98, 403–407.

Hedström, I., Monge-Najéra, J., 1998. Is sexually transmitted fungal infection evidence for size-related mating success in Neotropical guava fruit flies? Rev. Biol. Trop. 46, 1131–1134.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht.

Huldén, L., 1983. Laboulbeniales (Ascomycetes) of Finland and adjacent parts of the USSR. Karstenia, 23, 31–136.

Iperti, G., 1999. Biodiversity of predaceous coccinellidae in relation to bio-indication and economic importance. Agric. Ecosyst. Environ. 74, 323–342.

Kamburov, S.S., Nadel, D.J., Kenneth, R., 1967. Observations on *Hesperomyces virescens* Thaxter (Laboubeniales), a fungus associated with premature mortality of *Chilocorus bipustulatus* L. in Israel. Israel J. Agric. Res. 17, 131–134.

Kidd, K.A., Nalepa, C.A., Day, E.R., Waldvogel, M.G., 1995. Distribution of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in North Carolina and Virginia. Proc. Entomol. Soc. Wash. 97, 729–731.

Knell, R.J., Webberley, K.M., 2004. Sexually transmitted diseases of insects: distribution, evolution, ecology and host behaviour. Biol. Rev. 79, 557–581.

Kokko, H., Ranta, E., Ruxton, G., Lundberg, P., 2002. Sexually transmitted disease and the evolution of mating systems. Evolution 56, 1091–1100.

Lindroth, C.H., 1948. Notes on the ecology of Laboulbeniaceae infesting carabid beetles. Svensk Botan. Tidskr. 42, 34–41.

Majerus, M.E., 1997. Interspecific hybridisation in ladybirds (Col.: Coccinellidae. Entomol. Rec. 109, 11–23.

Nalepa, C.A., Kidd, K.A., Ahlstrom, K.R., 1996. Biology of *Harmonia axyridis* (Coleoptera: Coccinellidae) in winter aggregations. Ann. Entomol. Soc. Am. 89, 681–685.

Obata, S., 1987. Mating behavior and sperm transfer in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Appl. Entomol. Zool. 22, 434–442.

Obata, S., 1988a. Mating behavior and sperm transfer in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). In: Nie-mczyk, E., Dixon, A.F.G. (Eds.), Ecology and Effectiveness of Aphidophaga. SPB Academic Publishing, The Hague, pp. 39–42.

Obata, S., 1988b. Mating refusal and its significance in females of the ladybird beetle, *Harmonia axyridis*. Physiol. Entomol. 13, 193–199.

Obata, S., Johki, Y., 1991. Comparative study on copulatory behaviour in four species of aphidophagous ladybirds. In: Polgár, L., Chambers, R.J., Dixon, A.F.G., Hodek, I. (Eds.), Behaviour and Impact of Aphidophaga. SPB Academic Publishing, The Hague, pp. 207–212.

Obata, S., Johki, Y., Hidaka, T., 1986. Location of hibernation sites in the ladybird beetle, *Harmonia axyridis*. In: Hodek, I. (Ed.), Ecology of Aphidophaga. Academia, Prague, pp. 193–198.

Osawa, N., 1994. The occurrence of multiple mating in a wild population of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). J. Ethol. 12, 63–66.

Osawa, N., Nishida, T., 1992. Seasonal variation in elytral colour polymorphism in *Harmonia axyridis* (the ladybird beetle): the role of non-random mating. Heredity 69, 297–307.

Riddick, E.W., Schaefer, P.W., 2005. Occurrence, density, and distribution of parasitic fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on multicolored Asian lady beetle (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 98, 615–624.

SAS Institute. 2001. User's Manual, version 8.2. SAS Institute, Cary, NC.

Scheloske, H.-W., 1976. Morphologische Anpassungen eines ektoparasitischen Pilzes (Ascomycetes: Laboulbeniales: *Misgomyces coneglanensis*) an Körperbau und Fortpflanzungsverhalten seines Wirtes (Coleoptera: Hydrophilidae: *Laccobius minutus*). Entomol. Germ. 3, 227–241.

Tavares, I.I., 1985. Laboulbeniales (Fungi, Ascomycetes). Mycol. Mem. 9, 1–627.

Thaxter, R., 1891. Supplementary note on North American Laboulbeniaceae. Proc. Am. Acad. Arts Sci. 25, 261–270.

Ueno, H., 1994. Intraspecific variation of P2 value in a coccinellid beetle, *Harmonia axyridis*. J. Ethol. 12, 169–174.

Ueno, H., 1996. Estimate of multiple insemination in a natural population of *Harmonia axyridis* (Coleoptera: Coccinellidae). Appl. Entomol. Zool. 31, 621–623.

Weir, A., Beakes, G.W., 1995. An introduction to the Laboulbeniales: a fascinating group of entomogenous fungi. Mycologist 9 (Part 1), 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. Ecol. Entomol. 26, 547–550.

Whisler, H.C., 1968. Experimental studies with a new species of *Stigmatomyces* (Laboulbeniales). Mycologia 60, 65–75.

Yoder, J.A., Hobbs, H.H.I., Hazelton, M.C., 2002. Aggregate protection against dehydration in adult females of the cave cricket, *Hadenoecus cumberlandicus* (Orthoptera, Rhaphidophoridae). J. Cave Karst Stud. 64, 140–144.