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Research Article

Limited Transmission of the Ectoparasitic Fungus *Hesperomyces virescens* between Lady Beetles

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The ectoparasitic fungus *Hesperomyces virescens* Thaxter (Ascomycota: Laboulbeniales) commonly infects the invasive lady beetle *Harmonia axyridis* (Pallas) and several other aphidophagous lady beetles in North America and Europe. We tested the hypothesis that bodily contact between adults of different lady beetle species supports horizontal transmission of *H. virescens*. We used laboratory assays to determine whether *H. axyridis* or *Olla v-nigrum* (Mulsant) harboring *H. virescens* (i.e., source beetles) transmit the fungus to noninfected target beetles *H. axyridis*, *O. v-nigrum*, *Coccinella septempunctata* L., *Coleomegilla maculata* (De Geer), or *Hippodamia convergens* Guerin-Meneville. Results indicate that intraspecific transmission (i.e., for the source beetles *H. axyridis* and *O. v-nigrum*) was common but interspecific transmission (i.e., from source *H. axyridis* or *O. v-nigrum* to target species) was low. Interspecific transmission occurred at low rates from *H. axyridis* to both *C. septempunctata* and *O. v-nigrum* and from *O. v-nigrum* to both *C. septempunctata* and *H. convergens*. Based upon our laboratory assays of forced pairings/groupings of source and target beetles, we predict that horizontal transmission of *H. virescens* between species of aphidophagous coccinellids is possible but likely rare.

1. Introduction

Laboulbeniales (Ascomycota) are ectoparasitic fungi and nearly all 2,000 described species are obligate parasites that grow on the integument of living arthropods, mostly insects, and usually on the adult stage [1, 2]. Within the ten insect orders that contain host species, about 80% of these parasitic fungi are on beetles (Coleoptera) [2]. Of particular interest are Laboulbeniales that infect Coccinellidae (Coleoptera). Four species of *Hesperomyces* (*H. chilomenis*, *H. coccinelloides*, *H. hyperaspidis*, and *H. virescens*) attack entomophagous Coccinellidae [1, 3, 4]. Of these species, *H. virescens* Thaxter infects more coccinellid species than the other three species [4]. Negative impacts of parasitism by *H. virescens* on lady beetle populations are not well defined, but Kamburov et al. [5] found that infected *Chilocorus bipustulatus* L. adults suffered premature mortality.

Known coccinellid hosts of *H. virescens* include *Adalia bipunctata* (L.), *Brachiacantha quadripunctata* Melsheimer, *Chilocorus stigma* (Say), *Chilocorus bipustulatus* (L.), *Eriopis connexa* Germar, *Cycloneda munda* (Say), *Cycloneda sanguinea* (L.), *Coccinula crotchi* (Lewis), *Coccinula sinensis* Weise, *Coccinella septempunctata* L., *Hippodamia convergens* Guerin-Meneville, *Harmonia axyridis* (Pallas), *Olla v-nigrum* (Mulsant), and *Psyllobora vigintimaculata* (Say) [1, 3–9]. Although *H. virescens* may occur on these species, it may not occur on some other coccinellid species found within the same habitat at the same time. For example, Harwood et al. [8] sampled lady beetles using Malaise traps and recorded *H. virescens* from *B. quadripunctata*, *C. munda*, *H. axyridis*, and *P. vigintimaculata*. The fungus was not on *Coleomegilla maculata* (De Geer) or *Hyperaspis signata* (Olivier) in those samples. Riddick and Cottrell [10] found *H. virescens* infecting *H. axyridis*, *H. convergens*, and *O. v-nigrum* when

beetles were collected using sweep nets. At the same time, *H. virescens* was not on *C. septempunctata*, *C. maculata*, *C. munda*, *Scymnus loewii* Mulsant, or *S. socer* LeConte. Harwood et al. [8] and Riddick and Cottrell [10] reported that the exotic *H. axyridis* had the highest percentage of infected individuals (82.3 and 50.1%, resp.) among the species sampled. Additionally Riddick and Cottrell [10] reported that *H. virescens* infected 33.1% of *O. v-nigrum* adults but only 4.7% of other species of adult lady beetles.

Horizontal transmission between adult Coccinellidae is via direct contact, usually during copulation but also within overwintering aggregations [11–15]. Indirect transmission of Laboulbeniales, that is, beetles infected from ascospores discharged onto a substrate is not likely [2].

Our goal was to use laboratory assays to test the hypothesis that bodily contact between different species of lady beetles provides an avenue for horizontal transmission of *H. virescens*. We paired infected beetles (i.e., source beetles) with noninfected beetles (i.e., target beetles) for varying times to determine whether transmission, within or between species, occurred. Additionally, we examined whether transmission within species occurred via indirect transmission under laboratory conditions.

2. Materials and Methods

2.1. Insects. We used adult beetles from laboratory colonies or field collections in experiments. We established laboratory colonies of *H. axyridis*, *C. maculata*, and *O. v-nigrum* from individual beetles collected at the USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA, USA. Colonies were maintained on a diet of pecan aphids (Hemiptera: Aphididae), frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, and a meat-based diet (Beneficial Insectary, Redding, CA, USA) similarly as described by Cottrell [16]. Adult *H. convergens* and *C. septempunctata* were field-collected and maintained on a similar diet for 2-3 wk before use in experiments. Holding field-collected beetles in the laboratory before using them in assays permitted time to detect any infected individuals.

2.2. Ectoparasitic Fungus. We initiated separate colonies of *H. virescens*-infected *H. axyridis* and *O. v-nigrum* by collecting infected beetles from the field, confirming infection on beetles using a stereomicroscope, and maintaining beetles in the laboratory. We housed groups of infected beetles in containers ($19 \times 13.5 \times 9$ cm) and provided food and water as previously described. We perpetuated each of the infected colonies by the periodic addition of noninfected, laboratory-reared adult *H. axyridis* or *O. v-nigrum*. These infected beetles served as *H. virescens* source beetles used in fungus transmission studies. We did not consider beetle age or whether the beetle was field-collected or laboratory-reared when used in assays. Rather, we ascertained that mature thalli were present on the source beetles used in experiments and placed source and target beetles together, in the first transmission experiment (see below), with regard to thalli density on source beetles. Furthermore, we did not determine sex of beetles in order to reduce handling and potential spore dispersal prior to experimentation.

2.3. Transmission Studies

2.3.1. Seven-Day Exposure Experiment. We conducted this experiment using two separate trials, one in January and another in April 2010. For each trial, source *H. axyridis* and *O. v-nigrum* were tested separately against laboratory reared, target *H. axyridis*, *O. v-nigrum*, and *C. maculata*. Before trials began, source beetles were observed using a stereomicroscope, mature thalli were counted and each beetle was designated with high ($\geq$24), moderate (14 to 23), or low ($\leq$13) thalli density (range = 35). In both trials, we placed a source beetle (i.e., *H. axyridis* or *O. v-nigrum*) in a 9 cm diameter Petri dish with a target beetle (i.e., *H. axyridis*, *O. v-nigrum*, or *C. maculata*). Each trial consisted of three replicates of all possible source-target species combinations at a 1 : 1 ratio of source : target. Thus, we used five target beetles of one species per source species in each replicate for a total of 15 targets per trial and 30 targets for the experiment.

In the first trial, *O. v-nigrum* source beetles with similar thalli densities were lacking; therefore, not all five target beetles of each species (i.e., *H. axyridis*, *O. v-nigrum*, and *C. maculata*) were matched with *O. v-nigrum* source beetles harboring similar densities of thalli. However, similar treatments between target species within each replicate were achieved by using three targets each paired with a low thalli density source *O. v-nigrum*, a fourth target paired with a moderate thalli density source beetle and a fifth target paired with a high thalli density source beetle. Additionally during the first trial, all *H. axyridis* source beetles had low thalli densities thus two source beetles were placed with each target beetle (ratio of source : target = 2 : 1) to insure that an outcome showing a lack of transmission was not solely due to low thalli density of a single source beetle.

In the second trial, thalli density varied on both source species but source and targets were paired such that each of the five targets in each replicate was exposed to source beetles with similar thalli density. In both trials, Petri dishes containing source and target beetles were housed in an environmental chamber ($25 \pm 1^{\circ}$C and 14 : 10 [L : D]h) for seven days and provided food and water. After seven days, we removed source beetle(s) but kept the target beetle in that same Petri dish for one month. During this time, we examined target beetles three times per week under a stereomicroscope for development of mature (or at least nearly mature and identifiable) *H. virescens* thalli. We documented any target beetles containing at least one mature thallus and placed them into 70% ethanol for later confirmation of *H. virescens*.

2.3.2. Tumbled Beetles Experiment. In the previous experiment, pairs of same sex or interspecific source and target beetles may have hindered beetle interaction and affected *H. virescens* transmission. Additionally, thalli distribution on source beetles and the behavior of source beetles (e.g., not attempting to mate) could hinder *H. virescens* transmission. This experiment attempted to negate these factors by forcing

interaction between the source and target. Again, *H. axyridis* and *O. v-nigrum* were the source beetles. The target species were *C. septempunctata*, *C. maculata, H. axyridis*, *H. convergens*, and *O. v-nigrum*. We paired source and target beetles in 2-dram vials and then placed the vials on an automated roller (Nostalgia Electrics HRD-565 Hot Dog Roller, Nostalgia Products Group, LLC, http://www.nostalgiaelectrics.com/), as used in insecticide assays to coat the inner walls of vials, and rolled them at 2.6 revolutions/min for 1 h. Paired beetles within the rolling vial were active and tumbling and thus became entwined while attempting to remain upright and maintain their footing on the rotating glass vial (TEC, personal observations). After being tumbled, we placed target beetles into Petri dishes and maintained them in an environmental chamber with food and water for one month. We observed beetles three times per week under a stereomicroscope to detect any mature thallus, documented its presence and preserved infected beetles in 70% ethanol.

2.3.3. Extended Exposure Experiment. We attempted to facilitate transmission of *H. virescens* between source and target beetles by keeping them in close contact for an extended period. We grouped a single source beetle with three individuals of the same target species in a Petri dish and replicated four times ($n = 12$ target beetles per species). This was done using *H. axyridis* and *O. v-nigrum* source beetles with the target species *C. septempunctata*, *C. maculata*, *H. axyridis*, *H. convergens*, and *O. v-nigrum*. These beetles remained together for 6 wk and were fed, watered and observed for development of *H. virescens* thalli. When source and target beetles were of the same species, we easily differentiated the single source beetle and the targets by presence/absence of *H. virescens* and later by thalli density when we detected the first mature *H. virescens* thallus on a target beetle.

2.3.4. Substrate Borne Transmission Experiment. Temporal and spatial overlap of coccinellids in the field might allow for substrate borne transmission as potential hosts contact *H. virescens* ascospores. We placed two source *H. axyridis* adults in a Petri dish with food and water then removed them after 24 hr. Density of thalli on source beetles varied from low to high. Immediately following removal of the source beetles, five target *H. axyridis* beetles were added to the same dish and maintained in that dish, with food and water, for 7 d. This procedure was replicated using five dishes ($n = 10$ source and 25 target *H. axyridis*). After 7 d, we transferred the target beetles into a clean dish (and thereafter on an as-needed basis) and maintained them with food and water for the next three weeks. We used the same experimental design to examine substrate borne transmission when source *H. axyridis* remained in the Petri dish for 120 h. Additionally, we examined the potential of substrate-borne transmission of *H. virescens* by source *O. v-nigrum* to target *O. v-nigrum* using the same experimental design; source beetles were exposed to Petri dishes for 24 and 120 h. For both target species, we examined individual beetles three times per week, during weeks 2–4 of the study, for mature thalli using a stereomicroscope.

Table 1: Previously noninfected target lady beetles infected with the ectoparasitic fungus *H. virescens* when exposed in a Petri dish for 7 d to already infected source *H. axyridis* or *O. v-nigrum* lady beetles.

Source beetle	Target beetle	Proportion infected (±SE)*
H. axyridis	*C. maculata*	0 b
H. axyridis	*H. axyridis*	0.46 ± 0.11 a
H. axyridis	*O. v-nigrum*	0 b
O. v-nigrum	*C. maculata*	0 b
O. v-nigrum	*H. axyridis*	0 b
O. v-nigrum	*O. v-nigrum*	0.87 ± 0.06 a

*Within each source beetle, different letters following the proportion infected indicate a significant difference ($P < 0.05$) between target beetles.

2.4. Statistical Analysis. Within source species, we combined data from both trials of the seven-day exposure experiment. For both the seven-day exposure and tumbled beetles experiments, we used the nonparametric Kruskal-Wallis analysis of variance by ranks to analyze the proportions of infected beetles. We did this separately for when *H. axyridis* or *O. v-nigrum* was the source beetle. We analyzed proportions to take into consideration that some target beetles died before it was conceivable that successful transmission could have been identified and thus these individuals were not included in analyses. When a significant difference between species was found for the proportions infected, the Tukey-Kramer Honestly Significant Difference multiple comparison was used [17, 18].

3. Results

3.1. Seven-Day Exposure Experiment. When *H. axyridis* was the source, the only target species found with mature thalli was *H. axyridis*, and the proportion of infected beetles was significantly higher than no infection observed for *C. maculata* or *O. v-nigrum* ($\chi^2_{0.05,2} = 10.59$; $P = 0.0050$) (Table 1). From the January 2010 trial when source *H. axyridis* had different thalli density, 25, 50, and 25% of the newly infected target beetles had been housed with source beetles rated with low, moderate, and high thalli densities, respectively. Additionally, the average time (±SE) to detect mature thalli on target *H. axyridis* when exposed to source *H. axyridis* was 25.8 ± 1.5 d.

When source *O. v-nigrum* beetles were paired with target *H. axyridis*, *C. maculata*, or *O. v-nigrum,* the only target observed with mature thalli was *O. v-nigrum* and the proportion of infected beetles was significantly greater than for either *C. maculata* or *H. axyridis* ($\chi^2_{0.05,2} = 11.74$; $P = 0.0028$) (Table 1). From both trials, infection of target beetles exposed to *O. v-nigrum* source beetles with low, moderate and high thalli densities was 45, 30, and 25%, respectively. The average time (±SE) to detect mature thalli on target *O. v-nigrum* when exposed to source *O. v-nigrum* was 15.0 ± 0.4 d.

3.2. Tumbled Beetles Experiment. When we paired source *H. axyridis* in a glass vial with the target beetles *C. maculata*, *C. septempunctata*, *H. axyridis*, *H. convergens*, or *O. v-nigrum*

Table 2: Previously noninfected target lady beetles infected with the ectoparasitic fungus *H. virescens* when a pair of target and source lady beetles were placed in a vial and tumbled on a vial roller for 1 h. Source and target beetles were then separated and target beetles observed for mature thalli over the next month.

Source beetle	Target beetle	Proportion infected (±SE)*
H. axyridis	*C. septempunctata*	0.11 ± 0.06 b
H. axyridis	*C. maculata*	0 b
H. axyridis	*H. axyridis*	0.52 ± 0.11 a
H. axyridis	*H. convergens*	0 b
H. axyridis	*O. v-nigrum*	0 b
O. v-nigrum	*C. septempunctata*	0.14 ± 0.10 b
O. v-nigrum	*C. maculata*	0 b
O. v-nigrum	*H. axyridis*	0 b
O. v-nigrum	*H. convergens*	0 b
O. v-nigrum	*O. v-nigrum*	0.61 ± 0.11 a

*Within each source beetle, different letters following the proportion infected indicates a significant difference ($P < 0.05$) between target beetles.

for 1 h and observed the targets for the next month, the only targets observed with mature thalli were *H. axyridis* and *C. septempunctata*. However, horizontal transmission of *H. virescens* was significantly higher between beetles of the same, rather than different, species ($\chi^2_{0.05,4} = 10.28$; $P = 0.0360$) (Table 2). The average number of days before we observed mature thalli on *H. axyridis* and *C. septempunctata* targets was 17.9 ± 0.9 and 21.0 ± 0.0 d, respectively. When *O. v-nigrum* was the source, only *O. v-nigrum* and *C. septempunctata* targets were infected. Transmission of *H. virescens* to *O. v-nigrum* was significantly higher between individuals of the same species than between different species ($\chi^2_{0.05,4} = 10.28$; $P = 0.0360$) (Table 2). As an interesting note, the nonparametric statistics for the analysis of variance by ranks was identical for when *H. axyridis* or *O. v-nigrum* was the source. The average number of days before we observed mature thalli on *O. v-nigrum* and *C. septempunctata* targets was 14.5 ± 0.33 and 22.5 ± 1.5 d, respectively.

3.3. Extended Exposure. Not all beetles (source or target) survived to the end of this experiment. When *H. axyridis* and *O. v-nigrum* source beetles were housed with *H. axyridis* and *O. v-nigrum* target beetles, respectively, most source beetles either survived longer than respective target beetles or past the average time required, as reported in the prior experiments, to detect a mature *H. virescens* thallus on a target beetle (Table 3). As such, insufficient time to transmit the fungus was not of concern. We found a mature thallus on most *H. axyridis* targets (i.e., 83%) after 19 ± 1 d of contact with *H. axyridis* source beetles. Two noninfected target *H. axyridis* did not survive this long and likely died before a thallus could mature and be recorded (Table 3). Average survival time of other noninfected target species, except *O. v-nigrum*, exposed to source *H. axyridis* was longer than 19 d (Table 3). We did not find mature thalli on *C. septempunctata*, *C. maculata*, and *H. convergens* after confinement with source *H. axyridis*. Only one *O. v-nigrum* target, confined with a source *H. axyridis*, harbored a mature thallus at 21 d (a longer period of thallus development than previously noted for target *O. v-nigrum* infected by source *O. v-nigrum*). Three other target *O. v-nigrum* surviving longer than 21 d with the source *H. axyridis* beetles were not infected.

Table 3: Average days (±SE) that *H. virescens* source lady beetles and target lady beetles (that were not infected during the experiment) survived when one source lady beetle and three target lady beetles were housed together in Petri dishes for 44 days.

Source beetle	Target beetle	Average days (± SE) source survived	Average days (± SE) noninfected target survived
H. axyridis	*C. septempunctata*	21 ± 8	39 ± 4
H. axyridis	*C. maculata*	32 ± 7	38 ± 3
H. axyridis	*H. axyridis*	N/A[a]	9 ± 4[b]
H. axyridis	*H. convergens*	23 ± 2	30 ± 5
H. axyridis	*O. v-nigrum*	29 ± 9	16 ± 5
O. v-nigrum	*C. septempunctata*	20 ± 2	44 ± 0
O. v-nigrum	*C. maculata*	20 ± 7	33 ± 5
O. v-nigrum	*H. axyridis*	17 ± 4	34 ± 4
O. v-nigrum	*H. convergens*	22 ± 7	35 ± 4
O. v-nigrum	*O. v-nigrum*	5[c]	7 ± 1[d]

[a]All source beetles either survived longer than target beetles or past the time when a mature thallus was detected on infected target beetles.
[b]Two beetles survived for 5 or 13 d. A mature thallus was detected on all other target beetles between 15 and 21 d.
[c]One beetle survived only 5 days and others either survived longer than target beetles or survived past the time when infection was detected on target beetles.
[d]Most beetles (75%) died before mature thalli were likely to have been observed. Two of three that survived longer than 7 d were observed infected.

We found two target *O. v-nigrum* infected after 13 ± 0 d when housed with source *O. v-nigrum* and these two infected targets represent 100% of available *O. v-nigrum*. The other *O. v-nigrum* targets (i.e., 83%) did not survive long enough for *H. virescens* thalli to mature (Table 3). The only other target found infected by source *O. v-nigrum* was *H. convergens*. A mature thallus was found on three (i.e., 30%) *H. convergens* after 17 ± 2 d (excluding two beetles that survived for only 13 d). Noninfected targets (i.e., *C. septempunctata*, *C. maculata,* and *H. axyridis*) survived longer than the time required before detection of a mature thallus on target *O. v-nigrum* or *H. convergens* (Table 3).

3.4. Substrate-Borne Transmission. We found no evidence of substrate-borne transmission of *H. virescens* (as suggested by a mature thallus on a target beetle) between adults of the same species, for either *H. axyridis* or *O. v-nigrum*. Mortality of target *H. axyridis* (mean ± SE) that remained alive for at least 15 d was 8 ± 8 and 12 ± 8% whether source beetles were exposed to Petri dishes for 24 or 120 h, respectively; similarly, mortality of target *O. v-nigrum* was and 8 ± 5 and 16 ± 10%, respectively.

4. Discussion

Transmission of *H. virescens* from a source to a target beetle was successful when a mature (or nearly mature) *H. virescens* thallus was on the target beetle [19]. In the absence of a mature thallus on a target beetle, we made no observations regarding whether ascospores transferred from source to target beetles. As such, we do not comment on whether absence of transmission resulted from transferred ascospores that germinated on the target beetle but failed to develop to maturity. Many physical, chemical, and biological factors affect adhesion of fungal spores to surfaces with subsequent attachment to and germination [20], any or all of which may have affected successful transmission in this study.

Direct transmission of Laboulbeniales, generally via sexual contact and within overwintering aggregations, is likely the primary mode of dispersal within the Coccinellidae [6, 11, 12, 14]. Overall, substrate-borne transmission of Laboulbeniales is rare considering that the ascospore is short lived [2]. We did not attempt to group beetles to determine evidence of transmission via sexual contact. Rather, our study grouped beetles in situations unlikely to occur naturally but very likely to result in considerable contact between source and target beetles. Under these conditions, we observed transmission (as denoted by target beetles with mature *H. virescens* thalli) between source and target beetles when both occupied the same container at the same time. In contrast, we did not observe transmission when target beetles occupied a container after removal of source beetles. Results presented here support the hypothesis that direct bodily contact between coccinellid hosts is necessary for transmission of *H. virescens* [2] and other Laboulbeniales [19, 21].

Transmission of *H. virescens* between coccinellids of the same species was more common than between different species in this study. Note that the tumbling experiment forced all paired source and target beetles, regardless of sex or species, to make considerable contact and allowed for horizontal transmission of *H. virescens* spores. Despite this, transmission between coccinellids of the same species still dominated. In the tumbling experiment, we only documented transmission between different species with target *C. septempunctata* exposed to *O. v-nigrum* or *H. axyridis* source beetles. Tumbling that led to the discharge of spores (onto the glass vial), which were transmitted to hosts is unlikely but cannot be ruled out from the results provided here. In addition to *C. septempunctata*, the only other instances of transmission between species was with target *H. convergens* exposed to source *O. v-nigrum* and target *O. v-nigrum* exposed to source *H. axyridis*.

In general, a higher rate of successful pathogen transmission within the same species is not surprising. Even though some entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff) can infect a broad range of host insect species, isolates within each of these species can have a high degree of host specificity [22]. In fact, Cottrell and Shapiro-Ilan [23] found relatively high host specificity of *B. bassiana* isolates from source *O. v-nigrum* when tested against target *O. v-nigrum* and target *H. axyridis*. A similar scenario appears likely for *H. virescens* infecting different species of Coccinellidae. Isolates/strains of *H. virescens* may exist under field conditions and only infect closely related Coccinellidae or even a single species. At present, isolates/strains of *H. virescens* are unknown. Perhaps *H. virescens* that has had several passes on the same species is more virulent toward that species than to other species, similarly as Steinhaus [24] described increasing virulence of an entomopathogen.

In this study, the time required to detect a mature thallus on a target was less when the source and target were both *O. v-nigrum* than when the source and target were both *H. axyridis*. The separation in time to detect mature thalli on these two species is not explained simply by our schedule of observing specimens for mature thalli three times per week as opposed to daily. Even when we paired source and targets for one hour or seven days, detection of mature thalli occurred earlier when transmission was from *O. v-nigrum* to *O. v-nigrum* than from *H. axyridis* to *H. axyridis*. If there are no *H. virescens* isolates/strains infecting *O. v-nigrum* and *H. axyridis*, this time differential could be explained by nutritional quality of the two hosts and/or that more time is required for the ascospore to germinate and for the "foot" and haustoria to develop on *H. axyridis*. Although *H. virescens* may be pathogenic to numerous species of Coccinellidae, virulence against some species may be attenuated depending upon the number of passes it has gone through on other species, for example, *H. axyridis*. This could also explain why more time was required to detect a mature thallus on target *C. septempunctata*, infected from a source *O. v-nigrum*, than for *O. v-nigrum* intraspecific transmission.

As stated previously, our assay conditions used forced pairings/groupings not likely to occur in the field, yet we documented limited transmission between species. Direct contact between coccinellids of different species can occur in the field as promiscuous males attempt to mate with females of other coccinellid species [25, 26] (EWR, personal observation). Copulation (or mating attempts) between different coccinellid species has been observed in field cage tests among the phytophagous lady beetles *Henosepilachna yasutomii* Katakura and *H. niponica* Lewis [27]. Additionally, copulation attempts by a male *C. maculata* with an unidentified beetle species (Coleoptera: Cleridae) has been observed in the field (TEC, personal observation). Although we did not attempt to document copulation when source and target lady beetles were in Petri dishes for 7 d, we only observed transmission of *H. virescens* within the same host species. When source and target beetles were confined for an extended interval, mortality of source and target beetles was problematic in some groupings. Nonetheless, the results were similar as previously observed, that is, transmission of fungus occurred within the same host species with the exception of one target *O. v-nigrum* becoming infected from source *H. axyridis* and three *H. convergens* becoming infected from source *O. v-nigrum*. Infection through contact with substrate borne ascospores is rare among Laboulbeniales because the ascospore is short lived [2]. We did not find evidence of substrate-borne transmission between coccinellids in this study.

It is not clear why some coccinellid species sampled by Harwood et al. [8] and Riddick and Cottrell [10] had a relatively low prevalence of *H. virescens* infection. Their sampling methods, species abundance, or host specificity of *H. virescens* could have been influential. Further transmission studies on other species of Coccinellidae could provide insight regarding host specificity of *H. virescens*.

Interestingly, *H. virescens* represents one of the first parasites to infect *H. axyridis* in North America. Another parasite found on *H. axyridis* in North America is the ectoparasitic podapolipid mite *Coccipolipus hippodamiae* (McDaniel and Morrill) [28]. After *H. axyridis* established and quickly dispersed across North America, natural enemies may now be adapting to it. What impact *H. virescens* has on the dynamics of any coccinellid population is yet to be determined given that it is reported to cause anywhere from little impact to premature mortality [5, 6, 29].

In conclusion, it is likely that high host specificity and an apparent need for substantial periods of close contact between potential hosts will limit transmission of *H. virescens* by *H. axyridis* and *O. v-nigrum* with other coccinellids in the field [8, 10].

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