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Do Biotic and Abiotic Factors Influence the Prevalence of a Common Parasite of the Invasive Alien Ladybird *Harmonia axyridis*?

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Hesperomyces virescens (Ascomycota, Laboulbeniales), a fungal ectoparasite, is thus far reported on *Harmonia axyridis* from five continents: North and South America, Europe, Africa, and Asia. While it is known that *He. virescens* can cause mortality of *Ha. axyridis* under laboratory conditions, the role of biotic and abiotic factors in influencing the distribution of *He. virescens* in the field is unknown. We collected and screened 3,568 adult *Ha. axyridis* from 23 locations in seven countries in Central Europe between October and November 2018 to test the effect of selected host characters and climate and landscape variables on the infection probability with *He. virescens*. Mean parasite prevalence of *He. virescens* on *Ha. axyridis* was 17.9%, ranging among samples from 0 to 46.4%. Host sex, climate, and landscape composition did not have any significant effect on the infection probability of *He. virescens* on *Ha. axyridis*. Two color forms, f. *conspicua* and f. *spectabilis*, had a significantly lower parasite prevalence compared to the common *Ha. axyridis* f. *novemdecimsignata*.

Keywords: community ecology, *Hesperomyces*, Laboulbeniales, parasite prevalence, precipitation, temperature, spatial modeling, agricultural landscape

INTRODUCTION

Parasites may be the least studied life form on the planet (Price, 1980; Windsor, 1990, 1995). In their call for a "global parasite conservation plan," Carlson et al. (2020) proposed 12 major goals within four themes. These themes are data collection and synthesis (aimed at describing parasites and incorporating them into biodiversity surveys, among others), risk assessment and prioritization (documenting drivers of parasite declines and develop regional and global Red

Lists), conservation practice (e.g., building parasite conservation capacity), and outreach and education. One of the most common parasites of the globally invasive harlequin ladybird *Harmonia axyridis* (Coleoptera, Coccinellidae) is *Hesperomyces virescens*, which was for the first time observed on this host in 2002 (Garcés and Williams, 2004).

Hesperomyces virescens (Ascomycota, Laboulbeniales) is a biotrophic fungus that has a very wide distribution with confirmed reports in North and South America, Europe, Africa, and Asia (Haelewaters et al., 2017). Based on the results of an integrative taxonomic approach, we know that *He. virescens* is a complex of multiple species segregated by hosts (Haelewaters et al., 2018a). Thus far, two species have been formally described in the complex, *He. halyziae* (Haelewaters and De Kesel, 2020) and *He. parexochomi* (Crous et al., 2021). The other species, including the one associated with *Ha. axyridis*, are awaiting formal description. The parasite prevalence of *He. virescens* on *Ha. axyridis* differs among geographic regions and, exceptionally, may be as high as 96.5% in a given ladybird population, as reported in Meise, Belgium (February 2012, *n* = 107) and in Westmoreland, New Hampshire, United States (December 2012; *n* = 83) (Haelewaters et al., 2017). As a result, *He. virescens* has recently gained traction among entomologists as a potential biological control agent against *Ha. axyridis*.

Little information is available about the ecology of *He. virescens*. A recent experimental study demonstrated that infection with *He. virescens* affects the survival of *Ha. axyridis* on its own and when ladybirds are co-infected with either of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium brunneum* (Haelewaters et al., 2020). Thus far, however, potential effects of biotic and abiotic factors on the distribution of *He. virescens* and its parasite prevalence on *Ha. axyridis* are unknown. This information is fundamental for studies in conservation, applied ecology, and biocontrol strategies (Ferrier, 2002; Rushton et al., 2004; Magan, 2021). In this study, we collected adult specimens of *Ha. axyridis* across Central Europe and evaluated how selected host traits and climate and landscape variables affect infection patterns with *He. virescens*. Variables tested included host sex, host color form, color of elytra, proportion of agricultural and forested areas (European Environment Agency, 2020), temperature, and precipitation (Fick and Hijmans, 2017).

MATERIALS AND METHODS

Ladybirds were collected either by hand or using a mouth-operated aspirator from October to November 2018 in different Central European countries (**Figure 1**): Croatia (Istria County), the Czech Republic (Central Bohemian Region, South Bohemian Region, Plzeň Region), Germany (State of Bavaria), Hungary (Hajdú-Bihar County), Poland (Mazovian Voivodeship), Slovakia (Nitra Region), and Switzerland (Canton of Jura). Contributors were asked to collect at least 100 specimens from each ladybird population. Geographic coordinates were recorded and can be found in **Supplementary File 1**. Specimens were preserved in 70% ethanol until examination in the laboratory.

Ladybirds were screened under 40–50× magnification for the presence of non-hyphal thalli of *He. virescens* (sensu De Kesel, 2011; Haelewaters et al., 2018a). For each ladybird, the following traits were recorded: sex (see McCornack et al., 2007); color form [non-melanic f. *novemdecimsignata* (also referred to as *succinea*), and melanic f. *conspicua*, f. *axyridis*, and f. *spectabilis*]; color of elytra (for non-melanics) or spots (for melanics) (yellow, orange, red; Fiedler and Nedvěd, 2019). For *novemdecimsignata* specimens, we described whether spots were well-circumscribed (0), missing or fewer in number than typically present (−), or large and touching each other (+) (Fiedler and Nedvěd, 2019). For each population, no matter the number of sampled ladybirds, we screened 100 randomly selected specimens. When available, we screened and processed additional specimens of the melanic forms to avoid statistical restrictions due to these forming in low percentages. Screening results for all processed ladybirds are available in **Supplementary File 1**. After processing, voucher specimens were deposited in the Purdue Entomology Research Collection (West Lafayette, IN, United States) under the following accession numbers: PERC 0147670–0147680.

All statistical analyses were performed using the R software, version 3.6.3 (R Core Team, 2020). To identify the variables influencing the infection probability of *He. virescens* on *Ha. axyridis*, we used generalized mixed effect models (GMEM) with a binomial data distribution (infected yes/no) [function glmer(), R package *lme4*; Bates et al., 2015]. We included host sex, host color form, color of elytra, the proportion of agricultural, and forested areas in a buffer surrounding each sampling location (100 m, 300 m, 600 m, 1 km, and 2.5 km), and climate variables. Urban area was excluded from the analysis because it was highly collinear with the other predictor variables. For each buffer radius, a separate model was calculated, resulting in five distinct models. The landscape variables were obtained by extracting the landscape composition of Copernicus Corine Land Cover images taken in 2018 (European Environment Agency, 2020) with the help of R package *raster*, using the function extract (Hijmans et al., 2020). We pooled values in the categories "broad-leaved forest," "coniferous forest," and "mixed forest" into the forested area variable, whereas the agricultural area variable consisted of values for the categories "non-irrigated arable land," "fruit trees and berry plantations," "pastures," "complex cultivation patterns," and "land principally occupied by agriculture, with significant areas of natural vegetation" (**Supplementary File 2**). We further calculated three models using a "ring buffer" or annulus (sensu Rey et al., 2020), one for each of the following radius combinations: 100 m inner and 300 m outer radius, 300 m inner and 600 m outer radius, and 600 m inner and 1 km outer radius.

Climate variables were extracted from WorldClim with a resolution of 30 arc seconds (ca. 1 × 1 km) (Fick and Hijmans, 2017) (**Supplementary File 2**). As variables for temperature and humidity are generally collinear, we decided to summarize the following variables using a principal component analysis (PCA) [prcomp(), R package *stats*; R Core Team, 2020]: annual mean temperature, mean maximum temperature in the hottest month, mean minimum temperature in the coldest month,
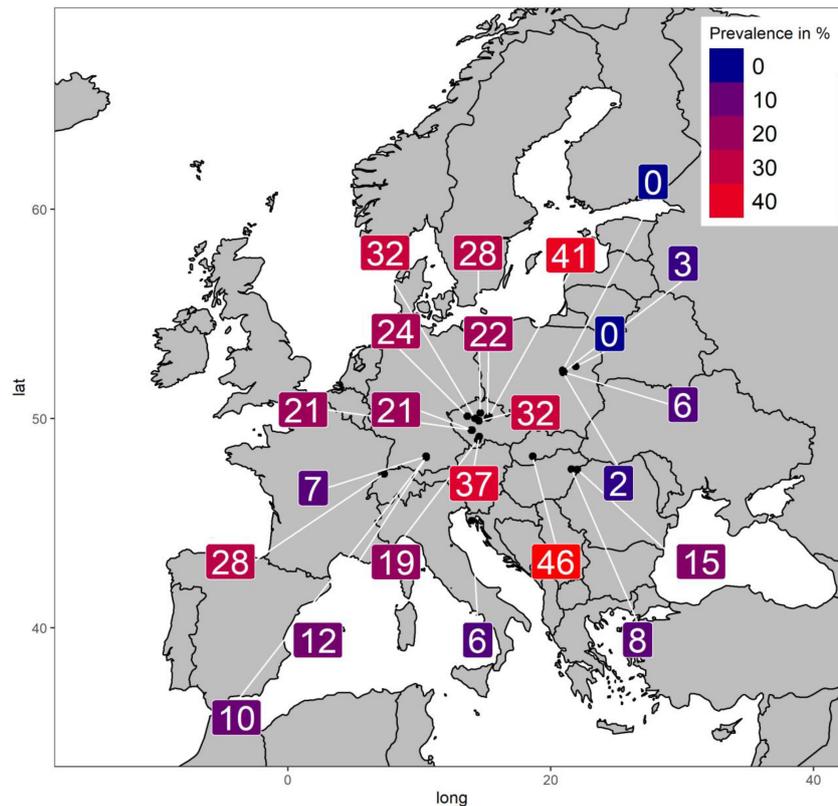
FIGURE 1 | Parasite prevalence by sampled population.

annual precipitation, precipitation during the wettest month, and precipitation during the driest month. We extracted the values for the first two dimensions for all sampling sites (**Supplementary File 2**). Statistical processing revealed two principal dimensions that explained 64 and 30.1% of the climatic variation, respectively. Finally, we included the random intercept *collection* nested within *sampling region* to address repeated sampling at the same location and also spatial autocorrelation as suggested by Zuur et al. (2010).

Hypothesis testing was done using likelihood ratio tests, with p values calculated based on χ^2 distributions, declaring an effect significant when $p \leq 0.05$. Nine models were compared, namely, the Null Model and the model with variables of interest within the different buffer radii (100 m, 300 m, 600 m, 1 km, and 2.5 km) and annulus radii (100–300 m, 300–600 m, and 600 m– 1 km). Model selection happened using the Akaike Information Criterion (Akaike, 1974). For all models, we calculated pseudo-R^2 values to estimate model fit by accounting for the variation explained by both fixed and random effects [function r2(), R package *performance*; Lüdecke et al., 2020].

RESULTS

We screened a total of 3,568 ladybirds, resulting in a mean infection prevalence of 17.9%, ranging from 0 to 46.4% among sampled populations (**Figure 1**). The population with the highest prevalence of *He. virescens* was from Levice in southwestern Slovakia. Two populations showed no visible signs of *He. virescens* infection, both of which were from Poland. Forty-seven individuals were excluded from statistical analyses, including a single f. *intermedia* specimen from Warsaw, Poland and 46 specimens with missing information on elytral color. Likelihood ratio tests confirmed that each of the eight models explained the observed variance better than chance (**Table 1**), while the conditional pseudo R^2-values estimated model fit at around 0.33, indicating good fit (**Table 1**). We only found variables on host individual characters to have significant effects on the parasite prevalence of *He. virescens* on *Ha. axyridis* (**Tables 2**, **3**). The significant effect of color form was consistent in all five models, with the color forms f. *conspicua* and f. *spectabilis* being less often infected compared to the common form f. *novemdecimsignata*(0). There was a trend for f. *novemdecimsignata*(-) to be less likely infected by *He. virescens* compared to the common form f. *novemdecimsignata*(0). This trend was consistent over all candidate models but not significant. Finally, the color of elytra had a significant effect on the infection probability, with individuals with red elytra being more likely and individuals with yellow elytra less likely infected compared to individuals with orange elytra. Host sex, climate, and habitat composition resulted in not having any significant effect on the infection probability of *He. virescens* on *Ha. axyridis*.

TABLE 1 | Results of model (dependency of *Hesperomyces virescens* prevalence on *Harmonia axyridis* on coloration and climate and habitat properties) evaluation showing candidate models being significantly better than the null model and estimated pseudo-R² values indicating model fit.

Model	AIC	χ^2	*p* value	pseudo-R^2
Null Model	2,934.0			
Buffer 100 m	2,880.7	77.3	<0.001***	0.35
Buffer 300 m	2,880.1	77.9	<0.001***	0.33
Buffer 600 m	2,879.3	78.6	<0.001***	0.32
Buffer 1 km	2,879.6	78.4	<0.001***	0.32
Buffer 2.5 km	2,880.8	77.2	<0.001***	0.35
Annulus 100–300 m	2,880.1	77.9	<0.001***	NA
Annulus 300–600 m	2,879.2	78.8	<0.001***	0.32
Annulus 600 m–1 km	2,879.8	78.2	<0.001***	0.33

*Significance levels at: ***p < 0.001.*

DISCUSSION

Ha. axyridis–He. virescens

The first published record of *He. virescens* on *Ha. axyridis* in Europe was made in the winter of 2006–2007, from Meise in Belgium (De Kesel, 2011). Other country records followed quickly, from the Netherlands (2008), Germany (2008–2009), Croatia and the Czech Republic (2013), Hungary and Poland (2014), Slovakia (2015), Bulgaria and Greece (2017), and most recently European Russia and Switzerland (2018) (Herz and Kleespies, 2012; Ceryngier and Twardowska, 2013; Ceryngier et al., 2013; Pfliegler, 2014; Gorczak et al., 2016; Ceryngier and Romanowski, 2017; Haelewaters et al., 2017; van Wielink, 2017; Orlova-Bienkowskaja et al., 2018; this paper). Many of these papers show that the parasite prevalence of *He. virescens* differs significantly over time and in space (Raak-van den Berg et al., 2014; Haelewaters et al., 2017). Differences in Laboulbeniales prevalence among locations have been attributed to host population density and habitat type (Scheloske, 1969; De Kesel, 1996), but to date, no data were thus far available with regard to the *He. virescens–Ha. axyridis* association.

Effect of Biotic Factors

A remarkable finding from our study is that the melanic color forms f. *conspicua* and f. *spectabilis* were less often infected with *He. virescens* compared to the common nineteen-spotted f. *novemdecimsignata*(0). Two other studies investigated the relationship between the degree of melanization and infection patterns. Haelewaters et al. (2018b) observed a slight trend to higher intensity of parasitism in more melanic males of *Ha. axyridis* f. *novemdecimsignata*. Fiedler and Nedvěd (2019) found (i) a positive association between putative age groups of *Ha. axyridis* estimated as carotenoid content and infection with *He. virescens* and (ii) a negative association between elytral melanization of *Ha. axyridis* f. *novemdecimsignata* specimens and infection. The latter was explained by the fact that younger ladybirds emerged later in the year, with lower temperatures inducing extensive melanization; since they were younger, they had less opportunities to be parasitized by *He. virescens*. Our

results are in line with the findings of Fiedler and Nedvěd (2019), but a definitive answer as to how elytral melanization affects the susceptibility to infection with *He. virescens* remains unclear. The black coloration of melanic forms of *Ha. axyridis* is negatively correlated with the total content of alkaloids (Bezzerides et al., 2007), which serve as defense against predators and pathogens (Röhrich et al., 2011). Then, heavier melanization should result in more infection with *He. virescens*, but our results are inconsistent with this hypothesis. Future work—performing bioassays in controlled settings and analyzing expression levels of immune genes—is needed to shed light on the susceptibility of different color morphs to *He. virescens*.

The results of our modeling approach show a significant correlation between elytral color and the infection probability of *Ha. axyridis* with *He. virescens*. Carotenoid accumulation, and thus red color intensity, is a function of ladybird age (Bezzerides et al., 2007; Nedvěd et al., 2019). In our study, older individuals of *Ha. axyridis* accumulated more *He. virescens* inoculum. Similar observations were made by Fiedler and Nedvěd (2019), particularly that individuals with red elytra are more likely to be infected. *Hesperomyces virescens* transmits among ladybirds through physical contacts (during mating and in overwintering aggregations), but auto-transmission by grooming or cleaning also occurs. These factors contribute to parasite prevalence (at the level of population) and thallus density (at the individual level) being positively correlated with host age (Riddick and Schaefer, 2005; Nalepa and Weir, 2007; Haelewaters et al., 2017) and thus, incidentally, with elytral color.

Effect of Abiotic Factors

Mean parasite prevalence did not significantly change according to any of the temperature variables tested [Kruskal–Wallis test, function kruskal.test(), R package stats; R Core Team, 2020], but our sampling scheme was somewhat limited, with only localities in Central European countries. For a broader understanding of the associations between *He. virescens* and *Ha. axyridis* within Europe, we recommend incorporation of data from northern and southern European countries in order to compile a dataset with a larger range in temperature. Collections also need to be expanded to city centers (e.g., in parks, community gardens, edges of playgrounds). A negative correlation was found between parasite prevalence of *He. virescens* sensu lato on *Adalia bipunctata* and distance from the city center of London (Welch et al., 2001). Prevalence in central London was as high as 40% (*n* = 105), whereas it was 0% outside of the urban area at a distance of 25 km. This could be linked to increased temperatures in urban environments (urban heat island effect), but this has not yet been tested. Adriaens et al. (2008) highlighted the idea that *Ha. axyridis* is less frequently found in natural landscapes compared to more urbanized and anthropogenic landscapes. Habitat preference may also be an important factor in the parasitism with *He. virescens*. Factors promoting *Ha. axyridis* may indirectly promote *He. virescens*. Particularly, a higher dominance of *Ha. axyridis* in a given ladybird community will be beneficial for ascospore transmission among individuals. The number of generations of *Ha. axyridis* in Central Europe varies from two to three and is probably dependent on habitat summer temperatures

TABLE 2 | Obtained parameters of each candidate model addressing the prevalence of infection by *Hesperomyces virescens* for different buffer radii (100 m, 300 m, 600 m, 1 km, 2.5 km).

	100 m buffer					300 m buffer				
	Estimate	Std. Error	z value	p value		Estimate	Std. Error	z value	p value	
(Intercept)	−1.597	0.507	−3.149	0.002	**	−1.652	0.480	−3.441	0.001	***
f. *axyridis*	0.299	0.768	0.389	0.697		0.303	0.769	0.394	0.693	
f. *conspicua*	−0.944	0.282	−3.347	0.001	***	−0.942	0.282	−3.344	0.001	***
f. *spectabilis*	−0.469	0.156	−2.996	0.003	**	−0.469	0.156	−2.999	0.003	**
f. *novemdecimsignata*(-)	−0.224	0.122	−1.846	0.065	.	−0.225	0.121	−1.853	0.064	.
f. *novemdecimsignata*(+)	0.020	0.170	0.117	0.907		0.022	0.170	0.128	0.898	
red	0.599	0.124	4.839	0.000	***	0.594	0.124	4.797	0.000	***
yellow	−0.663	0.154	−4.310	0.000	***	−0.665	0.154	−4.318	0.000	***
sex m	−0.003	0.102	−0.032	0.975		−0.003	0.102	−0.030	0.976	
agricultural	0.036	0.123	0.293	0.769		0.090	0.108	0.835	0.403	
forest	0.029	0.097	0.304	0.761		0.044	0.096	0.453	0.651	
Dim1	0.327	0.210	1.556	0.120		0.279	0.205	1.360	0.174	
Dim2	−0.090	0.127	−0.713	0.476		−0.100	0.122	−0.822	0.411	

	600 m buffer					1 km buffer				
	Estimate	Std. Error	z value	p value		Estimate	Std. Error	z value	p value	
(Intercept)	−1.679	0.461	−3.644	0.000	***	−1.654	0.466	−3.549	0.000	***
f. *axyridis*	0.303	0.768	0.395	0.693		0.302	0.768	0.393	0.694	
f. *conspicua*	−0.944	0.282	−3.352	0.001	***	−0.948	0.282	−3.364	0.001	***
f. *spectabilis*	−0.469	0.156	−3.005	0.003	**	−0.471	0.156	−3.017	0.003	**
f. *novemdecimsignata*(-)	−0.223	0.121	−1.839	0.066	.	−0.221	0.121	−1.824	0.068	.
f. *novemdecimsignata*(+)	0.023	0.170	0.136	0.892		0.023	0.170	0.133	0.894	
red	0.591	0.124	4.771	0.000	***	0.593	0.124	4.780	0.000	***
yellow	−0.666	0.154	−4.331	0.000	***	−0.668	0.154	−4.341	0.000	***
sex m	−0.002	0.101	−0.018	0.985		−0.002	0.101	−0.016	0.987	
agricultural	0.135	0.105	1.291	0.197		0.123	0.104	1.178	0.239	
forest	0.039	0.090	0.432	0.666		0.027	0.088	0.304	0.761	
Dim1	0.248	0.198	1.249	0.212		0.264	0.200	1.321	0.187	
Dim2	−0.113	0.120	−0.946	0.344		−0.120	0.122	−0.981	0.327	

	2.5 km buffer				
	Estimate	Std. Error	z value	p value	
(Intercept)	−1.571	0.502	−3.129	0.002	**
f. *axyridis*	0.300	0.769	0.390	0.697	
f. *conspicua*	−0.946	0.282	−3.356	0.001	***
f. *spectabilis*	−0.470	0.156	−3.005	0.003	**
f. *novemdecimsignata*(-)	−0.224	0.121	−1.841	0.066	.
f. *novemdecimsignata*(+)	0.021	0.170	0.122	0.903	
red	0.598	0.124	4.808	0.000	***
yellow	−0.665	0.154	−4.319	0.000	***
Sex m	−0.002	0.101	−0.020	0.984	
Agricultural	0.030	0.157	0.189	0.850	
Forest	0.034	0.113	0.299	0.765	
Dim1	0.334	0.216	1.544	0.123	
Dim2	−0.086	0.136	−0.634	0.526	

Significance levels at: ·$p < 0.1$, **$p < 0.01$, ***$p < 0.001$.

and prey availability. Population densities can increase due to the use of various prey patches enabled by high mobility in combination with the weak tendency for diapause extending the breeding period (Honek et al., 2018). In urbanized areas, the breeding season of *Ha. axyridis* is advanced by 2–3 weeks (Honek et al., 2021).

In addition to our buffer radius models, which are often used for forest management on a landscape scale (Brouwers et al., 2010), we also employed annulus radii to better model the behavior of *Ha. axyridis* in autumn. Our sampling localities are not where ladybirds became infected with *He. virescens*. As a result, the habitat variables might not be accurate for the habitats

TABLE 3 | Obtained parameters of each candidate model addressing the prevalence of infection by *Hesperomyces virescens* for different annulus radii (100–300 m, 300–600 m, 600 m–1 km).

	100–300 m					300–600 m					600 m–1 km				
	Estimate	Std. Error	z value	p value		Estimate	Std. Error	z value	p value		Estimate	Std. Error	z value	p value	
(Intercept)	−1.651	0.478	−3.454	0.001	***	−1.671	0.460	−3.636	0.000	***	−1.634	0.472	−3.464	0.001	***
f. *axyridis*	0.304	0.769	0.395	0.693		0.302	0.768	0.393	0.694		0.301	0.768	0.393	0.695	
f. *conspicua*	−0.943	0.282	−3.346	0.001	***	−0.946	0.282	−3.357	0.001	***	−0.949	0.282	−3.369	0.001	***
f. *spectabilis*	−0.469	0.156	−3.001	0.003	**	−0.470	0.156	−3.010	0.003	**	−0.472	0.156	−3.021	0.003	**
f. *novemdecimsignata*(-)	−0.225	0.121	−1.853	0.064	.	−0.222	0.121	−1.832	0.067	.	−0.221	0.121	−1.820	0.069	.
f. *novemdecimsignata*(+)	0.022	0.170	0.129	0.898		0.023	0.170	0.135	0.892		0.022	0.170	0.130	0.897	
red	0.594	0.124	4.795	0.000	***	0.591	0.124	4.773	0.000	***	0.594	0.124	4.789	0.000	***
yellow	−0.665	0.154	−4.320	0.000	***	−0.667	0.154	−4.333	0.000	***	−0.668	0.154	−4.341	0.000	***
sex m	−0.003	0.101	−0.030	0.976		−0.002	0.102	−0.015	0.988		−0.002	0.101	−0.016	0.987	
agricultural	0.093	0.106	0.877	0.381		0.139	0.104	1.336	0.181		0.111	0.105	1.058	0.290	
forest	0.046	0.097	0.472	0.637		0.037	0.089	0.419	0.675		0.024	0.087	0.269	0.788	
Dim1	0.275	0.204	1.348	0.178		0.249	0.197	1.262	0.207		0.279	0.201	1.388	0.165	
Dim2	−0.101	0.121	−0.829	0.407		−0.114	0.119	−0.957	0.338		−0.119	0.124	−0.964	0.335	

*Significance levels at: ·p < 0.1, **p < 0.01, ***p < 0.001.*

that the ladybirds occupied when they became infected with the fungus. Generally, we estimate that *Ha. axyridis* ladybirds fly 500 m during autumn migration. Detailed observations of ladybirds in České Budějovice, Czech Republic allowed the exact measurement of migration distance from before flight and after flight, which was 200–500 m toward the north (O. Nedvěd, unpublished). The longest flights measured were around 1,800 m in laboratory flight mills (Růžička, 1984). When Nalepa et al. (2005) conducted their experiments to test the role of visual contrast in autumn behavior of *Ha. axyridis* in 4 ha of open pasture, the required flight was about 200 m (Nalepa et al., 2005). In open fields in Japan, the median flight distance was around 400 m (Seko et al., 2008). We tested three different annulus radii (100–300 m, 300–600 m, and 600 m–1 km), but the results of our candidate models were highly similar to the buffer radius models. Aggregations of ladybirds are often formed on walls of building oriented toward the south or west (Kidd et al., 1995; Raak-van den Berg et al., 2012; Haelewaters et al., 2018b). As a result, we suggest that the annulus modeling approach could be made even more specific by obtaining landscape variables for an annulus section (or ring pie chart) facing the direction where ladybirds likely migrated from. This is methodologically complex and out of the scope of this paper, but is a consideration for future studies.

Community ecology research of Laboulbeniales is still in its infancy, with thus far only two published studies. Szentiványi et al. (2019) investigated whether climatic variables (temperature, humidity) influenced the distribution of ant- and bat fly-associated Laboulbeniales. They found that both the presence and prevalence of Laboulbeniales on their hosts were positively associated with low annual mean temperature and humidity. In addition, based on the study of more than 9,374 workers of the invasive ant *Lasius neglectus* in 66 colonies, Gippet et al. (2021) found that the presence of *Laboulbenia formicarum* on the ants was positively linked to warmer and dryer conditions at lower elevations. These

are seemingly contrary results, and our data render drawing general conclusions for these microfungi even more complex. However, the direction of the effects of some of these variables may be species-specific, as suggested by Dumolein (2021). One could make the case that the combined analysis of presence/absence data in Szentiványi et al. (2019) may obscure true interactions, and thus that separate analyses would give a more accurate picture of how bioclimatic variables affect the distribution of the two assessed study systems—the bat fly-associated *Arthrorhynchus* spp. vs. the ant-associated *Rickia wasmannii*. We note that the studies of Szentiványi et al. (2019) and Gippet et al. (2021) used outside climatic data as obtained from MERRAclim, but many of these ant–Laboulbeniales and bat fly–Laboulbeniales interactions and the host dynamics resulting in fungal transmission take place in ant nests and bat roosts, respectively. Ant nest and bat roosting environments are characterized by their own microclimatic conditions, which likely play a role in shaping the distribution of these species of Laboulbeniales. Efforts should be redirected at collecting temperature and relative humidity data (e.g., through automated readers) within these environments to test for the effect of these microclimate-specific abiotic traits on parasitism with Laboulbeniales.

Our results are the first for the *Ha. axyridis–He. virescens* study system based on specimens collected during autumn migration. Understanding the factors influencing the infection of Laboulbeniales on invasive ladybirds—including climatic and landscape variables as well as seasonality and host behavior (e.g., Raak-van den Berg et al., 2014; Haelewaters et al., 2015, 2017; Markó et al., 2016)—will help understand their global spread as they cross many different ecosystems and environmental conditions. Resolving this question will also inform potential biocontrol strategies because it will inform us under which conditions *He. virescens* may (or may not) thrive. The collection of standardized multi-year, multi-site field data will help in this regard in addition to controlled laboratory experiments.

DATA AVAILABILITY STATEMENT

The original data from this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

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