

Abiotic factors influencing the presence and prevalence of parasitic microfungi on an invasive ladybird

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Part 1: Introduction

1.1. Invasive species

1.1.1. *What is an invasive species?*

Non-native species (NNS) are organisms that have been introduced outside of their native ranges due to anthropogenic factors, such as trade and tourism. Increased globalization and climate change have caused NNS to be introduced at a far more rapid pace than ever before (Walther *et al.*, 2009; Seebens *et al.*, 2017). For example, from the 1800s to the present, the Arno River in Tuscany (Italy) saw an almost complete turnover from native to non-native fish and macroinvertebrate species mainly due to intentional introductions of NNS and anthropogenic-induced climate change (Haubrock *et al.*, 2021). Non-native species have the potential to colonize and become established in non-native ranges and spread as invasive species. Invasive species are a major cause of biodiversity loss (Clavero and Garciaberthou, 2005; Butchart *et al.*, 2010; Linders *et al.*, 2019), and, due to the fact that many become pests, also cause significant economic harm (Pimentel *et al.*, 2005; Gren *et al.*, 2009; Hoffmann and Broadhurst, 2016).

Invasive insects, in particular, make up some of the most costly and ecologically harmful invaders (Bradshaw *et al.*, 2016; Gruber *et al.*, 2022). They outcompete native insects for the same resources (Kfir, 1997; Bentivenha *et al.*, 2017; Hailu *et al.*, 2021), alter interactions between natural enemies and native hosts (Hoogendoorn and Heimpel, 2002; Fortuna *et al.*, 2022), and, in some cases, directly attack native species (O'Dowd *et al.*, 2003; Cremer *et al.*, 2006; Plentovich *et al.*, 2018). One of the main reasons why invasive insects are so disruptive to ecosystems is that many are generalists, meaning that they feed on many resources. (Snyder and Evans, 2006; Crowder and Snyder, 2010). They can often outcompete native species that are specialized on certain resources. In the case of the invasive ant *Lasius neglectus*, large densities allow them to easily overwhelm and dominate native *Lasius* species in terms of resource competition (Santaralasci *et al.*, 2014). Managing invasive insects, and species in general, is one of the most tricky and difficult problems that scientists face today as it requires intimate knowledge about how species interact, what determines their distributions, and their impact on non-native habitats (Dueñas *et al.*, 2021).

1.1.2. *Harmonia axyridis*

Harmonia axyridis (Coccinellidae, Coleoptera), commonly known as the multicolored or harlequin ladybird, is a highly polymorphic, globally invasive generalist insect (Roy *et al.*, 2016). Its resilient nature, large appetite, and success as a predator made it a popular biological control agent against aphids and coccids on crops (Camacho-Cervantes *et al.*, 2017). From the early 1900s to 1995, it was intentionally introduced from its native range in East Asia to North America and Europe as a biocontrol agent (Koch, 2003). While *Ha. axyridis* was intentionally released several times, it only became established in North America in 1985 (Gordon, 1985) and in Europe in the late 1990s (Brown *et al.*, 2008). Invasive populations in South America and Africa are thought to be the result of unintentional releases (i.e. through trading routes or tourism), likely originating from an invasive population in North America (Lombaert *et al.*, 2014). In 2016, *Ha. axyridis* was found in New Zealand (likely due to an unintentional release), making it now established in every continent except Antarctica (Camacho-Cervantes *et al.*, 2017, Figure 1).

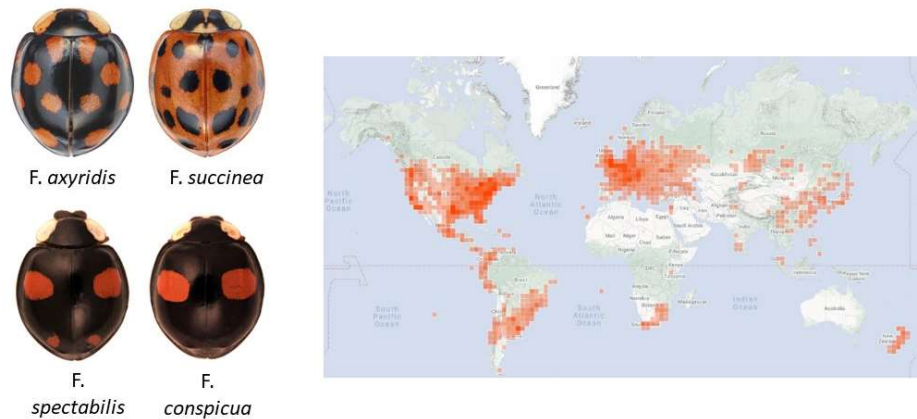


Figure 1. *Harmonia axyridis* most common color morphs and current distribution taken from INaturalist May 2nd, 2023.

After establishment, *Ha. axyridis* spreads rapidly (Brown *et al.*, 2011). From 2004 to 2006, after its initial release, *Ha. axyridis* abundance increased by an average factor of 2.8 in Great Britain (Brown *et al.*, 2008). In its non-native range, *Ha. axyridis* dramatically changes ladybird community structures by outcompeting native beetle species and through intraguild cannibalism (Roy *et al.*, 2016). It is thought to be responsible for a decrease in seven out of eight native ladybird populations found in the United Kingdom (Roy *et al.*, 2012). In Belgium, it has caused a 57% decline in the occurrence of the native ladybird *Adalia bipunctata* (Adriaens *et al.*, 2015). In addition to the ecological harm *Ha. axyridis* inflicts, it is also considered a pest in many areas. *Harmonia axyridis* commonly overwinters in large aggregates inside buildings and homes where it is considered a nuisance (Nalepa, 2007). During autumn, *Ha. axyridis* clusters around wine grapes in vineyards just before they are ready to be harvested. When the grapes are harvested, *Ha. axyridis* reflex bleeds onto the wine grapes, which subsequently taints the wine made from those grapes (Botezatu *et al.*, 2013). While *Ha. axyridis* has been the topic of numerous invasive species studies, there are still many questions about how it affects ecosystems. A recently published roadmap to ladybird conservation identifies several key threats to ladybirds, one of which are invasive species, and highlights the need for an increased understanding of its impacts on native populations (Soares *et al.*, 2023).

The fact that *Ha. axyridis* thrives in non-native environments may in part be attributed to the enemy release hypothesis, which states that invasive species rapidly increase in abundance and distribution in invaded ranges due to a lack of specialized natural enemies (Jeffries and Lawton, 1984; Keane and Crawley, 2002; Roy *et al.*, 2011). Populations of *Ha. axyridis* have been shown to be more resistant to natural enemies than native ladybirds (Shapiro-Ilan and Cottrell, 2005; Roy *et al.*, 2008; Berkvens *et al.*, 2010; Gross *et al.*, 2010). However, invasive species may lose their ability to resist natural enemies as natural enemies adapt to their presence over time (Siemann *et al.*, 2006; Schultheis *et al.*, 2015). *Harmonia axyridis* is parasitized by several organisms, including nematodes, wasps, mites, and fungi in its non-native ranges (Haelewaters *et al.*, 2017; Knapp *et al.*, 2019). While some advances have been made, there are still large knowledge gaps about the enemies affecting *Ha. axyridis* and how they might relate to its wide expansion and future distribution.

1.2. Laboulbeniomycetes

1.2.1. General biology

Laboulbeniomycetes is an enigmatic class of fungi consisting of three orders (Pyxidiophorales, Herpomycetales, and Laboulbeniales) and two unnamed clades (Haelewaters *et al.*, 2021). Pyxidiophorales are mycoparasitic, while Herpomycetales and Laboulbeniales are obligate ectotrophs on arthropods. Historically, Herpomycetales was placed within Laboulbeniales because of their similar characteristics, but based on developmental, micromorphological, and host usage data, Herpomycetales was eventually placed into a separate order (Haelewaters *et al.*, 2019). Instead of hyphae, representatives of Herpomycetales and Laboulbeniales form three-dimensional structures, thalli (Blackwell *et al.* 2020), directly from 2-celled ascospores that attach to the integuments of their hosts. These thalli exhibit determinate growth, unlike most fungi that grow indeterminately. Some species form penetrating structures known as haustoria to attach to their hosts and extract nutrients, while others do not. Herpomycetales contains only one genus (*Herpomyces*) that exclusively infects cockroaches and termites (Blattodea), while Laboulbeniales currently includes 145 genera of which representatives infect numerous orders of arthropods (Haelewaters *et al.*, 2021, Figure 2).

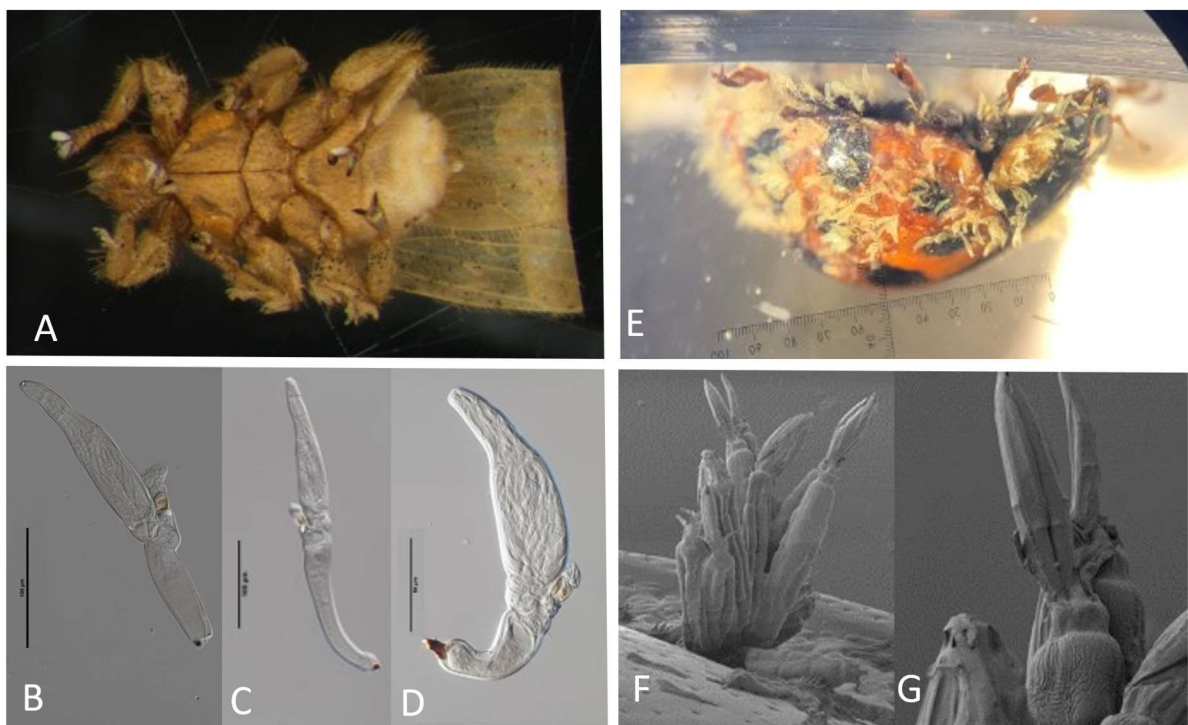


Figure 2. (A) Bat fly *Trichobius uniformis* infected by multiple *Gloeandromyces cusucoensis* on legs and wings (B-D). *Gloeandromyces diversiformis* f. *diversiformis*, f. *vanillicarpiformis*, and f. *musiformis* (E). *Harmonia axyridis* infected by *Hesperomyces harmoniae* (F-G). Scanning electron microscope pictures of sporulating *Hesperomyces adaliae* nom. prov. taken in the Laboratory of Electron Microscopy at the University of South Bohemia, České Budějovice. Scales: B-C = 100 μ m, D = 50 μ m.

Laboulbeniales fungi spread mainly by physical contact between an infected and uninfected host, but it can also spread through auto-infection caused by mature thalli already present on the host (De Kesel, 1993). Spores are secreted from the perithecium, a larger cell forming the sticky foot upwards, and attach

onto the host integument, where they then develop to maturity. Laboulbeniales species that have a haustorium are believed to be host-specific whereas those without these structures are more generalist (Haelewaters *et al.*, 2022).

The class Laboulbeniomycetes has been an enigma to mycologists and biologists. Some believed that the group broke off from floridean red algae (Karsten, 1869; Cohn, 1872; Sachs, 1874), while others thought of them as parasitic worms (Kolenati, 1857). Roland Thaxter (1890) was one of the first mycologists to identify members of these groups as fungi belonging to Ascomycota (historically ascomycetes). In addition, he was the first to observe the direct growth of a thallus from an ascospore (Thaxter 1896). Thaxter's *Contribution towards a monograph of the Laboulbeniaceae* was an important foundation in the study of Laboulbeniomycetes (Thaxter, 1895, 1908, 1924, 1926, 1931). Today, recent advances in molecular methods have helped uncover many of the mysteries that plagued early laboulbeniologists and have given us a greater insight into the world of Laboulbeniomycetes (Haelewaters *et al.*, 2015, 2019; Haelewaters *et al.*, 2018; Sundberg *et al.*, 2018).

1.2.2. *Hesperomyces harmoniae*

Hesperomyces harmoniae is a parasitic fungus in the order Laboulbeniales that infects members of the genus *Harmonia*, including *Ha. axyridis* (figure 3). *Hesperomyces harmoniae* is part of the species complex *He. virescens* that consists of multiple species that are specific to various ladybird genera. Discovered in 2002 on *Ha. axyridis* in Ohio (Garcés and Williams, 2004), it was referred to as *He. virescens* until its recent formal description based on host, morphology, DNA sequencing data, and species delimitation analyses (Haelewaters *et al.*, 2018; Haelewaters *et al.*, 2022).

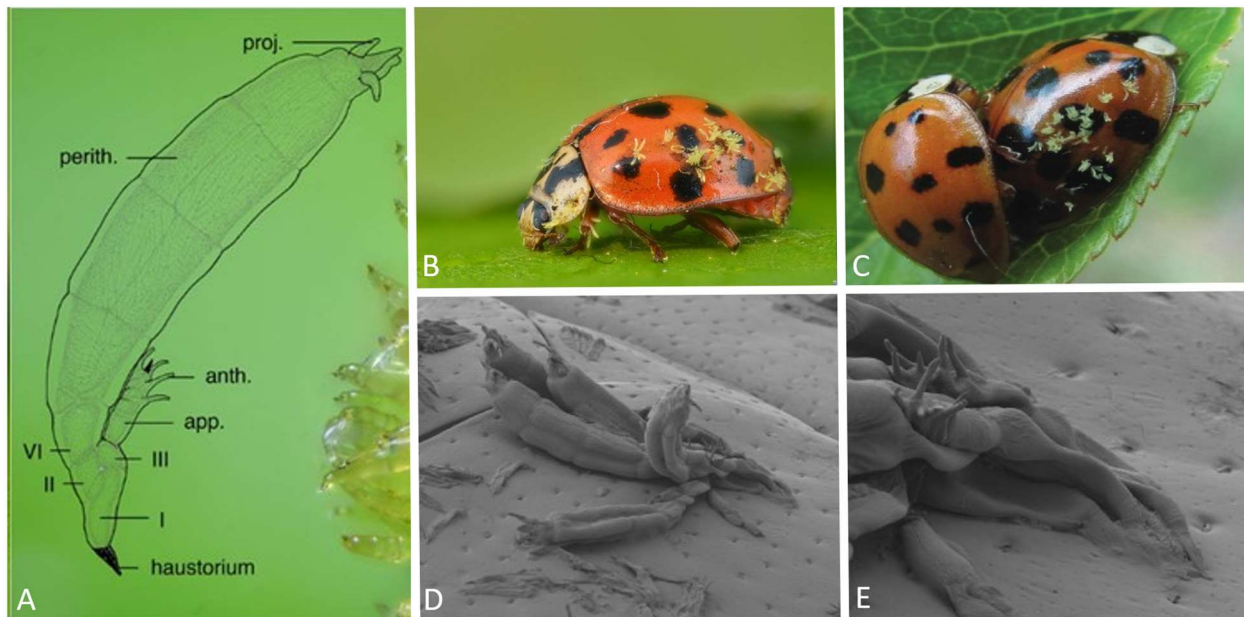


Figure 3. (A) Anatomical drawing of *He. harmoniae* with the following structures annotated: haustorium; cells I, II, III, and VI; the appendage (app.) with antheridia (anth.); and the perithecium (perith.) with its terminal projections (proj.). Drawing by André De Kesel. (B-C) *Harmonia axyridis* infected by *Hesperomyces harmoniae*. Photos by G. San Martin and iNaturalist #43924977 by G. Okatov, 27 April 2020, respectively. (D-E) Scanning Electron Microscope

image of *He. harmoniae* infecting *Ha. axyridis* with attachment point. Photo taken in the Laboratory of Electron Microscopy at the University of South Bohemia, České Budějovice.

The prevalence of *He. harmoniae* on *Ha. axyridis* differs depending on the locality, but it can be as high as 90-100% (Haelewaters *et al.*, 2017, supplemental information). Because *He. harmoniae* requires direct contact to infect *Ha. axyridis*, it is mainly spread through mating or contact in overwintering aggregations. This can lead to differential rates of infection and distribution of thalli depending on the season, behavior, and gender of the ladybirds. In autumn and late winter, ladybirds are more likely to be infected on their elytra than other parts (Riddick and Schaefer, 2005; Riddick, 2006), although other infection locations can be observed such as between claws, antennae, or head (Nalepa and Weir, 2007, personal observations). In summer, females were found to be more infected on their elytra than males who had infections on the meso- and metathorax, abdomen, midlegs, hindlegs, and elytra (Riddick, 2006). In spring, fungal thalli were commonly found on the head, antennae, and mouthparts of ladybirds (Nalepa and Weir, 2007). Due to the high level of promiscuity in *Ha. axyridis*, the large prevalence of thalli on elytra is most likely due to females mating with infected males, and uninfected males unsuccessfully attempting to mate with infected males (Ceryngier and Twardowska, 2013). Older ladybirds tend to be more heavily infected than younger ones, likely due to increased exposure with *He. harmoniae* throughout their lives (Fiedler and Nedvěd, 2019). Infection status can also vary between color morphs, with melanized morphs (*conspicua* and *spectabilis*) being less infected than non-melanized forms (*novemdecimsignata*) (Haelewaters *et al.*, 2022).

Hesperomyces harmoniae negatively affects *Ha. axyridis* in several ways. Thalli of *He. harmoniae* penetrate their ladybird hosts with a haustorium to make contact with the body cavity (hemocoel) and draw nutrients from it (Weir and Beakes, 1996). Depending on the position of the infection on the integument, individuals may experience decreased mobility and sensory capabilities (Nalepa and Weir, 2007; Haelewaters *et al.*, 2017), although this has never been formally tested. During winter, infected males experience increased mortality (Riddick, 2010; Knapp *et al.*, 2022). Interestingly, Knapp *et al.* [2022] found that *He. harmoniae* does not have a large impact on the immune system of *Ha. axyridis*, but it did significantly lower body mass post-overwintering (contrasting Nalepa and Weir, 2007), suggesting that mortality may be due to energy exhaustion.

Co-infections with *He. harmoniae* and other parasites show varying results. *Hesperomyces harmoniae* infection causes an increase in antimicrobial activity, suggesting that it could be somewhat beneficial (Knapp *et al.*, 2022). However, Awad *et al.* [2023] found that *Spiroplasma* bacteria and *He. harmoniae* infections were independent of each other, and female ladybirds who were double-infected with both had significantly lower fecundity and hatchability compared to ladybirds only infected with one parasite. Ladybirds infected with the parasitic mite *Coccipolipus hippodamiae*, and *He. harmoniae* had lower survival rates compared to uninfected beetles (Riddick, 2010). Double infections with the parasitic nematode *Parasitylenchus bifurcatus* and *He. harmoniae* are also found in several regions on *Ha. axyridis*, however, the consequences of double infection are still unknown (reviewed in: de Groot and Haelewaters, 2022). Experiments with the North American native ladybird *Olla v-nigrum* and *Ha. axyridis* co-infected with *He. harmoniae* and either entomopathogenic fungus *Beauveria bassiana* or *Metarhizium brunneum* found that *O. v-nigrum* suffered increased mortality when double-infected, but *Ha. axyridis* did not (Haelewaters *et al.*, 2020).

1.3. Parasitology and Laboulbeniology

There is often a negative connotation regarding parasites and their interactions with other organisms. As a result, parasites have historically been understudied and also underrepresented in biodiversity conservation (Brooks and Hoberg, 2001; Carlson *et al.*, 2017). However, parasites are examples of successful biological evolution and are essential in the functioning of natural ecosystems. Thus, parasites as a group represent both one of the most successful evolutionary experiments while at the same time are also among the most threatened groups of organisms in the face of global change: they must not only adapt to a changing climate but also to the disappearance of their host organisms. An increasing scarcity of host species accompanied by rapid environmental change may result in the extinction of many parasites.

In their paper “A Global Parasite Conservation Plan”, Carlson *et al.* [2020] outlined 12 goals that researchers should follow in the next decade to protect and conserve parasites. Four of these goals are dedicated to collecting, analyzing, and recording (meta-) data on parasites and their hosts to correct the current lack of documentation (Carlson *et al.*, 2020). It is estimated that the majority of parasites living on earth have yet to be discovered and documented. This is especially true with regard to fungal parasites. It is estimated that there are 2.2-3.8 million species of fungi in the world and that we have only named and described 3-8% of the total biodiversity (Hawksworth and Lücking, 2017). For insect-associated fungi, in particular, we only know an estimate of 1.5% of their total diversity (Mueller and Schmit, 2007). Therefore, the foremost important step in protecting fungal parasites from extinction is gathering as much knowledge about them as possible.

Laboulbeniales are difficult to work with because of their small sizes, heavily-melanized cells that interfere with PCR, and inability to be cultured on axenic media (Haelewaters *et al.*, 2015; Sundberg, *et al.*, 2018; Haelewaters *et al.*, 2021), making them a severely understudied group of organisms. Most studies have focused on species delimitation (Haelewaters *et al.*, 2018; Haelewaters and Pfister, 2019), alpha taxonomy, and evolutionary relationships within the Laboulbeniales (Goldmann and Weir, 2018; Haelewaters *et al.*, 2022). Also, only preliminary, and sometimes contradictory, data are available on how variations in local abiotic factors such as temperature and humidity affect the prevalence of parasitism with Laboulbeniales fungi (Markó *et al.*, 2016; Szentiványi *et al.*, 2019; Gippet *et al.*, 2021; Haelewaters *et al.*, 2022). Thus, the goal of this research is to further our understanding of how variation in abiotic factors affect Laboulbeniales using *He. harmoniae*. This work is significant as it can help increase our overall understanding of fungal parasites, and how invasive species (both *Ha. axyridis* and potentially *He. harmoniae*) spread and interact in ecosystems.

Part 2: Objective

The primary objective of this research is to test the effects of different humidities and temperatures on the growth of *Hesperomyces harmoniae* on *Harmonia axyridis*. The following hypotheses have been proposed:

Hypothesis 1: *Hesperomyces harmoniae* will mature more quickly at low temperatures.

Rationale 1: Szentiványi *et al.*, [2019] found that the prevalence of two distantly related Laboulbeniales species, *Arthrorhynchus* sp. on *Nycteribia schmidlii* bat flies and *Rickia wasmannii* on *Myrmica scabrinodis* ants, was highest in habitats with low annual mean temperature. This suggests that Laboulbeniales species prefer lower temperatures. If *He. harmoniae* follows this trend, then it should show increased growth at lower temperatures.

Hypothesis 2: Humidity will not have a significant effect on *Hesperomyces harmoniae* growth.

Rationale 2: *He. harmoniae* penetrates *Ha. axyridis* with a haustorium and uses it to draw nutrients (Haelewaters *et al.*, 2021). As long as *Ha. axyridis* has access to water, even at low humidity conditions *He. harmoniae* will have a sufficient supply of water to grow and mature. Similarly, if *He. harmoniae* relies solely on *Ha. axyridis* for hydration, high humidity will not affect its growth.

Hypothesis 3: There will be no differences in *He. harmoniae* infection between male and female *Ha. axyridis*.

Rationale 3: Field studies so far have shown that there is no difference in the probability of *He. harmoniae* infection between male and female *Ha. axyridis* (Riddick and Schaefer, 2005; Riddick, 2006; Haelewaters *et al.*, 2018, 2022). Following this logic, there should be no difference in the growth of *He. harmoniae* between male and female ladybirds.

Hypothesis 4: *Hesperomyces harmoniae* will mature more quickly on melanic forms of *Ha. axyridis*.

Rationale 4: Melanic *Ha. axyridis* produce less defensive alkaloids than non-melanic forms (Bezzerides *et al.*, 2007). Higher levels of these alkaloids may inhibit the growth of *He. harmoniae*. Therefore, *He. harmoniae* should mature and grow faster on melanic forms of *Ha. axyridis* compared to non-melanic forms because of their lower alkaloid concentration.

Part 3: Materials and Methods

3.1. Rearing

Laboratory colonies of *Ha. axyridis* were used to rear uninfected, *target*, ladybirds for infection. All ladybirds were fed three days a week with pea aphids (*Acyrtosiphon pisum*) that were reared on *Vicia faba* and a 1:2 mix of *Ephestia* eggs + pollen. Larvae were kept in a photoperiod of 18L:6D at 22 °C, 27 °C, and 22 °C (six hours period at each temperature) during the photophase and 17 °C during the scotophase. Pupae, adults, and infected ladybirds were kept at a constant 17 °C.

3.2. Infection

Target ladybirds were chosen from a new generation of reared, two month old ladybirds for infection via tumbling using a hot dog roller (Royal Catering, <http://www.cateringroyal.de/en/content/hot-dog-grills>), following procedures by Cottrell and Riddick [2012] and Haelewaters *et al.* [2020]. Using forceps, highly infected (≥ 50 thalli) *source* and non-infected *target* ladybirds were added to a glass vial, coated in fluon. For trial one, each vial was labelled with the intended treatment and contained a target/source ratio of 4:1. For trial two, a target/source ratio of 6:2 was added to each vial. Vials were sealed with parafilm and tumbled with a hot dog roller to force the ladybirds to touch one another and ensure sufficient contact with thalli. The vials were tumbled for one hour and repeatedly tapped every 20 minutes to keep source and target beetles in contact. In trial one, target ladybirds were immediately transferred to a separate Petri dish each with a wet cotton strip and *Ephestia* eggs + pollen mix, and placed in their respective treatment group. In trial two, target ladybirds were randomly placed into a treatment group and kept with source ladybirds in large Petri dishes for 13 days before being separated into individual dishes. For trials one and two, 12 ladybirds were used for each treatment group. In trial one, each group contained eight non-melanic (*succinea*) and four melanic ladybirds (*axyridis* and *spectabilis*). In trial two, ladybirds in all treatment groups were composed of *succinea*. An extra treatment group consisting of 12 non-melanic *spectabilis* ladybirds were added to the control conditions to test the differences in melanization on *He. harmoniae* growth.

3.3. Monitoring

1.3.1. 3.3.1. Trial one

Target ladybirds were assigned to five treatment groups: high humidity (17 °C/90% RH), low humidity (17 °C/30% RH), high temperature (25 °C/60% RH), low temperature (5 °C/60% RH), and control (17 °C/60% RH) (Figure 4). Humidities were adjusted using salt water and potassium hydroxide (KOH). Each incubator had a temperature and humidity probe to ensure conditions were constant. Probes were randomly swapped between different incubators every week. To keep ladybirds exposed to incubator conditions, the lids of Petri dishes used for housing target ladybirds were cut open and covered with a mesh film. Ladybirds were fed three times a week using pea aphids and *Ephestia* eggs + pollen mix and given a wet cotton cloth to drink from. Ladybirds in the low humidity group were given glass vials filled with water and cotton instead as the cotton cloth often dried too quickly. All trials except the low-temperature condition were checked every day for mature thalli using a stereomicroscope until day 42, afterward they were checked

roughly every three days until day 72. As the ladybirds were still alive during monitoring, they were kept still by placing them on the underside of a Petri dish and with a lid over them. A thallus was determined to be mature if it had fully formed projections on its perithecium (Figure 3). The low temperature condition was monitored twice a week using a hand lens to prevent disturbance of the ladybirds.

1.3.2. 3.3.2. Trial two

In trial two, the temperatures were raised to 25 °C for the control, 17 °C for the low temperature, and 30 °C for the high temperature treatments (Figure 4). The humidities were kept the same as in trial one. Only ladybirds in the low humidity group (25 °C/30% RH) were kept in mesh covered Petri dishes while all others were transferred to unaltered Petri dishes. Feeding frequency did not change, but all ladybirds were only fed with pea aphids. All ladybirds were screened for mature thalli every day with a stereomicroscope after being separated into individual Petri dishes for 28 days. The technique used to immobilize ladybirds for screening in trial one was determined to be too harsh for the ladybirds, so they were instead enclosed in a Petri dish filled with cotton. The sex of the ladybirds used in this trial was determined by following the methods of McCornack *et al.* [2007]. As sex was not a variable that was taken into account when selecting ladybirds for the experiments, each group had an unequal ratio of males and females.

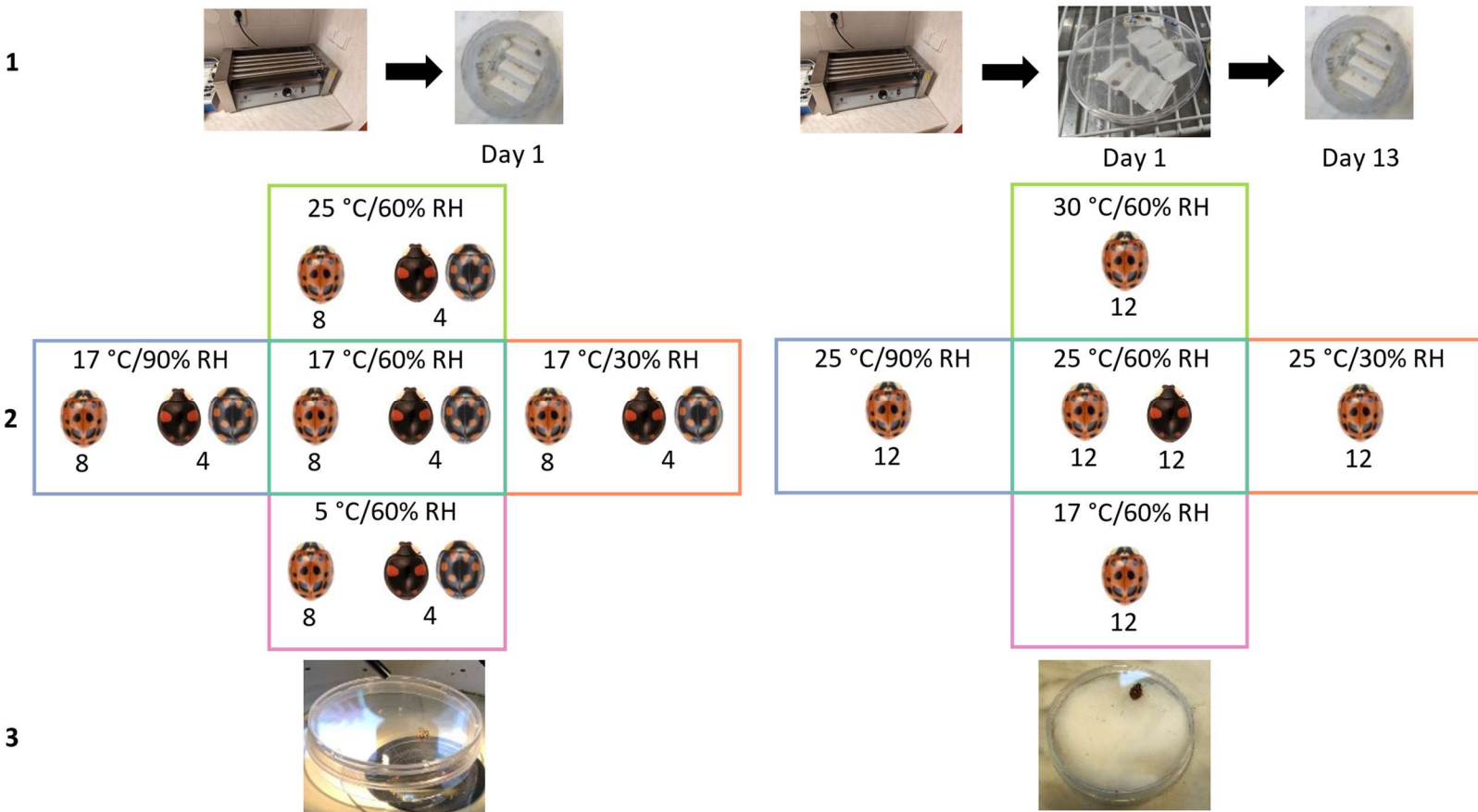


Figure 4. Graphical summary of methods used in trial 1 (left) and trial 2 (right). The following steps are labeled: **(1)** Infection and housing, **(2)** Incubator setup, and **(3)** Monitoring.

3.4. Statistical analyses

The updated methods in trial two resulted in a greater number of mature and immature infections, so only the results of that trial were included in the statistical analyses. Data were analyzed in R using the package *survival* (Therneau and Grambsch, 2000). To test whether there was a significant difference in time to infection, multivariate analyses were performed using the Cox proportional hazards test (Kaplan and Meier, 1958; Cox, 1972; Agresti, 2002). The influence of sex, treatment group, and melanic vs. non-melanic forms was included in this analysis. Model assumptions were checked with the package *survival* and figures were produced using the package *ggplot2* (Wickham, 2016).

Differences between the curves were assessed using the 'compareGrowthCurves' function of the *statmod* software package for R (<http://bioinf.wehi.edu.au/software/compareCurves>). This function performs pairwise comparisons using a permutation test for all treatments (Phipson and Smyth, 2010). The number of permutations performed was 1000. P-values were adjusted for multiple testing using the Bonferroni–Holm method.

Part 4: Results

4.1. Overview of results

1.3.3. 4.1.1. Trial one

The low temperature treatment (5 °C/60% RH) was omitted from the experiment on day 42 due to difficulties in maintaining the ladybirds at that temperature while also screening them. None of the ladybirds showed signs of infection before they were omitted. Statistical analyses were not performed on this trial because of the poor infection rates compared to trial two. The results of trial one are presented in Figure 5.

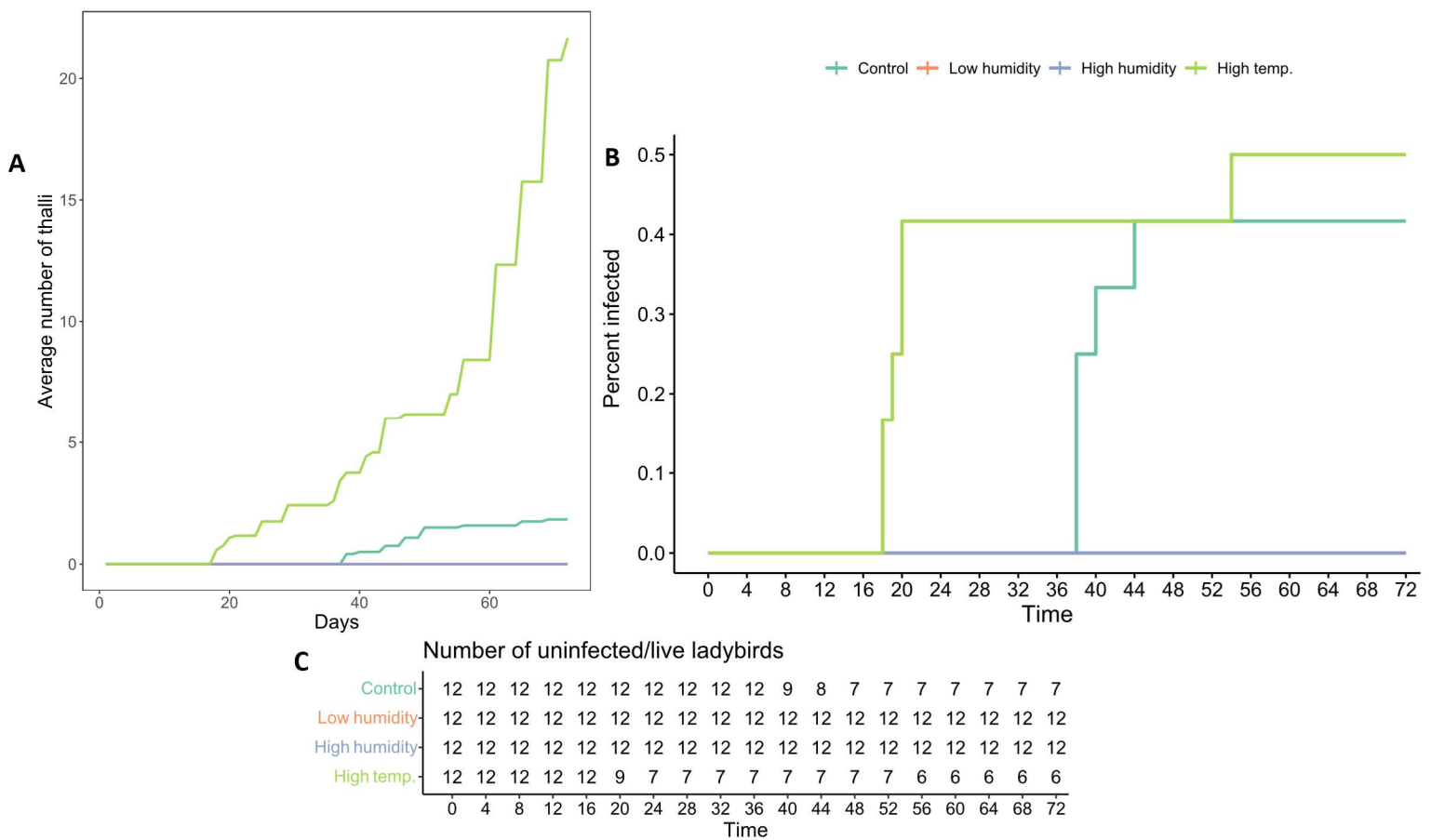


Figure 5. (A) Average number of mature thalli per ladybird found over time in each treatment group **(B)** Infection curves showing the percentage of ladybirds in each group infected over time. **(C)** Table showing the number of uninfected and live ladybirds at each time point depicted in the graphs.

1.3.4. 4.1.2. Trial two

Observations for trial two began on day 14 after the ladybirds were separated into individual containers. Before separation the following deaths occurred: one ladybird in the control, one in the high humidity, and five in the melanic group. All dead ladybirds were screened and determined to be unaffected. Additionally, the following infected source ladybirds died before separation: two in high temperature, two in high humidity, one in low temperature, three in non-melanic, and three in melanic. An extra infected ladybird, likely due to a counting error, was found in the low temperature treatment and kept in the experiment. An overview of the results is presented in Figure 6.

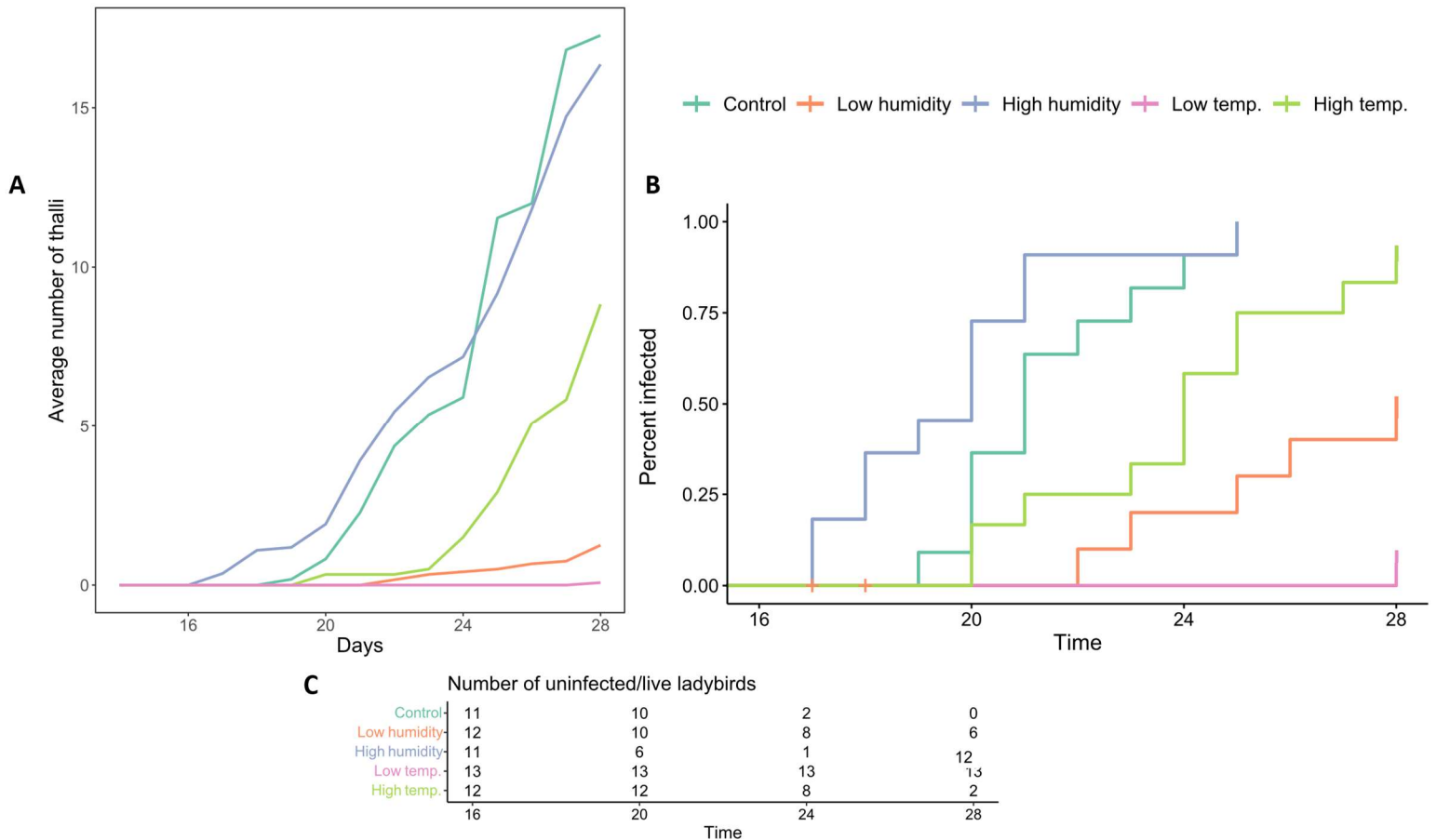


Figure 6. (A) Average number of mature thalli per ladybird found over time in each treatment group. Time starts on day 14 and ends on day 28. **(B)** Infection curves showing the percentage of ladybirds in each group infected over time. Pluses represent dead, uninfected ladybirds. **(C)** Table showing the number of uninfected and live ladybirds at each time point depicted in the graphs.

4.2. Growth curve analyses

Using pairwise comparisons, the differences between growth curves (figure 6A) were assessed. The control, high humidity, and high temperature groups were not significantly different from each other ($p = 0.062-0.97$), although the high temperature had the lowest p -values in all comparisons with the control and high humidity ($p = 0.062$). All other groups were significantly different ($p = 0.005-0.007$). There were no differences between the melanic and non-melanic groups ($p = 0.9$).

4.3. Cox proportional hazards

The Cox proportional hazards model tests how specified factors influence the rate of a particular event happening (e.g., infection) at a particular point in time (Fox and Weisberg, 2018). The results of these tests are represented in a hazard ratio (HR) that compares the probability of infection to the control group. An HR of smaller than one indicates a lower chance of infection, above one means that there is a higher chance, and exactly one means there is no difference in time to infection. The results of these tests are summarized in Table 1. Additionally, not listed in the table, there were no significant effects of melanization on infection probability in the control conditions ($p = 0.32$).

	Hazard ratio
Low humidity	-2.368*** (-3.607, -1.130)
High humidity	0.540 (-0.384, 1.464)
Low temperature	-4.514*** (-6.660, -2.369)
High temperature	-1.094** (-2.010, -0.177)
Sex (male)	-0.116 (-0.827, 0.594)
Observations	59
R2	0.642
Max. Possible R2	0.991
Score (Logrank) Test	65.610*** (df = 5)
Note:	* $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$

Table 1. Summary of Cox proportional hazards test

Part 5: Discussion

5.1. Infection success: Trial one vs. trial two

Trial two was undoubtedly more successful than trial one at producing infected ladybirds. In trial one, only a total of 10 out of the 60 ladybirds in all the treatments showed signs of infection, whereas, in trial two, a majority of the ladybirds in most treatments were infected (Figure 5 and 6). The major differences in infection success were most likely due to the fact that in trial two, target ladybirds were kept in the same dishes as source ladybirds for two weeks, and all treatment temperatures were raised. The increased exposure time to *He. harmoniae* resulted in more infections throughout the experiment. The effects of temperature on *He. harmoniae* growth is discussed in section 5.2.

The downside to keeping target and source ladybirds together is that it is more difficult to determine the exact amount of time a *He. harmoniae* spore takes to reach maturity after attachment. We assumed that the earliest mature thalli attached during tumbling and all subsequent mature thalli attached in the shared dishes. Thus, the time between the start of the experiment and the first observed mature thallus represents the total amount of time a thallus takes to develop. For some conditions, namely low temperature (17 °C /60% RH), spores could still be seen attached to the integument of the ladybirds even after two weeks inside the shared containers. Assuming the only variation in the development of thalli at different temperatures is time, the development of *He. harmoniae* can be tracked clearly in low-temperature conditions. So far, growth observations in Laboulbeniales have been limited to what can be stained on a slide (Faull, 1911; Tavares, 1966). It could be beneficial for future research to observe how a thallus grows directly on its host.

5.2. Effects of abiotic factors

Obligate parasites are highly dependent on their hosts for growth, survival, and reproduction. Laboulbeniomycetes, cannot grow without a living host (Richards and Smith, 1954; Whisler, 1968) meaning that changes in the conditions of their host species will affect their fitness. There was a significantly lower probability of infection at 30 °C (HR = -1.094 (-2.010, -0.177)) compared to 25 °C, but there were no differences between their growth curves. This means that there was a lag time in the appearance of mature thalli in the 30 °C conditions, but they grew at the same rate as the control. This lag time could be due to a decrease in fitness in *He. harmoniae*, *Ha. axyridis*, or both at 30 °C.

While *Ha. axyridis* is capable of occupying many areas outside of its native range (Koch *et al.*, 2006; Lombaert *et al.*, 2014) in high temperatures (>30 °C) they suffer from lower survival, dry body mass, and size during developmental stages (Lamana and Miller, 1998; Knapp and Nedvěd, 2013; Zhang *et al.*, 2014; Benelli *et al.*, 2015). Additionally, *Ha. axyridis* individuals have been found in Saudi Arabia, a region with summer temperatures often 35 °C and above, but there has never been an established population (Biranvand *et al.*, 2019). High temperature stress causes a decrease in fitness in *Ha. axyridis* which could lead to negative effects for *He. harmoniae*.

Compared to *Ha. axyridis*, data on the climatic distribution of *He. harmoniae* is sparse. Most of the studies on the prevalence of *He. harmoniae* on *Ha. axyridis* come from North America and Europe in

areas that are mainly temperate. While there have been reports of infections in South America, Africa, and Asia (Haelewaters *et al.*, 2014; Haelewaters *et al.*, 2016; Haelewaters *et al.* 2022), there is a lack of prevalence data in sub-tropical and tropical regions to compare the data that we have on temperate regions too. Other climatic studies on Laboulbeniales have been contradictory so far with some suggesting a preference for low annual mean temperature (Szentiványi *et al.*, 2019), or high temperatures (Gippet *et al.*, 2021). Coupled with the fact that it is impossible to grow *He. harmoniae* without its host, it is difficult to determine the exact effect of high temperatures on the growth of *He. harmoniae*.

The slowest growths were observed in the low temperature (17 °C/60% RH) and low humidity (25 °C /30% RH) conditions. These observations fail to support hypothesis one, which stated that lower temperatures would see faster growth, and two, which postulated that humidity would not affect the growth of *He. harmoniae*. These observations may have different mechanisms underlying them, however. The ladybirds in the low temperature conditions had a large number of visible immature thalli and spores whereas those in the low humidity conditions had a noticeable lack of both. This suggests that low humidity had a significant effect on the ability of *He. harmoniae* to infect *Ha. axyridis*. De Kesel [1997] found that Laboulbeniales species infecting Carabidae beetle species preferred hosts that inhabit moist soils over ones in dry or fresh forest soil, but their prevalence was not significantly affected by habitat humidity in most cases. From these results, it was concluded that in more humid environments, Laboulbeniales species have a better opportunity to infect hosts, but their growth was not hindered by dry conditions. In our experiment, the spores that remained on the ladybirds still reached maturity, meaning that the low humid conditions were not completely lethal to *He. harmoniae*. This suggests that humidity may only interfere with the ability of ascospores to stick onto the integument of the ladybirds, but not in the development of *He. harmoniae*. This hypothesis is also supported by the fact that high humidity did not have a significant effect on the timing nor growth of *He. harmoniae* infection compared to the control group in this experiment. In our experiment, we did not keep a count of how many spores and/or immature thalli were present in each treatment. To test how significant these differences are, future experiments should incorporate these counts.

Hesperomyces harmoniae matured the slowest in low temperature conditions. Unlike the low humidity treatment, 17 °C did not cause any noticeable hindrance to spore attachment. Given enough time, the immature thalli will most likely become fully mature, as shown in the results of the control conditions (17 °C/60% RH) in trial one. The reason for this slower growth may be tied to the life history of *Ha. axyridis*. *Harmonia axyridis* overwinters in aggregations for 3-6 months depending on seasonal conditions (Honek *et al.*, 2022). This behavior is regulated by both external (change in daylengths and temperature) and internal factors. *Hesperomyces harmoniae* could similarly react to changes in environmental and host conditions by slowing its growth. If *He. harmoniae* continued to grow at the same rate when *Ha. axyridis* is in diapause, it risks depleting its energy reserves and killing its host before winter is over. This could prevent *He. harmoniae* from infecting other ladybirds. Knapp *et al.*, [2022] found that *He. harmoniae* increases winter mortality in *Ha. axyridis* mainly through energy exhaustion, showing that *He. harmoniae* does pose a significant threat to the survival of *Ha. axyridis* during winter. Thus, *He. harmoniae* would benefit from reducing energy consumption so it can continue to infect other ladybirds after winter.

5.3. Effects of biotic factors

There were no significant differences in sex on time to infection. Previous studies have shown that there is no difference in the probability of *He. harmoniae* infection between male and female *Ha. axyridis* (Riddick and Schaefer, 2005; Riddick, 2006; Haelewaters *et al.*, 2018, 2022), so our results align with these findings and support hypothesis three. As *He. harmoniae* spread through contact, male and female ladybirds have an equal chance of becoming infected. Our observations suggest that there are no significant differences in immune responses between male and female *Ha. axyridis* that can prevent *He. harmoniae* infection and growth.

There were also no significant differences in melanization on time to infection between melanic and non-melanic growth curves, which does not give support to our fourth hypothesis that stated that melanic ladybirds will have mature infections earlier. The influence of melanization on *He. harmoniae* is still a mystery, however. Haelewaters *et al.* [2018] hypothesized that *He. harmoniae* infection would be positively associated with melanization because melanization is negatively correlated with alkaloid production (Bezzarides *et al.*, 2007). These alkaloids are heavily involved in defensive and anti-microbial activities (Röhrich *et al.*, 2012). Their data did not support this hypothesis, and future studies would find that non-melanic forms and less melanized *succinea* forms of *Ha. axyridis* actually carried more infections (Fiedler and Nedvěd, 2019; Haelewaters *et al.*, 2022). Our results showed that there is no difference in the time to infection nor growth of *He. harmoniae* between melanic and non-melanic ladybirds. This leads to the question: why are there more infections in non-melanic ladybirds in the wild if melanization does not affect thalli development?

Over the course of our experiment, 6 out of the 12 melanic ladybirds used for the experiment died. None of these ladybirds had mature thalli on them, but perhaps the beginning stages of infection posed a significant immune challenge which led to increased mortality in this group. As melanization is negatively correlated with alkaloid production, and therefore anti-microbial defenses, we hypothesize that penetration by spores of *He. harmoniae* could allow microbes to more easily infect melanic ladybirds leading to an increase in mortality. Knapp *et al.* [2022] tested the immune response of *He. harmoniae* infected *Ha. axyridis* during winter. They found that *He. harmoniae* only had a limited effect on the immune system of *Ha. axyridis*, except for an elevated response against *E. coli*. However, they did not compare non-melanic and melanic forms of *Ha. axyridis*. To find the effects of *He. harmoniae* on the immune system of melanic *Ha. axyridis*, I suggest repeating this experiment with an emphasis on differences in immune responses between melanic and non-melanic forms.

5.4. Wider implications

The largest impact this research has is on our ability to predict what could happen to *He. harmoniae* if climate became more extreme. *Harmonia axyridis* is a highly polymorphic species capable of adapting to climates outside of its preference (Grill *et al.*, 1997). CLIMEX models suggest that *Ha. axyridis* will increase in both abundance and geographic range as climate change worsens (Evans and Simpson, 2010). *Hesperomyces harmoniae* is negatively affected by temperatures 30 °C and above because of lower fitness in *Ha. axyridis*. Consistently higher temperatures could decrease the infectivity of *He. harmoniae*. However, longer and harsher winters may provide *He. harmoniae* with more opportunities to infect *Ha. axyridis* by increasing the time they spend overwintering in aggregations. This increased

overwintering time could offset the decreased growth experienced by *He. harmoniae* in cold temperatures. Increasingly humid conditions could provide better chances for *He. harmoniae* to spread in historically dry areas, allowing it to infect *Ha. axyridis* in previously unsuitable habitats. How *He. harmoniae* will respond to more extreme temperatures is still uncertain and will require further research into its physiological responses to different climates.

5.5. Future research

There are still many questions regarding *He. harmoniae* growth and maturation in different conditions. We suggest several ideas to improve future research in this field:

1. Increase sampling and prevalence studies outside of temperate areas.
2. Incorporate spore and immature thalli counts in future studies involving abiotic factors.
3. Include both melanic and non-melanic forms of *Ha. axyridis* in studies on the physiological responses to *He. harmoniae* infections.

It should be noted that the exact mechanisms behind these observations can only be inferred without knowledge of how *He. harmoniae* (and Laboulbeniales in general) derive energy from its host. *Hesperomyces harmoniae* creates a haustorium that penetrates the cuticle of *Ha. axyridis* and, presumably, either absorb nutrients through pore canals or feed on waxy lipids produced by epidermal cells (Weir and Beakes, 1996; Haelewaters *et al.*, 2021). However, this has yet to be formally tested. Staining techniques proposed by Haelewaters *et al.* [2022] could allow us to track the flow of nutrients from *Ha. axyridis* to *He. harmoniae*. Tracking the rate of nutrient absorption under different environmental conditions will inform us how exactly *He. harmoniae* reacts to both abiotic (temperature, humidity) and biotic (host stress) factors. It will also give us a greater understanding of how *Ha. axyridis* responds to infection by *He. harmoniae* under different environmental conditions.

Part 6: Conclusion

Our research is the first to explore the relationship between abiotic and biotic factors on *He. harmoniae* growth and development. *Hesperomyces harmoniae* experienced the fastest growth at 25 °C (control condition), the slowest growth at 17 °C (low temperature), and a decrease in infectivity at 30% RH compared to 60% RH. Surprisingly, we saw a lag time in infection at 30 °C (high temperature) compared to the control, implying that *He. harmoniae* is significantly affected by the changes in fitness in *Ha. axyridis*. Additionally, there were no differences in time to infection between sexes and color morphs. We also saw no difference in the growth of thalli between melanic and non-melanic infected ladybirds, despite non-melanic forms being significantly more infected in the wild. This research sheds further light on the mysteries surrounding *He. harmoniae* and Laboulbeniales in general and leads to interesting questions about the exact mechanisms behind parasite infections. These data can also help inform us about the global distribution patterns of *He. harmoniae* as well as how changing climate can affect these distributions. *Harmonia axyridis* has been proposed as a model invasive species to study the mechanisms behind biological invasions (Roy and Wajnberg, 2008). On a wider scale, the results generated in this study can aid in this endeavor by informing us how invasive species are controlled by parasites in non-native ranges and the effect changes in invasive species behavior and fitness have on their parasites.

Summary

Harmonia axyridis (Coccinellidae, Coleoptera), bekend als het Aziatisch lieveheersbeestje, is een zeer polymorf, wereldwijd invasief generalistisch insect. Zijn veerkrachtige aard, grote eetlust en succes als predator maakten hem tot een populair biologisch bestrijdingsmiddel tegen bladluizen en schildluizen op gewassen. Vanaf begin 1900 tot 1995 werd hij opzettelijk vanuit zijn oorspronkelijk verspreidingsgebied in Oost-Azië geïntroduceerd in Noord-Amerika en Europa als biocontrolemiddel, waar hij zich vervolgens vestigde als invasieve soort. (Gordon, 1985; Koch, 2003; Brown *et al.*, 2008). Invasieve populaties hebben zich ook gevestigd in Zuid-Amerika en Afrika door onopzettelijke verspreiding (Lombaert *et al.*, 2014). In 2016 werd *Ha. axyridis* aangetroffen in Nieuw-Zeeland (waarschijnlijk als gevolg van een onbedoelde introductie), waardoor de soort nu op elk continent behalve Antarctica is ingeburgerd (Camacho-Cervantes *et al.*, 2017). Na vestiging verspreidt *Ha. axyridis* zich snel (Brown *et al.*, 2011). In zijn niet-inheemse verspreidingsgebied verandert *Ha. axyridis* de structuur van de lieveheersbeestjesgemeenschap drastisch door inheemse keversoorten te verdringen, te bejagen, en op te eten (Roy *et al.*, 2016). Verschillende landen hebben melding gemaakt van kleinere aantallen inheemse lieveheersbeestjes na invasies van *Ha. axyridis* (Roy *et al.*, 2012; Adriaens *et al.*, 2015). *Harmonia axyridis* vormt een belangrijke bedreiging voor inheemse ecosystemen, maar is niet immuun voor invloeden van buitenaf. In niet-inheemse gebieden wordt *Ha. axyridis* geparasiteerd door verschillende organismen, waaronder nematoden, wespen, mijten en schimmels (Haelewaters *et al.*, 2017; Knapp *et al.*, 2019).

Eén van deze parasieten op *Ha. axyridis* is de zwam *Hesperomyces harmoniae*. *Hesperomyces harmoniae* behoort tot de orde Laboulbeniales (Ascomycota). Soorten in deze orde hebben een obligate associatie met geleedpotigen. Laboulbeniales verspreiden zich hoofdzakelijk door fysiek contact van een geïnfecteerde gastheer met een niet-geïnfecteerde gastheer, maar zij kunnen zich ook verspreiden door auto-infectie binnen één individu (De Kesel, 1993). Sporen worden uit het perithecium afgescheiden en hechten zich aan het integument van de gastheer, waar ze zich vervolgens ontwikkelen tot volwassen structuren die thalli worden genoemd. *Hesperomyces harmoniae* infecteert uitsluitend lieveheersbeestjes van het geslacht *Harmonia* (Haelewaters, *et al.*, 2018; Haelewaters *et al.*, De Kesel, 2022). De prevalentie van *He. harmoniae* op *Ha. axyridis* verschilt per locatie, maar kan oplopen tot 90-100% (Haelewaters *et al.*, 2017 supplemental information). De exacte mechanismen die de prevalentie van *He. harmoniae* op *Ha. axyridis* bepalen zijn tot nu toe onbekend. Het doel van dit onderzoek is om meer inzicht te krijgen in hoe variatie in abiotische factoren Laboulbeniales beïnvloedt met behulp van *He. harmoniae*. Dit werk is belangrijk gezien het kan bijdragen tot een beter algemeen begrip van parasitaire fungi, en ook meer inzicht kan bieden in hoe invasieve soorten (*Ha. axyridis* en mogelijk *He. harmoniae*) zich verspreiden en interageren in ecosystemen.

Om ons onderzoeksdoel te bereiken, gebruikten we *Ha. axyridis* die in een laboratorium waren gekweekt en besmetten we deze met *He. harmoniae*. Er waren twee proefperiodes, waarbij de tweede meer succes had dan de eerste. Alleen de resultaten van de tweede proef werden gebruikt in onze analyses. In deze proef werden de lieveheersbeestjes in de volgende omstandigheden geplaatst: 25 °C/60% RH (controle), 25 °C/30% RH (lage luchtvochtigheid), 25 °C/90% RH (hoge luchtvochtigheid), 17 °C/60% RH (lage temperatuur), en 30 °C/60% RH (hoge temperatuur). Een groep bestaande uit alleen melanistische vormen van *Ha. axyridis* werd aan de controleomstandigheden toegevoegd om ook verschillen in groei

van *He. harmoniae* tussen melanistische en niet-melanistische lieveheersbeestjes te testen. Alle lieveheersbeestjes werden gedurende 28 dagen gecontroleerd op de groei van *Hesperomyces harmoniae*. Uit deze gegevens werden groeicurven geconstrueerd en geanalyseerd met de functie 'compareGrowthCurves' van het statmod softwarepakket in R (<http://bioinf.wehi.edu.au/software/compareCurves>). Tijd tot infectie en multivariate analyses werden geanalyseerd met de Cox proportional hazards test (Kaplan en Meier, 1958; Cox, 1972; Agresti, 2002) in R met het pakket survival (Therneau en Grambsch, 2000). Er werden geen significante verschillen in groeicurves en tijd tot infectie gevonden tussen de controle- en de hoge vochtigheidscondities. Vergeleken met de controle deed de behandeling bij hoge temperatuur er langer over om tot maturiteit te komen, maar er waren geen verschillen in groei na rijpheid. Hoewel *Ha. axyridis* in staat is veel gebieden buiten zijn oorspronkelijk verspreidingsgebied te bezetten (Koch *et al.*, 2006; Lombaert *et al.*, 2014), hebben ze bij hoge temperaturen (>30 °C) te kampen met een lagere overleving, lagere drooggewichten en kleinere groottes tijdens hun larvale ontwikkeling (Lamana en Miller, 1998; Knapp en Nedvěd, 2013; Zhang *et al.*, 2014; Benelli *et al.*, 2015). Dit suggereert dat een afname van de fitness van de gastheer een afname van de fitness van *He. harmoniae* veroorzaakte.

De behandeling met lage vochtigheid had een aanzienlijk lagere kans op infectie en een tragere groei. Bovendien hadden lieveheersbeestjes in de omstandigheden met lage luchtvochtigheid een duidelijk gebrek aan immature thalli. Dit suggereert dat een lage luchtvochtigheid een significant effect heeft op het vermogen van *He. harmoniae* om *Ha. axyridis* te infecteren, hetgeen volgt uit wat De Kesel [1997] concludeert dat Laboulbeniales-soorten in vochtige omstandigheden hogere kansen hebben om gastheren te infecteren, maar dat hun groei niet wordt belemmerd. De lage temperatuur beïnvloedde ook aanzienlijk de timing en de groei van *He. harmoniae* infecties, maar in tegenstelling tot de behandeling met lage vochtigheid waren er nog steeds een merkbare hoeveelheid sporen en thalli. Aangezien *Ha. axyridis* tussen 3 en 6 maanden overwintert (Honek *et al.*, 2022) vertrouwt hij uitsluitend op opgeslagen energiereserves om te overleven. Wij veronderstellen dat als *He. harmoniae* tijdens de winter in hetzelfde tempo groeit als in de controleomstandigheden, hij het risico loopt deze energiereserves uit te putten en *Ha. axyridis* te doden voordat de winter voorbij is.

Ten slotte waren er geen verschillen in het tijdstip van infectie tussen de geslachten en de melanistische vormen. Bovendien was er geen verschil in groei tussen melanistische en niet-melanistische lieveheersbeestjes. Eerdere studies hebben aangetoond dat er geen verschil is in de kans op besmetting met *He. harmoniae* tussen mannelijke en vrouwelijke *Ha. axyridis* (Riddick en Schaefer, 2005; Riddick, 2006; Haelewaters *et al.*, 2018, 2022), dus onze resultaten komen overeen met deze bevindingen. In het wild zijn niet-melanistisch lieveheersbeestjes echter vaker besmet dan melanistische lieveheersbeestjes (Fiedler en Nedvěd, 2019; Haelewaters *et al.*, 2022). Dit roept de vraag op hoe *He. harmoniae*-infectie verschillende vormen van *Ha. axyridis* beïnvloedt.

Onze resultaten helpt ons een duidelijker beeld te krijgen van hoe *He. harmoniae* kan reageren onder klimaatveranderingsomstandigheden. CLIMEX-modellen suggereren dat *Ha. axyridis* zowel in abundantie als in geografisch bereik zal toenemen naarmate de klimaatverandering doorgaat (Evans en Simpson, 2010). Consequent hogere temperaturen zouden de infectiegraad van *He. harmoniae* op *Ha. axyridis* kunnen verminderen, wat op termijn tot minder infecties zou kunnen leiden. Langere en strengere

winters kunnen *He. harmoniae* echter meer mogelijkheden bieden om *Ha. axyridis* te infecteren doordat ze langer in aggregaties overwinteren. Deze langere overwinteringstijd zou de verminderde groei van *He. harmoniae* bij koude temperaturen kunnen compenseren. Toenemende vochtige omstandigheden zouden *He. harmoniae* betere kansen kunnen bieden om zich te verspreiden in historisch droge gebieden, waardoor het *Ha. axyridis* kan infecteren in voorheen ongeschikte habitats.

Onze resultaten roepen ook vragen op over de fysiologische en evolutionaire mechanismen van onze observaties. Wij stellen verschillende methoden voor om deze vragen te helpen beantwoorden. Ten eerste stellen wij voor om meer bemonsterings- en prevalentiestudies uit te voeren buiten de gematigde zones. Dit zal helpen om te bepalen hoe hoge temperaturen de prevalentie van *He. harmoniae* beïnvloeden, en niet alleen *Ha. axyridis*. Ten tweede zou het voor toekomstige incubatie-experimenten nuttig zijn om tellingen van sporen en onvolgroeide thalli te incorporeren om het effect van milieuomstandigheden op de overleving van sporen statistisch te testen. Ten slotte moeten zowel melanistische als niet-melanistische vormen van *Ha. axyridis* worden opgenomen in *He. harmoniae* studies die de fysiologische reacties op infectie onderzoeken.

Ons onderzoek is het eerste dat de relatie nagaat tussen abiotische en biotische factoren op de groei en ontwikkeling van *He. harmoniae*. Deze gegevens kunnen ook gebruikt worden om inzicht te krijgen in de wereldwijde distributiepatronen van *H. harmoniae* en hoe een veranderend klimaat deze distributies kan beïnvloeden. Op grotere schaal kunnen deze gegevens ons vertellen over hoe invasieve soorten worden gecontroleerd door parasieten in niet-inheemse gebieden en welk effect veranderingen in het gedrag en de fitness van invasieve soorten hebben op hun parasieten.

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