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Control of *Harmonia axyridis* by the ectoparasitic fungus *Hesperomyces harmoniae*

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Abstract: *Harmonia axyridis* (Coccinellidae, Coleoptera) is an invasive ladybird that is known to outcompete native species, inflict economic harm, and act as a significant pest species. *Harmonia axyridis* is parasitized by the ectobiotrophic fungus *Hesperomyces virescens* (Ascomycota, Laboulbeniales). Recently, integrative taxonomy has shown that *He. virescens* is a species complex of which delimitation is determined by ladybird host. The fungus associated with *Ha. axyridis* was recently formally described as *He. harmoniae*. Here, we review the effects of *He. harmoniae* on its invasive host. During winter, *He. harmoniae* increases mortality in male ladybirds by exhausting their energy reserves despite a limited effect on their immune system. Co-infection experiments have had varying results. When *Ha. axyridis* was infected with *He. harmoniae* and either *Beauveria bassiana* or *Metarhizium brunneum*, there was no significant increase in mortality compared to infection by *He. harmoniae*-only infection. This may be explained by the enemy release hypothesis, which states that invasive species in new geographic areas have reduced regulatory effects from natural enemies. When co-infected with either *Spiroplasma* or *Wolbachia* bacteria and *He. harmoniae*, both fecundity and hatchability were significantly lowered in female ladybirds, but there was no increase in mortality. *Hesperomyces harmoniae* thus shows potential as a biocontrol agent for *Ha. axyridis*, especially when co-infected with other natural enemies.

Key words: biocontrol, co-infection, complex interactions, ectoparasite, entomopathogen, invasive alien species

Introduction

Harmonia axyridis (Coleoptera: Coccinellidae), often referred to as the harlequin ladybird, is a globally invasive pest species. It is known to outcompete native ladybirds and act as a significant pest species in agricultural and anthropogenic settings (Botezatu et al., 2013; Adriaens et al., 2015; Roy et al., 2016). In the early 1900s, *Ha. axyridis* was intentionally introduced from its native range in East Asia to North America and Europe as a biocontrol agent. Aided by anthropogenic factors, invasive colonies of *Ha. axyridis* established themselves in every continent except Australia and Antarctica (Lombaert et al., 2014).

Harmonia axyridis is responsible for a noticeable decrease in seven out of eight native ladybird species found in the UK (Roy et al., 2012). During autumn, it clusters around wine grapes in vineyards just before they are ready to be harvested and subsequently taints the wine made from those grapes (Botezatu et al., 2013). *Harmonia axyridis* has been shown to pose a

significant threat to biodiversity and agriculture, leading to a need for ways to control its invasive populations.

Hesperomyces virescens (Ascomycota, Laboulbeniales) is an ectobiotrophic fungus known to infect over 30 ladybird species including *Ha. axyridis* (Haelewaters and de Kesel, 2017). Instead of hyphae, *He. virescens* forms a thallus on the integument of its ladybird hosts and penetrates the integument with a root-like structure called haustorium. The fungus spreads primarily through direct transmission, i. e., resulting from physical contact with other thalli during mating or social behavior of their hosts. Using integrative taxonomy methods, it was shown that *He. virescens* is a complex of multiple species, with each member segregated by by ladybird host genus (Haelewaters et al., 2018). Since this key paper, several species have been described within *He. virescens* sensu lato (s.l.) – among others *He. harmoniae* that is a parasite of *Ha. axyridis* (Haelewaters et al., 2022 b). Its genus-specificity along with its relatively high rates of infection make it a promising biological control agent (Haelewaters et al., 2022 a).

The goal of this review is to summarize the effects of *He. harmoniae* on *Ha. axyridis* and discuss its use as a potential biocontrol.

Materials and methods

We reviewed the results of three recent bioassays involving both *Ha. axyridis* and *He. harmoniae*. We were interested in the direct effects of *He. harmoniae* on its host, especially in combination with other natural enemies.

Results and discussion

Knapp et al. (2022) collected *Ha. axyridis* from overwintering sites and screened ladybirds for the presence of *He. harmoniae*. Heavily infected and non-infected ladybirds were then used in two separate experiments. In the first experiment, body mass change from autumn to spring and post-overwintering longevity were tested. In the second experiment, haemolymph was sampled from ladybirds during and after overwintering. The authors demonstrated that during winter conditions *He. harmoniae* caused increased mortality in *Ha. axyridis*. Ladybird body mass was significantly lower in ladybirds infected through winter than in those that were not infected. However, the measured haemolymph parameters were not significant affected by *He. harmoniae* infection suggesting that the fungus had little effect on the ladybird's immune systems. The significant decrease in body mass suggests that energy exhaustion is the reason for increased mortality.

Haelewaters et al. (2020) performed double infection experiments using *He. virescens* s.l. and an entomopathogenic fungus, *Beauveria bassiana* or *Metarhizium brunneum*, on *Ha. axyridis* and the North American-native ladybird *Olla v-nigrum*. *Harmonia axyridis* and *O. v-nigrum* were collected from overwintering sites and reared in the laboratory. Field-collected ladybirds infected with *Hesperomyces* were then used to infect reared ladybirds through a tumbling technique (Cottrell and Riddick, 2012). Newly *Hesperomyces*-infected ladybirds were co-infected with either *B. bassiana* or *M. brunneum*. The authors found that, compared to *Hesperomyces*-only infection, mortality in *O. v-nigrum* was significantly affected by dual infections while mortality in *Ha. axyridis* did not increase. These results fall in line with the enemy release hypothesis that states that invasive species experience less regulatory predation pressure than native species in a new geographic location (Jeffries and Lawton, 1984).

Note, however, that the (currently undescribed) *Hesperomyces* species associated with *O. v-nigrum* is different than *He. harmoniae* on *Ha. axyridis* (Haelewaters et al., 2018).

Awad et al. (in review) collected *Ha. axyridis* from the field in autumn and kept them in simulated overwintering conditions in the laboratory. The ladybirds were checked for dual infections with *He. harmoniae* and male-killing bacteria *Rickettsia*, *Spiroplasma*, and *Wolbachia*. No individuals were infected with *Rickettsia* and only two out of 379 individuals examined were infected with *Wolbachia*. Double infections with *Spiroplasma* and *He. harmoniae* in living female ladybirds resulted in lower fecundity and lower hatchability of their eggs. Infections with both *Spiroplasma* and *He. harmoniae* were independent of each other; the bacterium did not have any effect on *He. harmoniae* infection.

In summary, laboratory bioassays and field observations have shown that *He. harmoniae* has a significant effect on the mortality of *Ha. axyridis* likely due to energy exhaustion. Many questions are still left about potential non-target effects of *He. harmoniae* on native ladybird species. Co-infections of *Hesperomyces* and an entomopathogenic fungus do not affect mortality in *Ha. axyridis*, while they result in significantly increased mortality in native ladybirds. It was shown that *He. harmoniae* can be transmitted to non-target ladybird species (Cottrell and Riddick, 2012) but no studies have tested mortality rates of non-target hosts infected by *He. harmoniae*. There are also questions about the general physiology of these laboulbenialean microfungi that still need answering such as how they take up nutrients (Haelewaters et al., 2021). Overall, *He. harmoniae* shows potential as a biocontrol for invasive populations of *Ha. axyridis*.

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