CZECH UNIVERSITY OF LIFE SCIENCES

Faculty of Environmental Sciences

Department of Ecology

Diploma Thesis

Host choice in braconid wasp Dinocampus coccinellae: laboratory experiments to identify relevant factors

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Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

Bc. Nikola Gramanová

Nature Conservation

Thesis title

Host choice in braconid wasp Dinocampus coccinellae: laboratory experiments to identify relevant factors

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Objectives of thesis

The proposed Master thesis will consist of literature review gathering relevant information on host choice in parasitoids. Main aim of the thesis is to perform a set of laboratory experiments investigating diverse factors potentially influencing parasitoid preferences for hosts. Specifically, effects of host sex, body size, movement activity and presence of ectoparasites will be investigated for study system consisting of braconid wasp Dinocampus coccinellae (parasitoid) and ladybird Harmonia axyridis (host).

Methodology

The literature review will be performed based on scientific papers gather by search on Web of Science. It is supposed that at least 40 relevant papers will be utilized as source of information.

Laboratory experiments will be performed in several consecutive years (2020-2022) in ecophysiological laboratory at FŽP ČZU in Prague. Pairs of compared ladybirds, e.g., male vs female, large vs small, infected vs uninfected or with different movement activity, will be exposed to one Dinocampus wasp in Petri dish (9 cm in diameter). Behaviour of Dinocampus coccinellae (and also ladybirds in case of movement activity) will be recorded (video) and analysed.

The proposed extent of the thesis

ca. 40-50 pages

Keywords

behaviour, body size, Dinocampus coccinellae, host choice, movement activity, parasitoid, Harmonia axyridis

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Recommended information sources

Ceryngier P et al (2018) Predators and parasitoids of the harlequin ladybird, Harmonia axyridis, in its native range and invaded areas. Biological Invasions 20: 1009–1031.

Colinet H et al (2005) Host age and fitness-related traits in a koinobiont aphid parasitoid. Ecological Entomology 30: 473-479.

Fracanti S (2018) Rearing of parasitoid braconid wasp Dinocampus coccinellae in a simplified tritrophic system. Bulletin of Insectology 71: 287–293.

Knapp et al (2019) Invasive host caught up with a native parasitoid: field data reveal high parasitism of Harmonia axyridis by Dinocampus coccinellae in Central Europe. Biological Invasions 21: 2795–2802.

Koyama S, Majerus MEN (2008) Interactions between the parasitoid wasp Dinocampus coccinellae and two species of coccinellid from Japan and Britain. Biocontrol 53: 253–264.

Wolf S et al. (2021) Host searching and host preference of resident pupal parasitoids of Drosophila suzukii in the invaded regions. Pest Management Science 77: 243-252.

Expected date of thesis defence

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The Diploma Thesis Supervisor

doc. Ing. Michal Knapp, Ph.D.

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prof. Mgr. Bohumil Mandák, Ph.D.	**prof. RNDr. Vladimír Bejček, CSc.**
Head of department	Dean

Prague on 30. 03. 2023

Declaration

I declare that I have written a bachelor thesis on: Preferences during host selection procedure in parasitoid wasp *Dinocampus coccinellae* was prepared independently and I have cited all the information sources I have used in the thesis, and which I have also listed at the end of the thesis in the list of information sources used.

I am aware that my bachelor thesis is fully covered by the No. 121/2000 Coll., on copyright, on rights related to copyright and on amendments to certain acts, as amended, in particular the provisions of § Section 35(3) of this Act, i.e., on the use of this work.

I am aware that by submitting my bachelor's thesis I agree to its publication in accordance with Act No. 111/1998 Coll., on the Universities and on Amendments and Additions to Other Acts, as amended, regardless of the outcome of its defence.

By my signature I also declare that the electronic version of the thesis is identical to the printed version and that the data contained in the thesis have been handled in the context of the GDPR.

Prague, 31.3.2023

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Abstract

The first part of this thesis involves a literature review focused on the relationship between parasitoids and their hosts, including the way of life of parasitoids, parasitoid-host coevolution, their possible adaptation over time, and a description of the biology of *Dinocampus coccinellae* and *Harmonia axyridis*. The focus of the investigation was to determine the parasitoid's potential preference for specific host species, including their ability to select a more suitable host based on factors such as age, size, or gender, in order to optimize parasitization success. The thesis also includes two laboratory experiments on the success of parasitization of the ladybird *H. axyridis* by the parasitoid wasp *D. coccinellae* and host preferences of the parasitoid wasp. In the first experiment, we tested whether the ladybird age, body size, or the presence of the *Hesperomyces virescens* in *H. axyridis* influenced parasitiod preference and the parasitisation success of *D. coccinellae*. On the first day after hatching, the adult wasp was subsequently placed in four arenas (Petri dishes), spending ten minutes in each. Each arena contained one pair of ladybirds of the same sex (small x large, infected x uninfected, young x old individual). The findings revealed that parasitoid wasps did not display any discernible preference for larger body size, younger age, or uninfected hosts (i.e., hosts without *Hesperomyces virescens*). Host selection was ‘random’ in this case.

In the second experiment the 20-second videos before the parasitoid attack were investigated if the *Dinocampus* wasp preferred more active (moving) *H. axyridis* individuals over less active ones. On the first day after hatching, the wasp was placed in a Petri dish where one of the combinations of ladybird pairs (F x F, M x M) was prepared. Each of the combinations of ladybird pairs (F x F, M x M) was observed for ten minutes by the parasitoid, and the entire process was recorded on camera for later analysis of movement. It was demonstrated that the wasp attacked moving ladybirds with higher probability than less active ones. At the same time, the behavior of the ladybird 3 seconds before the attack was observed. It was found that the highest number of attacks occurred on ladybirds that were moving quickly or at least slowly just before the attack.

Keywords: behavior, body size, *Dinocampus coccinellae,* host choice, movement activity, parasitoid, *Harmonia axyridis*

Abstrakt v ČJ

První část této práce zahrnuje rešerši zaměřenou na vztah mezi parazitoidy a jejich hostiteli, včetně životního stylu parazitoidů, ko-evoluce mezi parazitoidem a hostitelem, jejich možné adaptace v průběhu času a popisu biologie *Dinocampus coccinellae* a *Harmonia axyridis*. Cílem této práce bylo zjistit potenciální preference parazitoidů pro konkrétní druhy hostitelů, včetně jejich schopnosti vybrat si vhodnějšího hostitele na základě faktorů, jako jsou věk, velikost nebo pohlaví, za účelem maximalizace úspěchu parazitace. Práce také zahrnuje dva laboratorní experimenty zaměřené na úspěšnost parazitace slunéčka *H. axyridis* parazitoidní vosou *D. coccinellae* a preference parazitoidní vosy vůči hostitelům.

V prvním experimentu jsme testovali, zda věk, velikost těla nebo přítomnost *Hesperomyces virescens* u *H. axyridis* ovlivňuje preference parazitoidů a úspěšnost parazitace *D. coccinellae*. První den po vylíhnutí byl dospělý parazitoid umístěn do čtyř arén (Petriho misek), kde v každé strávil deset minut. Každá aréna obsahovala jeden pár slunéček stejného pohlaví (malý x velký, infikovaný x neinfikovaný, mladý x starý jedinec). Zjištění ukázala, že parazitoidní vosy neprojevovaly žádné zřejmé preference pro větší velikost těla, mladší věk nebo neinfikované hostitele (tj. hostitele bez *H. virescens*). V tomto případě byl výběr hostitele "náhodný".

V druhém experimentu byla zkoumána 20sekundová videa před útokem parazitoida, aby se zjistilo, zda preferuje aktivnější (pohybující se) jedince *H. axyridis* před méně aktivními. První den po vylíhnutí byl parazitoid umístěn do Petriho misky, kde byla připravena jedna z kombinací párů slunéček (F x F, M x M). Každá kombinace párů slunéček byla společně s parazitoidem pozorována po dobu deseti minut a celý proces byl zaznamenán na kameru pro pozdější analýzu pohybu. Bylo prokázáno, že parazitoid útočil s vyšší pravděpodobností na pohybující se slunéčka než na méně aktivní. Zároveň bylo pozorováno chování slunéčka 3 sekundy před útokem. Zjistilo se, že nejvyšší počet útoků se vyskytoval u slunéček, která se pohybovala rychleji nebo alespoň pomalu před útokem.

Klíčová slova: chování, velikost těla, *Dinocampus coccinellae,* volba hostitele, pohybová aktivita, parazitoid, *Harmonia axyridis*

General

1. Introduction and goals

Parasitoids and their hosts are distributed all over the world and account for up to 10% of described insect species. Parasitoids are an integral part of many functioning ecosystems. Humans use their services for the biological control of many important insect pests. This method is very efficient, especially from a financial point of view, and often has greater benefits than the use of agrochemicals in the form of pesticides.

Parasitoids, by their way of life, can reduce the abundance of host populations at a short time scale. From an evolutionary perspective, hosts and their parasitoids are in a constant race.

The goal of this thesis is to conduct a literature search and introduce the topic of the relationship between parasitoids and their hosts. The work includes a general overview about the history of their relationship, coevolution, and a description of the life cycles of parasitoids as well as their importance to humanity. The experimental part focuses in more detail on the relationship between *Dinocampus coccinellae* and *Harmonia axyridis*. The main objective of the work is to gather relevant information on host selection in parasitoids and perform a set of laboratory experiments investigating various factors that may influence parasitoid preferences for hosts. Specifically, the effects of host sex, body size, movement activity, and the presence of ectoparasites will be examined.

In the second experiment, the idea was to see if the wasp preferred *H. axyridis* that were more active. And whether the behavior of the ladybird just before the parasitoid attack has an impact on its selection.

2. Introduction

2.1 Definition of parasitoid

A parasitoid is an organism whose juvenile stage develops on or within the host upon which it feeds and eventually kills the host directly or indirectly (Lenteren & Godfray, 2005). Parasitoids rely on their hosts for their reproduction and development, but if suitable hosts are not available, they may feed on other food sources or settle for a limited number of hosts. Some parasitoids may even exist in nature as inactive forms (e.g., in the embryonic stage) and only activate when they find suitable hosts, for example, by using chemical signals (Weinersmith, 2019). There are exceptions where parasitoids only paralyze their host for the entire developmental process and the host either survives or dies when the parasitism is finished. Townsend (2003) described the parasitoid has only one host, which it typically kills at the end of larval development. There are also cases where the parasitoids have another parasitoid as its host, this is called hyperparasitoidism, here three different species are in coevolution (Vinson, 1976).

Parasitoids are divided into two general groups, endoparasitoids and ectoparasitoids. Endoparasitoids hatch from eggs inside of the host body. After which, they feed and develop inside the host cavity. Ectoparasitoids paralyse their host (carried out by the parent or the first larval instar itself) and develop on or near the surface of its body. They feed by aspirating body fluid through the host's cuticle (Strand, 2014).

Parasitoids are characterized by two types of developmental strategies: idiobiont and koinobiont. The developmental strategy of idiobiont means that a parasitoid prevents some part of development of the host. This strategy may also inhibit the host's ability to move (Desneux et al., 2009). This is mainly to prevent the host from harming the parasitoid when moving or flying. Conversely, koinobionts allow the host to further develop and move around. The most common for ectoparasites is the idiobiont strategy, while the endoparasitoids frequently utilize the koinobionts strategy (Harvey et al., 2016).

Some parasitoids can even influence the behavior of their host to increase the success rate of parasitoid development (Weinersmith, 2019). One example of a parasitoid that manipulates the behavior of its host is

Hymenoepimecis argyraphaga, a parasitoid of spiders. When a female of this parasitoid decides to infect a spider, she lays an egg inside its body. When the larva hatches, it starts to manipulate the spider's behavior using chemical signals. The larva settles on the underside of the spider's abdomen and begins to produce chemicals that affect the spider's nervous system. The spider then starts to behave in a peculiar way, as if it were drunk, and begins to weave complex webs instead of the usual shape. After the spider completes the web, the parasitoid larva kills the spider and uses the spider's web as a place for its cocoon. This type of host behavior manipulation helps to increase the success of the parasitoid's development (Eberhard, 2001)

2.1.1 Taxonomic overview

A total of up to 10% of described insect species are parasitoids (Vilhelmsen, 2003). The vast majority of parasitoids are found in the orders Hymenoptera, which includes ca. 50,000 described species of parasitoids, and Diptera, which includes ca. 15,000 described species of parasitoids. Some examples of well-known parasitoid families in Hymenoptera are Ichneumonidae (ichneumon wasps), Braconidae (braconid wasps), and Chalcidoidea (chalcid wasps) (Aigbedion-Atalor et al., 2020). The most well-known parasitoid species in Diptera are from the family Tachinidae (tachinid flies). Parasitoids can also be found in other insect orders, e.g., Coleoptera (beetles), Lepidoptera (moths), and Neuroptera (lacewings) (Vilhelmsen, 2003).

Braconidae is one of the largest families of parasitoids, with over 18,000 described species, and is among the most diverse groups of parasitoids. Braconids are mostly small parasitoids that can range in size from less than 1 mm to over 10 mm. The color of their body can range from black to golden (Aigbedion-Atalor et al., 2020).

The host organisms of braconids include many different species of insects, especially beetles, flies, and caterpillars, but also moths, wasps, and other parasitoids. The parasitoid eggs are laid inside the body of the host organism. The larvae then feed on the body of their host, ultimately leading to its death. Braconids are koinobiont, which means that the parasitoid larvae can develop together with the host organism. According to Shaw and Hochberg (2011), some braconid species have very specific hosts, while others are less specialized and can parasitize a wide range of host organisms. For example, Belokobylskij and Maeto (2009) describe the species *Cotesia glomerata*, which is specialized to parasitize the caterpillars of the small white butterfly, a serious pest of agricultural crops. Braconids parasitize various stages of the host organism, including eggs, larvae, pupae, and even adult individuals of some insect species, such as wasps or flies (Miko, 2011).

The Ichneumonidae is one of the largest families of parasitoid wasps, with more than 60,000 described species worldwide. They are known for their long ovipositors, which can be as long as their body or even longer. Ichneumonids are usually small to medium-sized parasitoids, ranging from less than 1 mm to more than 50 mm in length (Quicke, 2015).

Ichneumonids are parasitoids of a wide range of insects, including caterpillars, beetles, flies, and even other parasitoid wasps (Wharton et al., 1997). Their hosts can be found in various habitats, such as forests, fields, and wetlands. The female wasp lays her eggs either on or in the host insect, and the resulting larvae feed on the host's tissues, eventually killing it (Quicke, 2015). They are typically koinobiont, meaning that their larvae continue to feed on the host insect even as it develops and grows. However, some species are idiobiont, which means that their larvae paralyze the host insect and prevent it from further development before consuming it (Shaw and Hochberg, 2001).

The diversity of host insects and the adaptability of ichneumonids to exploit different habitats have led to the evolution of a wide range of life history strategies within the family. For example, some species are highly specialized and have very specific host requirements, while others are generalists that can use a wide range of hosts (Wharton et al., 1997).

Chalcididae is a parasitoid wasp, which includes more than 23,000 described species and is one of the largest families in the Hymenoptera order (Noyes, 2019). The size of chalcidids can range from less than 1 mm to over 10 mm, but most species are small to medium-sized (Noyes, 2019). Chalcidids parasitize a wide range of hosts, including insects such as beetles, flies, and moths, as well as spiders and plant galls (Heraty et al., 2013). They typically lay their eggs inside or on the surface of the host's body, depending on the species (Heraty et al., 2013). The larvae then feed on the host's tissues, ultimately leading to the host's death. Chalcidids can parasitize the host at various stages of its life cycle, including eggs, larvae, and pupae, depending on the species (Noyes, 2019).

Tachinidae is a large group of parasitoid flies, with over 10,000 described species worldwide. They are generally small to medium-sized flies, ranging in size from 2 to 20 mm (O'Hara et al., 2021). Tachinid parasitoids have a wide range of hosts, including insects from many different orders such as Lepidoptera, Coleoptera, Diptera, Hymenoptera, Orthoptera, Hemiptera, and even spiders (O'Hara et al., 2021). Tachinid flies typically lay their eggs on the outside of the host's body, and the larvae burrow into the host and feed on its tissues, eventually killing it (James et al., 2011). Some species of Tachinidae are known to parasitize agricultural pests, making them important in biological control programs (Stireman et al., 2006). They are known for their high diversity and wide range of host species, making them ecologically important parasitoids in many ecosystems (O'Hara et al., 2021).

2.1.2 Life cycles

For reproduction, parasitoids use the ovipositor, a tubular organ used to lay or inject eggs into the host (Vilhelmsen, 2003). The female lays her eggs inside the body of the (often immature) host or in its immediate vicinity (Vansant, et al., 2019).

However, the immature parasitoid stages are lethal, feeding directly on hosts and often killing them. At first, they can feed on non-vital organs, so they don't kill the host, but eventually they get to vital organs, which is fatal to the host (Lenteren & Godfray, 2005).

The life cycles of parasitoids are characterized by a unique strategy in which the immature stages develop at the expense of a single host individual, ultimately leading to its death. This strategy has evolved as a highly efficient way of exploiting host resources, as evidenced by the high conversion efficiency of some parasitoids. According to Brodeur et al. (2011), parasitoids can convert host biomass into their own biomass at a rate of up to 50%, making them highly effective at utilizing limited resources. To achieve such efficiency, parasitoids have evolved complex life cycle strategies that involve a variety of host manipulation tactics. For instance, many parasitoids manipulate their host's behavior in order to ensure that their offspring are not attacked by the host's immune system (Heimpel & de Boer, 2008). This can involve the injection of toxins or hormones that alter the host's behavior or physiology, or the production of physical structures such as cocoons that protect the developing parasitoid from the host's immune defenses (Whitfield & Asgari, 2003).

The development of parasitoids is also highly synchronized with that of their hosts, allowing them to optimize their resource use. According to Ellers and Jervis (2003), parasitoids have evolved a range of developmental strategies that are synchronized with those of their hosts, including those that involve diapause, or a state of arrested development, which allows the parasitoid to emerge at a time when its host is most abundant.

Overall, the highly efficient life cycle strategies of parasitoids have allowed them to become important regulators of host populations, and their unique biology and behavior continue to fascinate and intrigue biologists and ecologists alike.

According to Vilhelmsen (2003), egg parasitoids are a group of parasitoid insects that lay their eggs directly into the eggs of their host insects. The developing larva feeds on the contents of the host egg and finally emerges from the host egg as a mature larva, spins a cocoon, and pupates within it. According to Vansant et al. (2019), egg parasitoids parasitize a wide range of insect pests, such as moths, butterflies, leafhoppers, and planthoppers. Egg parasitoids can effectively reduce the number of host individuals, particularly in the early stages of host development, such as eggs, and play an important role in regulating insect pest populations. For instance, the wasp *Dolichomitus mesocentrus* searches tree trunks for carpenter beetle larvae and lays an egg on them. The parasitoid larva gradually consumes the carpenter beetle larva during its development and overwinters as a larva before hatching in the spring (Zwakhals, 2010).)

2.1.3 Importance for humanity

Parasitoids are key species for the biological control of many insect pests (Waage & Hassell, 1982). They thus provide a valuable ecosystem service to farmers. Farmers use them as natural enemies of the pests they need to control. This often involves the deliberate release of reared parasitoids at a designated site, with the aim of controlling pest abundance at that site (Scheiner & Martin, 2020). This method is very effective, especially from a financial point of view, and often has several times greater benefits compared to the use of agrochemicals in the form of pesticides. From an environmental point of view, the use of natural enemies and a far more sustainable approach, as parasitoids are rarely harmful to non-target organisms (Stanley & Preetha, 2016).

The disadvantage of biological plant protection is the time-consuming nature and usually the impossibility to combine it with pesticides. The process is much longer in time than the use of chemicals, but will be worth the longer term results (Gaigher et al., 2015). Many parasitoids are susceptible to commonly used insecticides, especially broad-spectrum toxins (Stanley & Preetha, 2016). In addition to lethal effects, insecticides also result in sublethal effects that are observed in surviving parasitoids and can negatively affect their reproduction and offspring sex ratio (Teder & Knapp, 2019).

The invasion of new parasitoids can pose a risk to agricultural crops, trees, or native species. If a new parasitoid species is not regulated in its native environment, it can cause excessive damage to native species, which can have a negative impact on overall ecosystem stability. In some cases, parasitoids become invasive species and threaten the native fauna and flora (Blackburn & Copp, 2014).

Many countries have programs for controlling and regulating invasive species, including parasitoids. For example, in the EU, there is a regulation on invasive species that regulates the import and spread of invasive species. Upon arrival in the destination country, trucks, containers, and packages are also inspected to prevent the unintended introduction of invasive species. Scientists also study and monitor parasitoids that could have the potential to become invasive species and take measures for their management and regulation (Hulme, 2009).

Interestingly, some plant species deliberately produce chemicals that attract parasitoids to their immediate vicinity. In doing so, they provide themselves with protection from herbivore insects that could damage or kill them (Scheiner & Martin, 2020).

Prior to any parasitoid introduction, the species of pest that will be exterminated must be known. It is also important to know its bionomy, which includes its mode of nutrition, reproduction or suitable habitat (biotic and abiotic factors) such as climate, habitat or food sources (Hackett-Jones et al., 2008).

2.2 Parasitoid-host coevolution

Parasitoids must continuously be adapting to new conditions and changes in host’s traits (for example by elongation of the ovipositor or increase in its strength). Most parasitoids are highly specialised to a specific host species (Forbes et al., 2018) and thus have an innate ability to detect this specific host, typically using chemical signals. In addition to chemical cues, some parasitoids are able to utilize visual or acoustic signals from the host (Gross, 1993).

From an evolutionary perspective, parasitoids and hosts are in a constant arms race that ranges from immune responses to different behavioral strategies (Dicke et al., 2020). Within this evolutionary conflict, both parasitoids and hosts have the ability to learn new signals (Leavell et al., 2019). An example would be the change in parasitoid and host behavior due to climate change (Snell-Rood et al., 2019). Changes in temperature and precipitation patterns can affect seasonality, development time, reproductive cycle and other biological factors that influence parasitoid-host interactions. These changes can lead to new interactions and adaptations to new conditions, which can affect the population and biodiversity in the ecosystem (Dury et al., 2011).

Phenology also plays an important role in host-parasitoid interactions. Proper timing of development (oviposition) is crucial for the parasitoid. In addition to successful oviposition, proper timing provides parasitoids with the opportunity for multiple species to coexist on a single host (Hackett-Jones et al., 2008).

In the moment a parasitoid finds a host, behavioral, morphological, physiological, or immune defences of the host are triggered (Gross, 1993). It is always more advantageous for the host to employ multiple types of defence simultaneously (Weseloh, 1976).

Nasonia vitripennis is a known parasitoid of *Musca domestica* (housefly). It is a small chalcidoid wasp that attacks the pupae of the housefly and other related species. Over a long period of several experiments, Pimentel et al. (1963) investigated the coevolutionary interactions of hosts and parasitoids under laboratory conditions. The main change that occurred during the experiments was that the host pupae became heavier and thicker. Overall, the fly pupa spent less time in the most vulnerable phase of the life cycle. The more robust pupa is a clear defensive adaptation against wasps. The shorter pupal period is consistent with past theories that development should accelerate during life stages when the risks of parasitoid-induced mortality are high (Pimentel et al., 1963).

The theory of tritrophic interactions was introduced by Price et al. (1980). They described the mutual communication between three organisms: a plant, a herbivore and associated natural enemies, including arthropod predators and parasitoids (Price et al., 1980). The tritrophic interaction is the ability of a plant to enlist the help of a parasitoid to attack the phytophagous insect that is damaging it, using odorants. When plants are attacked and injured by phytophagous insects, they produce a mixture of odorous compounds called herbivore induced volatiles (HIPVs). These compounds act either as repellents for herbivores (Landolt, 1993; Signoretti et al., 2012) or as attractants for parasitoids (Anderson & Alborn, 1999; Shiojiri & Takabayashi, 2003). HIPVs have been shown to attract more than 30 species of parasitoids, including several representatives of the family Braconidae (Hymenoptera) - (Geervliet et al., 1996). Given the potential utility of parasitoid wasps for pest control, understanding the olfactory mechanisms by which herbivores and parasitoid wasps detect plant life could facilitate the use of parasitoids for effective control of cultivated plants (Guo & Wang, 2019).

2.2.1 Host quality

If the parasitoid lays eggs in a host that has already been parasitized, the survival or the number and quality of the resulting offspring is compromised. Host occupancy thus has a direct effect on the further development of the parasitoid (Van Alphen & Nell, 1982). For this reason, the ability of the parasitoid to recognize whether a given host has already been infested plays a major role. The rejection of a host by a parasitoid in which it has discovered the eggs of another individual (or traces thereof) is called host discrimination. This phenomenon is widespread among parasitoids of the order Hymenoptera (Hofsvang, 1988). Host discrimination is common also in the superfamily Chalcidoidea.

Parasitoids often also influence host behavior, usually in a manipulative style. The aim of behavioral manipulation might be to reduce predation pressure on the parasitoid (Tanaka & Ohsaki, 2006), or to alter the internal environment of the host (Kaeslin et al., 2005). If the probability of the further parasitoid evolution in the host body increases, we speak of an adaptation to host behavior (Weseloh, 1976). It is not always easy to determine whether this is a deliberate manipulation by the parasitoid or just a response by the host to the parasitoid's evolution (Leavell, et al., 2019).

Many of parasitoids in the families Ichneumonidae and Braconidae use symbiotic viruses, among others, to influence the host. These are polydnaviruses that replicate in the ovary cells of the female (Kaeslin et al., 2005) and are injected during oviposition, providing passive protection of the eggs from the host immune response for a period of time. These symbiotic viruses are absolutely crucial for the successful life cycle of the parasitoid (Asgari & Schmidt, 1994).

2.2.2 Parasitoid preference

Parasitoids have different mechanisms for recognizing and preferring hosts (Harvey & Vet, 2017). The primary sense they rely on is vision. Many species of parasitoids prefer hosts based on visual signals such as color, shape, size, and texture. For example, some species of wasp parasitoids prefer insect eggs that are the same color as their own eggs. Other species prefer hosts with a certain texture that allows for easier egg-laying or facilitates the hatching of offspring (Alphen et al., 1986).

Another sense by which parasitoids can prefer hosts is through smell (Harvey et al., 2013). Many species of parasitoids use chemosensory signals to recognize potential hosts. These signals may come from host plants or from host males or females. For example, some species of fly parasitoids use the scent of host male insects to recognize a suitable host.

Some parasitoid species may prefer hosts based on temperature. For instance, some beetle species parasitize insect larvae in decaying wood and prefer temperatures in the range of 25 to 30 °C (Harvey & Vet, 2017).

An example of a parasitoid species with excellent host recognition abilities are *Cotesia* wasps. They are parasitizing moth caterpillars and can precisely recognize a suitable host using chemical signals emitted by different caterpillar species. Thanks to this ability, *Cotesia* has become an important tool in biological pest control against Noctuidae pests (Heil et al., 1991).

All these factors may be important for parasitoids to gain as much energy and resources as possible for their offspring. However, parasitoid preferences may change depending on environmental changes and host availability (Wang et at., 2017).

2.3 Parasitoid wasp *Dinocampus coccinellae*

Dinocampus coccinellae is a parasitoid wasp that belongs to the order Hymenoptera, family Braconidae and genus Dinocampus. It most commonly parasitizes ladybirds in the tribe Coccinellini. Adult *D. coccinellae* have transparent membranous wings. The forewings are longer than the hind ones (Silva et al., 2012). The adult wasp is very small and rarely seen in the wild.

The parasitoid *D. coccinellae* lays its eggs directly into the abdomen of the ladybird *Harmonia axyridis*, specifically in the area between the first and second abdominal segments. Female *D. coccinellae* insert the eggs using a specialized ovipositor, which penetrates through the host's cuticle (i.e. the outermost layer) and deposits the egg into the host's body (Vansant, et al., 2019). It is interesting to note that the parasitoid survives in the pupal stage (during cocoon formation) beneath the body of a host that has been paralyzed by the DcPV virus, which serves as protection for the parasitoid against predators (Geoghegan et al., 1998).

Fig. 1: The ladybird *Harmonia axyridis* with the cocoon of the parasitoid wasp *Dinocampus coccinellae* (Baugnée, 2018).

Existing data about parasitism and abundance of *D. coccinellae* in several species of ladybird suggest that the wasp prefers larger ladybird species as hosts because they likely provide sufficient food resources for larval growth (Ceryngier et al., 2017).

The smallest hosts, *Hippodamia parenthesis* produced the smallest adult wasps. Similarly, the largest hosts (*Coccinella septempunctata*), were able to produce the largest larvae of *D. coccinellae* (Ceryngier et al., 2017).

The wasp is not a completely new parasitoid for the eastern ladybird either, both species co-occur in their native range in Asia. Here, it is considered a marginal parasitoid of *Harmonia axyridis* (Koyama & Majerus, 2007). Newly measured values in the Czech population of *H. axyridis* indicate much greater parasitism by the wasp than populations collected elsewhere around the world. These results point to an adaptation of the Central European population of *D. coccinellidae* to *H. axyridis*. The high level of parasitism indicates the possibility of reducing the invasive ladybird species by natural enemies (wasp). It is possible that some Central European populations of *D. coccinellae* have evolved to overcome the immune system of *H. axyridis*, which was previously thought to be responsible for the high larval mortality of *D. coccinellae*. Further lab studies are needed to test the hypothesis (Knapp et al., 2019).

2.3.1 Life cycle of *Dinocampus coccinellae*

Dinocampus coccinellae starts to lay eggs just one hour after hatching from the pupa. The wasp usually has at least two or three generations in a season and most often overwinters as a first instar larva in the host (Majerus, 1997). As soon as the wasp sees the host, it tries to get into its immediate vicinity. It moves its rump back and forth. The eggs are inserted into the host body by the ovipositor. One egg is planted in the host's soft underbelly. Not every single attempt by a wasp to insert an egg into the body of ladybird is successful. So, it often repeats it several times. However, only one larva develops each time in the body of the selected host (Silva et al., 2012).

The larvae use specific nutritional cells, teratocytes, for their survival inside the host. These are cells sent to the host's haemolymph, from which it obtains nutrients (Strand, 2014). Another source for the larval nutrition is the host's lipid reserves. Case studies also demonstrated that reactions triggered by the presence of *D. coccinellae* in the host body can induce increased storage of energy resources in the body. This increases the likelihood of the wasp surviving through the winter in the host (Ceryngier et al., 2017).

The incubation period of the larva inside the body of the ladybird is around 18 - 27 days. Generally, the incubation period of the parasitoid larva can vary among different ladybird species and populations of the same species, and it can also change depending on temperature and other factors (Boivin & Brodeur, 2006). The larvae undergo 3 instars in the host body.

They are yellowish in colour, cylindrical and slightly flattened, without legs but with a distinct head (Balduf, 1926; Strand, 2014). When the larva is ready to pupate, it causes the host to become paralysed and exist the body using the posterior body tentacles. The paralysis is caused by an RNA virus named Dinocampus coccinellae paralysis virus (DcPV). The viruses first multiply in the body of the larva and later enter the body of the host. They paralyse its nerval cells. The ladybird is alive but cannot move very well (Dheilly et al., 2015). The larva pupates within a few hours in a cocoon fixed to the underside of the ladybird´s body using the produced filaments (Geoghegan et al., 1998). The aposematic colouration of the host provides even more security for the larval wasp. A study showed that parasitoid cocoons involving live and manipulated ladybirds suffered less predation than single cocoons or cocoons under dead ladybirds. But that depends on the age of the dead individual (Maure et al., 2011). The ladybird will come out of paralysis only after a few days when the RNA virus has disappeared. This happens after the adult wasp of the species hatches from the pupa. (Dheilly et al., 2015). Whether the ladybird survives parasitoid infestation depends on many factors. According to the study by Roy et al. (2016) approximately 80% of *Harmonia axyridis* survive the development of the parasitoid *Dinocampus coccinellae* in their body. On the other hand, there are cases where the development of the larva in the host has been so drastic for the host that the host dies from the effects (Ceryngier et al., 2017). An experimental study from 2016 suggests that the fate of parasitized ladybirds is also related to food availability. Ladybirds such as *H. axyridis*, *Adalia bipunctata*, or *Coccinella septempunctata*, which feed on aphids or a combination of aphids and pollen, are more likely to recover from parasitism (65% to 81%) than those that feed only on pollen (Maure et al., 2016).

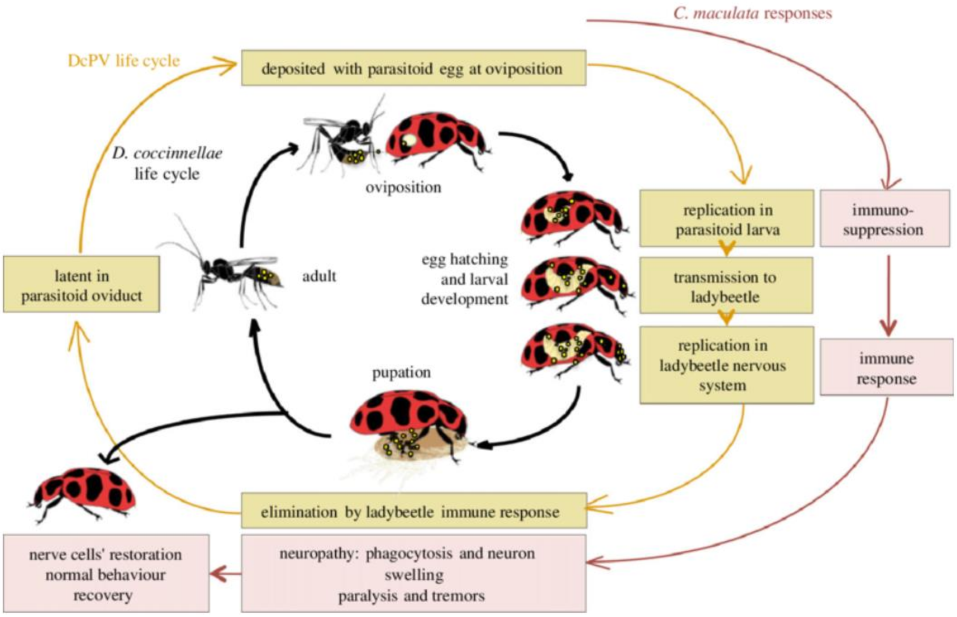

Fig. 2: The life cycle of *Dinocampus coccinellae* (Dheilly et al., 2015)

A laboratory study according to Wright et al. (1979) observed mating of parasitoids, the offspring of the original parasitoids here were only females. Males were observed only very rarely. The male has a more slender and generally smaller body than the female and tends to be darker in colour. The male does not have an ovipositor (Koyama et al., 2007).

2.3.2 Host relationship

Dinocampus coccinellae is a species capable of parasitizing about 50 species of ladybirds, mainly from the tribe Coccinellini, and less from Chilocorini (Ceryngier et al. 2018*)*. The most parasitized is the tribe Coccinellini (Hoogendoorn & Heimpel, 2002).

The prevalence of *D. coccinellae* varies among host species (Majerus, 1997), even within a particular host species. Wasps preferentially attack adults of ladybird species rather than juvenile stages (Geoghegan et al., 1998), but laboratory studies have documented that they can also develop in ladybird larvae or pupae (Vansant et al., 2019). *D. coccinellae* more often chooses larger Coccinellidae species as its hosts (Silva et al., 2012). In nature, the wasp more often chooses female ladybirds. Females tend to be larger than males, and this provides the parasitoid with greater resources for the larva (Barron & Wilson, 1998). In addition, larvae of *D. coccinellae* in the body of a female host may be able to exploit the resources from the ovary and fat body. Males can only draw energy from fatty tissue (Geoghegan et al., 1997).

3. Experimental part

3.1 Biology and ecology of the studied species *Harmonia axyridis*

Harmonia axyridis, a multi-coloured Asian beetle, is native to Central and East Asia from temperate to subtropical climatic zones (Chapin et al., 1991). The western boundary of the natural range is around Kazakhstan (Roy et al. 2016).

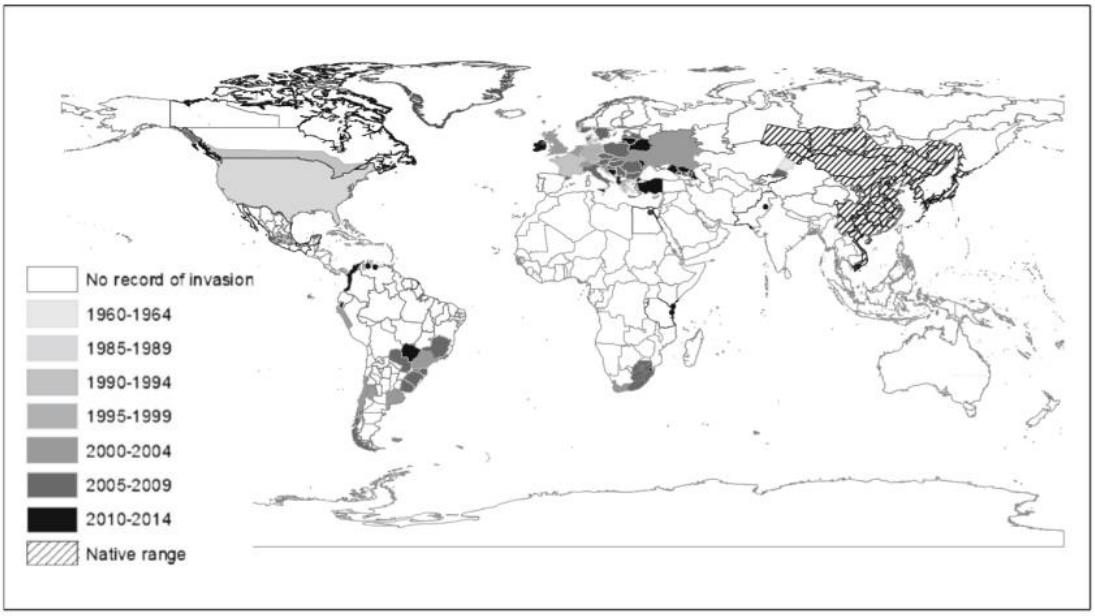

Fig. 3: The global distribution of *Harmonia axyridis* (Roy et al. 2016).

The invasive form of *H. axyridis* originated probably due to the crossing of two subspecies imported from geographically separate native ranges (Roy et al. 2016). Especially during the second half of the 20th century, *H. axyridis* were repeatedly transported to the United States of America and later to Europe because they are excellent aphid predators and have high fecundity. The first invasive population was established around 1988 in the USA. Around the millennium, the first invasive individuals were also recorded in Europe. Among the first countries with the invasive form of *H. axyridis* were Belgium and the Netherlands. In the Czech Republic, there are verified records from 2006 from České Budějovice (Nedvěd et al. 2011). *H. axyridis* is currently spreading in the following areas across Europe, Asia and the USA, sometimes with mass occurrences (Roy et al. 2016).

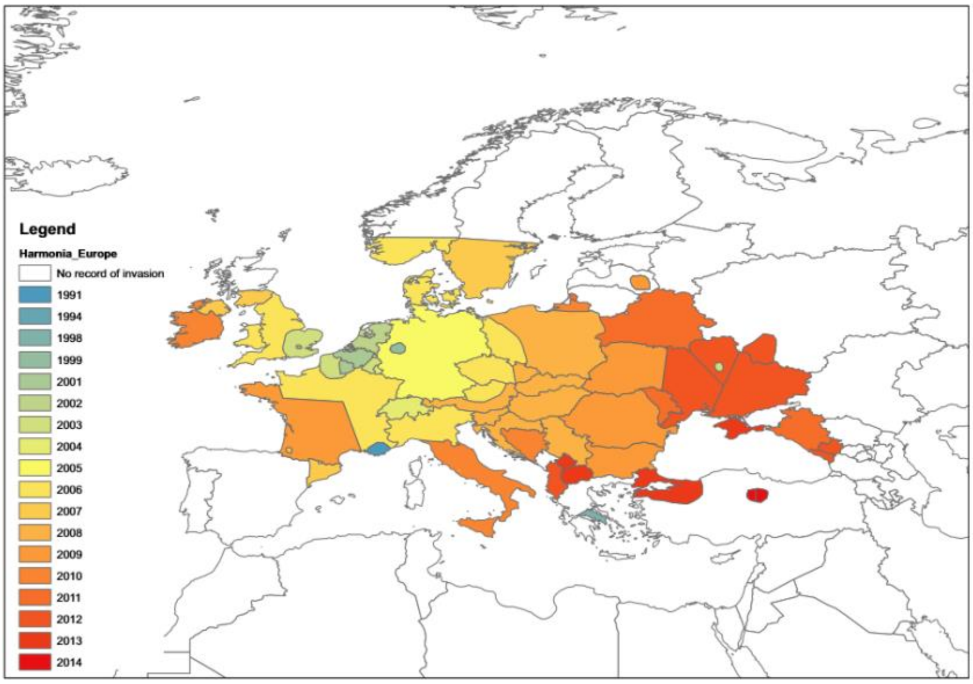

Fig. 4: The distribution of *Harmonia axyridis* in Europe (Roy et al., 2016).

The entire taxonomic history of *H. axyridis* is quite complicated and long. In 1773, this species was originally described as *Coccinella axyridis* Pallas. Currently, it is named *H. axyridis* and is placed in the tribe Coccinellini of the family Coccinellidae (Escalona et al. 2017). One can encounter the fairly common name Halloween beetle. This nickname probably originates from the massive migrations of *H. axyridis* that occur around Halloween (i.e., late October in North America) - (Verheggen et al., 2017).

The colouration of ladybirds is highly variable, with elytra that can be black, yellow, or black with yellow markings. Generally, the elytra can range in colour from yellow to orange and red, and the number of spots can vary from none to nineteen black spots (Chapin & Brou, 1991). The polymorphism in *H. axyridis* colour is heritable and likely associated with multiple alleles (Osawa, 1993).

Fig. 5: Polymorphic coloration of *Harmonia axyridis* (wikipedia.org, online 15. 3. 2023)

3.1.1 Immune system *Harmonia axyridis*

Harmonia axyridis has several defense mechanisms that protect it against natural enemies (Firlej et al., 2012). Cellular immunity in *H. axyridis* involves the activation of immune system cells, such as phagocytes and cytotoxic T lymphocytes, which can recognize and kill cells infected by pathogens (Nguyen et al, 2012). Humoral immunity in *H. axyridis* involves the production of antibodies (immunoglobulins) against foreign substances. Antibodies are produced by B lymphocytes, which can recognize and bind to antigenic determinants of foreign substances and subsequently differentiate into plasma cells that produce antibodies. These antibodies then bind to pathogens and help eliminate them. A potent alkaloid, harmonin, also forms in the hemolymph of the eastern ladybug. Due to this, hemolymph is likely to have very strong antibacterial effects, and this may increase the ability to resist certain pathogens (Wang, 2019). Both types of immunity are important for protecting *H. axyridis* against infectious diseases and ensuring its health and survival.

Ontogenesis has been studied in *H. axyridis*, in which the lowest hemocyte concentration was measured in the larval stages. During ontogenesis, haemocyte concentration increased (Řeřicha et al., 2018). During *H. axyridis* development, the concentration of proteins present in the haemolymph, which contribute to humoral-based immune responses, also increased (Řeřicha et al., 2018).

Studies suggest that the immune system of *H. axyridis* is able to eliminate a large proportion of *Dinocampus coccinellae* eggs (Firlej et al., 2012). *D. coccinellae* is unable to recognize hosts based on their fitness. Females lay similar numbers of eggs in suitable and unsuitable hosts (Ceryngier et al., 2017).

3.1.2 Parasitization of *Dinocampus coccinellae* in the body of *Harmonia axyridis*

During a field survey in Belgium in 2005, three adult *Harmonia axyridis* were found successfully parasitized by the parasitoid *Dinocampus coccinellae*, representing the first record of a wasp parasitizing *H. axyridis* in Europe (Berkvens et al., 2010).

The parasitoid attacked adults and fourth instars of ladybird´s more frequently than younger instar of larvae (Vansant et al., 2019). Based on its size, *H. axyridis* would be an ideal species for a *D. coccinellae* (Obrycki et al., 1985; Ceryngier et al., 2017). However, field data suggest that, consistent with previous studies, *H. axyridis* is a marginal host for *D. coccinellae*, even within its native range (Hoogendoorn & Heimpel, 2002).

The incidence of parasitism of the wasp is lower than for ladybird species of the same size (Ceryngier et al., 2017). This may be related to the fact that the wasp has not yet adapted to the invasive *H. axyridis* (Knapp et al., 2019). This phenomenon has also been attributed to the inability of the wasp to overcome the well-functioning humoral part of the *H. axyridis* immune system (including the alkaloid harmonin; cit) (Firlej et al., 2012).

The recent occurrence of *D. coccinellae* in Czech populations of *H. axyridis* is much higher compared to invasive populations sampled elsewhere in the world. Further experimental data are needed to test the hypothesis that Central European populations of *D. coccinellae* have adapted to use *H. axyridis* as their preferred host (Knapp et al., 2019).

3.2 Experiment 1

3.2.1 Experiment preparation

The experiment was performed at the Faculty of Environmental Sciences (Czech University of Life Sciences Prague) in the Ecophysiological laboratory D414. Under the Supervision Michal Řeřicha and doc. Ing. Michal Knapp, Ph.D.

The first part of this experiment started with the collection of eggs of *Harmonia axyridis*. The eggs were collected at the end of March and the beginning of April 2020. For the purpose of the experiment, ladybirds were raised in the lab to exclude the effect of age, and the influence of biotic and abiotic conditions and possible parasitism of *Dinocampus Coccinellae* in nature. The site of egg collection was located in the campus of Faculty of Environmental Sciences – Czech University of Life Sciences in Prague. The eggs were collected on the underside of trees and smaller shrubs (poplar, alder, birch or, for example, blackberry). A total of 30 egg clutches were collected. In the laboratory, each clutch was placed in a separate petri dish. Hatching of the first instar larvae usually lasted one week. Just before hatching, the entire clutch of eggs darkened. At that point, a cotton ball of water and food was added to the petri dish. The eggs of the *Ephestia kuehniella* were given as standardized food.

The second instar larvae were divided into petri dishes of five individuals per petri dish. Water and food were given *ad libitum* to avoid cannibalism. Once a week, the entire petri dish was changed, and the larvae were placed in completely new and clean dishes. At the end of the fourth instar the larvae pupated and after a week the adults hatched.

The adults were divided by gender using a microscope and placed in petri dishes of up to ten individuals each. They were supplemented with water and food every two days.

In a second step of preparation, a large number of adult ladybirds were collected in nature and monitored in the laboratory for parasitism by *D. coccinellae*. Ladybirds that developed a *D. coccinellae* cocoon on their ventral side were immediately separated from the others.

In the last step, a large number of ladybirds infected with the fungus *Hesperomyces virescens* were collected. The infected ladybirds were used in the experiment part.

3.2.2 Course of the experiment

On the day after *Dinocampus coccinellae* hatching, a test environment was prepared. Multiple pairs of ladybirds were prepared: young male and old male, young female and old female, large male and small male, large female and small female, infected male and uninfected male, infected female and uninfected female. The wasp was always placed in a petri dish where one pair of ladybirds were prepared. On the first day, wasps were successively placed with four different pairs of ladybirds. I observed and recorded when and how many times the wasp poked the ladybirds with their ovipositor. Each pair was observed for 10 minutes, after which the wasp was separated into a petri dish and given water and honey. The wasp was kept undisturbed like this until the next day. The used ladybirds were placed into separate petri dishes with food and water. The ladybirds were observed over the next five weeks to see, whether the parasitisation was successful, and a second generation of wasp hatched.

On the second day, four new petri dishes were prepared to hold another pair of ladybirds. The experiment was repeated as on the first day. The wasp was placed into four petri dishes one by one. Each time I recorded two variables: 1) when wasp and 2) how many times the wasp successfully attacked ladybirds. At the end of the experiment the wasp was again separated and provided with water and honey until the next day.

On the third day, the last four petri dishes were prepared and another combination of pairs of ladybirds were placed in them. The experiment was repeated as on the first and second day. After the last repetition was completed, the wasp was placed in a small eppendorf, with 96% ethanol and placed in the freezer along with the ladybird from which they hatched. This step was done for possible molecular analysis in the future.

The pairs of ladybirds were regularly rotated for different days. This whole process was repeated with 20 wasps and a total of 200 *H. axyridis*.

3.2.3 Data analysis

All the data were analyzed using the statistical program R (version 2023-03-09) and paired t-tests were used for comparing two related datasets. The main objective of the paired t-test is to determine whether the means of the two datasets are statistically significantly different from each other. A total of three tests were conducted.

The first test focused on the preference of a wasp towards the body size of a ladybirds. Two categories of ladybirds (small and large) were compared. The first category contained the time (in seconds) it took for the wasp to attack the small ladybird for the first time, while the second category contained the times of the first attacks of the wasp on the large ladybird. The aim was to determine if the wasp prefered one of the two ladybirds more.

The second test was focused on the preference of a wasp towards the age of a ladybird. Again, two categories were compared, where one contained the time taken in seconds for the first attack of the wasp on a one-day-old ladybird and the other containing the attack times on a ladybird that was several weeks old. The aim was to determine if the wasp prefers one of the two ladybirds more.

The last test was focused on whether the wasp can recognize a ladybird infected with the *Hesperomyces virescens* fungus, and therefore choose the other healthy ladybird. The first categories contained the time taken in seconds for the first attack of the wasp on the infected ladybird, while the second one contained the attack times on the uninfected ladybird.

The gender of ladybirds was not taken into account. In my Bachelor's Thesis, I proved that the parasitoid wasp, *Dinocampus coccinellae*, does not show any preference towards the gender of ladybirds.

3.2.4 Results

The first result of my experiment was that the wasp did not prefer larger or smaller ladybirds ($P = 0.7$). In Figure 6, we can see that the average first attack of the wasp occurred slightly more on smaller ladybirds, but it was not statistically proven. Interestingly, I would have expected the wasp to prefer larger ladybirds because they offer more resources and therefore a greater chance of successful parasitism.

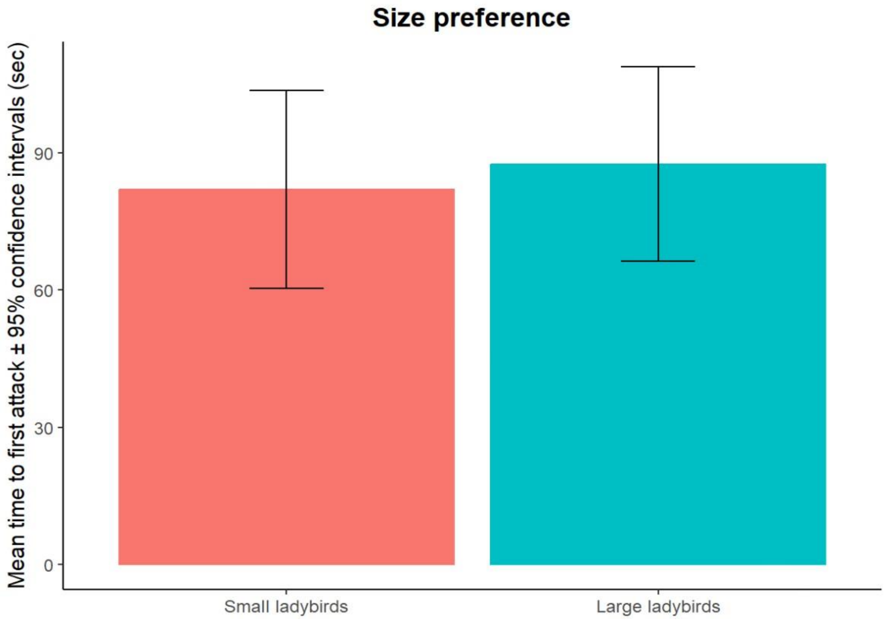

Fig. 6: A graph representing the time in seconds of the first attack of *Dinocampus coccinellae*. The y-axis shows the average time of the first attack, and the x-axis shows the size of the ladybird.

Figure 7 shows that there was also no statistical evidence that the wasp preferred young or old ladybirds. (P = 0.6). If the wasp cannot recognize a healthy or infected ladybird, then it should pick both healthy and infected ladybirds. The graph shows a slight trend in preference for non-infectious ladybirds, but this has not been statistically confirmed (P = 0.3; picture 8). This may be due, for example, to the use of a small number of ladybirds examined (a total of 70 ladybirds). The data show us that the parasitoid wasp does not prefer any of the selected factors in ladybirds, and its selection is purely random. However, this statement needs to be confirmed by further experiments in the future.

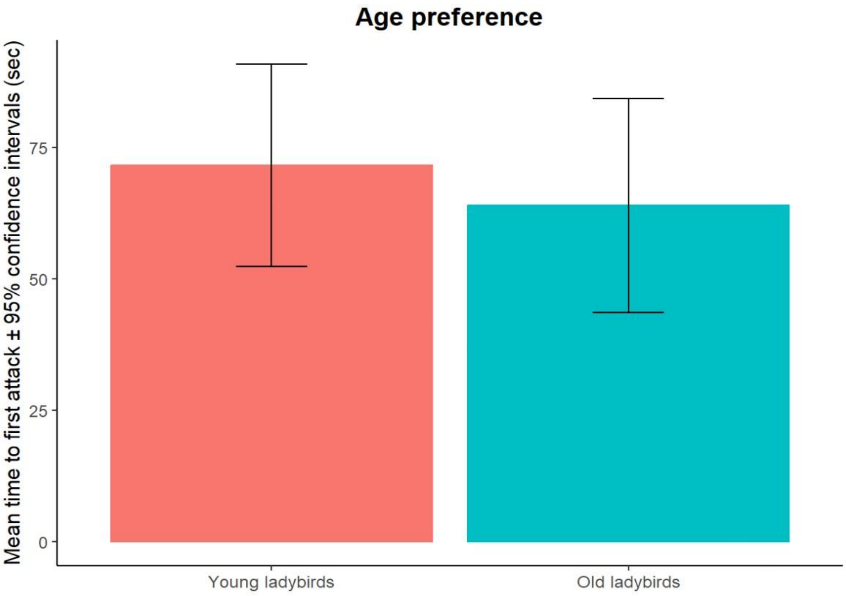

Fig. 7: Graph representing the time in seconds of the first attack of Dinocampus coccinellae. The y-axis shows the average time of the first attack and the x-axis shows the age of ladybirds.

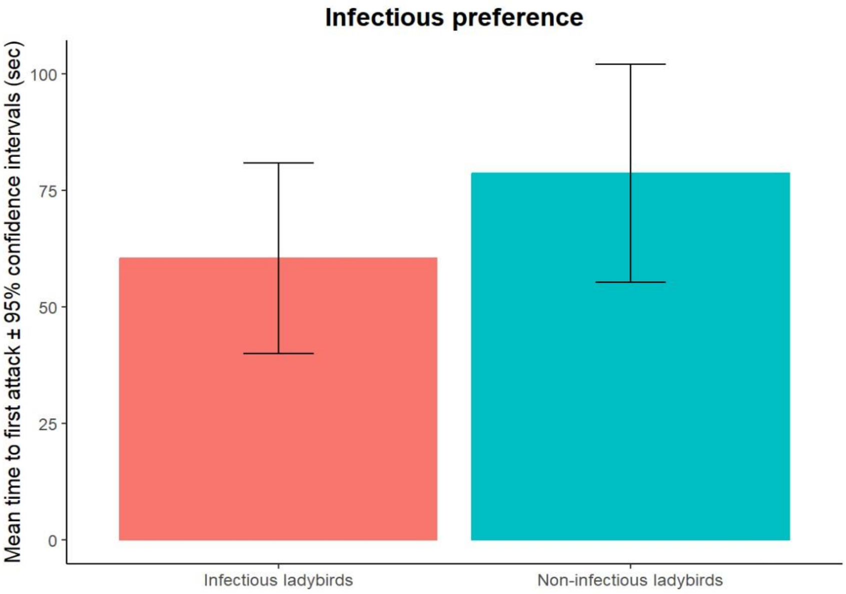

Fig. 8: Graph depicting the time in seconds of the first attack by *Dinocampus coccinellae*. The y-axis represents the average time of the first attack and the x-axis represents the infectivity of ladybirds.

3.3 Experiment 2

3.3.1 Experiment preparation

The experiment was performed at the Faculty of Environmental Sciences (Czech University of Life Sciences Prague) in the Ecophysiological laboratory D414. Under Supervision Ing. Michal Řeřicha, PhD. and doc. Ing. Michal Knapp, Ph.D.

The first step in preparation was collecting large quantities of *Harmonia axyridis*. The ladybirds were separated by sex and placed in Petri dishes containing ten individuals each. Water was added to the petri dishes, and *Ephestia kuehniella* eggs were provided as standardized food. The ladybirds were then kept in the laboratory for three weeks to avoid potential parasitization from the natural environment.

In the second step, a large number of ladybirds infected with the parasitoid *Dinocampus coccinellae* were collected. After hatching, the wasps were placed in separate petri dishes with a cotton swab soaked in water and honey.

3.3.2 Course of the experiment

On the day following the hatching of *Dinocampus coccinellae*, a testing environment was prepared. The wasp was placed in a Petri dish with one pair of ladybirds prepared in each dish, consisting of either a female and female, female and male, or male and male pairing. Each wasp was placed with only one pair of ladybirds, and I observed and recorded on camera when and how many times the wasp stung the ladybirds. Each pair was monitored for 10 minutes, and all wasp attacks were recorded. From this video, short video sequences (20 seconds before and 10 seconds after each attack) were created. The behavior of both the ladybirds and the wasp was observed before and after each attack. The movement of both ladybirds was measured for 20 seconds before the attack to evaluate whether the wasp preferred active or inactive ladybirds.

At the end of the experiment, the wasp was placed in a small Eppendorf tube containing 96% ethanol, along with the ladybird from which it hatched. Both were stored in the freezer for potential future molecular analyses. The used ladybirds were divided into separate Petri dishes and provided with water as food. The ladybirds were observed for another five weeks to determine whether parasitization was successful and whether a second generation of wasps hatched.

3.3.3 Data analysis

Initially, I wanted to use the Tracktor software to analyze the data. Tracktor is an object tracking software based on OpenCV (Pergola & Guckenheimer, 2019). The software is capable of tracking a single object in a noisy environment or multiple objects in uniform environments while maintaining individual identity. The software can evaluate the motion activity of the tracked objects (ladybirds). However, the automatic detection of tracked objects did not work reliably and therefore it could not be used further.

The data were analyzed using the statistical program R (version 2023-03-09), and both a paired t-test and a generalized linear model were used. I was particularly interested in whether the parasitoid prefers ladybirds that are more active. The test compared the movement activity of both ladybirds 20 seconds before the parasitoid attacked, and I wanted to see if the parasitoid indeed always attacked the more active ladybird or not.

The model focused on the effect of the ladybird's behavior 3 seconds before the parasitoid attack. The dependent variable was the attack/non-attack of the individual *Dinocampus coccinellae* on the ladybird. The independent variable was the behavior of the ladybird (running, slow running, defense, standing) and the sex of the ladybird (female/male) was used only as an interaction. The model included paired interactions between the main variables, and a binomial distribution was used.

3.3.4 Results

Ladybirds that were actually attacked by the parasitoid had on average more seconds of movement than ladybirds that were not attacked. It was also statistically significant (P = 0.01). Figure 9 shows the average number of seconds of movement of attacked and non-attacked ladybirds.

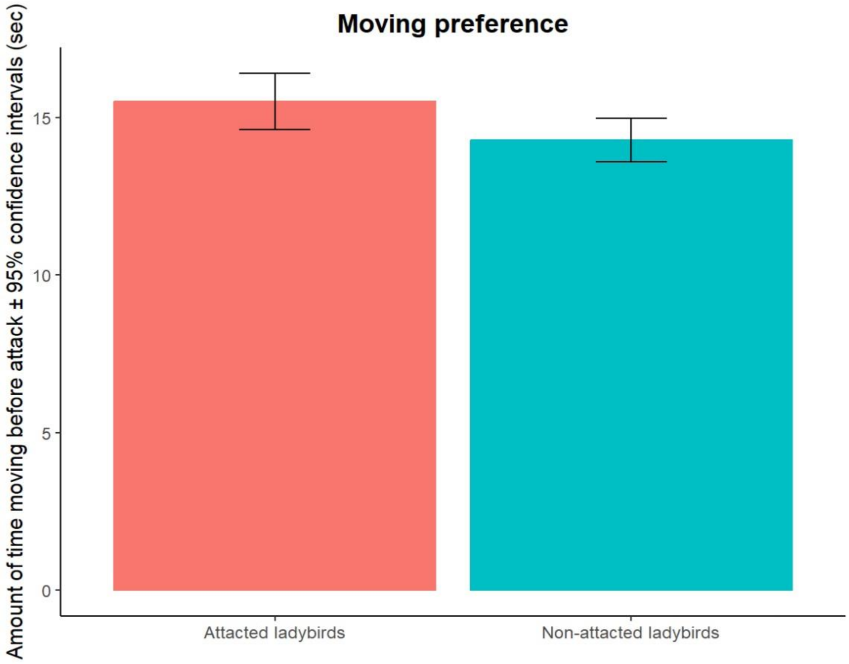

Fig. 9: Graph showing the average time of attack of *Dinocampus coccinellae* on ladybirds. The y-axis represents the average time of the first attack, and the x-axis represents ladybirds that were either attacked or not attacked.

In figure 10, we can see the total number of attacks on ladybirds based on their behavior. It was statistically significant (P = 2.459e-07) that the ladybird's behavior 3 seconds before the attack had an effect on whether the parasitoid attacked or not. From the graph, we can see that the highest number of attacks occurred on ladybirds that were moving or at least slowly moving. Ladybirds that were defending themselves or standing still had a similar number of attacks, but overall, there were very few attacks on them.

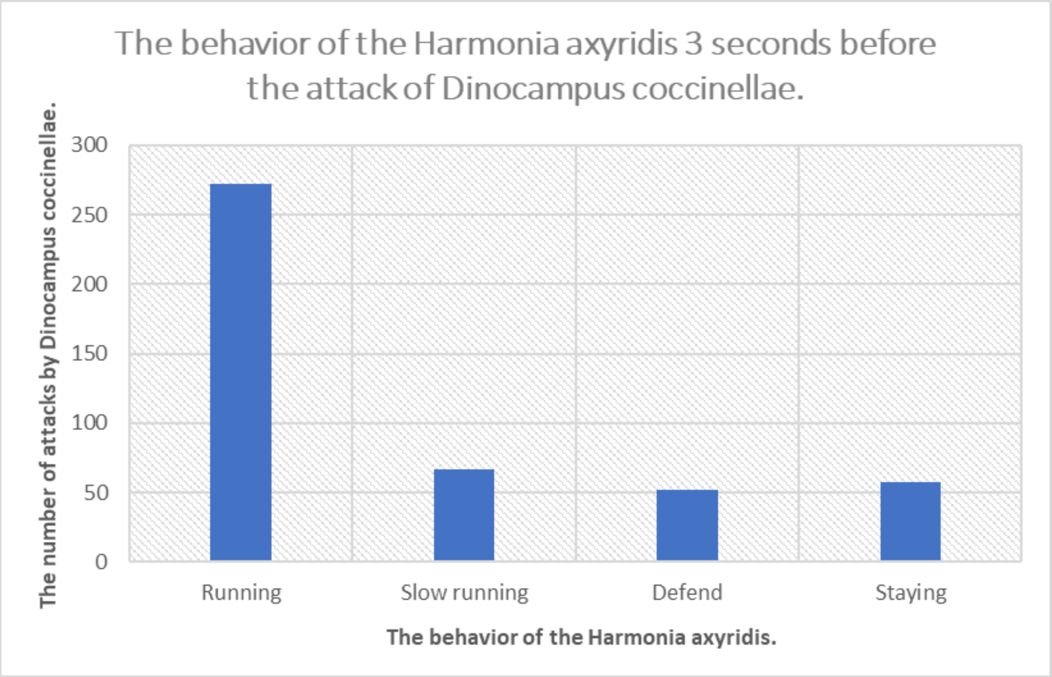

Fig. 10: The graph shows the behavior of *Harmonia axyridis* 3 seconds before the attack of *Dinocampus coccinellae*. The x-axis shows the ladybird's behavior, and the y-axis shows the total number of attacks.

4. Discussion

The relationship between parasitoids and their hosts has undergone a long evolutionary process. Both parasitoids and hosts rely on their innate defense mechanisms (Gross, 1993). Over the years of coexistence, many adaptations have occurred. Hosts try to defend themselves as much as possible from parasitoid attack, while parasitoids try to outsmart their hosts. This leads to an endless race of "outsmarting" each other (Dicke et al., 2020).

Parasitoids are valuable for biological control of insect pests, providing a sustainable and effective alternative to pesticides (Waage & Hassell, 1982). Farmers release reared parasitoids to control pests, which has several times greater benefits than using agrochemicals. This approach is also environmentally friendly, as parasitoids are rarely harmful to non-target organisms (Stanley & Preetha, 2016). In my case, a parasitoid would be beneficial if it could reduce the population of the invasive species *Harmonia axyridis*, which has not yet been clearly demonstrated and requires further study.

There are published studies describing the possible preference of *Dinocampus coccinellae* for females of different ladybird species. There could be several reasons for this. Females are often larger and heavier than males, providing parasitoids with more resources for their larvae (Barron & Wilson, 1998). Parasitoids can use resources from the ovaries and fat body of the ladybird female (Geoghegan et al., 1997).

In the previous experiment of my Bachelor's Thesis, it was not statistically proven that the parasitoids preferred one sex of *H. axyridis* over the other, so this factor was not given much emphasis in the experimental part of this Master's Thesis.

D. coccinellae prefers and reacts more to moving ladybirds (Silva et al., 2012), often due to its antennae which are very sensitive to touch and movement. Another study published in the journal Ecological Entomology in 2020 also showed that some parasitoids do indeed prefer hosts with certain levels of activity (Nouhuys et al., 2020). This phenomenon was confirmed during the experiment. Whenever the ladybird was not moving, the parasitoid generally did not attack it. During the observation, it appeared that the ladybird deliberately remained still so that the parasitoid would not notice it. It is possible that the ladybirds have adapted in this regard as well. In the experimental part, it was significant that the wasp attacked ladybirds that were moving more times on average. It was also investigated whether the behavior of ladybirds just before the parasitoid attack had an impact. It was statistically proven that the behavior of ladybirds has an effect on the attack of the parasitoid. Most attacks occurred on ladybirds that were in fast or slow movement 3 seconds before the attack. The least attacks occurred on ladybirds that were defending themselves or standing still. There are also other parasitoids that choose their hosts based on their activity. For example, parasitoids of the genus Trichogramma also prefer hosts with high locomotor activity (Consoli et al., 2010). The specific behavior of the parasitoid *D. coccinellae*, which utilizes adult hosts in motion, suggests that ladybirds are extremely armored and the parasitoid cannot access them except from below and during movement. The way *D. coccinellae* detects the movement of its host is not yet fully understood. It is assumed that the parasitoid primarily relies on visual cues, but also chemical signals (Koyama & Majerus, 2007).

The wasp tries to lay its eggs into an unprotected part of the ladybird's body using its ovipositor (Silva et al., 2012). It was difficult to determine in the experiment whether the ovipositor insertion was successful or not. Parasitoids are known for their ability to select their hosts based on various factors, such as color, shape, size, or texture of the host (Alphen et al., 1986). Research shows that some parasitoid species have the ability to actively search for and attract their hosts using various attractants. However, in my thesis, I investigated the parasitoid *D. coccinellae* and its host *H. axyridis* and found that this parasitoid does not intentionally choose its host. We analyzed whether the success of parasitism depended on the age, size, and possible infection by a *Hesperomyces virescens* in the ladybird. It was completely random which individual was attacked in the experiment. It seems that this behavior is not exceptional and is found in many species of parasitoids. According to a study published in the Journal of Animal Ecology in 2013, parasitoids typically focus on smaller and less armored hosts (Citron & Bernstein). This result may be important for further research on the host selection of parasitoids, as it shows that some parasitoid species may not have highly specialized preferences for their hosts.

An interesting result was shown in my Bachelor Thesis, which confirms that the geographical area (country of origin) of *D. coccinellae* has a significant impact on the average number of hatched parasitoids in *H. axyridis*. Individuals of the first generation of *D. coccinellae* were collected from different locations in the Czech Republic and Estonia (collected from *Coccinella septempunctata*). No second-generation parasitoids hatched from the Estonian ladybird population (where *Harmonia axyridis* is probably not yet present) after laboratory infection with this parasitoid in *Harmonia axyridis*, whereas many hatched from the Czech population. This would demonstrate the adaptation of the parasitoid to the eastern ladybird in areas where these two species naturally occur. In the Czech Republic, ten years ago, the parasitoid was not adapted to *H. axyridis* and its parasitism success was very low. Today, based on scientific experiments, it has been shown that it has successfully adapted to *H. axyridis* and parasitized it (Knapp et al., 2019).

5. Conclusion

The aim of the thesis was to describe the relationship between parasitoids and their hosts and to highlight the ability of parasitoids to choose their hosts. The main goal was to conduct a set of experiments investigating various factors influencing parasitoid preference for hosts.

The entire methodology was divided into two main experiments. I investigated whether the parasitoid *Dinocampus coccinellae* prefers different factors in its hosts. Specifically, in the first experiment, the effects of host body size, age or the presence of *Hesperomyces virescens* were examined. It was not shown that the parasitoid preferred any ladybirds more based on these factors. With a slight trend towards the first attack on smaller ladybirds. On the contrary, I would expect it to rather prefer larger ladybirds, which could indicate their greater amount of nutrients and higher probability of successful parasitism, but it was not statistically proven. In the case of aging ladybirds and *Hesperomyces virescens* infection, it has not been statistically proven that wasps prefer anyone more.

In the second experiment, it was examined whether the host's movement activity had an impact on its selection by the parasitoid. It was statistically proven that the parasitoid attacks more on ladybirds that are in motion. This result was not surprising to me, as I had this theory already after processing the experimental part of my Bachelor's Thesis. Therefore, I am glad that this experiment could be carried out and statistically confirmed.

Overall, it can be said that the results of this work provide valuable information on the relationship between parasitoids and their hosts. Although no significant preference of *D. coccinellae* was demonstrated based on host size, age, and infection status, it was shown that the host's movement activity has an impact on parasitoid selection. These findings can be useful for studying the relationships between parasitoids and their hosts and can be applied to further research in the field of parasitoid biology.

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7. List of figures

8. Attachments

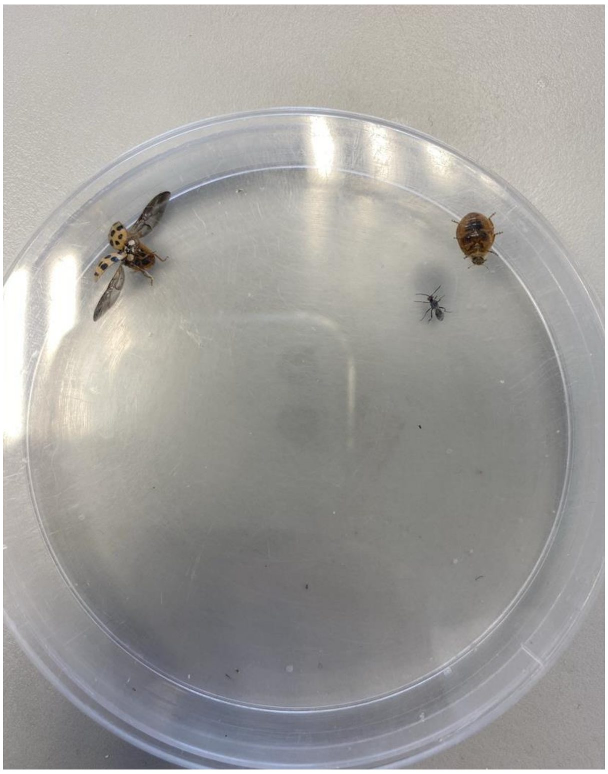
Attachments 1: *Harmonia axyridis* just emerged from the pupa.

Attachments 2: *Dinocampus coccinellae* placed in a Petri dish with ladybirds.

Attachments 3: *Dinocampus coccinellae* has its ovipositor ready and attacking the ladybirds.

Attachments 4: A freshly emerged larva of *Dinocampus coccinellae* from the body of *Harmonia axyridis.*

Attachments 1: *Harmonia axyridis* just emerged from the pupa.



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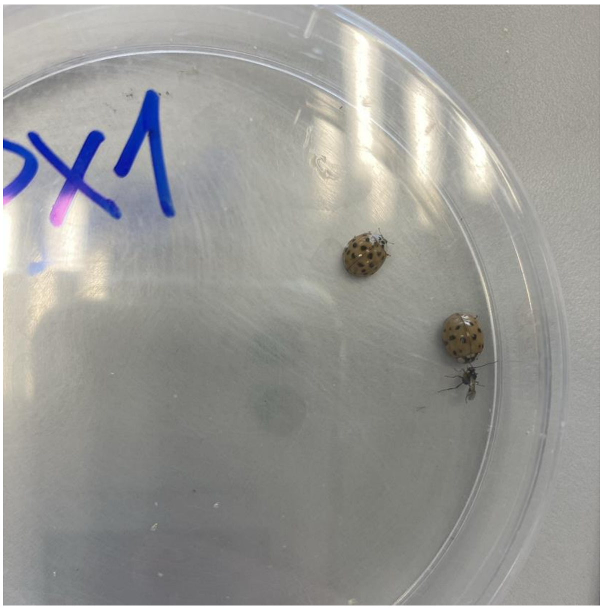

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