Chapter 1

General introduction and thesis overview

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In October 2012, a new species was detected in the Netherlands identified as the fruit fly, *Drosophila suzukii* (NPPO, 2012). It had spread beyond its native range in South-East Asia (Hauser, 2011) and was placed on the European and Mediterranean Plant Protection Organisation (EPPO) A2 list as a pest species recommended for regulation for which quarantine is recommended. Yet, at that time, the pest status was officially declared as “present, at low prevalence” and no control measures were being considered in the Netherlands (NPPO, 2012). Three years later, however, the number of this exotic fruit fly had exploded and caused major damage to farmers of a wide range of soft and stone fruits, including cherries, blueberries, strawberries and raspberries, with an estimated economic loss of 20 million euro (Nijland & Helsen, 2015). At the same time, in 2015, the pest was also recorded from 9 other European countries, from Spain and Italy to Austria and Croatia, and had established along the western part of North America up to Canada (Calabria, Máca, Bächli, Serra, & Pascual, 2012; Adrion et al., 2014; Cini et al., 2014), likely facilitated by the international trade of soft fruits (Fraimout et al., 2017). By 2020 it had spread to most regions in Europe, Africa, Russia, and North and South America (EPPO, 2020) causing major economic losses to fruit growers (Bolda, Goodhue, & Zalom, 2010; Farnsworth et al., 2017; Mazzi, Bravin, Meraner, Finger, & Kuske, 2017; Yeh, Drummond, Gómez, & Fan, 2020).

The morphology of the invasive fruit fly seems very similar to its well-studied, ‘famous’, and closely related species, *D. melanogaster* (Ometto et al., 2013). However, unlike other *Drosophila* that lay eggs in overripe decaying rotting material, *D. suzukii* exhibit a serrated ovipositor enabling it to pierce through the hard skin of undamaged fruits (Atallah, Teixeira, Salazar, Zaragoza, & Kopp, 2014). This causes direct damage by the fruit fly larvae feeding from pulp, and indirectly by allowing entry of other pathogens (Rombaut et al., 2017; Ioriatti et al., 2018). This, together with its polyphagous feeding on fruit crops and wild host plants (Lee et al., 2011; Poyet et al., 2015; Kenis et al., 2016), makes the fly destructive to a wide range of fruits at the moment of ripening (Walsh et al., 2011).
The similarity to other *Drosophila* species and limited knowledge of its taxonomy and ecology impaired immediate detection and hampers implementation of effective control actions (Hauser, 2011; Cini, Ioriatti, & Anfora, 2012). With zero tolerance of insect infestation, farmers often take a pro-active approach by applying broad-spectrum pesticides to protect their crops (Van Timmeren & Isaacs, 2013; Diepenbrock, Rosensteel, Hardin, Sial, & Burrack, 2016; Farnsworth et al., 2017). This is however not a long-term sustainable solution, as these chemicals are harmful to non-target species and their repetitive application increases the risk of selecting for insecticide resistance (Desneux, Decourtye, & Delpuech, 2007; Van Timmeren, Mota-Sanchez, Wise, & Isaacs, 2018; Van Timmeren, Sial, Lanka, Spaulding, & Isaacs, 2019). This initiated a global research effort to increase understanding of the biology of this pest species and use this knowledge for development of pest management strategies.

**The story of *D. suzukii* is just one of many**

The range-expansion of *D. suzukii* does not stand on its own. Over the last decades an increasing number of new species has been reported to have established outside their native range (Lambdon et al., 2008; Butchart et al., 2010; McGeoch et al., 2010; Early et al., 2016) and those able to establish can have large negative impact on biodiversity (Powell, Chase, & Knight, 2013) and economies (Pimentel, Zuniga, & Morrison, 2005; McNeely, 2006). However, international trade and climate change are expected to continue to enhance the invasion process and facilitate the prevalence of species range shift in the future (Dukes & Mooney, 1999; Levine & D'Antonio, 2003; Meyerson & Mooney, 2007; Bellard et al., 2013; Early et al., 2016). A unified framework is therefore needed for understanding factors that facilitate the establishment and spread of exotic species and for development of general approaches to efficiently reduce the occurrence and impact of invasive species for long-term management (Keller, Geist, Jeschke, & Kühn, 2011; Robertson et al., 2020).

An important factor by which exotic species can establish and thrive in the invasive area is known as the ‘enemy release hypothesis’, which posits that species can spread and proliferate due to scarcity of natural enemies in introduced ranges (Keane & Crawley, 2002; Colautti, Ricciardi, Grigorovich, & MacIsaac, 2004). The lack of such ‘top-down’ control might be due to the inability of native species to detect the exotic prey/host, their inability to circumvent its defence mechanisms, or their strong preference for exploitation of native over invasive species. Consequently, the control of biological invasions by natural enemies requires a change in their resource acquisition, either by a niche expansion or shift. Interestingly, exotic species can represent an under-exploited new niche and can give individuals a fitness advantage when they are able to exploit it (Carlsson, Sarnelle, & Strayer, 2009). As such, exotic species can exert a new selection pressure on native enemies, such as predators, parasites and parasitoids. When at least some individuals are able to successfully attack the invader, native species might adapt and provide biotic resistance and augment long-term control of the invader. Yet, although evolutionary responses have been documented, little is known about the frequency and underlying genetic mechanisms of such evolution (Strauss, Lau, & Carroll, 2006).
Can the impact of invasive species be reduced by evolution?

Rapid evolution entails the increase in frequency of alleles over generations (micro-evolution). This requires heritable genetic variation in traits enabling exploitation of the invader and requires that these genotypes differ in fitness, i.e., individuals should obtain a fitness benefit when they can consume/parasitize the novel species. Hence, if these requirements are met, evolution of exploitation might occur. There is however one problem; the speed at which this can take place.

The case of *Drosophila suzukii* illustrates that the establishment, spread and growth rate of exotic species beyond their native range can occur over relative short time. The evolution of exploitation of the invader in native species might therefore (assuming presence of heritable variation in exploitation) not keep up with speed of invasion to provide biotic resistance. Hence, the difference in time scale of evolutionary and ecological processes can cause a problem when the speed of adaptation to the invader of native species does not meet the timely demand for adequate prevention and control. Several (interacting) genetic and ecological factors can impede rapid evolutionary response. For example, selection pressure can change through time due to e.g., seasonal variation in abundances of the invasive species and alternative resources, which can change the direction of selection, and thus the speed of evolution. Also, rapid evolution likely selects mainly on pre-existing genetic variation. However, species might lack this variation, or adaptive genotypes may occur at a low frequency in a population making them vulnerable to be lost by genetic drift. In addition, gene expression might substantially be influenced by environmental conditions (i.e., have a relative low heritability) and therefore not be subject to selection. Another problem might occur when the invader evolves counter adaptations to escape from native natural enemies (co-evolution). Hence, although native species might adapt and augment control of invasive species in the future, i.e. if genetic and ecological conditions allow evolutionary change, this might not always meet the urgent need for management strategies in time.

Instead of waiting for native species to evolve virulence, natural enemies already adapted to the pest in its area of origin might be imported for biological control. Although such planned species introductions in ‘classical’ biocontrol programs can reduce (economic) damage of the pest (Naranjo, Ellsworth, & Frisvold, 2015), it has been shown to pose severe environmental risks and thus requires careful evaluation before implantation (Howarth, 1991; Louda, Pemberton, Johnson, & Follett, 2003; Heimpel & Cock, 2018). This, together with new regulations of the import of species (Cock et al., 2010; van Lenteren et al., 2011) impede the development of classical biocontrol programs and raises the question whether there are alternative biological control methods.

Can humans give evolution a helping hand?

The high demand for sustainable pest management solutions and reduced availability of potential control agents has led to the revival of and old concept within biocontrol: speed up and direct the evolution of native species (Lommen, de Jong, & Pannebakker, 2017). This involves selecting and breeding native natural enemies with desirable characteristics for
controlling the invasive pest and releasing them in the field. Despite the large body of theoretical research and its successful application in plant and animal breeding for agriculture, artificial selection has not widely been applied to select natural enemies for biological pest control. Yet, the use of already existing natural genetic variation might be a promising environmentally friendly approach to improve biological pest control. The application of this method, however, also challenges the integration of fundamental and applied research to answer questions about evolutionary processes and biological pest control: what determines phenotypic variation and the outcome of biotic interactions for both agents involved, how can we direct and predict evolution, what factors might hamper adaptation of native species towards novel invasive species in the field and what conditions would facilitate such adaptation?

**Thesis outline**

*How will organisms respond to something they do not know and what are the consequences?*

The objective of this PhD research was to investigate how native natural enemies respond to invasive species via evolution. Deeper knowledge of the ecology and evolution of bio-interactions is important for the advancement of basic scientific understanding and of improving biological control to reduce the impact of exotic species invasions. I examined this by focussing on native natural enemies, parasitoids with the potential to parasitize the invasive pest *Drosophila suzukii*. First, we investigate through literature research the amount of natural variation within and between parasitoid species in exploiting the non-native *D. suzukii* as host (Chapter 2) and by testing different parasitoid populations in the laboratory of one of the most promising species, *Leptopilina heterotoma* (Chapter 3). Next, I aimed to understand what determines the observed phenotypic variation in parasitization in *L. heterotoma*. Therefore, I investigated the role of genetics (Chapter 3), endosymbionts and developmental conditions (Chapter 4) in shaping the individuals’ phenotype: host killing and reproductive success. Given the presence of genetic variation, I then tested the potential of *L. heterotoma* to adapt to the novel host under laboratory conditions by artificial selection (Chapter 5) and in a two host - one parasitoid system through an individual-based model study (Chapter 6).

In **Chapter 2** we outline the importance of finding new ways to improve natural enemies for controlling invasive pests. We therefore propose a four-step approach for development of biocontrol methods by exploitation of intra-specific variation in native enemies: investigate the amount of (1) inter- and (2) intra- specific variation, (3) understand which genetic and environmental factors shape this variation and (4) exploit variation by selective breeding. This approach is then illustrated with the parasitoids – *D. suzukii* interaction. This shows that *L. heterotoma* is one of the few European parasitoid species parasitizing the pest.

Following the described four-step approach in Chapter 2, in **Chapter 3** I then test and compare seven European *L. heterotoma* lines to determine the amount of intra-specific variation to parasitize *D. suzukii*. I report significant variation between lines in
parasitization performances, indicating presence of genetic variation. I then crossed these populations to make a genetically variable population and quantified the amount of additive genetic variation and heritability of different parasitization performances. I found significant genetic effects in attack rate, killing rate and killing efficiency, but not in reproductive success.

In Chapter 4 I aimed to further investigate non-genetic factors shaping parasitization ability of *D. suzukii* in *L. heterotoma*, namely the role of the endosymbiont *Wolbachia*, and developmental conditions to improve the wasps’ killing performance. I found that the developmental host plays a major role in the wasps’ fitness: wasps cultured on relatively low-quality hosts produced fewer offspring, these offspring were smaller and exhibited a lower killing rate. Three successive generations of heat-shock treatments did, however, not influence *Wolbachia* presence in the wasps nor host-killing ability of their offspring, indicating that the *Wolbachia*–*L. heterotoma* interaction is heat tolerant.

Based on reported genetic variation in non-reproductive host killing in Chapter 3, I conducted an artificial selection experiment for increased host killing in Chapter 5. I selected parasitoids for seven generations, and found that despite previously significant genetic effects (Chapter 3), there was a small and inconsistent response to selection in the three selection lines relative to the control lines. There was, however, a strong and consistent response to selection in a correlated trait, the attack rate, after 7 generations. Moreover, we found that survival of the *D. suzukii* stock population in absence of parasitoids significantly increased over the course of this one-year selection experiment. Hence, I conclude that non-reproductive host killing might have evolved through selective breeding but detection was hampered due to increased fitness of the host.

In Chapter 6 I further explore the evolution of the parasitoid, but instead of human imposed selection in the laboratory, I use an agent-based simulation model to investigate whether and how resident parasitoids might evolve in response to selection imposed by the invasive pest in a two host - one parasitoid system. If parasitoid populations exhibit genetic variation in host performance and preference, what conditions would favour adaptation and/or avoidance to the (initially) unsuitable host? To answer this question, I simulate the evolution of the parasitoid for host species preference and parasitization strategy in a model that is inspired by the *D. suzukii* – *L. heterotoma* interaction.

In the final Chapter 7 I synthesize the results of my three data chapters, the literature review and the modelling study, and discuss future research directions.