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THESIS

From Asia to Europe, evaluation of parasitoids for the biological control of the invasive fruit pest *Drosophila suzukii*

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“ From Asia to Europe, evaluation of parasitoids for the biological control of the invasive fruit pest *Drosophila suzukii* ”

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Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.

Louis Pasteur, Free Lance of Science (1960) by René Jules Dubos

RÉSUMÉ

La production agricole est en constante évolution afin d'améliorer le rendement. Actuellement, 40% de la production agricole est perdue à cause des ravageurs des cultures (majoritairement des insectes exotiques). Leur contrôle est une des priorités majeures à laquelle les chercheurs font face aujourd'hui. Le commerce international et le changement climatique ont accéléré la dissémination de nouvelles espèces exotiques à travers le monde. L'une de ces espèces récemment introduite, est la Drosophile à ailes tachetées, *Drosophila suzukii.* Cette mouche originaire d'Asie orientale a été recensée en Europe et en Amérique du Nord en 2008 et depuis, génère une attention particulière car elle cause de sérieuses pertes économiques dans les productions maraichères des petits fruits. A contrario des autres Drosophilidae qui habituellement pondent dans la matière en décomposition, *D. suzukii* pond ses œufs dans les fruits frais. La larve en s'alimentant, entraine alors la dégradation du fruit. Actuellement, le contrôle de *D. suzukii* consiste à utiliser des traitements chimiques et à mettre en place des pratiques culturales adaptées. Des évaluations sur l'utilisation potentielle de la lutte biologique visant à utiliser des parasitoïdes de drosophiles dans les zones envahies ont été menées, cependant la majorité de ces espèces indigènes n'ont pu se développer dans *D. suzukii* car elles n'étaient pas adaptées. C'est pourquoi, l'introduction d'ennemis naturels de la région native de *D. suzukii* est envisagée. Ainsi l'objectif de cette thèse était d'évaluer le potentiel de différents parasitoïdes larvaires (généralement plus spécifique) Asiatique de *D. suzukii* en tant qu'agent de lutte biologique. Ce projet a débuté par la collection de parasitoïdes en Asie (Chapitre 1) permettant ainsi d'évaluer leur efficacité. Au moins huit espèces de parasitoïdes ont été recensées, dont certaines nouvelles espèces. Les taux de parasitismes en Asie sont très variables (0-80%) mais, dans chaque région le complexe de parasitoïdes est dominé par deux Hyménoptères (Famille: Figitidae), *Ganaspis* sp. et *Leptopilina japonica*. De nombreuses souches de ces espèces ainsi qu'un troisième Hyménoptère (Famille: Braconidae) *Asobara japonica* ont été collecté et importé en Suisse afin de conduire des expériences de laboratoire en quarantaine. Différents aspects de leur biologie ont été étudiés (Chapitre 2) et comparés à une espèce européenne *Leptopilina heterotoma*. La période de pré-oviposition et le temps de développement ont été mesurés, ainsi que la capacité à se développer dans *D. suzukii* dans le fruit (myrtille) ou sur substrat artificiel ont été comparé. Les trois espèces asiatiques ont été capables de se développer sur *D. suzukii*, alors que les œufs et les larves de *L. heterotoma* ont été majoritairement encapsulés par *D. suzukii*. *Asobara japonica* et *L. japonica* ont réussi à se développer sur *D. suzukii* sur les deux substrats, alors que *Ganaspis* sp. a pondu très peu d'œufs dans les larves sur substrat artificiel, suggérant ainsi qu'il est peut-être spécialisé dans les drosophiles vivant dans un habitat « fruit frais ». Dans un second temps (Chapitre 3), la spécificité de ces mêmes parasitoïdes a été évaluée lors de tests en non-choix sur *D. suzukii*, cinq espèces de drosophiles européennes et une Tephritidae sur myrtilles et/ou deux milieux artificiels. D'une part, ces tests ont montré que *A. japonica* était le plus généraliste et d'autre part, que *Ganaspis* sp. était l'espèce la plus spécifique. Cependant, d'importantes variations entre les deux souches de *Ganaspis* sp. ont été observées. La souche japonaise étant strictement spécifique à *D. suzukii* dans les myrtilles, alors que la souche chinoise a bien parasité *D. suzukii* mais également une espèce non-cible *D. melanogaster* sur un substrat artificiel enrichi en fruit mixés. La souche de *L. heterotoma* européen a attaqué *D. suzukii* mais étant non adapté à cet hôte, quasiment tous les œufs et les larves ont été encapsulés au contraire de ceux pondus dans les drosophiles européennes. Dans une dernière étude (Chapitre 4), les tests d'olfactométrie ont confirmé les tests en non-choix. La souche japonaise de *Ganaspis* sp. montrant une forte attractivité pour *D. suzukii* dans les fruits frais en comparaison des fruits en décomposition et du substrat artificiel enrichi en fruit, au contraire de la souche chinoise. Pris dans leur ensemble, ces résultats sont prometteurs pour le contrôle biologique de *D. suzukii* en Europe et ont montré que *Ganaspis* sp. est le candidat le plus prometteur. Cependant, des variations intra-spécifiques de la spécificité de l'hôte ont été observées. D'autres études seront nécessaires sur son statut taxonomique et sur l'existence de biotypes ou d'espèces cryptiques avant que des lâchers sur le terrain puissent être envisagés en Europe.

MOTS CLES: Drosophile à ailes tachetées, parasitoïdes, test de spécificité d'hôte, olfactométrie, lutte biologique classique.

ABSTRACT

Agricultural processes are constantly improved to improve crop yields. However, 40% of crop productions are currently lost to pests each year. Insect pests are one of the main factors of these losses and their management is one of the top priorities that researchers are facing worldwide. An important part of these losses are caused by pest with alien origins. Globalization and climate change speed up the spread of new invasive pests. One of these recent invasive pests is the spotted wing Drosophila, *Drosophila suzukii.* This fly of East Asian origin was first found in Europe and North America in 2008, and since then, it has generated much attention due to severe economic losses in berry and stone fruit crops. Unlike other Drosophilidae that usually develop in decaying matters, *D. suzukii* lays its eggs inside ripening fruits and damages are mainly caused by larval feeding, resulting in the degradation of fruits. Currently, the management of *D. suzukii* relies on chemical treatments and cultural methods. Studies have been undertaken to investigate the potential of biological control using native parasitoid species associated with *D. suzukii* in its invaded regions, but the majority of these species failed to develop as they were not able to locate the host in ripening fruits. Therefore, the introduction of natural enemies from the native region of the pests is envisaged. In Drosophilidae, the most abundant and specific natural enemies are usually larval parasitoids. Thus, the objective of this thesis was to assess the potential of Asian larval parasitoids of *D. suzukii* as biological control agents in Europe. The project started with surveys in China and Japan (Chapter 1) to study the larval parasitoid complex of the fly in its region of origin and assess parasitism. At least eight parasitoid species were collected, including some new to science. Parasitism rates in Asia were highly variable (0-80%) but, in all investigated regions, the parasitoid complex was dominated by two hymenopterans of the family Figitidae, *Ganaspis* sp. and *Leptopilina japonica*. Several strains of these two species and a third species, the Braconidae *Asobara japonica*, were imported to Switzerland for laboratory experiments in quarantine conditions. Several aspects of their biology were investigated (Chapter 2) and compared with the European species *Leptopilina heterotoma*. The pre-oviposition period and their development time were measured, and their ability to parasitise *D. suzukii* in fruit (blueberry) and artificial diet was compared. The three Asian species were successfully reared on *D. suzukii* larvae, in contrast to *L. heterotoma* whose eggs and larvae were encapsulated by the host larvae. *Asobara japonica* and *L. japonica* were highly successful in both media, while *Ganaspis* sp. laid very few eggs in larvae in the artificial diet, suggesting that it may be specialised in *Drosophila* species living in fresh fruits. In a second step (Chapter 3), the specificity of the same parasitoids was assessed through no-choice tests on *D. suzukii,* five European *Drosophila* spp. and one Tephritidae, in blueberry and/or two different artificial diets. On the one hand, these tests showed that *A. japonica* was the most polyphagous species. On the other hand *Ganaspis* sp. showed the highest specificity. However, important variations between two tested *Ganaspis* sp. strains were observed. The Japanese strain was strictly specific to *D. suzukii* in blueberry, whereas another strain from China parasitised *D. suzukii* and the non-target *D. melanogaster* in a diet enriched with blended fruit. The European *L. heterotoma* massively attacked *D. suzukii* but almost all eggs and larvae were encapsulated, in contrast to eggs laid in European *Drosophila* spp. In a last study (chapter 4), olfactometer tests confirmed the no-choice tests. *Ganaspis* sp. from Japan showed a strong attractiveness towards *D. suzukii* in fresh fruits compared to decaying fruits and to diet enriched with fruit, in contrast to the Chinese strain. Taken all together, these results are promising for the biological control of *D. suzukii* in Europe and showed that *Ganaspis* sp. is the most promising candidate. It is both the most important parasitoid of *D. suzukii* in Asia and the most specific one in laboratory tests. However, important intra-specific variations in host specificity have been observed. More studies are needed on its taxonomic status and the existence of biotypes or cryptic species before field releases can be envisaged in Europe.

KEY WORDS: Spotted wing Drosophila, host range test, parasitoids, specificity test, olfactometer bioassays, classical biological control

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GENERAL INTRODUCTION

OVERVIEW

Species naturally expand their native range to new territories; it is the main goal of any living organism to increase its fitness. Geographical barriers (*e.g.* mountains, sea barriers), competition and predation are some challenges that have reduced and controlled this expansion for billions of years of evolution. In recent decades, human activities and in particular the intensification of world trade have favoured the breakdown of geographical barriers, which previously limited the expansion of species. As a result, many exotic species, introduced accidentally or voluntarily, settled in new geographical areas (Lambdon et al., 2008; Hulme, 2009) and some generated disturbances within these ecosystems (Blackburn et al., 2011). Those that create disturbances are considered “invasive species”. It is estimated that the 50,000 non-native species established in the United States would have cost more than $137 billion in agricultural losses and treatments. Within the European Union, it is estimated that invasive species of arthropods do represent a loss of €10 billion per year (Kenis & Branco, 2010). In agriculture, 40% of crop productions are currently lost due to pests each year and a fair amount due to invasive species.

BIOLOGICAL INVASION: FACTORS & THEORIES

A biological invasion is the result of three successive stages: the introduction, establishment and proliferation of an organism in a new geographical area (Mack et al., 2000). Several factors may explain the success of an invasion: i) climatic and/or ecological similarities between native and invaded areas may facilitate the establishment of the species, ii) life history traits may confer increased invasiveness capacity (*e.g.* fertility, reproductive mode, time development) and iii) high genetic diversity of the introduced population would provide better chances to adapt to new environmental constraints (Phillips et al., 2008). Invasive species are often more abundant and may have a higher impact than in their region of origin and, in general, the theory of "Enemy Release Hypothesis: ERH" (Colautti et al., 2004) is often provided as the main explanation for this phenomenon. The likelihood of success of an invasive species is greatly increased with the absence of natural enemies in the invaded area. An indirect effect of the ERH theory is the possibility of re-allocating energy usually spent on defense to other life traits, which would further increase the fitness of the invasive species (related theory of " Evolution of Increased Competitive Ability hypothesis ": EICA) (Blossey & Notzold, 1995). The absence of enemies in the introduced area also leads to a difference in genetic composition between the species in its native and in its introduced area due to differences in the selection pressures on the different genotypes.

BIOLOGICAL CONTROL: PRINCIPLE & OBJECTIVES

The economic and environmental impacts of invasive species (the estimated cost of invasive species worldwide is more than $1.4 trillion per year - Pimentel et al., 2001) justify the use of eradication or control methods such as mass trapping, cultural or chemical control (pesticides, herbicides, etc.). However, chemical methods are often ecologically and societally unsatisfactory (risk to human health and the environment). It implies an additional economic cost for the producers and does not follow global political objectives of developing sustainable agriculture. In addition, many invasive species may use non-crop habitats and are therefore difficult to treat at landscape level. Some may already express resistance to some chemical products or may develop resistance in the foreseeable future.

An alternative to manage invasive species is biological control - *i.e.* the use of an organism (biocontrol agent) to control the population density of another organism (pest). Biocontrol agents can be used in many ways and it is important here to identify and define the three different categories of biological control strategies: augmentation, conservation and introduction (or classical). Biological control by augmentation implies regular releases of biological agents that may already be present in the habitat or not, for a temporary control. The second strategy (conservation), tries to promote an area wide approach by preserving or by recreating the habitat of the naturally-occurring biological control agents within the crops system or natural lands in order to enhance their populations. Finally the last approach is called “classical biological control” (CBC) and defined as: *“the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control”* (Eilenberg et al., 2001). Biological control is commonly less controversial than chemical control because of the direct benefits (*e.g.* on human health, food safety and maintenance of ecosystem services) and effectiveness of controlling agricultural pests and invasive aliens. However, In CBC and biological control by augmentation involving exotic agents, potential adverse ecological effects have to be considered prior to the introduction (Heimpel & Mills, 2017). From the 6,000 introductions of biological control agents conducted since the 1890s to control crop pests, about 25% have been effective to date (Van Lenteren et al., 2006). The most commonly used biocontrol agents are parasitoids belonging to the orders Hymenoptera or Diptera. This type of organism lays and develops in or on another arthropod (host) and consumes its tissues, effectively killing it. The choice of the biological agent that will be introduced is a crucial step. It is generally quested in the native area of the pest and chosen accordingly to its effectiveness and host specificity, *i.e.* its capacity to develop almost exclusively on the target pest. The choice of agent must take into account many factors in order to prevent any non-target impacts that could be deleterious for the ecosystem in the introduced area.

UNINTENTIONAL EFFECTS IN CLASSICAL BIOLOGICAL CONTROL

As classical biological control is a deliberate introduction of exotic species to a new environment, it may have unintended consequences, which have to be studied prior the release of any biological control agents. About 1.7% of introductions of parasitoids or predators for biological control purpose have had adverse consequences on non-susceptible species with a minor effect and 0.34% have had severe impacts and population reduction (Lynch & Thomas, 2000; Van Driesche & Hoddle, 2017). This occurred from a genetic level, that can leads to hybridization with local species to a demographic level with negative effects on non-target species population dynamics through changes in its host range, which could lead to non-target species extinction (Van Lenteren et al., 2006). For example, in the 1970s, the introduction of *Coccinella septempunctata* in the United States to control the population of *Diuraphis noxia* (Russian wheat aphid) resulted in the displacement of native ladybird populations. The decrease of these local predators ultimately led to less control of aphid communities (Louda et al., 2003). Introductions of biological control agents can also lead to the displacement and decline of native competitors such as the displacement of native ladybirds observed following the introduction of another ladybird, *Harmonia axyridis*, in Europe and North America (Roy et al., 2012). It is therefore necessary to consider the possibility of any possible non-intentional impacts before any introduction of an exotic natural enemy (parasitoid, predator or pathogen) for the management of a pest. Host specificity assessment is usually the main task in the evaluation of non-target effects, but other non-target effects such as competition with native organisms and potential indirect effects on the environment need to be considered. The potential environment impacts of introduced biological control agents are various. i) Direct attacks on native species populations; instead of focusing on the target host or prey for which the biological agent was introduced for, it switches to a native host or prey. ii) Negative food-web impact (competition or displacement of native species): within the food-web, the newly introduced agent over-competes with native species, which decline or are displaced to geographical areas where the biological control agent is not yet present. iii) Positive food-web effects that are beneficial to non-target species: it could happen that a decrease of the pest population by the introduced biological control agent conducts to an increase of native species populations. iv) Hybridization with native species: interbreeding may occur when biological control agents are introduced in areas where closely related species are present. If hybrids are fertile, this may lead to the genetic decline of the native species. v) Attacks on previously introduced biocontrol agents: in some cases the newly introduced agents switches its host range and attack another biological control agent previously introduced, *e.g.* to control an invasive weed (Van Driesche & Hoddle, 2017). Finally, at a later stage, it is important to conduct post-release studies in order to evaluate the impact, establishment and dispersion of the released agent.

Because of stricter regulations and increasing concerns for non-target effects, the number of entomophagous insects introduced for biological control purposes has decreased since the 1980s (Cock et al., 2016). In order to better control the introduction of exotic species and preserve biodiversity, many "precautionary principles" have been listed in many countries to give a better frame for this research activity. This imposes an assessment of the risks associated with the introduction of any macro-organisms in the environment outside its native area. This regulation, which applies to biological control, requires an application for authorization to enter the territory for non-indigenous organisms and an assessment of the non-intentional effects of their introduction. In most countries approval for release of classical biological control agents is based on a risk assessment determined from a petition detailing outcomes of studies on host specificity and other information (Mason et al., 2013). However, no harmonized procedures have been decided within the European Union for example. Current practices usually include laboratory host specificity tests as a first step to define the fundamental host range of the potential biological control agent (Van Driesche & Murray, 2004; Bigler et al., 2005, 2006; Van Lenteren et al., 2006).

Each government has the choice to use or not to use a biological agent. However, it is impossible after the release to control the geographical expansion of the population of a biological agent. It has already been demonstrated that, after the release, biological agents crossed the border toward a country that originally denied the use of this specific insect in its lands. *i.e. Torymus chinensis* was allowed to be used in Italy against *Dryocosmus kuriphilus* but not in Switzerland. A few years after Italian releases, the first specimens were collected in Switzerland and saved the chestnut production in the country.

CONTEXT – THE INVASION OF *DROSOPHILA SUZUKII*

Recently, an Asian fly pest, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) (Figure 1A) invaded and rapidly spread in North America and Europe simultaneously (Figure 2), where it now seriously threatens small fruit productions (Fraimout et al., 2017). This fly is also known under the name "Spotted Wing Drosophila" (SWD), in reference to the dark spot on the wing tips of the male. In Europe, this species was first reported in Spain and Italy in 2008 and then spread very quickly throughout most of Europe. It invaded Switzerland within a couple of years (2010-2012) (Cini et al., 2014).

In contrast to the vast majority of Drosophilidae, which feed on rotting fruits and other organic matters, *D. suzukii* can lay eggs through the skin of healthy fruits at maturity using its serrated ovipositor, which makes it a pest of many fruit crops, including berries and stone fruits (Figure 1B) (Atallah et al., 2014). This highly polyphagous fly also develops in wild fruits (blackberry, elderberry, etc.), which serve as a reservoir (Kenis et al., 2016). Like other Drosophilidae, *D. suzukii*'s life cycle is composed of 4 different stages: egg, larvae (3 stages), pupae and adults; at 22 ± 2°C, 55 ± 5 % RH, 16:8 hours (L:D) it takes 14 days to complete a generation (Figure 3A).

The ovipositor is expanded and has two rows of highly sclerotized teeth. Thanks to this adaptation, *D. suzukii* is able to exploit a different ecological niche (fresh fruits) than the local Drosophilidae, which potentially reduces competition with closely related species, but also causes massive agricultural damages and economic concerns (Walsh et al., 2011). The damage generated in berries crops (strawberries, raspberries, blueberries, etc.) and stone fruits (cherries, apricots, etc.) demands the development of new integrated pest management (IPM) methods, which will vary depending on the fruit crop. The presence of *D. suzukii* larvae in the harvested fruit leads to the rotting of the fruits and enhances the development of secondary infections (micro-organisms and fungi), which may prevent commercialization, resulting in heavy losses. For example, costs of pest management measures (monitoring and control measures) against *D. suzukii* in sweet cherry production in Switzerland are estimated around CHF 1,857 per hectare and per year (Mazzi et al., 2016). In the province of Trento, Italy, annual losses in small fruit production were estimated at €2,8 million per year (De Ros et al., 2013). Annual costs to the US fruit production may exceed $500 milion (Bolda et al., 2010).

Figure 1. Pictures of Drosophila suzukii A. male and female, B. Eggs and larvae in blueberry and cherry. (© Photo: G. Arakelian, T. Haye, A. Lucchi & S. Revadi)

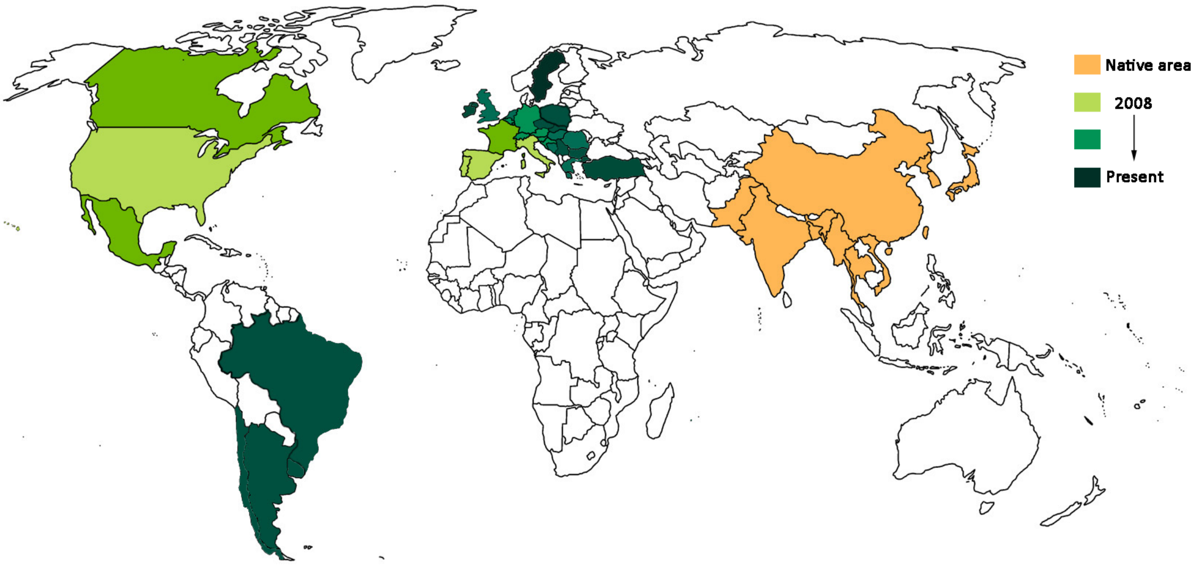

Figure 2. Worldwide distribution of *Drosophila suzukii*. Native area: orange, light green to dark green: invaded area over time (2008 to present) (Data from EPPO global database & CABI Invasive Species Compendium)

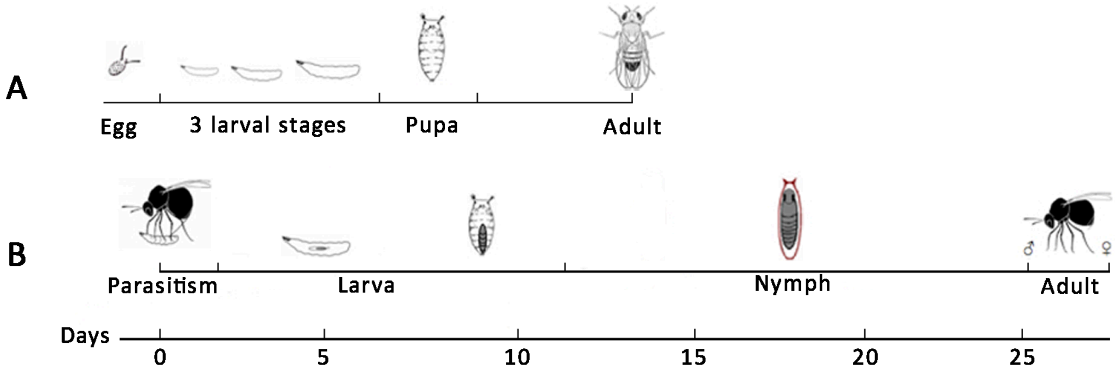

Figure 3. Life cycle for A. *Drosophila suzukii* and B. *Leptopilina heterotoma* at 22 ± 2°C, 55 ± 5 % RH, 16:8 h (L:D).

The closely-related *D. melanogaster* is one of the most studied insects worldwide, and knowledge about this local fly may help to better understand the invasion of *D. suzukii* and could inform the development of new control methods (Iacovone et al., 2015). *Drosophila suzukii* was not well known before the invasion, and scientists had first to increase knowledge on the biology and ecology of this fly. 90% of the 317 publications (after 2003) found in the Web of Science have been published since 2011 (Figure 4). Prior to developing appropriate IPM methods and classical biological control against this new invasive pest, basic knowledge of the biology and ecology of the fly were required.

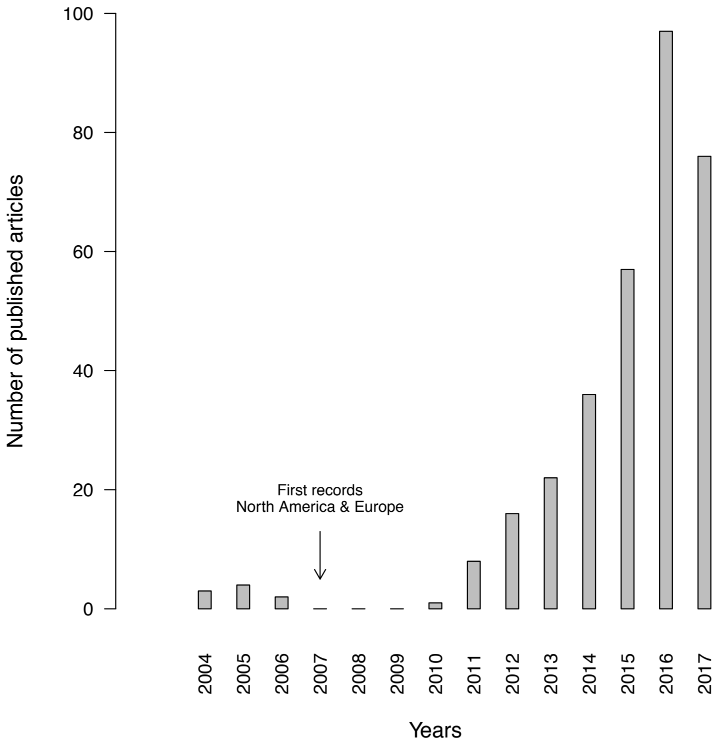

Figure 4. Number of published articles on *Drosophila suzukii* since 2004 (Data from Web of Science, October 2017) Total records: 317 articles (20 articles published prior 2004)

CONTROL METHODS

Many different methods are currently used and are still being developed internationally in many different projects to fight against *D. suzukii*. As with all crop insect pests, the three major ways to control insects in orchards are: chemical, cultural and biological control methods (Haye et al., 2016).

Chemical control

Evaluations in the laboratory or in the field of the efficacy of insecticides for the control of *D. suzukii* have been done in most of the fruit growing regions where the fly is now distributed. The limitation of the use of chemicals is that fruits have to be harvested fresh and ripe just a few days before being sold. At this stage, the presence of chemical residues is restricted for obvious food safety reasons for customers. The current effective insecticides used for managing SWD are principally conventional broad-spectrum products, which are not always compatible with IPM programs, such as new pyrethroids and organophosphates (Beers et al., 2011; Haviland & Beers, 2012; Van Timmeren & Isaacs, 2013). Neonicotinoids have been used, but they are perceived to be less effective (Bruck et al., 2011). Spinosyns (spinosad and spinetoram) currently seem to be one of the most effective solutions against *D. suzukii* with less adverse impact on human health than other compounds (Beers et al., 2011; Bruck et al., 2011; Haviland & Beers, 2012). For resistance management, the number of applications per year of a chemical control needs to be limited on a given crop. Currently, there is limited published information regarding the resistance of *D. suzukii* to chemicals, but resistance will likely become a major problem in the future. Knowledge about how *D. melanogaster* has developed resistance to insecticides would likely help chemists to develop new strategies (Remnant et al., 2014; Wan et al., 2014).

Cultural methods

Cultural methods such as sanitation (reduction of the covering vegetation, removal of dropped and over-ripe fruits during the harvest period) (Lee et al., 2011), destruction without oxygen or solarisation (infested fruits are placed in plastic bags and exposed for at least 2 days to the sun), leaf thinning (to reduce humidity around fruits) have been suggested and are presently used in various ways in different crops. For example, short harvest intervals may help to reduce the number of infested fruits during harvest. Using insect proof nets (mesh size varies between 0.5 x 0.8, 1 x 1 and 1 x 1.6 mm (Gamper, 2015; Cormier et al., 2015) seems to have clear positive results (Kawase & Uchino, 2005), but before their installation, growers need to be sure that there is absolutely no *D. suzukii* in the field. Mass trapping (sticky or bait trap) is now a commonly practiced cultural method, but its efficiency is variable. Application of lime solution is tested in Switzerland as an alternative of chemical compounds and results show its positive effect as a repellent, which reduce fruits attractiveness for *D. suzukii* (Baroffio et al, 2017). In the same way, kaolinite could be used to reduce visual attractiveness of ripe fruits. Traps are often used to monitor populations and plan other control methods.

Biological control: native and exotic parasitoids

It is well known that parasitoids play an important role in the regulation of Drosophilidae populations (Carton et al., 1986; Fleury et al., 2009). An important consideration is whether parasitoids native to Europe will be able to control *D. suzukii* in Europe or whether it is needed to envisage the introduction of Asian parasitoids in invaded areas. Parasitoids of Drosophilidae are Hymenoptera that either attack larvae (larval parasitoids) or pupae (pupal parasitoids). Larval parasitoids are usually emerging from pupae. In most cases larval parasitoids are more specific than pupal parasitoids. Larval parasitoids of *D. melanogaster* that are present in Europe and/or North America, have been extensively studied, in particular *Leptopilina heterotoma*, *L. boulardi* (Hymenoptera: Figitidae) and *Asobara tabida* (Hymenoptera: Braconidae) (Iacovone et al., 2015, Fleury et al., 2009). However, their impact on *D. suzukii* in the field is close to zero (Kenis et al. 2016). In the laboratory, tests have been made but quasi no emergence was observed on this new invading species (Chabert et al., 2012; Poyet et al., 2013; Gabarra et al., 2015) except for an Italian strain that was more successful (Rossi-Stacconi et al., 2015)). On the other hand, local European generalist pupal parasitoids such as *Trichopria drosophilae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) can successfully develop in *D. suzukii*, but their impact in the field is limited and their ability to attack in fresh fruits is not yet demonstrated (Rossi- Stacconi et al., 2017). Differences in the ability of killing their host can be partly explained by the immune system of *Drosophila* spp. that is able to resist parasitism. It is characterized by the formation of a cellular capsule that melanizes the egg or larvae of the parasitoid, resulting in their death (Carton & Nappi, 1997). Parasitoids that co-evolve with their host develop the ability to prevent encapsulation by injecting venom during parasitism. This venom contains virulence factors that suppress the immune response of the host (Fleury et al., 2004). For its own development, the parasitoid keeps its host alive until the host pupates, and then kills its host (Figure 3B). Parasitism trials may lead to 4 outcomes (Figure 5): A) if the parasitoid does not oviposit, then a fly emerges; B) if the parasitoid is avirulent and the host resistant, then a fly emerges with a dead encapsulated parasitoid egg, C) if the parasitoid is virulent and the host susceptible, then a parasitoid emerges as the host is killed; and D) if the parasitoid is virulent and the host resistant then, the death of both organisms may occur.

The absence of effective larval parasitoids attacking *D. suzukii* in Europe and the need to find a management measure that can control the pest at landscape level rather than at crop level to limit re-infestations of crops from the surrounding areas suggests that Asian larval parasitoids could be introduced, provided that they are sufficiently specific to limit non target effects on native biodiversity. At the beginning of the thesis, the knowledge on larval parasitoids was limited to a few studies in Japan (Mitsui et al., 2007; Kasuya et al., 2013). Since the invasion of *D. suzukii* in Europe and North America, parasitism has been further studied in Japan (Matsuura et al., 2017; Nomano et al., 2015; 2017) and one survey for parasitoids of *D. suzukii* has been carried out in South Korea (Daane et al., 2016). These studies all showed that the parasitoid complex is dominated by a Figitidae of the genus *Ganaspis*. However, even now hardly anything is known on parasitism of *D. suzukii* in the largest part of its native range, in particular in China and no clear experiments were performed to demonstrate the specificity on *D. suzukii* of the Asian parasitoids complex.

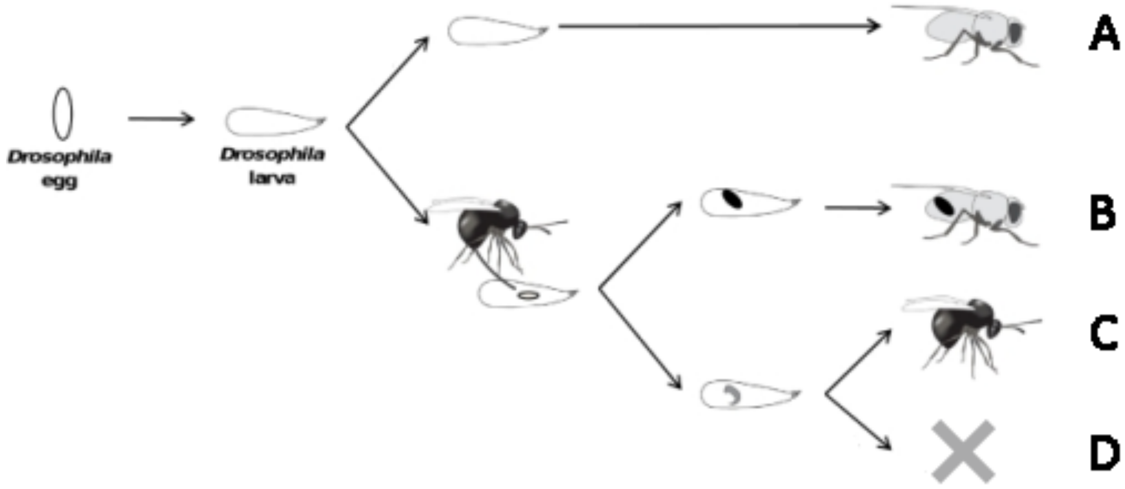

Figure 5. Temporal sequence of the drosophila-parasitoid interaction (modified from (Martinez et al. 2012)) A. if no parasitoid, fly emergence. If parasitoid, B. encapsulation or C. parasitoid emergence or D. death of both organisms.

Thesis outlines

The main objective of this thesis was to study Asian parasitoids of *D. suzukii* and to characterize host-parasitoid interactions in order to develop an efficient biological control programme. It includes three research hypotheses, each of them being tested in one or two specific studies.

Hypothesis 1. Drosophila suzukii is attacked, in its native range, by a complex of larval parasitoids that could be considered for introduction into Europe

The first step of a classical biological control programme is to gather information on natural enemies in the native range of the invasive pest. Research focused on larval parasitoids since extensive surveys had shown that they are totally absent from invasive populations of *D. suzukii* in Europe (Kenis et al., 2016). In addition, they are known for being more specific than pupal parasitoids and predators of Drosophilidae. Surveys were carried out from 2015 to 2017 in 12 provinces in China and 5 Prefectures in Japan. This was a work conducted with other partners as INRA (Sophia-Antipolis, France), MoA-CABI (Beijing, China), Yunnan Agricultural University (Kunming, China) and University of Hokkaido (Hokkaido, Japan). Results of these surveys are presented in Chapter 1. With the campaign of Daane et al., (2016) in South Korea, they are so far the only surveys in continental Asia reported in the literature. Some of the parasitoids collected during these surveys were brought to Switzerland and used for the studies described in Chapters 2, 3 and 4.

Hypothesis 2. Asian parasitoids are sufficiently specific to D. suzukii to be introduced

The main parasitoids collected in China and Japan were tested for their specificity and potential use in classical biological control. In Chapter 2, we made no-choice tests on *D. suzukii* in blueberry and artificial diet, with six strains of three Asian parasitoids (*Ganaspis* sp., *Leptopilina japonica* and *Asobara japonica*). This study provided the first baseline data for the assessments of the host specificity of Asian parasitoids of *D. suzukii*. We tested to what extent the success of parasitism varied between the three candidate parasitoids on *D. suzukii*, and if this success is affected by the host's diet (fresh fruits and artificial diet). The study also provided data on the pre-oviposition time of the parasitoids and their development time in the laboratory, which is important information for conducting specificity studies successfully. Then, in Chapter 3, to assess their specificity, the same parasitoids were tested on several European *Drosophila spp.* and one Tephritidae, on different diets and fresh fruits. Our results showed that *A. japonica* is the most polyphagous species, and *Ganaspis* sp. the most specific one. It also highlighted important intra-specific variations in *Ganaspis* sp., a Japanese strain being totally specific to *D. suzukii* in fruits whereas a Chinese strain also successfully parasitized *D. melanogaster* and *D. suzukii* in diet.

Hypothesis 3. Variations in specificity between parasitoids are due to different reactions to volatiles emitted by fresh and decaying fruits.

To better understand the attractiveness and specificity of Asian and European larval parasitoids towards *D. suzukii* and host habitats, olfactometer bioassays were carried out with the two strains of *Ganaspis* sp. having shown different behaviour in specificity tests, as well as with the Asian *Leptopilina japonica* and the European *Leptopilina heterotoma* in Chapter 4 with various odour sources (*e.g.* fresh fruits, decaying fruits, artificial diet or *D. suzukii* infested fruits). These assays confirmed the specificity tests, *i.e.* the *Ganaspis* sp. strain from Japan was the only parasitoid to show a strong preference for *D. suzukii* in fresh fruits as compared to decaying fruits and diet.

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CHAPTER 1: PARASITOID COMPLEX OF *D. SUZUKII* AND OTHER FRUIT-FEEDING DROSOPHILA SPP. IN ASIA

In preparation for:

SCIENTIFIC REPORT

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ABSTRACT

The spotted wing Drosophila, *Drosophila suzukii*, is an invasive insect of East Asian origin that has become a serious fruit pest worldwide. Classical biological control through the introduction of parasitoids from its region of origin could help reducing populations at landscape level and, thereby, decrease the need for management in cropping systems. However, little is known in the parasitoid complex of the fly in its region of origin, especially in China, which shares the largest part of its native distribution. Therefore, surveys for larval parasitoids of *D. suzukii* were carried out in 12 Chinese provinces and five Japanese prefectures in the period 2015-2017. Parasitoids of *D. suzukii* and other fruit-inhabiting drosophilids were found at 28 sites in four provinces in China and four prefectures in Japan. Larval parasitoids were obtained at most sites where *D. suzukii* was found, with parasitism varying from 0.0 to 75.6 %. At least eight parasitoid species were reared out. The most abundant and frequent parasitoids were the Figitidae *Ganaspis* sp. and *Leptopilina japonica*, but two other *Leptopilina* species and at least four undetermined Braconidae of the genera *Asobara* and *Tanycarpa* were obtained. In most samples, *D. suzukii* was accompanied by *D. pulchrella* or *D. subpulchrella*, two other Drosophilidae that attack fresh fruits in Asia and are probably attacked by the same parasitoids. The most promising parasitoid for biological control is *Ganaspis* sp. because of its abundance in Asia and its likely restricted host range. However, its exact specificity and taxonomic status still need to be resolved.

KEY WORDS

Spotted wing Drosophila, biological control, invasive species, Braconidae, Figitidae

INTRODUCTION

The spotted wing Drosophila, *Drosophila suzukii* Matsumura (Diptera, Drosophilidae), is a native fly of Asian origin that recently invaded several regions and continents including Europe, North and South America, Réunion Island and Central Asia (Asplen et al., 2015, Fraimout et al., 2017). The economic impact of this invasive fly is increasing proportionally to its geographic range. Unlike most other Drosophilidae, *D. suzukii* is able to lay eggs in fresh fruits thanks to a serrated ovipositor. With this feature, *D. suzukii* has become a major pest of small and stone fruits on most invaded regions (Walsh et al., 2011; De Ros et al., 2015; Mazzi et al., 2017). It has also a very wide host range comprising many cultivated fruits as well as fruits from ornamental and wild plants (Lee et al., 2015; Kenis et al., 2016; Briem 2016) and a short development time, which allows the development of several generations per year (Asplen et al., 2015). As a result, crops are constantly reinvaded from neighbouring habitats, which complicates management strategies in the crops and at landscape level (Haye et al., 2016). In addition, in invaded regions, *D. suzukii* encounters very few competitors. It is attacked by generalist predators (Woltz and Lee, 2017) and, to a much lower extend, generalist pupal parasitoids (Gabarra et a. 2015; Miller et al., 2015; Rossi Stacconi et al., 2015; Wang et al., 2016). In contrast, larval parasitoids, which are often considered as major mortality factors in Drosophilidae (Carton et al., 1986; Fleury et al., 2009) are, so far, totally absent from the natural enemy complex of *D. suzukii* in invaded regions (Haye et al., 2016; Kenis et al., 2016). Indeed, larval parasitoids of local Drosophilidae either do not show interest for *D. suzukii* or are not able to develop successfully in *D. suzukii* larvae, partly because of the strong host immune response of the fly larvae (Chabert et al., 2012; Kacsoh and Schlenke, 2012; Poyet et al., 2013; Knoll et al., 2017; Rossi Stacconi et al., 2017). Current control methods rely on chemical sprays or good cultural practices (*e.g.* sanitation, leaf thinning, bait traps and insect proof nets). However these methods do not take into account the of the invasion of *D. suzukii* in both cultivated and natural lands.

In this respect, the introduction of larval parasitoids from the region of origin of the pest that are specialised in parasitizing *D. suzukii* could help reducing populations at landscape level and, consequently, decrease the need for management. However, little is known from larval parasitoids of *D. suzukii* in Asia. The most comprehensive studies have been recently carried out in Japan but these often focused on specific parasitoid species or genera and rarely provided quantitative data on the role of parasitoids in the natural control of *D. suzukii* (Novkovic et al., 2011; Kasuya et al., 2013, Nomano et al., 2015, 2017; Matsuura et al., 2017). They concluded that the most promising biological control agent would be a Figitidae of the genus *Ganaspis* of unclear taxonomic status (named *G. xantophoda* in Kasuya et al. 2013 and *G. brasiliensis* in Matsuura et al. 2017 and Nomano et al. 2017). This species is the most abundant species of parasitoid and a specific strain seems to be specialised on *D. suzukii*. On the Asian continent, data on larval parasitism are restricted to surveys by Daane et al. (2016) in South Korea and by Guerrieri et al. (2016) in the Yunnan Province of China and South Korea, the latter being restricted to the braconid genus *Asobara*. Both studies used fruit collection and traps baited with uninfested fruits and suggest that field collection of suitable fresh fruits is a more reliable method to collect parasitoids. The most abundant larval parasitoids collected in South Korea were the Braconidae *Asobara japonica* and the Figitidae *Ganaspis brasiliensis* and *Leptopilina japonica*.

In this publication, we report on surveys made from 2015 to 2017 in 12 Chinese Provinces and five Japanese prefectures to gather quantitative data on larval parasitism of *D. suzukii*. These surveys were made by sampling potentially suitable fresh fruits and, thus, also collected a two other *Drosophila* spp. living in the same habitat, *D. pulchrella* and *D. subpulchrella*. The parasitoid complex of these two species is totally unknown.

METHODS

Collection sites and methods

Surveys for *D. suzukii* and parasitoids were carried out in China and Japan from 2015 to 2017. In China, fruits that could potentially host *D. suzukii* were collected at more than 100 sites in five prefectures in Japan and 12 provinces in China. Among these, seven sites in Japan (5 prefectures) and 29 sites in China (8 Provinces) provided a sufficient number of *D. suzukii* and parasitoids (arbitrarily set at 10) to assess parasitism (Figure 1 & 2 and Supplementary material). Only fresh fruits that were still on the plant when collected were sampled to avoid the collection of *Drosophila* spp. that prefer rotten or damaged fruits on the ground. The protocol to obtain *Drosophila* spp. and parasitoids slightly varied with years and regions but, in general, was as follows. Collected fruits were placed in a cooler box during their transport to the laboratory. Record of the location (GPS coordinates) and name of the collected fruit were annotated for each sample. Fruits were then counted and placed in plastic containers of various sizes, on a layer of slightly moist cellulose paper. The containers were closed with ventilated lids. The boxes were inspected daily for emergence of *Drosophila* spp. and parasitoids that were collected and reared in a cage or placed in alcohol. The cellulose paper was checked and moistened if necessary. After about a week, the paper and each fruit were inspected to collect the remaining drosophilid pupae. All pupae were placed in Petri-dishes on slightly moist cellulose paper. The Petri-dishes were then inspected daily. Emerged drosophilids and parasitoids were either put directly in alcohol or first placed in cages for laboratory rearing.

No permissions were needed to sample in all sampling locations. Local growers and Universities allowed us to sample in their properties. No protected or endangered species have been threatened in those surveys. Some of the Asian parasitoids have been transported to CABI's quarantine facility under the Swiss Federal Office for the Environment agreement: A-141345-3.

Identification of Drosophila spp. and parasitoids

Drosophila suzukii, *D. pulchrella* and *D. subpulchrella* were identified using Takamori et al. (2006) and EPPO (2013). Other *Drosophila* spp. were not determined to species level. Parasitoids were identified using morphological characters by MB and MK and Francisco Javier Peris Felipo (Basel). In parallel of the entomological identification, Asian parasitoids were also identified through molecular characterization, at INRA (Sophia-Antipolis, France) and, to a lower extend, at the University of Neuchâtel (Suisse), using classical barcoding methods (C01 and ITS genes) and comparison with published sequences in GenBank, NCBI (at this stage molecular analyzes are still underway and not presented in this manuscript).

Calculation of parasitism rates

Parasitism rates were calculated, for each sample, as the division of the number of parasitoids of one or all parasitoid species by the total number of parasitoid and *Drosophila* spp. adults emerged from the sample. Samples that contained more than 1% of *Drosophila* spp. unable to attack fresh, undamaged fruits, *i.e.* other than *D. suzukii*, *D. pulchrella* and *D. subpulchrella*, were discarded from the calculation because we supposed that they had attacked rotten or damaged fruits before the sampling or during the manipulation of the fruits in the field or in the laboratory. These species are supposed to have their cohorts

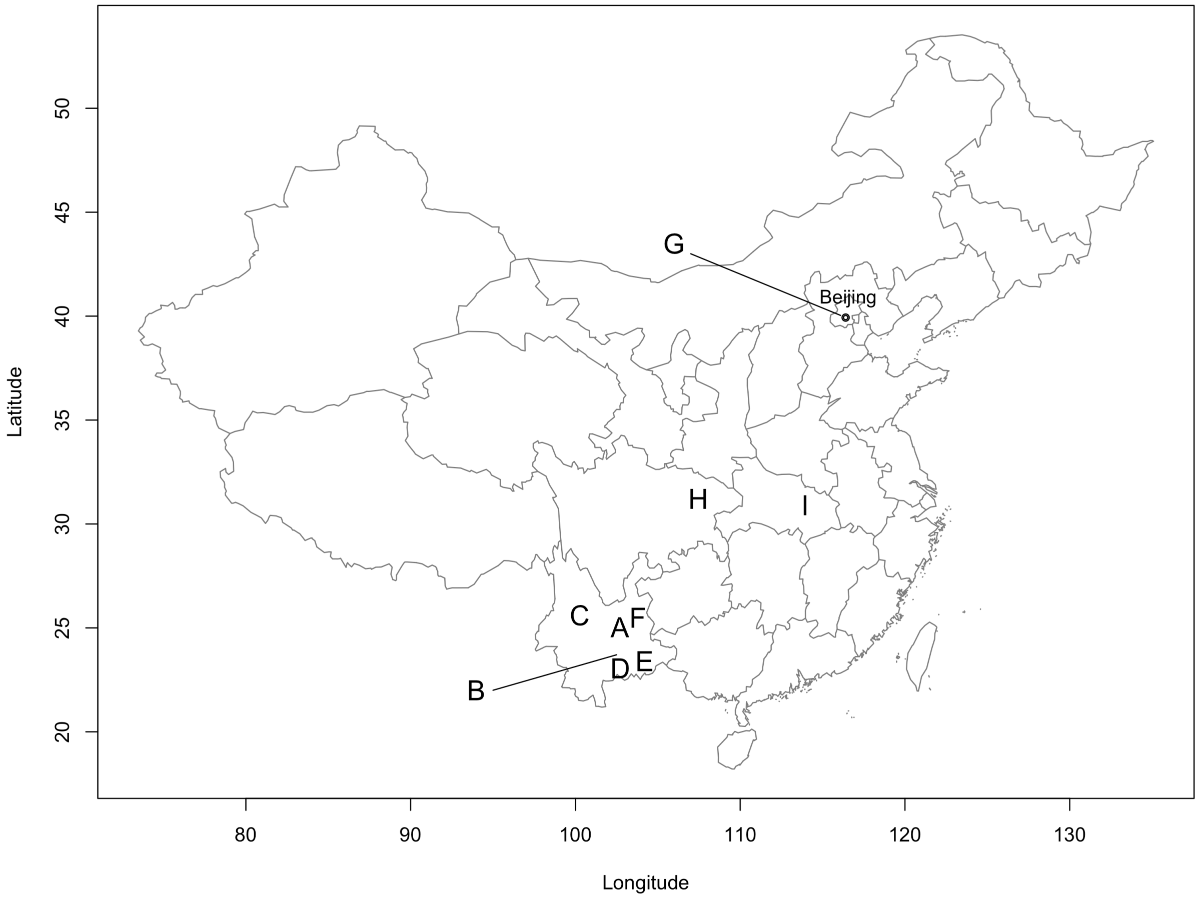

Figure 1. Geographic distribution of the successful sampling sites in China. A: Kunming - Fumin, B: Shiping, C: Dali, D: Panzihua, E: Wenshan, F:Qujing, G: Jiu Mountain - Lija Farm - Yiangtai Mountain, H: Dazhou and I: Xiaoguan.

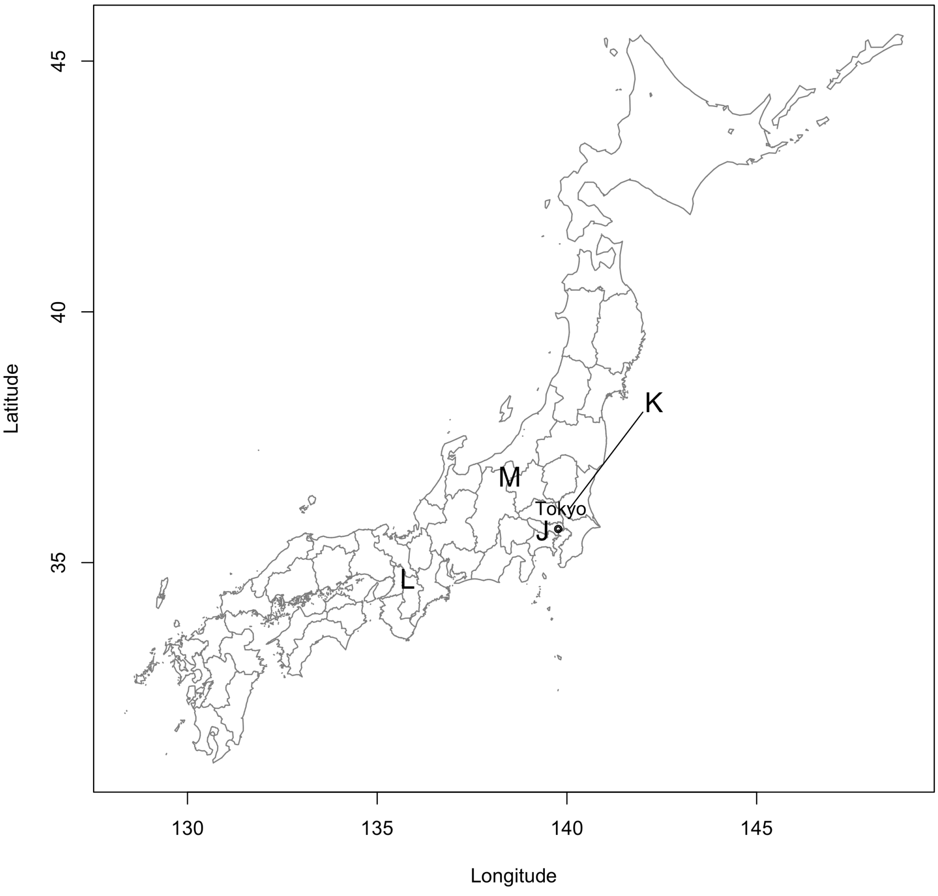

Figure 2. Geographic distribution of the successful sampling sites in Japan. J: Tokyo, K: Tsukuba, L: Nara and M: Hasuike.

of parasitoids, specialised in rotten habitats whereas the aim of this study was to assess the parasitoid complex of *Drosophila* spp. specialised in fresh fruits.

RESULTS AND DISCUSSION

Parasitism of Drosophila spp.

The results of the larval parasitoid surveys, with parasitism rates, are summarised in Table 1. For 2015 and 2016, only samples that produced at least one larval parasitoid are shown because, in some cases, a high mortality occurred in the host pupal stage and the lack of parasitism may be due to samples deterioration in the period between fly and parasitoid emergence. For 2017, samples without parasitoid emergence were added because the rearing techniques had improved and we were more confident that all samples could be kept clean and healthy until parasitoid emergence. In any case, parasitism rates provided in Table 1 should be considered with caution. These rates are based on fly and parasitoid adult emergence and the rearing and transport conditions experienced during these surveys may have affected *D. suzukii* and its parasitoids very differently. On the one hand, the most abundant parasitoids emerge about two weeks after the *D. suzukii* adults. During this period, fruits became covered by fungi, which surely prevented parasitoids from emerging. On the other hand, *D. suzukii* pupae are extremely sensitive to high temperatures and drought. Unpublished observations by the authors showed that no fly emerges when pupae are exposed to temperature above 30°C or humidity below 50% RH. It is possible that parasitoids in the host pupa are less sensitive than their host, however, nothing is known yet on the climatic requirements of the parasitoid species.

Larval parasitism rates were highly variable, from 0 to 75.6%. The highest rates of parasitism were observed in Yunnan Province (China) and Nara prefecture (Japan). In contrast, parasitism seems to be lower in northern China, as shown by collections in Beijing and Jilin Provinces, but also samples from 2015 and 2016 in Inner Mongolia, Zhejiang and Jiangsu (China) did not provide parasitoids. A possible explanation could be that *D. suzukii* is likely non-native in these areas and parasitoids may be less well adapted to severe winter conditions experienced in these regions. Strong variations in parasitism were observed between nearby sites but also from year to year at the same sites. For example, parasitism in Japan on the same *Prunus serrulata* trees in Tokyo climbed from 9% in 2015 to 27.5% in 2016 at the same period of the year. These strong variations in parasitism are typical for insects that have short development times and many annual generations. Abiotic factors may affect hosts and parasitoids differently, or affect them similarly but hosts and parasitoids have very different capacities to recover from these adverse effects. Another factor that may affect the estimation of parasitism rates is the fact that parasitoids attack young larvae whereas we probably collected a part of the *Drosophila* spp. as eggs, *i.e.* before the attack of the parasitoids, which may result in an underestimation of the parasitism rate. On the other hand, parasitoids emerge later than *D. suzukii* and it cannot be ruled out that some samples contained emerged fly pupae and non emerged parasitoids in host pupae. This, however, is rather unlikely because most larvae leave the fruits for pupation (Woltz and Lee 2017) and we specifically sampled fruits on the plant that looked undamaged, to avoid collecting other Drosophila species.

In the majority of samples, *D. suzukii* was accompanied by the congeneric species that are also able to attack fresh fruits, *D. pulchrella* at high altitudes in Yunnan and Sichuan Provinces (China) and *D. subpulchrella* in Japan and Beijing and Hubei Provinces (China) and elsewhere. This confirms the geographic range of the two species described by Takamori et al. (2006). Since pupae of the three Drosophila species are morphologically indistinguishable, it was impossible to determine from which host the parasitoids emerged. Several samples, including very large ones, contained only *D. suzukii*, from which we deducted that the parasitoids emerged from this host. In contrast, only one small sample gave rise to *D. subpulchrella* only and no sample provided exclusively D*. pulchrella* and, thus, the association of the parasitoids with these two species could not be ascertained.

Other data on parasitism rates of *D. suzukii* in Asia are scarce. Data on parasitism in South Korea have been recently published by Daane et al (2016) who have observed rates of parasitism of 0-17%, *i.e.* in line with our results from Northern/Eastern China. Guerrieri et al. (2016) focused on the genus *Asobara* (Braconidae) in South Korea and Yunnan, and found two species, *A. japonica* and *A. leveri*, associated with *D. suzukii*, but they do not provide parasitism rates. Parasitism of *D. suzukii* has been studied more extensively in Japan but the information provided in the literature is mostly qualitative. Quantitative data were published by Matsuura et al. (2017) from *Prunus serrulata* in Tokyo in 2015 and 2016. These results are very similar to our collections because fruits were collected at the same moment and the same time. Other quantitative data from the same site can be found in Kasuya et al. (2013).

To our knowledge, this is the first time that *D. pulchrella* and *D. subpulchrella* have been sampled for parasitism but since these two species occur nearly always together with *D. suzukii* and their pupae is indistinguishable, it is difficult to assess their parasitism rate and parasitoid complex separately. The only solution would be to keep all pupae singly and to barcode pupae from which parasitoids have emerged, as species can be distinguished through the nucleotide sequence of the CO1 gene.

Larval parasitoid species

Ganaspis sp. (Hymenoptera, Figitidae)

Ganaspis sp. (Figure 3) was the most frequently reared parasitoid of *D. suzukii* in China and Japan, being present in all samples from where parasitoids emerged. It was also the species that reached the highest levels of parasitism in both countries. All specimens seem to belong to a species that was morphologically identified as *G. brasiliensis* (Ihering) as previously noticed by Buffington and Forshage (2016) who examined Asian specimens emerged from *D. suzukii*. However, *G. brasiliensis* was originally described as a fruit fly parasitoid from Brazil and was also found in the Caribbean and Central America. Nomano et al. (2017), using molecular tools and behavioural studies, showed that *G. brasiliensis* is likely a complex of cryptic species with different distributions and various degrees of specificity. Therefore, in this paper, our samples will be referred as *Ganaspis* sp.

Ganaspis brasiliensis was also the most abundant Figitidae parasitoids in surveys in South Korea by Daane et al. (2016) and Japan by Matsuura et al. (2017). Nomano et al. (2017) also showed that the strain, or sibling species attacking *D. suzukii* is the ‘suzukii-specialised’ type of *Ganaspis xanthopoda* cited in Kasuya et al. (2013). This study and Nomano et al. (2017) suggest that this strain, or cryptic species, only attacks on, and develops in *D. suzukii* in fresh fruits, which is partly confirmed by our studies in quarantine conditions in Switzerland (Girod et al., this thesis Ch. 2). However, Girod et al. (2018) showed some variability in host suitability, some populations being able to develop on *D. melanogaster* and some not. *Ganaspis* sp. was also reared from the only sample from which *D. subpulchrella* emerged without *D. suzukii*.

Figure 3. Pictures of *Ganaspis* sp. Tokyo female (left) and male (right). (© Photo: P. Girod)

Leptopilina japonica Novkovic & Kimura (Hymenoptera, Figitidae)

Leptopilina japonica (Figure 4) has been found in all regions of China and Japan, but rarely reached high parasitism rates. However, it was at least as abundant as *Ganaspis* sp, in Beijing and more abundant in the single sample from Sichuan (Table 1). This parasitoid was already known from *D. suzukii* in Japan and Taiwan (Novkovic et al., 2011; Matsuura et al., 2017) and was also reared frequently from *D. suzukii* in South Korea (Daane et al., 2016). However, to our knowledge this is the first record of *L. japonica* in the People's Republic of China. Novkovic et al. (2011) mention two sub-species, *L. japonica japonica* and *L. japonica formosana*, the former from Japan and the latter from Taiwan. Both sub-species were also found in South Korea (Daane et al., 2016). Molecular analyses and morphological observations of specimens from Kunming and Beijing suggest that they belong to the subspecies *L. japonica japonica* but not all specimens reared during this study were identified at subspecies level. *Leptopilina japonica* was successfully reared on *D. suzukii* in the laboratory (Girod et al., this thesis Ch. 2) and is also known to successfully develop in *Drosophila biauraria* and *D. rufa* in Japan under natural conditions and in *D. simulans* in the laboratory.

Figure 4. Picture of *Leptopilina japonica* Beijing female parasitizing *Drosophila suzukii* larve in blueberry. (© Photo: T. Haye)

Table 1. Larval parasitoids and parasitism rates observed from fruit collections in China and Japan in 2015, 2016 and 2017. Only samples that produced at least one larval parasitoid and more than 10 adults (*D. suzukii* + parasitoids) and for which quantitative data on parasitism are available are shown. Samples with no parasitism are shown for 2017 only. Parasitism rates are calculated as the division of the number of parasitoids of one or all species by the total number of parasitoid and *Drosophila* spp. adults emerged from the sample.

Locality	Fruit	Month of collection	Other *Drosophila* sp. (%)*	Larval parasitoids and parasitism rates (%)						
				Ganaspis sp.	*Leptopilina japonica*	*Leptopilina* sp.	*Asobara* spp.	*Tanycarpa* spp.	Opiinae	Total parasitism (n insects)
Yunnan										
Kunming - YAU	*Prunus cerasoides*	June 15	5.1 (Dp)	42.0	10.9	-	-	-	-	52.9 (174)
Shiping	*Myrica rubra*	June 15	3.5 (Dp)	1.7	0.7	-	-	-	-	2.4 (537)
Dali	*Prunus cerasoides*	May 16	1.8 (Dp)	1.8	-	-	-	-	-	1.8 (224)
Kunming – West Mountain	*Prunus* (*Cerasus*) sp.	May 16	6.7 (Dp)	1.4	-	-	-	-	-	1.4 (142)
Panzihua	*Prunus* (*Cerasus*) sp.	Apr 16	0.0	2.1	-	-	-	-	-	2.1 (373)
Fumin	*Myrica rubra*	July 16	74.5 (Dp)	0.4	11.8	35.9	6	-	-	54.1 (566)
Wenshan	*Myrica rubra*	June 16	0.0	12.6	34.5	-	-	-	6.9	54.0 (87)
Kunming – West Mountain	*Myrica rubra*	July 16	0.0	25.0	17.2	7.2	1.8	-	-	51.2 (615)
Dali	*Sambucus williamsii*	July 16	55.3 (Dp)	1.2	-	-	1.0	-	-	2.3 (483)
Kunming - Snake Mountain	*Solanum nigrum*	July 16	0.0	5.9	5.9	-	29.4	-	-	41.2 (17)
Wenshan	*Rubus* sp.	Sept 16	73.5 (Dp)	2.0	7.0	-	-	-	-	9 (199)
Qujing	*Lonicera maacki*	Sept 16	83.7 (Dp)	1.8	1.8	-	-	-	0.6	4.3 (326)
Kunming	*Lonicera maacki*	Sept 16	65.0 (Dp)	8.7	4.3	-	-	-	-	13 (23)
Kunming – West mountain	*Prunus* (*Cerasus*) sp.	May 17	14.0 (Dp)	-	-	-	-	-	-	0 (150)
Kunming - Snake mountain	*Rubus sp.*	May 17	26.7 (Dp)	-	-	-	-	-	-	0 (56)
Kunming - YAU	*Rubus ellipticus* (?)	May 17	90.0 (Dp)	18.2	4.5	-	-	-	-	22.7 (22)
Kunming – Xining temple	*Prunus* (*Cerasus*) sp.	May 17	8.4 (Dp)	5.2	4.6	-	-	-	-	9.8 (504)
Fumin	*Myrica rubra*	June 17	0.0	6.8	2.3	-			-	9.1 (88)
Fumin mountain	*Prunus* (*Cerasus*) sp.	June 17	92.2 (Dp)	16.8	3.6	-	0.2	-	-	20.6 (552)
Fumin mountain	*Princepia utilis*	June 17	92.5 (Dp)	9.5	-	-	-	-	-	9.5 (74)
Beijing										
Jiu Mountain	*Prunus* (*Cerasus*) sp.	June 16	2.3 (Dsp)	0.2	1.0	-	0.04	-	-	1.2 (8114)
Lija Farm	*Prunus* (*Cerasus*) sp.	June 16	0.0	1.6	1.6	-	-	-	-	3.3 (366)
Yiangtai Mountain	*Prunus* (*Cerasus*) sp.	June 16	0.0	10.8	19.9	-	-	-	-	30.7 (251)
Sichuan										
Dazhou	*Prunus* (*Cerasus*) sp.	May 16	- (Dp)#	2.7	15.2	-	0.4	2.1	-	20.4 (816)
Hubei										
Xiaoguan	*Coriaria nepalensis*	June 16	100.0 (Dsp)	9.7	-	-	-	3.2	-	12.9 (31)
Jilin										
Changchun	*Vaccinium* spp.	Aug 17	0.0	-	-	-	-	-	-	0.0 (2298)
Wanliang	*Rubus* sp.	Aug 17	0.0	-	-	-	-	-	-	0.0 (15)
Quanyang	*Rubus* sp.	Aug 17	0.0	-	-	-	-	-	-	0.0 (116)
Liaoyuan	*Vaccinium* sp.	Aug 17	0.0	-	-	-	-	-	-	0.0 (147)

Locality	Fruit	Month of collection	Other *Drosophila* sp. (%)*	Larval parasitoids and parasitism rates (%)						
				Ganaspis sp.	*Leptopilina japonica*	*Leptopilina* ? sp.	*Asobara* spp.	*Tanycarpa* spp.	Opiinae	Total parasitism (n insects)
Japan										
Tokyo	*Prunus serrulata*	June 15	0.0	7.5	0.2	-	1.2	-	-	8.9 (402)
Tokyo	*Prunus serrulata*	June 16	0.0	26.3	-	-		-	-	27.5 (205)
Nara	*Morus* sp.	June 16	0.0	75.6	-	-	-	-	-	75.6 (127)
Tsukuba	*Prunus serrulata*	June 16	0.0	4.4	-	-	-	-	-	4.4 (45)
Yoshigadaira	*Vaccinium* spp.	August 17	0.0	-	-	-	-	-	-	0.0 (95)
Hasuike	*Vaccinium* spp.	August 17	24.0 (Dsp)	9.9	1.8	-		2.5	-	14.2 (566)
Yamanouchi	*Prunus* (*Padus*) sp.	August 17	7.7 (Dsp)	-	-	-	-	-	-	0.0 (13)

*Percentage of other *Drosophila* spp. in the sample, based on emerging adult flies.

Dp = *Drosophila pulchrella*

Dsp = *Drosophila subpulchrella*. Other *Drosophila* spp. accounted for less than 1% in all samples.

$^{\#}$*D. pulchrella* present but not quantified

Leptopilina sp. (Hymenoptera, Figitidae)

Another Figitidae was reared in high numbers from bayberry fruits collected at two sites in Yunnan. While a sample provided a mixture of *D. suzukii* and *D. pulchrella,* only *D. suzukii* emerged from the other. This parasitoid has also been successfully reared on *D. suzukii* in the CABI laboratory in Beijing (J. Zhang, unpublished data). It is morphologically very different from *L. japonica* and does not cluster with any known species in our molecular analyses. It is, however, morphologically and genetically closer to the genus *Leptopilina* than to *Ganaspis*, but could also potentially be a new genus.

Asobara spp. (Hymenoptera, Braconidae)

Asobara is the third important genus of larval parasitoids in Drosophilidae worldwide (Carton et al., 1986). At least three species of *Asobara* spp. have been collected at many sites in several Chinese Provinces and Japan, but usually in very low numbers. The only high rate of parasitism was obtained at Kunming, Snake Mountain, where six specimens were reared from a small amount of *D. suzukii* pupae. Both the morphological examination and molecular characterization have to be completed. Nomano et al. (2015) studied the capacity of eight *Asobara* species associated with *Drosophila* spp. in Japan to parasitize *D. suzukii*. An undescribed species, *Asobara* sp. TK1, could be specific to *D. suzukii*. Guerrieri et al. (2016) suggest that *Asobara* sp. TK1 could be a newly described species, *A. triangulata*, based on the molecular analysis of one specimen from Yunnan, China. The other species are polyphagous and while at least two other species also showed their capacity to parasitize *D. suzukii* (*A. japonica* and *Asobara* sp. TS1), other species fail to attack or develop in this host. However, *Asobara* sp. TK1 has only been collected in very low numbers, and only in Tokyo (Nomano et al., 2015; Matsuura et al. 2017). A few specimens were obtained during this study at the same site in the 2015 survey. Guerrieri et al. (2016) and Daane et al. (2016) surveyed for parasitoids of Drosophilidae in Yunnan (China) and South Korea and found *Asobara japonica* and possibly *A. brevicauda* and *A. leveri* associated with *D. suzukii.*

Other Braconidae

One or two undetermined species of the genus *Tanycarpa* have been collected in China at two sites in Sichuan and Hubei as well as in Japan, in the mountains of Central Honshu. The sample from Hubei only provided *D. subpulchrella* and the other sites provided a mixture of *D. suzukii* and *D. pulchrella* or *D. subpulchrella*. At least two species of the genus *Tanycarpa* are known as parasitoid of Drosophilidae (Carton et al., 1986) but no species has ever been recorded from *D. suzukii, D. pulchrella* or *D. subpulchrella.* Yao et al. (2015) provide a key to the 20 described species of *Tanycarpa*. Fourteen species have been recorded from China but none from Japan so far.

Undetermined specimens of a species of the sub-family Opiinae emerged from pupae obtained at two sites in Yunnan. This species is presently under molecular and taxonomic identifications. Opiinae are very common larval parasitoids of Diptera, but attack more frequently fruit-infesting Tephritidae and mining Agromyzidae. (Li et al., 2013). However, parasitism of Drosophilidae has been occasionally reported (Wharton et al., 2007).

CONCLUSIONS - PROSPECTS FOR BIOLOGICAL CONTROL

For the first time, a large survey for larval parasitoids of *D. suzukii* was carried out in several Chinese Provinces. These surveys, and those made in Japan, revealed that most populations are parasitized by a complex of at least eight parasitoids. The main species, *i.e. Ganaspis* sp. and *L. japonica* are similar to those observed by previous surveys in Japan (Kasyua et al., 2013; Matsuura et al., 2017) and South Korea (Daane et al., 2016). Other species, *e.g. Leptopilina* spp. and *Tanycarpa* sp. have been recorded for the first time from *D. suzukii* and would deserve further investigations. However, studies presently being carried out on the biology of the parasitoids (Girod et al., this thesis Ch. 2-3) are in accordance with Kasuya et al. (2013) and Matsuura et al. (2017) and suggest that *Ganaspis* sp. (as *G. brasiliensis* or "*suzukii*-specialised type of *Ganaspis*) is the most specific parasitoid. Since it is also the most abundant parasitoid of *D. suzukii* in Asia, it is clearly the first candidate for introduction into Europe, North America and other regions invaded by *D. suzukii*. The fact that it also probably attacks two other species also found in fresh fruits in Asia, *D. pulchrella* and *D. subpulchrella* suggests that it may be specific to fresh fruits rather than purely host specific. This would not prevent its introduction in Europe and North America since native Drosophilidae in these regions are not able to attack fresh, undamaged fruits. These surveys also showed that, in some East Asian regions, *D. pulchrella* and *D. subpulchrella* are nearly as common in fresh fruits as *D. suzukii*, and their introduction to other continents should be avoided at all costs. It remains to be seen whether *Ganaspis* sp. would be able to colonise all invaded regions or whether it would be limited by climatic constraints. The highest parasitism rates were observed in sub-tropical and warm-temperate climates in Yunnan (China) and Japan. However, the fact that it was also rather abundant at a high elevation site in Nagano Prefecture (Central Japan: Hasuike, 1400m) is promising for temperate regions in Europe and North America. Since larval parasitism does not occur in invaded regions, any additional parasitism by an introduced parasitoid may help lowering populations at a landscape level and, thus, limit the need for other management methods. Nevertheless, before its introduction, further efforts are needed to complete specificity tests and resolve its taxonomic status.

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Supplementary material. Details of the collections of *D. suzukii*, *D. pulchrella* and *D. subpulchrella* in China and japan, 2015-2017. In includes all collections in Table 1 plus collections in 2016 with no parasitoids but which (1) did not include more than 3% of other Drosophila spp. and (2) mortality of pupae was low (-20%).

Locality	Province/Prefecture	Country	Altitude	Latitude N	Longitude E	Date of collection	Fruit	No. Fruits	*D. suzukii*	*D. pulchrella*	*D. subpulchrella*	Parasitoids	In Table 1
Kunming - YAU Campus	Yunnan	China	1950	25.13210	102.7484	03/06/15	*Prunus cerasoides*	200	Yes	Yes	No	Yes	Yes
Shiping	Yunnan	China	1500	23.743734	102.4816375	06/06/15	*Myrica rubra*	250	Yes	Yes	No	Yes	Yes
Kunming - Snake mountain	Yunnan	China	2000	25.13222222	102.7161111	03/06/15	*Corialia nepalensis*	1400	Yes	Yes	No	No	No
Dali	Yunnan	China	2020	25.61027778	100.2411111	19/05/16	*Prunus cerasoides*	1450	Yes	Yes	No	Yes	Yes
Kunming – West Mountain	Yunnan	China	2200	24.96833333	102.6225	23/05/16	*Prunus (Cerasus)* sp.	1360	Yes	Yes	No	Yes	Yes
Kunming - Snake mountain	Yunnan	China	2000	25.11833333	102.7161111	25/05/16	*Corialia nepalensis*	1200	Yes	Yes	No	No	No
Panzihua	Yunnan	China	1270	23.05194444	102.7394444	31/03/16	*Prunus (Cerasus)* sp.	572	Yes	No	No	Yes	Yes
Daheishan	Yunnan	China	1250	23.00805556	102.065	01/04/16	*Prunus (Cerasus)* sp.	310	Yes	No	No	No	No
Majiang	Yunnan	China	1550	22.69861111	101.2719444	07/04/16	*Prunus (Cerasus)* sp.	304	Yes	No	No	No	No
Fumin	Yunnan	China	1980	25.1475	102.5288889	02/07/16	*Myrica rubra*	189	Yes	Yes	No	Yes	Yes
Honghe Prefecture,Shiping county	Yunnan	China	1960	23.70583333	102.465	02/06/16	*Myrica rubra*	350	Yes	Yes	No	No	No
Wenshan, Yanshan county	Yunnan	China	1490	23.55416667	104.3475	25/06/16	*Myrica rubra*	295	Yes	No	No	Yes	Yes
Kunming – West Mountain, Dian Wei	Yunnan	China	2010	25.1475	102.5288889	02/07/16	*Myrica rubra*	388	Yes	No	No	Yes	Yes
Dali	Yunnan	China	2330	25.51	100.4413889	03/07/16	*Rubus sp.*	1852	Yes	Yes	No	No	No
Dali	Yunnan	China	2240	25.505	100.4355556	23/07/16	*Sambucus williamsii*	1393	Yes	Yes	No	Yes	Yes
Kunming - Snake Mountain	Yunnan	China	2070	25.12666667	102.7147222	28/07/16	*Solanum nigrum*	713	Yes	No	No	Yes	Yes
Kunming,Xundian county	Yunnan	China	1850	25.52805556	103.3294444	29/07/16	*Vaccinium spp.*	1666	Yes	Yes	No	No	No
Yuxi, Mopanshan national Forest Park	Yunnan	China	2530	23.93666667	101.9883333	18/08/16	*Fragaria nilgeerensis*	168	Yes	Yes	No	No	No
Wenshan, Yanshan county	Yunnan	China	1490	23.55416667	104.3475	22/08/16	*Rubus sp.*	26	Yes	Yes	No	No	No
Wenshan, Yanshan county	Yunnan	China	1490	23.55416667	104.3475	01/09/16	*Berchemia kulingensis*	357	Yes	Yes	No	No	No
Wenshan, Yanshan county	Yunnan	China	1490	23.55416667	104.3475	01/09/16	*Rubus* sp.	274	Yes	Yes	No	Yes	Yes
Qujing, Shizong county	Yunnan	China	2180	24.655	104.1705556	04/09/16	*Lonicera maacki*	198	Yes	Yes	No	Yes	Yes
Kunming - Botanical garden	Yunnan	China	1920	25.140000	102.7408333	23/09/16	*Lonicera maacki*	209	Yes	Yes	No	Yes	Yes
Midu	Yunnan	China	2370	25.50888889	100.4430556	03/07/16	*Rubus sp.*	214	Yes	Yes	No	No	No
Midu	Yunnan	China	2371	25.50888889	100.4430556	03/07/16	*Fragaria nilgeerensis*	370	No	Yes	No	No	No
Kunming – West mountain	Yunnan	China	1930-2200	24.78277778	102.6166667	30/05/17	*Prunus (Cerasus)* sp.	2300	Yes	Yes	No	No	Yes
Kunming - Snake mountain	Yunnan	China	2000	25.11833333	102.7161111	31/05/17	*Rubus* sp.	410	Yes	Yes	No	No	Yes
Kunming - YAU	Yunnan	China	1950	25.144444	102.7538889	31/05/17	*Rubus ellipticus* (?)	75	Yes	Yes	No	Yes	Yes
Kunming – Xining temple	Yunnan	China	1950	25.10722222	102.7166667	31/05/17	*Prunus (Cerasus)* sp.	1010	Yes	Yes	No	Yes	Yes
Fumin	Yunnan	China	unknown	From fruit market		01/06/17	*Myrica rubra*	300	Yes	No	No	Yes	Yes
Fumin mountain	Yunnan	China	2550	25.20527778	102.4316667	01/06/17	*Prunus (Cerasus)* sp.	1848	Yes	Yes	No	Yes	Yes
Fumin mountain	Yunnan	China	2550	25.20527778	102.4316667	01/06/17	*Princepia utilis*	500	Yes	Yes	No	Yes	Yes

Locality	Province/Prefecture	Country	Altitude	Latitude N	Longitude E	Date of collection	Fruit	No. Fruits	*D. suzukii*	*D. pulchrella*	*D. subpulchrella*	Parasitoids	In Table 1
Xishanlinyu	Beijing	China	50	40.035	116.2113889	21/06/16	*Prunus (Cerasus)* sp.	240	Yes	No	No	No	No
Jiu Mountain	Beijing	China	200	40.035	116.0927778	22/06/16	*Prunus (Cerasus)* sp.	5456	Yes	No	Yes	Yes	Yes
Lija Farm	Beijing	China	50	40.035	116.2113889	22/06/16	*Prunus (Cerasus)* sp.	1012	Yes	No	No	Yes	Yes
Yiangtai Mountain	Beijing	China	350	40.06861111	116.0688889	22/06/16	*Prunus (Cerasus)* sp.	219	Yes	No	No	Yes	Yes
Yiangtai Mountain	Beijing	China	350	40.06861111	116.0688889	22/06/16	*Morus* sp.	292	Yes	No	No	No	No
Miaofeng Mountain	Beijing	China	430	40.04305556	116.0380556	22/06/16	*Morus* sp.	223	Yes	No	No	No	No
Miaofeng Mountain	Beijing	China	430	40.04305556	116.0380556	22/06/16	*Prunus (Cerasus)* sp.	152	Yes	No	No	No	No
Dazhou	Sichuan	China	420	31.27388889	107.4552778	05/05/16	*Prunus (Cerasus)* sp.	?	Yes	Yes	No	Yes	Yes
Xiaoguan	Hubei	China	1000	29.93916667	109.38	08/06/16	*Coriaria nepalensis*	418	No	No	Yes	Yes	Yes
Nanjing, Yinhewan garden	Jiangsu	China	30	32.0525	118.925	07/06/16	*Myrica rubra*	120	Yes	No	No	No	No
Tongliao airport	Inner Mongolia	China	180	43.74638889	125.4075	14/07/16	*Prunus (Cerasus)* sp.	2411	Yes	No	No	No	No
Changchun - Jilin Agric. Univ.	Jilin	China	220	43.79638889	125.4075	02/08/17	*Vaccinium* spp.	?	Yes	No	No	No	Yes
Wanliang - Qingsheng village	Jilin	China	630	42.42527778	127.1858333	03/08/17	*Rubus* sp.	?	Yes	No	No	No	Yes
Quanyang -	Jilin	China	770	42.16944444	127.4733333	04/08/17	*Rubus* sp.	?	Yes	No	No	No	Yes
Liaoyuan	Jilin	China	270	42.88333333	125.1333333	04/08/17	*Vaccinium* sp.	?	Yes	No	No	No	Yes
Tokyo - Naganuma Park	Tokyo	Japan	150	35.6368	139.36473	03-07/06/2015	*Prunus serrulata*	?	Yes	No	No	Yes	Yes
Tokyo - Naganuma Park	Tokyo	Japan	150	35.6368	139.36473	01-02/06/2016	*Prunus serrulata*	?	Yes	No	No	Yes	Yes
Tokyo - Naganuma Park	Tokyo	Japan	150	35.6368	139.36473	31/05/2016	*Morus* sp.	?	Yes	No	No	No	No
Nara	Nara	Japan	170	34.66878	135.85211	07-09/06/2016	*Morus* sp.	?	Yes	No	No	Yes	Yes
Tsukuba	Ibaraki	Japan	30	36.07025	140.12085	09/06/2016	*Prunus serrulata*	?	Yes	No	No	Yes	Yes
Yoshigadaira - Shibu pass	Gunma	Japan	1840	36.6559	138.5483	01/08/2017	*Vaccinium* spp.	350	Yes	No	No	No	Yes
Hasuike - Shiga Kogen	Nagano	Japan	1490	36.7189	138.4935	01-02/08/2017	*Vaccinium* spp.	1560	Yes	No	Yes	Yes	Yes
Yamanouchi	Nagano	Japan	770	36.7282	138.4424	02/08/2017	*Prunus (Padus)* sp.	500	Yes	No	Yes	No	Yes

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CHAPTER 2: DEVELOPMENT OF ASIAN PARASITOIDS IN LARVAE OF *D. SUZUKII* FEEDING ON BLUEBERRY AND ARTIFICIAL DIET

Submitted to (accepted with minor revisions):
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ABSTRACT

In just a few years, the Asian fly *Drosophila suzukii* has invaded several continents and has become a very serious pest of many fruit crops worldwide. Current control methods rely on chemical insecticides or expensive and labour-intensive cultural practices. Classical biological control through the introduction of Asian parasitoids that have co-evolved with the pest may provide a sustainable solution on condition that they are sufficiently specific to avoid non-target effects on local biodiversity. Here we present the first study on the development of three larval parasitoids from China and Japan, the Braconidae *Asobara japonica* and the Figitidae *Leptopilina japonica* and *Ganaspis* sp., on *D. suzukii*. The Asian parasitoids were compared with *Leptopilina heterotoma*, a common parasitoid of several Drosophilidae worldwide. The three Asian species were successfully reared on *D. suzukii* larvae in both, blueberry and artificial diet, in contrast to *L. heterotoma* whose eggs and larvae were encapsulated by the host larvae. All parasitoids were able to oviposit one day after emergence. *Asobara japonica* laid as many eggs in larvae feeding in blueberry as in artificial diet, whereas *L. heterotoma* oviposited more in larvae on the artificial diet and the Asian figitids oviposited more in larvae feeding on blueberry. *Ganaspis* sp. laid very few eggs in larvae in the artificial diet, suggesting that it may be specialised in *Drosophila* species living in fresh fruits. These data will be used for the development of a host-range testing to assess the suitability of Asian parasitoids as biological control agents in invaded regions.

KEY WORDS

Spotted wing Drosophila, pre-oviposition period, invasive species, Asobara, Leptopilina, Ganaspis

INTRODUCTION

Since the second half of the twentieth century, around 30% of alien arthropod species established in Europe originate from Asia (Roques et al., 2009). Among these, *Drosophila suzukii* Matsumura (Diptera, Drosophilidae), or spotted wing drosophila, a fly of East Asian origin, was first found in 2008 in Europe and North America, from where it invaded several other regions (Fraimout et al., 2017). This fly quickly became a serious pest of small and stone fruits in the invaded regions because, in contrast to the majority of *Drosophila* species, which feed on rotting fruits and other organic matters, *D. suzukii* is able to lay eggs in fresh fruits (Asplen et al., 2015). Larval feeding causes rapid degradation of these fruits and reduces crop yields. *Drosophila suzukii* can attack and develop in a very large range of wild and cultivated fruits as well as in fruits of ornamental plants (Lee et al., 2015; Kenis et al., 2016). In addition to its broad host range, *D. suzukii* probably owes its invasive success to the lack of effective natural enemies and competitors in the invaded area (Haye et al., 2016), a high fecundity and a short development time (14 days at 22°C), enabling it to develop up to 13 generations per year (Asplen et al., 2015; Tochen et al., 2014, 2016).

Various pest management methods are currently used, with variable success. Insecticides may be temporarily efficient but due to the short development time of the fly, they have to be applied regularly and can cause severe environmental and health concerns (Bruck et al., 2011; Van Timmeren & Isaacs, 2013; Cuthbertson et al., 2014; Sampson et al., 2017; Smirle et al., 2017). Cultural management such as the use of insect-proof nets, mass trapping and removal or solarisation of infested fruits are also used, but these strategies are expensive and labour-intensive and have to be adjusted to each type of fruit crop or cultivation system (Haye et al., 2016). New control techniques focusing on long-term management are therefore urgently needed. Since wild host plants constitute a large reservoir of individuals that can potentially reinvade field crops, controlling the pest at a landscape level is essential.

A number of natural enemies are known to have an impact on the dynamics and regulation of *Drosophila* spp. and may provide an area-wide control of fly populations (Carton et al., 1986; Fleury et al., 2009). Two generalist pupal parasitoids, *Trichopria drosophilae* Perkins (Hymenoptera, Diapriidae) and *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera, Pteromalidae), are found attacking *D. suzukii* worldwide and are being studied as potential biological agents for inundative control in crops, however, their impact on natural populations is low (Gabarra, et al., 2015; Rossi Stacconi et al., 2015, 2017; Knoll et al., 2017). In the invaded regions, larval parasitoids such as *Leptopilina heterotoma* (Thompson) (Hymenoptera, Figitidae), *Leptopilina boulardi* Barbotin, Carton & Keiner-Pillault (Hymenoptera, Figitidae) and *Asobara tabida* (Nees) (Hymenoptera, Braconidae)*,* which are important natural enemies of local *Drosophila* spp., are rarely able to develop successfully in *D. suzukii* larvae, partly because of the strong host immune response of the fly larvae (Chabert et al., 2012; Poyet et al., 2013; Gabarra et al., 2015; Rossi Stacconi et al., 2015). The main immune defense observed on *Drosophila* spp. against larval endoparasitoids is encapsulation. This involves cells of the insects' hemolymph that attach to the surface of a parasitoid egg or larva and then melanise to form a capsule, leading to the death of the parasitoid (Carton & Nappi, 1997). In *D. suzukii*'s native range, larval parasitoids in the genera *Asobara*, *Leptopilina and Ganaspis* can successfully develop on the host (Mitsui & Kimura, 2010; Novković et al., 2011; Kasuya et al., 2013a; Nomano et al., 2015; Buffington & Forshage, 2016; Daane et al., 2016). In parasitoids of *Drosophila* spp., as in other systems, the success in host-parasitoid interaction and the evolution of this interaction are related to two major factors: i) the immune response of the host and the virulence of the parasitoid (Poyet et al., 2013); ii) host detection mechanisms at both long and short distances, which can be very different among parasitoids of *Drosophila* spp. (Vet & van Alphen, 1985).

Classical biological control, introducing Asian parasitoid wasps specialised in *D. suzukii,* may provide a sustainable and area-wide long-term solution. However, strict regulations require pre-release studies evaluating the host specificity of the potential biological control agents to minimise the risks of non-target effects (Hajek et al., 2016; Heimpel & Mills, 2017). Such studies require a good knowledge of the biology of the potential biological control agents and the development of efficient rearing methods. The present study presents the first baseline data for the assessments of the host specificity of Asian parasitoids of *D. suzukii*. We first provide data on the pre-oviposition time of the parasitoids and their development time in the laboratory, which is important information for conducting specificity studies successfully. Then, we tested to what extent the success of parasitism (measured as parasitism rates, host mortality rate and encapsulation of eggs and larvae) varies between the three candidate parasitoid genera on *D. suzukii*, and if this success is affected by the host's diet (fresh fruits and artificial diet).

MATERIAL AND METHODS

All insect rearing and experiments have been done under laboratory conditions at 22 ± 2°C, 60 ± 10 % RH and a photoperiod of 16:8 hours (L:D).

Collection and rearing of Drosophila spp. and parasitoids

Drosophila suzukii was obtained from a variety of wild fruits (*e.g. Rubus* sp. and *Fragaria* sp.) collected by S. Fischer (Agroscope Changins, Switzerland) from various sites in Switzerland in 2015. Adults were kept in gauze cages (47.5x47.5x47.5 cm) in groups of 300-500 individuals per cage and fed with sugar water provided on dental cotton rolls. Wet cellulose paper was provided as a water source. Two tubes (ø 50x100 mm) containing 10g of commercial fly diet (Formula 4-24 medium, Carolina Biological SupplyCo., Burlington, NC) with 40mL of 1.43 g/L of methyl-4-hydroxylbenzoate and a pinch of yeast to enhance egg laying were placed as food source and oviposition substrate in each cage. Tubes with *D. suzukii* eggs were changed twice a week and placed in incubators at similar conditions, as described above until emergence of adults, which were then added back in the rearing cage.

A colony of *Drosophila melanogaster* Meigen was obtained from the INRA Laboratory in Antibes (France). It was reared on the same diet as *D. suzukii* in tubes (ø 50x100 mm) placed in an incubator. Adults were provided with fresh diet twice a week and infested tubes were checked every second day for adult emergence.

In total, one European and six Asian parasitoid strains belonging to at least three species were used in this study. The European species *L. heterotoma* was obtained from a baited trap placed outdoors in Delémont, Switzerland, during summer 2015. It was maintained on *D. melanogaster*, reared in tubes as described above, by offering the wasps first instar larvae of *D. melanogaster* twice a week reared on the fly artificial diet. A drop of honey was added to the foam plug of each tube as food source. The tubes were checked twice a week to remove newly emerged parasitoid adults and start it over.

The following parasitoids were collected in Asia in June 2015 and brought to the quarantine facilities of CABI in Delémont, Switzerland:

(1) *Ganaspis* sp. (Hymenoptera, Figitidae) from *Prunus cerasoides* fruits infested by *D. suzukii*, Kunming, Yunnan, China.

(2) *Ganaspis* sp. from *Myrica rubra* fruits infested by *D. suzukii*, Shiping, Yunnan, China.

(3) *Ganaspis sp.* from *Prunus serrulata* fruits infested by *D. suzukii*, Tokyo, Japan.

(4) *Leptopilina japonica* Novkovic & Kimura (Hymenoptera, Figitidae) from *Prunus cerasoides* fruits infested by *D. suzukii*, Kunming, Yunnan, China.

(5) *Leptopilina japonica* from *Prunus* sp. fruits infested by *D. suzukii*, Beijing, China

(6) *Asobara japonica* Belokobylskij (Hymenoptera, Braconidae) collected as adults by sweeping grass with a net below a *Prunus serrulata* trees of which fruits were infested by *D. suzukii* and had dropped to the ground, Tokyo, Japan.

The Figitidae species were identified by Dr. Matthew Buffington (Systematic Entomology Laboratory, USDA ARS, Washington, USA). Using morphological characters *Ganaspis* sp. was initially identified as *Ganaspis brasiliensis* Ihering; but recent studies have shown that *G. brasiliensis*, which has been recorded from various continents (Buffington & Forshage, 2016), is likely a complex of cryptic species with different distributions and various degrees of specificity (Nomano et al., 2017). Therefore, in this paper, our samples will be referred as *Ganaspis* sp. *Asobara japonica* was identified through molecular analyses (CO1 barcoding) at the INRA Sophia-Antipolis laboratory in Antibes, France.

The six parasitoid strains were reared in the CABI quarantine facility. The five Figitidae strains were kept in rearing boxes (ø 90x50 mm) (around 50-60 individuals per box). An Eppendorf tube with a wet cellulose paper was placed in all rearing boxes as a water source. Boxes were closed with a foam plug on which a drop of honey was placed as a food source. Fresh blueberries (*Vaccinium corymbosum*) were placed in each *D. suzukii* rearing cage for 48 hours and then the berries were distributed among the parasitoid rearing boxes for another 48 hours to allow female parasitoids to oviposit in the fly larvae. After the exposure fruits were removed and kept into rearing tubes (ø 50x100 mm) with a filter paper at the bottom to absorb leaking fruit juice. The rearing tubes were checked daily for newly emerged adults, which were transferred to new rearing boxes. The thelytokous species *A. japonica* was maintained on *D. suzukii* larvae in tubes with artificial diet, using the same method as described above for *L. heterotoma*.

Pre-oviposition and development time

Oviposition tests were carried out with the six Asian strains on young larvae of *D. suzukii* in blueberries. Blueberries were first placed for 8 hours in the *D. suzukii* rearing cage. Fruits were then inspected for *D. suzukii* eggs, which were counted using a stereomicroscope. Blueberries with 10 to 30 eggs were retained for the experiments and fruits were stored for 72 hours under laboratory conditions. Newly emerged (less than 12 hours old) males and females of *Ganaspis* sp. and *L. japonica* were kept together for 24 hours in a rearing box to allow mating. Females of the thelytokous *A. japonica* were kept in the same way. Then females of each strain were exposed individually for 8 hours to a single blueberry previously exposed to *D. suzukii* and containing 72 hours-old larvae, which were found to be highly suitable for parasitism by all species in preliminary rearing tests. For seven consecutive days, the same females were offered another blueberry for 8 hours. Twenty replicates were made per species, all performed within the same month. Tubes containing exposed blueberries were kept under laboratory conditions and the emergence of *D. suzukii* and parasitoids was checked daily. The first day of a successful oviposition event (indicated by the emergence of a parasitoid progeny) was noted for each female. The number of offspring per day and per female was recorded, as well as the time needed for parasitoid development.

Substrate suitability and performance

A performance experiment based on a 7 x 2 factorial design was set up with the seven parasitoid strains and two substrates, a standard *Drosophila* diet and fresh blueberries. The experiment was conducted in quarantine under laboratory conditions. For homogeneity, all females used in the experiments were of the exact same age and mated. To ensure that, only newly emerged male and female parasitoids (less than 8 hours old) from the rearing colonies were kept together for 3 days to allow mating prior the experiments. One day prior each test, tubes (ø 25x50 mm) filled with 5 mL of the commercial *Drosophila* diet (Formula 4-24 medium®, with blue dye to facilitate the counting of eggs) and Petri dishes filled with blueberries were exposed to *D. suzukii* oviposition for 8 hours. The number of eggs per blueberry or tube with artificial diet was counted using a stereomicroscope. Blueberries and artificial diet tubes containing 10 to 30 eggs were kept for the experiments. Depending on the number of eggs per fruit one or two blueberries were placed in a tube (ø 25x50 mm) filled with a piece of filter paper at the bottom to absorb leaking berry juice. Blueberries and artificial diet tubes were stored for 24 hours under the same conditions described above to allow eggs to hatch. On the day of testing, parasitoid females were exposed individually either to an infested blueberry or to diet containing fly larvae. Both groups were tested simultaneously and parasitoids were left in the tubes for 48 hours. In total, 30 females per strain and treatment were tested (total 420 females) in a period of less than two months. For each substrate, 19 controls (without parasitoids) were performed to ensure that the rearing conditions allowed the successful development of *D. suzukii*. After a 48 hours exposure period, females were removed and the tubes were stored under the same conditions until the flies and parasitoids had emerged. Emerged flies and parasitoids were counted and sexed. The number of flies with encapsulated parasitoid eggs or larvae was recorded by squeezing the fly between two microscope glass slides. The few tubes with female parasitoids that died during the exposure step were excluded from the analysis.

For each parasitoid strain and substrate (blueberry or diet), several parameters were measured: the total number of emerged *D. suzukii* (n_d) (with or without encapsulated egg or larva); the number of emerged *D. suzukii* with an encapsulated egg or larva (n_e); the number of emerged parasitoids (n_p); the total number of emerged insects ($n = n_d + n_p$); and the number of female parasitoids that attacked *D. suzukii* larvae (i.e. females for which at least one emerging parasitoid or fly with an encapsulated egg was counted (n_o)).

Five parameters of the host-parasitoid interaction were estimated for each strain and condition:

(1) The "Proportion of Ovipositing Females" (POF) corresponds to the number of female parasitoids which laid at least one egg in *D. suzukii* larvae (n_o) divided by the number of females tested (N). POF was calculated as $F = n_O/N$.

(2) The "Overall Parasitism Rate" (OPR), which is the proportion of parasitized hosts, i.e. the proportion of *D. suzukii* that contained an encapsulated egg or produced parasitoid offspring. It was calculated as $OPR = (n_p + n_e)/n$ for each parasitoid female.

(3) The "Apparent Parasitism Rate" (APR), which is estimated as the proportion of parasitoid offspring among the total number of insects that emerged. APR was calculated as

$APR = n_p/n$ for each parasitoid female.

(4) The "Encapsulation Rate" (ER), which corresponds to the proportion of adult flies that emerged with an encapsulated parasitoid egg or larva among the number of parasitized individuals (emerged parasitoids and flies with a capsule). ER was calculated as

$ER = n_e/(n_p + n_e)$ for each parasitoid female.

(5) The "Encapsulation Level" (EL), which is estimated as the proportion of parasitoid offspring among the total number of insects that emerged. EL was calculated as

$EL = n_e/n$ for each parasitoid female.

In addition, we recorded incidences of undetermined mortality of immature stages of *D. suzukii*, i.e. the rate of eggs that did not result in a fly or a parasitoid for blueberry, comparing parasitoid-exposed berries with controls without parasitoid exposure. Unspecific mortality was not assessed for treatments using diet because the accurate counting of eggs inserted into the diet was not possible.

Statistical analyzes

All statistical analyses were performed with the R studio software (version 3.3.3) (R CORE Team, 2017). The normality of residuals was checked with Shapiro's test for the overall parasitism rate, apparent parasitism rate and the encapsulation rate. A Chi square test was used to compare the oviposition rates among oviposition substrates and species. Overall parasitism rate, apparent parasitism rate and the encapsulation rate for each parasitoid species and substrate were compared using generalized linear models (Tweedie family). Pairwise comparisons among species were performed using the Tukey's post-hoc test.

RESULTS

Pre-oviposition and development time

The number of females that oviposited varied between 38 and 90%. For all strains, only a small proportion of females oviposited on the first day of exposure, i.e. when females were 24-44h old. The vast majority of the females started ovipositing within the first 4 days (Figure 1). For all parasitoid species, the number of offspring produced per female increased within the first 2-3 days (Table 1).

Asobara japonica had the shortest development time, averaging 24 days. Development times for *Ganaspis* sp. and *L. japonica* were 34 and 29 days, respectively. For *L. japonica* and *Ganaspis* sp. males emerged 3-4 days prior females (Table 2).

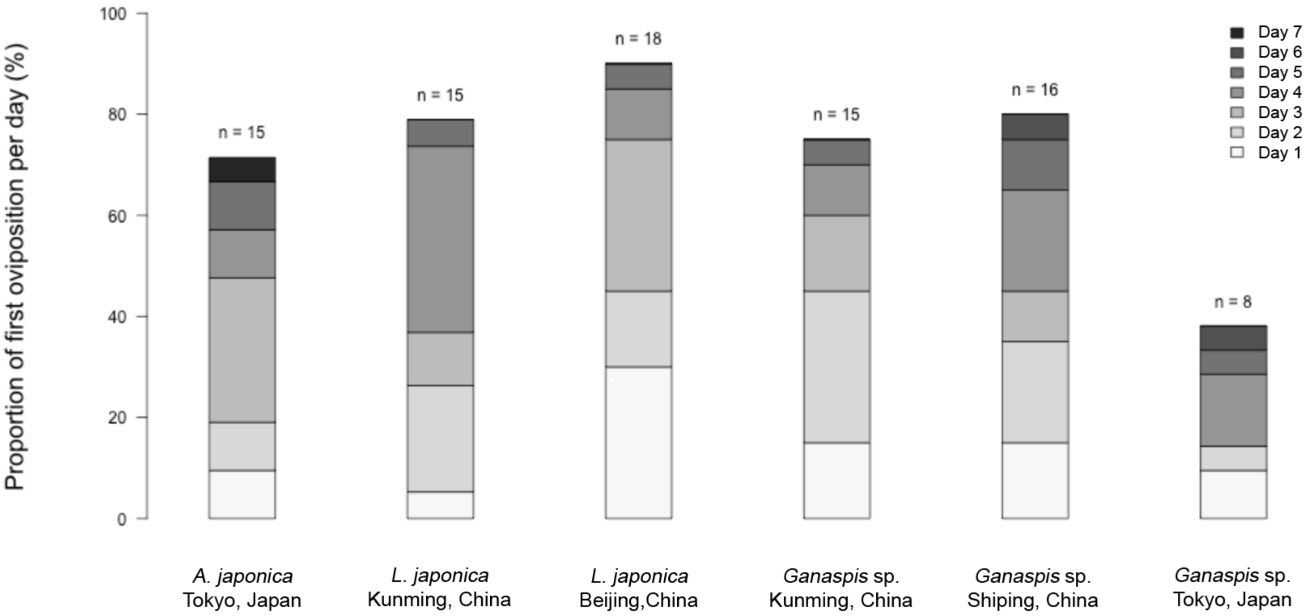

Figure 1. Proportion of first oviposition occurrence per day and per parasitoid species (n= number of responding females. Oviposition was recorded as successful when either an encapsulated egg or larva was found in the abdomen of the emerged fly, or when a wasp offspring was produced).

Substrate suitability and performance

Proportion of Ovipositing Females

The POF of *A. japonica* in the two different substrates was not significantly different (Chi square= 1,181, df = 1, P = 0.277). The two *L. japonica* strains oviposited in both substrates, but significantly more females of the Beijing strain oviposited in blueberries compared to diet (Chi square= 16,484, df = 1, P < 0,001). More females of the three *Ganaspis* sp. strains laid eggs in larvae feeding inside blueberries than in the diet, with only about 10 % of the tested females per strain ovipositing in the diet (Kunming and Shiping strains: Chi square= 32,411, df = 1, P < 0,001 for both strains; Tokyo strain: Chi square= 16,484, df = 1, P < 0,0001). Finally, *L. heterotoma* had a significantly higher POF on artificial diet, with 100% of females laying eggs in the diet and only 36 % in blueberries (Chi square= 27,805, df = 1, P < 0,001) (Figure 2).

Table 1. Mean number of emerging parasitoids per female, per day and per parasitoid species (± SE)

Species	Origin	Days						
		1	2	3	4	5	6	7
Asobara japonica	Tokyo, Japan	0.2 ± 0.14	0.267 ± 0.15	1.333 ± 0.43	0.733 ± 0.25	1.667 ± 0.55	0.733 ± 0.23	0.533 ± 0.27
Leptopilina japonica	Kunming, China	0.067 ± 0.06	0.667 ± 0.25	0.6 ± 0.27	0.933 ± 0.25	0.667 ± 0.21	0.733 ± 0.28	0.867 ± 0.27
Leptopilina japonica	Beijing, China	0.389 ± 0.14	0.389 ± 0.14	1.278 ± 0.51	0.833 ± 0.19	0.5 ± 0.18	0.722 ± 0.32	0.556 ± 0.38
Ganaspis sp.	Kunming, China	0.267 ± 0.15	1.4 ± 0.42	0.933 ± 0.21	1.667 ± 0.44	1.533 ± 0.53	0.933 ± 0.3	1.867 ± 0.45
Ganaspis sp.	Shipping, China	0.625 ± 0.34	1.188 ± 0.36	1.250 ± 0.39	1.188 ± 0.33	1.875 ± 0.42	1 ± 0.29	0.813 ± 0.21
Ganaspis sp.	Tokyo, Japan	0.25 ± 0.16	0.5 ± 0.33	1 ± 0.57	0.625 ± 0.32	0.5 ± 0.25	1.5 ± 0.57	0.125 ± 0.13

Table 2. Mean development time of Asian parasitoid species in days (±SE) at 22 ± 2°C (n=number of offspring)

Species Origin	*Asobara japonica* Tokyo, Japan	*Ganaspis* sp. Kunming, China	*Ganaspis* sp. Shiping, China	*Ganaspis* sp. Tokyo, Japan	*Leptopilina japonica* Kunming, China	*Leptopilina japonica* Beijing, China
Males	-	31.9 ± 0.5 (n = 80)	33.5 ± 0.6 (n = 107)	32.3 ± 0.2 (n = 12)	26.8 ± 0.4 (n = 46)	27.8 ± 0.4 (n = 51)
Females	23.9 ± 0.5 (n = 83)	35.3 ± 0.3 (n = 44)	36.5 ± 0.3 (n = 22)	36.2 ± 0.4 (n = 20)	31.0 ± 0.3 (n = 21)	32.1 ± 0.3 (n = 30)

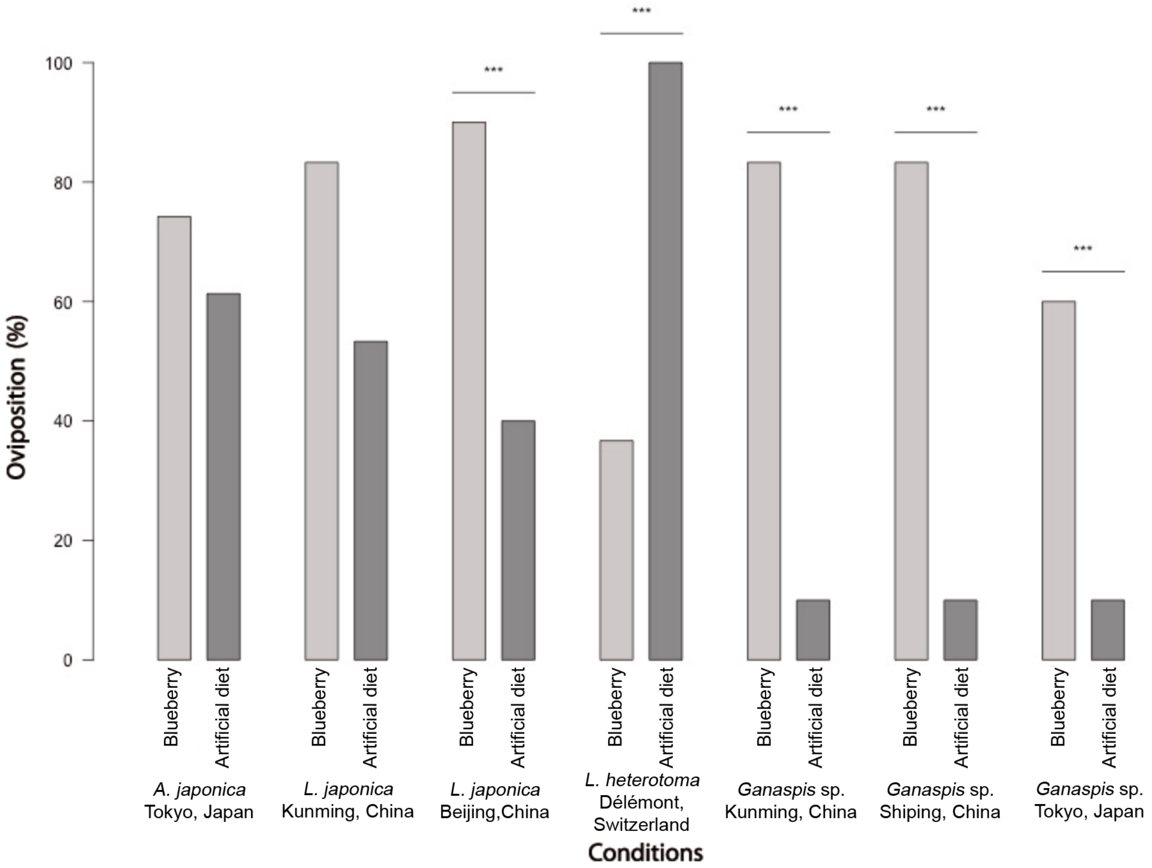

Figure 2. Proportion of Ovipositing Females (POF) proportion of parasitoid females that oviposited when exposed to larvae of *Drosophila suzukii* in artificial diet or blueberries. Oviposition was recorded as successful when either an encapsulated egg or larva was found in the abdomen of the emerged fly or a wasp offspring was produced (Chi-square test , *** p<0.001).

Overall parasitism rate

Asian parasitoid strains did not differ in the OPR on blueberry, with an average of ca. 40 % for all strains and species (Figure 3). However, the OPR of larvae in blueberries by *L. heterotoma* was significantly lower than the OPR of the other Asian parasitoids. Overall, the OPR was more variable among strains in the artificial diet, with *A. japonica* and *L. japonica* strains showing a much higher parasitism rate than the three strains of *Ganaspis* sp., which rarely attacked *D. suzukii* in the artificial diet.

When comparing the OPR on blueberry and artificial diet within each strain, the OPR was much higher on blueberry than on diet for all *Ganaspis* sp. strains. *Leptopilina heterotoma* showed the exact opposite*,* with a rate of 62 % parasitism in the diet compared to 7% on blueberry. *Asobara japonica* and the two Asian *L. japonica strains* showed no difference in the rate of parasitism in blueberry and artificial diet (Figure 3).

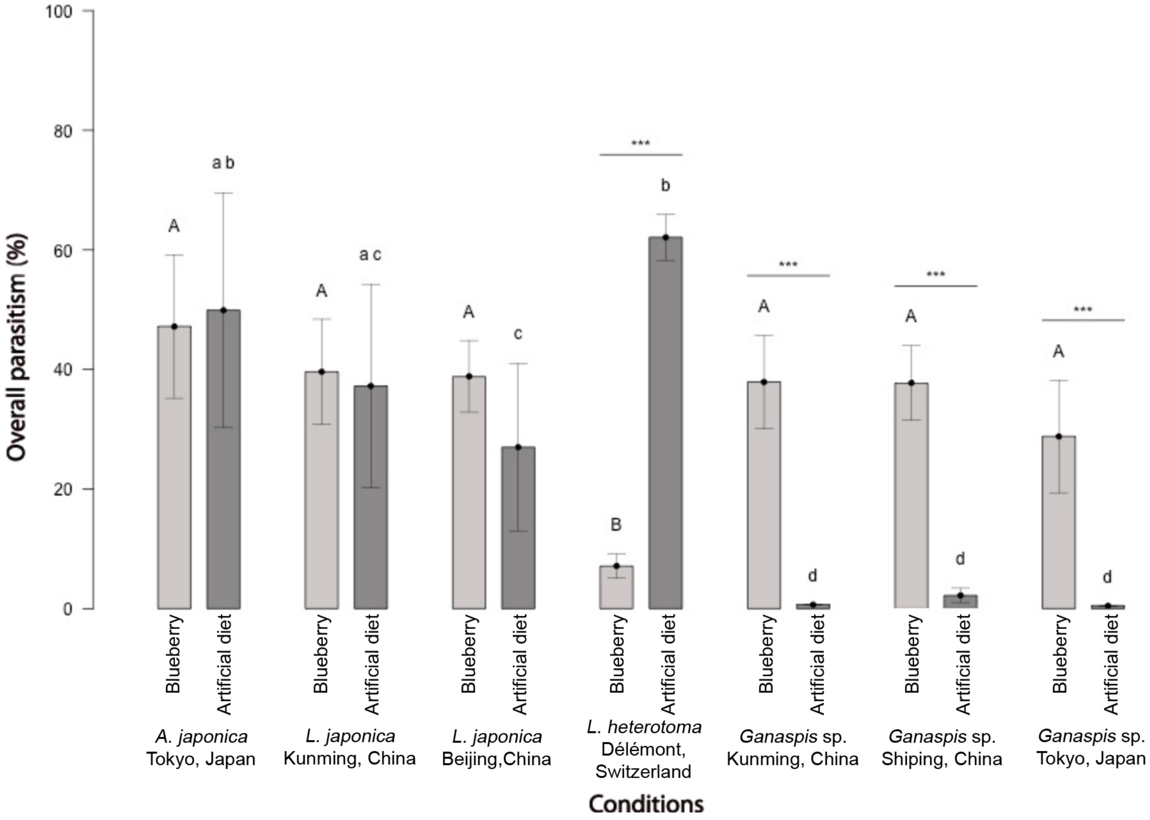

Figure 3. Mean of Overall Parasitism Rate (OPR) (±S.E.) caused by parasitoids exposed to larvae of *Drosophila suzukii* in artificial diet or blueberry. OPR is the proportion of parasitized hosts, i.e. the proportion of *D. suzukii* that contained an encapsulated egg or produced parasitoid offspring. Same letters above bars indicate no significant differences between conditions (upper case letters: comparison among the blueberry test condition; lower case letters: comparison among the artificial diet test condition; asterisks indicate significant differences between substrates, pairwise comparison per parasitoid species, (GLM (Tweedie family) Tukey post hoc, letters p<0.05 and ***p<0.001).

Apparent parasitism rate and encapsulation rate

All Asian parasitoids tested successfully developed in *D. suzukii* larvae feeding inside blueberries, and no significant difference was found among species tested. However, when larvae were exposed in artificial diet to the wasps, the *Ganaspis* sp. strains were barely effective in parasitizing *D.* suzukii, with an average APR less than 2% compared to an average of 36% for the other Asian wasps (Figure 4 & 5). Few eggs and larvae of the Asian parasitoids were found encapsulated, although ER was not negligible for *Ganaspis* sp. in diet, given the low amount of eggs laid in this substrate. Only five *Ganaspis* sp. from Shiping emerged from artificial diet (ER: 37.5%). For the two other strains ER were 100% with no emergence of parasitoids. In blueberry, ER were 6.48%, 5.45% and 6.58% for the strain Kunming, Shiping and Tokyo respectively. Numbers of emerged parasitoid wasps for those previous strains, were 101, 104 and 72 in blueberry (APR ca. 32.5% ± SD 4.1) (Figure 5). In contrast, *L.*

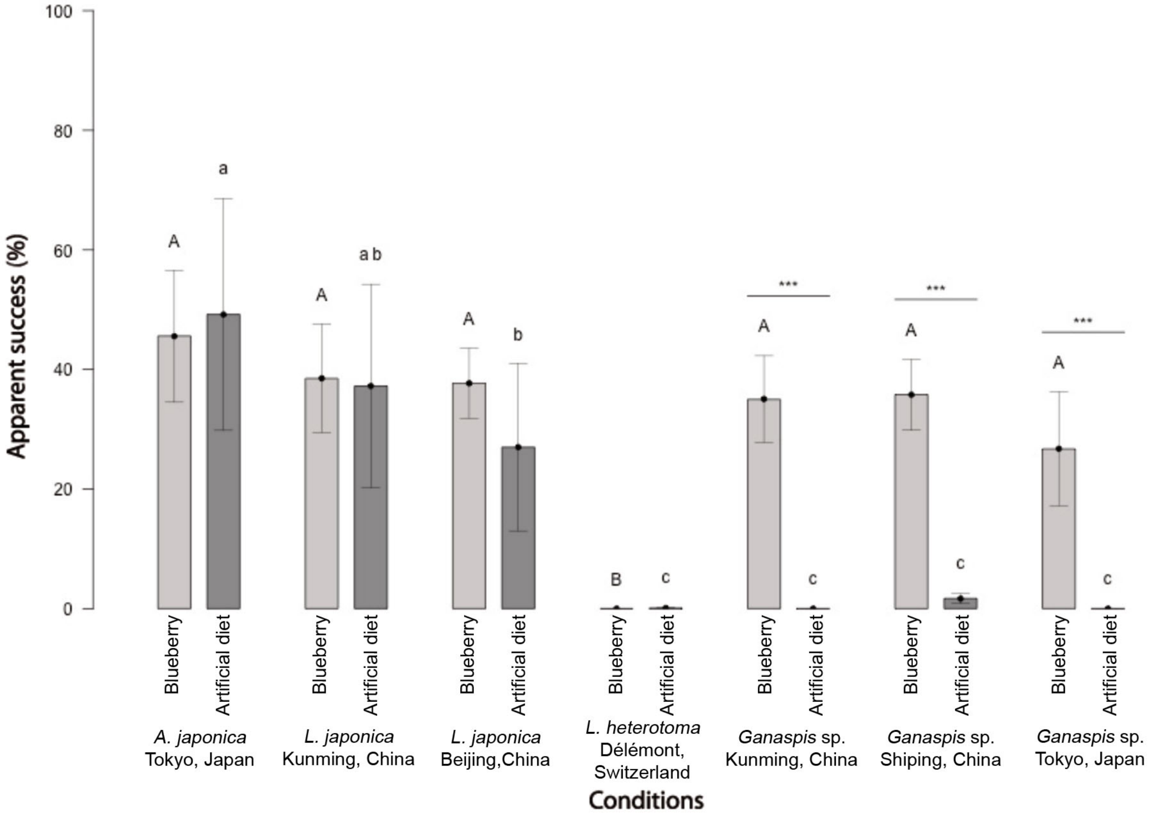

Figure 4. Mean of apparent parasitism rate (APR) (±S.E.) caused by parasitoids exposed to larvae of *Drosophila suzukii* in artificial diet or blueberry. APR was calculated as the proportion of parasitoid offspring among the total number of insects that emerged. Same letters above bars indicate no significant differences between conditions (upper case letters: comparison among the blueberry test condition, lower case letters: comparison among the artificial diet test condition; asterisks indicate significant differences between substrates ,pairwise comparison per parasitoid species, (GLM (Tweedie family) Tukey post hoc, letters p<0.05 and ***p<0.001).

heterotoma failed to successfully develop in *D. suzukii* larvae in blueberries and diet. *Leptopilina heterotoma* showed a much higher ER that was nearly 100% in both substrates (Figure 5). Only one *L. heterotoma* wasp emerged from artificial diet.

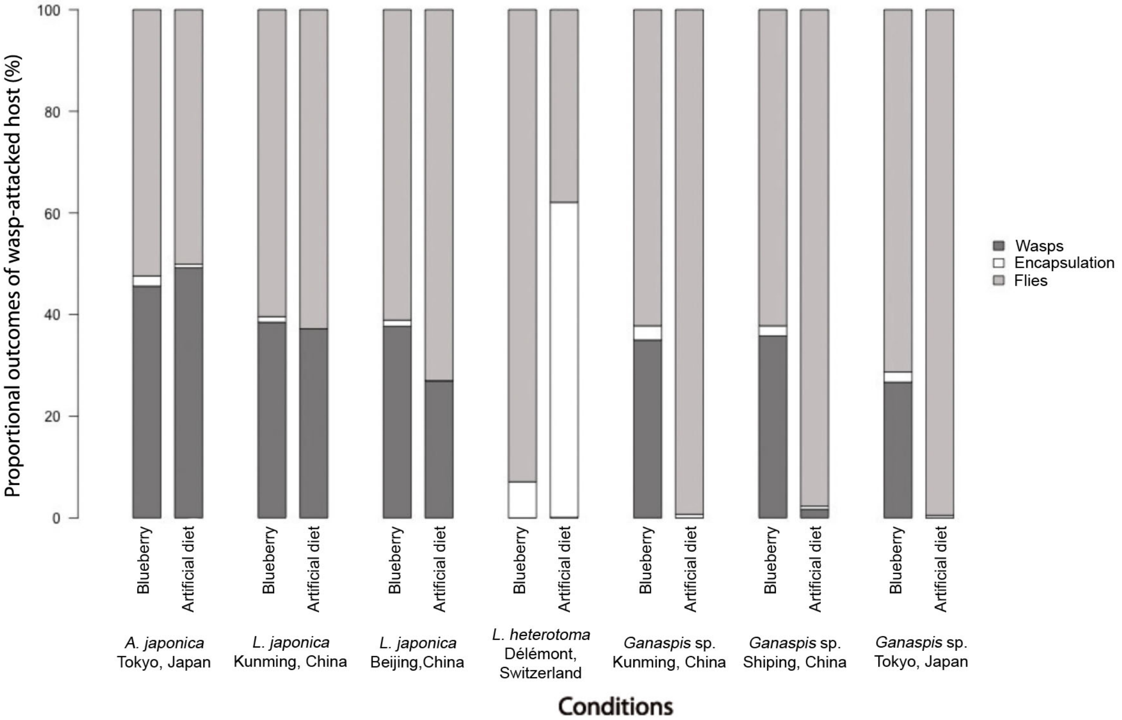

Figure 5. Mean proportion of emerged wasps (APR), encapsulation (EL) and flies (without capsule) per tested substrates (in artificial diet or blueberry) per parasitoid species.

Undetermined mortality

Undetermined mortality of immature stages of *D. suzukii*, i.e. the percentage of eggs that did not develop into flies or parasitoids, was 42% in control samples. In samples exposed to parasitoids, undetermined mortality rates were slightly, but not significantly higher compared to the control except for *A. japonica* (Table 3).

DISCUSSION

Figitidae and Braconidae larval parasitoids of *Drosophila* spp. are known to be partly proovigenic, (i.e. females emerge with mature eggs that are ready to be laid (Jervis et al., 2001), but the degree of proovigeny varies among species (Carton et al., 1986; Fleury et al., 2009). Our pre-oviposition experiments were not made to precisely assess ovarian development, but rather to help designing further oviposition tests (e.g. for specificity tests).

We showed that in most species tested, a small proportion of females laid eggs one or two days after emergence, but the majority started only after three to four days. Accordingly, more offspring was obtained from 3-4 days old females than from younger females. Therefore, we recommend that in future oviposition tests at least 4-day old females should be used to ensure high oviposition rates.

In blueberries, 39-54% of the immature stages of *D. suzukii* died of unknown causes. A similar mortality of 42% was observed in the control treatment. This suggests that parasitoids were not, or only partly responsible for this undetermined mortality. Mortality could be linked to a high density of eggs as suggested Burrack et al. (2013), who reported high mortality rates in blueberries when densities where above 20 eggs per fruit. For future tests, the number of hosts per fruit and also the type of fruit may have to be reconsidered to reduce pre-imaginal mortality. However, there are benefits to use blueberries, which stay much longer fresh and allow for easy counting of the fly eggs on the dark and smooth fruit surface.

Table 3. Undetermined mortality rate. Proportion (%) of immature stages of *D. suzukii* in blueberry that died for unknown reason, for each parasitoid strain and control without parasitoids. Only female parasitoids that produced at least one emerging parasitoid or capsule were included in the analysis. P-values based on Chi-square goodness-of-fit tests between each treatment and the control

Species	Origin	Eggs	Undetermined mortality (%)	P-value
Asobara japonica	Tokyo, Japan	336	53.27	0.007
Leptopilina japonica	Kunming, China	319	46.71	0.292
Leptopilina japonica	Beijing, China	449	41.20	0.869
Leptopilina heterotoma	Jura, Switzerland	193	39.38	0.617
Ganaspis sp.	Kunming, China	360	46.39	0.314
Ganaspis sp.	Shiping, China	430	45.12	0.473
Ganaspis sp.	Tokyo, Japan	284	46.83	0.294
Control	-	285	42.11	-

The present study indicates significant differences among Asian parasitoids in their ability or willingness to parasitize larvae in the two tested substrates. Such differences among species and between substrates could be explained by differences in searching behaviour (e.g. vibrotaxis, ovipositor searching and antennal searching) of the parasitoids and their ecology (host range and habitat selection), which could be related to the degree of specificity of each parasitoid (Vet & van Alphen, 1985). For most strains, more offspring was obtained from larvae feeding in blueberries than in the diet, which is possibly related to the situation in the area of origin, where *D. suzukii* attacks mainly fresh fruits (Nguyen et al., 2016). Among the three tested genera, *Ganaspis* sp. parasitized significantly more often larvae inside fresh blueberries than in artificial diet in a no choice situation. Indeed, only a very few *Ganaspis* sp. females actually laid eggs in larvae in artificial diet. These results indicate that these wasps may have a high degree of specialisation on *D. suzukii*, and may specifically use host finding cues associated with fresh fruits. In contrast, *A. japonica* and *L. japonica* are known to have a wider host range, attacking various species of *Drosophila* larvae on ripe and rotten fruits, mushrooms and decayed leaves (Ideo et al. 2008; Kasuya et al., 2013b). Therefore, females of this species could associate a wider variety of cues with the presence of *D. suzukii* larvae, allowing it to locate its hosts regardless of the substrate. Furthermore, *A. japonica* and *L. heterotoma* females emerged from diet, in contrast to the Asian Figitidae that were reared on blueberry. It is a well-known fact that parasitoids are able to learn olfactory cues during the pre-imaginal stages and at adult emergence (Turlings et al., 1993; Gandolfi et al., 2003) and it cannot be ruled out that variations in parasitism rates in the different substrates are due at least partly to the different rearing substrates. However, such effects are less likely to occur in no-choice conditions in tubes than in choice tests in larger environments.

Yet, the reason why *Ganaspis* sp. refrained from attacking host larvae in artificial diet remains unknown. It seems possible that *Ganaspis* sp. may not be able to detect larvae in artificial diet because chemical cues emitted by this substrate do not provide enough information for the females to stimulate foraging behaviour, even in a no-choice situation (Dicke et al., 1984). Furthermore, host localization could be hindered because host cues such as vibrations of the crawling larvae could be difficult to detect in the diet. Other *Ganaspis* spp. seem to use vibrotaxis to first detect the host and then orientate themselves toward the larvae, and then try to sting it with their ovipositor (ovipositor searching) (Vet & van Alphen, 1985). *Asobara* species also use vibrotaxis, whereas *Leptopilina* species appear to fully rely on chemical cues and ovipositor probing (Sokolowski & Turlings, 1987).

In contrast to the Asian species, the European *L. heterotoma* reproduced better in larvae that were offered in artificial diet rather than blueberries. This species is known to attack a wide range of *Drosophila* species in various substrates, such as fermenting fruits, fungi and decaying plant materials, but not fresh fruits. *Leptopilina heterotoma* is attracted by fermentation volatiles emitted by yeast, e.g. on decaying fruits (ethanol, ethyl acetate and acetaldehyde), which implies that cues emitted by fresh blueberries are unlikely to induce strong interest for host searching behaviours (Carton et al., 1986; Janssen et al., 1987; Mitsui et al., 2007). To locate host larvae in the substrate, females also rhythmically probe suitable substrates with the ovipositor while walking (Vet & van Alphen, 1985; Sokolowski & Turlings, 1987) and are unlikely to do this on fresh fruits. Finally, *L. heterotoma* females may be less adapted to pierce the skin of a fresh blueberry, which requires a stronger penetration force (Burrack et al., 2013) and is also more time-consuming than searching for hosts in decaying fruits or artificial diet. However, a small number of larvae in blueberries were parasitized, suggesting that the females were able to pierce the fruit skin.

Leptopilina heterotoma laid a large number of eggs in *D. suzukii* larvae feeding on artificial diet, but nearly all of these were encapsulated. These results suggest that *L. heterotoma*'s virulence is not strong enough to resist the immune reaction of *D. suzukii*. This was also observed in other studies (Chabert et al., 2012; Kacsoh & Schlenke, 2012; Poyet et al., 2013; Knoll et al., 2017; Rossi Stacconi et al., 2017). However, a single female succeeded to complete its development in this study, and higher rates of successful development were observed when testing various geographic strains of the parasitoid (P. Girod et al., unpublished data). Chabert et al. (2012) found only three parasitoid adults emerging from 180 parasitized larvae and Rossi Stacconi et al. (2015) demonstrated that in both artificial diet and blueberry *L. heterotoma* was able to successfully develop on *D. suzukii*, 10% in blueberry and up to 30% in artificial diet. Regarding all the results previously obtained, it cannot be ruled out that, in the future, *L. heterotoma* will naturally evolve and adapt to the exotic host, allowing it to successfully parasitize *D. suzukii* at least in decaying fruits.

In contrast to the European larval parasitoid, the Asian parasitoids tested in this study attacked and developed successfully in larvae in fresh fruits and could potentially be used as biological control agents to lower *D. suzukii* populations in the invaded range. Their low level of encapsulation indicates that these species have co-evolved with *D. suzukii* and are able to overcome the high haemocyte load of the host (Kacsoh & Schlenke, 2012). In contrast to *A. japonica* and *L. japonica* that attacked and developed in larvae in both substrates, *Ganaspis* sp. parasitized many more larvae in blueberries compared to artificial diet. *Ganaspis* sp. also showed a tendency to be more encapsulated in larvae that were feeding in artificial diet, although the low sample size (i.e. few eggs were laid in the host larvae in diet) does not allow us to draw a solid conclusion. Taken together, these data suggest that *Ganaspis* sp. is well adapted to *D. suzukii,* which mainly attacks fresh fruits. This needs further confirmation from host range tests with other *Drosophila* spp., which may be difficult to conduct with *Ganaspis* sp. since European and North American *Drosophila* spp. cannot be reared on fresh fruits. Similar specificity was observed for *G. xanthopoda* associated with *D. suzukii* in Japan (Kasuya et al., 2013a), which was subsequently identified as *G. brasiliensis* (Nomano et al., 2017) and could be the same species or biotype as the one tested in this study. A *Ganaspis* sp. reared from *D. suzukii* in South Korea was recently also identified as *G. brasiliensis* (Buffington & Forshage, 2016; Daane et al., 2016). However, Nomano et al. (2017) suggest that *G. brasiliensis* is a complex of cryptic species. Consequently, there is an urgent need to revise the taxonomy of the *G. brasiliensis* complex as this taxonomic uncertainty may prevent its use for biological control. *Asobara japonica* and *L. japonica* are both known to attack *D. suzukii* and other *Drosophila* species in Asia (Ideo et al., 2008; Mitsui & Kimura, 2010; Novković et al., 2011; Wachi et al., 2015; Daane et al., 2016; Guerrieri et al., 2016), but for these species too, the occurrence of biotypes or cryptic species, potentially showing different levels of host specificity, should be further investigated. Host range tests are currently underway with these Asian parasitoids and European *Drosophila* spp. to determine their degree of specificity and their potential for biological control of *D. suzukii* in Europe.

AUTHORS CONTRIBUTION

All authors contributed to the design of the research. The experiments and analyses were carried out by PG and LR, supervised by MK, TH and TT. The writing up of the manuscript was led by PG with contributions from all authors.

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CHAPTER 3: HOST SPECIFICITY OF ASIAN PARASITOIDS FOR POTENTIAL CLASSICAL BIOLOGICAL CONTROL OF THE INVASIVE FLY: *DROSOPHILA SUZUKII*

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ABSTRACT

The Asian spotted wing drosophila, *Drosophila suzukii* was first found in Europe and North America in 2008 and quickly became a serious pest in soft fruits crops. Current control strategies are based on chemical and cultural management, but since their efficacy, cost and impact on the environment raises concerns, alternative control methods are needed. Classical biological control, *i.e.* releasing larval parasitoids from Asia in areas invaded by the fly, may provide an environmentally friendly alternative. However, host specificity of such potential biological control agents has to be determined prior to releases to avoid unintended non-target impacts on native species. Five strains belonging to three larval parasitoids from China and Japan, *Asobara japonica*, *Leptopilina japonica* and *Ganaspis* sp. have been tested in quarantine on six different European flies on different substrate conditions (artificial diets and fresh blueberry). Similar tests were carried out with the European larval parasitoid *Leptopilina heterotoma*. *Asobara japonica* showed the lowest specificity, attacking and developing in all Drosophilidae offered to females. *Leptopilina japonica* successfully parasitized two non-target Drosophilidae: *D. melanogaster* and *D. subobscura*, with one singly progeny emerging from *D. immigrans*. *Ganaspis* sp. had the highest level of specificity but variations occurred between two geographical strains tested. A Japanese strain was strictly specific to *D. suzukii,* whereas another strain from China parasitized *D. suzukii*, *D. melanogaster* and sporadically *D. subobscura*. The European *L. heterotoma* successfully developed in *D. melanogaster*, *D. subobscura* and occasionally in *D. immigrans*, but nearly all eggs and larvae of *D. suzukii* were encapsulated. These results are promising for the biological control of *D. suzukii* in Europe and show that *Ganaspis* sp. from Japan is the species with the highest potential, but more studies are needed on its taxonomic status and the existence of biotypes or cryptic species varying in their specificity towards *D. suzukii* before field releases can be envisaged in Europe.

KEY WORDS

Spotted wing drosophila, biological control, non-target effects, host range, fruit flies, larval parasitoids

INTRODUCTION

Globalization and climate change speed up the spread of new invasive pests, causing an estimated agricultural loss of more than $1.4 trillion per year worldwide (Pimentel et al., 2001). Prevention, detection, and management are keys to reduce the impact of invasive species on the economy and biodiversity. Unintended and voluntary introductions can also potentially disrupt the balance of the ecosystem and lead to severe environmental disturbances (*e.g.* increasing competition, predation) or seriously impact evolutionary processes (*e.g.* species extinction or interbreeding) (Kenis et al., 2009). Since the second half of the twentieth century, around 30% of alien arthropod species established in Europe have originated from Asia (Roques et al., 2009). Among these, *Drosophila suzukii* Matsumura (Diptera, Drosophilidae), or spotted wing drosophila, a fly of East Asian origin, was first found in 2008 in Europe and North America, from where it invaded several other regions (Fraimout et al., 2017). In just a few years, *D. suzukii* has invaded several continents and become a very serious pest of many fruit crops worldwide.

Current control methods rely on chemical insecticides or expensive and labour-intensive cultural practices (Haye et al., 2016). An alternative approach to control *D. suzukii* would be to use classical biological control, *i.e.* introducing natural enemies from the native range of the pest. Compared to chemical control, this method is commonly less controversial because of its direct benefits on human health, food safety and maintenance of ecosystem services and its effectiveness in the long-term control of invasive species. However, potential adverse ecological effects of biological control introductions have to be considered prior to the introduction of an exotic biological control agent (Heimpel & Mills, 2017). A low number of unintended non-target impacts using parasitoids have been reported in the literature over the last 20 years (Boettner et al., 2000; Johnson et al., 2005; Barratt et al., 2007; Van Driesche & Hoddle, 2017) and, as a consequence, regulatory requirements have become more prescriptive. In most countries approval for release of classical biological control agents is based on a risk assessment determined from a petition detailing outcomes of studies on host specificity and other information (Mason et al., 2013). Current practices usually include laboratory host specificity tests as a first step to define the fundamental host range of the potential biological control agent (Van Driesche & Murray, 2004; Bigler et al., 2006; van Lenteren et al., 2006).

Prior conducting the management of *D. suzukii* which could be achieved through the introduction of Asian parasitoids that have co-evolved with the pest and which may provide a sustainable solution if they are sufficiently specific to the target, a full environmental risk assessment of these beneficial biological control agents is required. Recent studies indicated that natural enemies from Asia may provide a better area-wide control of *D.* suzukii's population in the invaded areas because native larval parasitoids in the genera *Leptopilina* and *Asobara* from Europe and North America are rarely able to complete their development in *D. suzukii* (Chabert et al., 2012; Poyet et al., 2013; Gabarra et al., 2015; Rossi-Stacconi et al., 2015; Daane et al., 2016). However, it has been described recently that local European generalist pupal parasitoids such as *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) can successfully develop in *D. suzukii* but their actual impact on fly populations when mass-released in infested orchards is not known yet (Knoll et al., 2017; Rossi-Stacconi et al., 2017).

In a first study (Girod et al., this thesis Ch. 2), we carried out preliminary tests with three larval parasitoids attacking *D. suzukii* in its native range in Asia, *Leptopilina japonica* Novkovic & Kimura*, Ganaspis* sp. (Hymenoptera, Figitidae) and *Asobara japonica* Belokobylskij (Hymenoptera, Braconidae). These tests allowed us to gather important information on development time, pre-oviposition period and preference for host substrates. In particular, it was shown that *Ganaspis* sp. was successfully reared in larvae in blueberry only but rejected larvae in diet, whereas *L. japonica* and *A. japonica* favourably attack and develop in host larvae feeding in artificial diet and blueberry. Here we present new insights on the assessments of the host specificity of the same larval parasitoids, by testing them on a variety of potential European hosts. These results could predict the possible non-target impacts of Asian parasitoids on native European *Drosophila* species if released for biological control of *D. suzukii*.

MATERIAL AND METHODS

Insect rearings

All insect rearing and experiments were conducted under laboratory conditions at 22 ± 2°C, 60 ± 10 % RH and a photoperiod of 16:8 h (L:D).

Target and non-target species

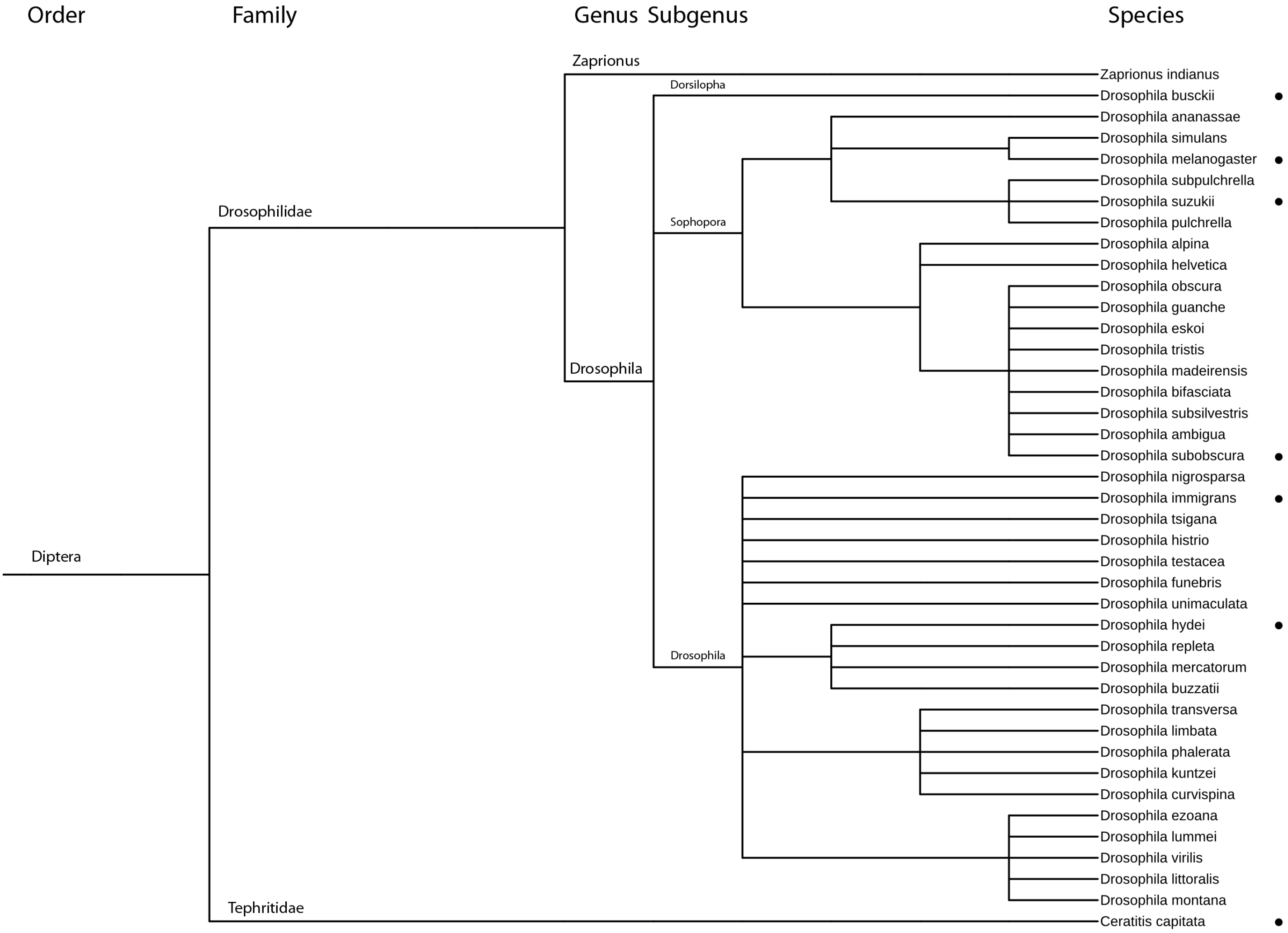
The original *D. suzukii* colony was obtained from wild fruits (*e.g. Rubus* sp. and *Fragaria* sp.) collected by S. Fischer (Agroscope Changins, Switzerland) from various sites in Switzerland in 2015. Around five hundred adults flies were reared in gauze cages (47.5x47.5x47.5 cm BugDorm-4©) and fed with sugar water provided on dental cotton rolls. Wet cellulose paper was also provided as water source. Two tubes (ø 50x100 mm) containing 10g of commercial artificial fly diet (Formula 4-24 medium©, Carolina Biological SupplyCo. Burlington, NC) with 40mL of 1.43 $g.L^{-1}$ of methyl-4-hydroxylbenzoate and a pinch of yeast to enhance egg laying were placed in each cage as a food source and oviposition substrate. Tubes with *D. suzukii* eggs were changed twice a week and placed in incubators at similar conditions as described above until emergence of adults, which were then randomly distributed among the rearing cage.

Five European *Drosophila* spp. (*D. busckii* Coquillett, *D. hydei* Sturtevant, *D. immigrans* Sturtevant*, D. melanogaster* Meigen and *D. subobscura* Collin) and one Tephritidae species (*Ceratitis capitata* Wiedemann) were selected as non-target test species.

Selection of non-target hosts was based on phylogenetic relatedness, sympatry of target and non-target species and information available from the literature (Kuhlmann et al., 2006; NCBI Taxonomy, https://www.ncbi.nlm.nih.gov/taxonomy; FlyBase, http://flybase.org) (Figure 1). *Ceratitis capitata* was selected as an out-group species as it is also able to oviposit into fresh berries as *D. suzukii,* in contrast to other European *Drosophila* spp. that usually attack decaying fruits and other organic matters. All non-target species were obtained from N. Ris (INRA, Sophia-Antipolis, France) in 2015. The *Drosophila* spp. were reared in tubes (ø50x100 mm) on the same artificial diet as *D. suzukii*. Tubes with *Drosophila* eggs were changed twice a week and placed in incubators at similar conditions, as described above until the emergence of adults.

Ceratitis capitata was reared on a homemade artificial diet (10g of Carolina© artificial fly diet, 10g of carrot powder and 10g of yeast powder with 40mL of 1.43 $g.L^{-1}$ of methyl-4-hydroxylbenzoate) in cages of two hundred adult flies (30x30x30 cm BugDorm-1©).

Figure 1. Phylogenetic tree of European Drosophila spp. with tested species marked with dots (•). Ceratitis capitata, tested in this study, and three exotic Drosophilidae attacking fresh fruits, Zaprionus indianus, Drosophila pulchrella and Drosophila subpulchrella, were added to the tree. The tree is based on Fauna Europaea and the phylogenetic tree was built with NCBI Taxonomy database, Software iTOL (Letunic, I., & Bork, P., 2016)



Parasitoid species

One European and five Asian strains of parasitoids were used in this study. The European species *Leptopilina heterotoma* Thompson (Hymenoptera, Figitidae) was obtained from a baited trap placed outdoors in Delémont, Switzerland, in the summer of 2015. It was maintained on *D. melanogaster* reared in tubes as described above, by offering the wasps first instar larvae for 3-4 days. A drop of honey was added to each tube as food source. The tubes were checked every second day to remove newly emerged parasitoid adults.

The following parasitoids were collected in Asia in June 2015 and reared in the quarantine facilities of CABI in Delémont, Switzerland:

(1) *Ganaspis* sp. from *Prunus cerasoides* fruits infested by *D. suzukii*, Kunming, Yunnan, China.

(2) *Ganaspis sp.* from *Prunus serrulata* fruits infested by *D. suzukii*, Tokyo, Japan.

(3) *Leptopilina japonica* from *Prunus cerasoides* fruits infested by *D. suzukii*, Kunming, Yunnan, China.

(4) *Leptopilina japonica* from *Prunus* sp. fruits infested by *D. suzukii*, Beijing, China.

(5) *Asobara japonica* collected by sweeping over *Prunus serrulata* fruits infested by *D. suzukii* that had dropped to the ground, Tokyo, Japan.

The Figitidae species were identified by Dr. Matthew Buffington (Systematic Entomology Laboratory, USDA ARS, Washington, USA). Using morphological characters, *Ganaspis* sp. was initially identified as *Ganaspis brasiliensis* Ihering, but recent studies have shown that *G. brasiliensis*, which has been recorded from various continents (Buffington and Forshage 2016; Nomano et al., 2017), is likely a complex of cryptic species with different distributions and various degrees of specificity (Nomano et al., 2017). Therefore, in this study, our samples will be referred as *Ganaspis* sp. *Asobara japonica* was identified through molecular analyses (CO1 barcoding) at the INRA Sophia-Antipolis laboratory in Antibes, France.

The five Figitidae strains were kept in rearing boxes (ø 90x50 mm), each containing approximately 50-60 individuals. An Eppendorf tube with a wet cellulose paper was placed in all rearing boxes as a water source. Boxes were closed with a foam plug on which a drop of honey was placed as a food source. Fresh blueberries (*Vaccinium corymbosum*) were placed in each *D. suzukii* rearing cage for 48 hours, and then the infested berries were distributed among the parasitoid rearing boxes for another 48 hours to allow female parasitoids to oviposit in the fly larvae. After the exposure infested fruits were removed and kept in rearing tubes (ø 50x100 mm) with a filter paper at the bottom to absorb leaking fruit juice. The rearing tubes were checked daily for newly emerged adults, which were transferred to new rearing boxes. The thelytokous species *Asobara japonica* was maintained on *D. suzukii* larvae in tubes with artificial diet, using the same method as described above for *L. heterotoma*.

Host specificity testing

Prior the host specificity tests, the oviposition substrates containing the fly larvae had to be modified due to their different attractiveness to the parasitoid and fly species. Indeed, a preliminary analysis had shown that the blended CAROLINA© diet used for rearing the *Drosophila* spp. is not accepted as substrate by *Ganaspis* sp. (Girod et al., this thesis Ch. 2). However, *D. suzukii* is the only *Drosophila* species being able to lay eggs in fresh fruits. Consequently, a blended diet was developed, which was accepted by all the Drosophilidae, Tephritidae and parasitoids tested (25g of blended fresh blueberry, 40mL of 1.43 $g.L^{-1}$ of methyl-4-hydroxylbenzoate and 20g of blended CAROLINA© diet).

The specificity tests were carried out in two steps. In experiment A, *A. japonica, L. japonica* and *L. heterotoma* were tested on *D. suzukii*, *D. melanogaster*, *D. immigrans*, *D. subobscura* and *D. busckii* in plain regular CAROLINA© diet (Table 1, Experiment A), because

Table 1. Experimental testing scheme for each parasitoid, diet, and host (* only tested hosts of *L. japonica* Beijing, China for experiment B).

Experiment #	Parasitoids		Oviposition substrate	Hosts		Nb. of replicates (n)
	Species	Origin				
A	*Asobara japonica* *Leptopilina japonica* *Leptopilina japonica* *Leptopilina heterotoma*	Tokyo, Japan Beijing, China Kunming, China Delémont, Switzerland	Artificial diet	*D. melanogaster*		30
				D. busckii		30
				D. subobscura		30
				D. immigrans		30
				D. suzukii		30
B	*Ganaspis* sp. Ganaspis sp. Leptopilina japonica*	Kunming, China Tokyo, Japan Beijing, China	Artificial diet + blended blueberry	*D. hydei*	*	30
				D. melanogaster		30
				D. busckii		60
				D. subobscura		30
				D. immigrans		30
				D. suzukii	*	60
				C. capitata	*	30
			Blueberry	*D. suzukii*	*	60
				C. capitata	*	30

Girod et al. (this thesis Ch. 2) demonstrated that these species would show the same parasitism behaviour with larvae in this artificial diet and blueberries. In experiment B, the two strains of *Ganaspis* sp. were tested on all potential host species in the diet containing blended blueberries. *Ganaspis* sp. was also tested on *D. suzukii* and *C. capitata* in fresh blueberries (Table 1, Exp. B). In Experiment B, *L. japonica* was also tested on *D. hydei* and *C. capitata* that were not assessed in experiment A, as well as on *D. suzukii* as control. In contrast, *A. japonica* was not assessed further because experiment A had shown that this species is highly polyphagous. In total, 43 different combinations of parasitoids, oviposition substrates and host were tested (Table 1).

From the laboratory colonies, 0-12 hours old female parasitoids were collected and set up in tubes with males (sex ratio female/male 2:1) for 72 hours to ensure that they were mated and mature at the time of the experiments (Girod et al., this thesis Ch. 2). After three days, single females were exposed to 12-20 hours old Drosophila larvae for 48 h. *Drosophila* spp. show small differences in their development time and exposing the hosts for 48 hours ensured that all *Drosophila* spp. were in their first instar and early second instars, a favourable stage for larval parasitoids of *Drosophila* spp. (Carton et al., 1986). Larvae of *C. capitata* were 24-32 h old at the beginning of the 48 h exposure to parasitoids because egg development for this species was slower. After two days of exposure, female parasitoids were removed. A total of 30 replicates (60 for *D. suzukii* and *D. busckii* in experiment B) and 20 controls (fly larvae without exposure to parasitoids) were performed for each species. Fly and parasitoid emergence was checked daily and all emerging individuals were counted and sexed. The number of flies with encapsulated parasitoid eggs or larvae was recorded by squeezing the fly between two microscope glass slides. The few female parasitoids that died during the experiments were excluded from the analysis, as were the tubes without any emergence of flies or parasitoids.

For each test with different parasitoid strains or substrates, a series of parameters was measured: the number of emerged *D. suzukii* (n_d), the number of emerged *D. suzukii* with an encapsulated parasitoid egg (n_e) among the total number of emerged *D. suzukii* (n_d), the number of emerged parasitoids (n_p), the total number of emerged individuals ($n = n_d + n_p$), and the number of female parasitoids that attacked *D. suzukii* larvae - *i.e.* females for which at least one emerging parasitoid or fly with an encapsulated egg was counted (n_o).

Five parameters of the host-parasitoid interaction were measured for each strain and condition:

(1) The “Proportion of Ovipositing Females” (POF) corresponds to the number of female parasitoids which laid at least one egg in *D. suzukii* larvae divided by the number of females tested (N). It was calculated as $POF = n_O/N$.

(2) The “Overall Parasitism Rate” (OPR), which is the proportion of parasitized hosts, *i.e.* the proportion of *D. suzukii* that contained an encapsulated egg or produced parasitoid offspring. It was calculated as $OPR = (n_P + n_e)/n$ for each parasitoid female.

(3) The “Apparent Parasitism Rate” (APR), which is estimated as the proportion of parasitoid offspring among the total number of insects that emerged. It was calculated as $APR = n_P/n$ for each parasitoid female.

(4) The “Encapsulation Rate” (ER), which corresponds to the proportion of adult flies that emerged with an encapsulated parasitoid egg or larva among the number of parasitized individuals (emerged parasitoids and flies with a capsule). It was calculated as $ER = n_e/(n_P + n_e)$ for each parasitoid female.

(5) The “Encapsulation Level” (EL), which is estimated as the proportion of parasitoid offspring among the total number of insects that emerged. EL was calculated as $EL = n_e/n$ for each parasitoid female.

Statistical analysis

Values of OPR and APR for each species and condition were compared with generalized linear models (Tweedie family), followed by pairwise comparisons, using the Tukey’s post-hoc test. All statistical analyses were performed with the R studio software (version 3.3.3) (R CORE Team 2017).

RESULTS

Females of the European parasitoid, *L. heterotoma*, attacked all tested hosts in artificial diet, except *D. busckii*. The proportion of ovipositing females (POF) ranged from 16.67% to 80.00% (Figure 2A). While this parasitoid managed to produce offspring on *D. melanogaster* and *D. subobscura* with successful parasitism (APR) of 46.65% and 30.41%, respectively, its APR was extremely low (<1%) on *D. immigrans* and *D. suzukii* (Figure 3A). Only three females produced offspring on the former and one on the latter. From *D. suzukii* larvae, only a single parasitoid emerged and the encapsulation rate (ER) was as high as 99.43%. In total, around 30% of the emerged flies presented a capsule (EL) in their abdomen (Table 2). Surprisingly, *L. heterotoma*, was highly attracted to *D. suzukii* with 73.33% of the females laying eggs (POF) in the *D. suzukii* larvae.

The two *L. japonica* strains (Beijing and Kunming) showed similar responses to non-target hosts. The proportions of females from the Beijing strain that laid eggs (POF) on *D. melanogaster* and *D. suzukii* were high, *i.e.* 51.85% and 63.33% respectively, whereas it was 40% and 31.03% for the Kunming strain. Lower proportions were observed on *D. subobscura* (7.41% and 3.70% for the Beijing strain and the Kunming strain) and *D. immigrans* (6.67% and 7.14%) (Figure 2A). Successful parasitism (APR) for the Beijing strain on *D. melanogaster* and *D. suzukii* was high, *i.e.* 36.26% and 35.07% respectively, and 21.97% and 15% for the Kunming strain. APR was much lower on *D. subobscura* (4.32% for the Beijing strain and 2.78% for the Beijing strain) and only one progeny emerged from *D. immigrans* (APR = 0.12% in the Beijing strain) (Figure 3A). *Leptopilina japonica* females did not attack *D. busckii* in artificial diet and *D. hydei* in blended diet. *Ceratitis capitata* larvae were parasitized neither in blended diet nor in fresh blueberries. However, on *D. suzukii*, the proportions of females laying eggs on blended diet and blueberries were 62.96% and 61.90%, respectively. In both conditions, successful parasitism was high, reaching 33.10% and 24.13% respectively (Figure 2B, 3B). Encapsulation levels were low for the two *L. japonica* strains in all parasitized hosts.

Asobara japonica females successfully attacked all tested European fly species and *D. suzukii* with a proportion of ovipositing females (POF) ranging from 12% to 85.71%. Of all tested parasitoids, it showed the highest successful parasitism (APR) on *D. melanogaster* with 54.31%*, D. busckii* with 2.87%, *D. subobscura* with 38.74%, *D. immigrans* with 1.17% and *D. suzukii* with 58.08 % in artificial diet (Figure 2A & 3A). *Asobara japonica* eggs and larvae were rarely encapsulated except on *D. immigrans* (ER = 33.33%). Of all parasitoids tested, *A. japonica* was most attracted to *D. suzukii,* with 85.71% of the females laying eggs (POF) in the *D. suzukii* larvae.

Both *Ganaspis* sp. strains hardly attacked larvae of *D. busckii*, *D. hydei*, *D. immigrans* and *C. capitata,* and no progeny emerged from these hosts (Figure 2B, 3B). Due to low emergence of flies in tubes of *D. busckii* in experiment B using blended died, results could not be analysed with confidence and therefore were not integrated in the statistical analyses. Overall, the proportion of females that oviposited (POF) was higher for the

Kunming strain (ranging from 3.33% to 74%) than the Tokyo strain (ranging from 3.45% to 46.15%) (Figure 2B). No emergence of parasitoids was observed for the *Ganaspis* sp. strain from Tokyo on *D. melanogaster* and *D. subobscura,* and the few attempts on the later host (OPR < 1%) were all encapsulated (ER = 100%). In contrast, *Ganaspis* sp. from Kunming did manage to develop in high numbers on *D. melanogaster,* and two specimens emerged from *D. subobscura,* with ER 14.78% and 25.00%, respectively. Even on its natural host, *i.e. D. suzukii*, *Ganaspis* sp. (Tokyo strain) rarely successfully developed on blended diet, with APR as low as 0.16% and ER of 85.00%; however, in blueberry, APR was up to 19.60% and ER was down to 15.63%. The Kunming strain performed much better on *D. suzukii* in blended diet*,* with APR of 15.98% and ER of 18.34%. In blueberry, APR was 32.46% and ER was 15.09% (Figure 3B).

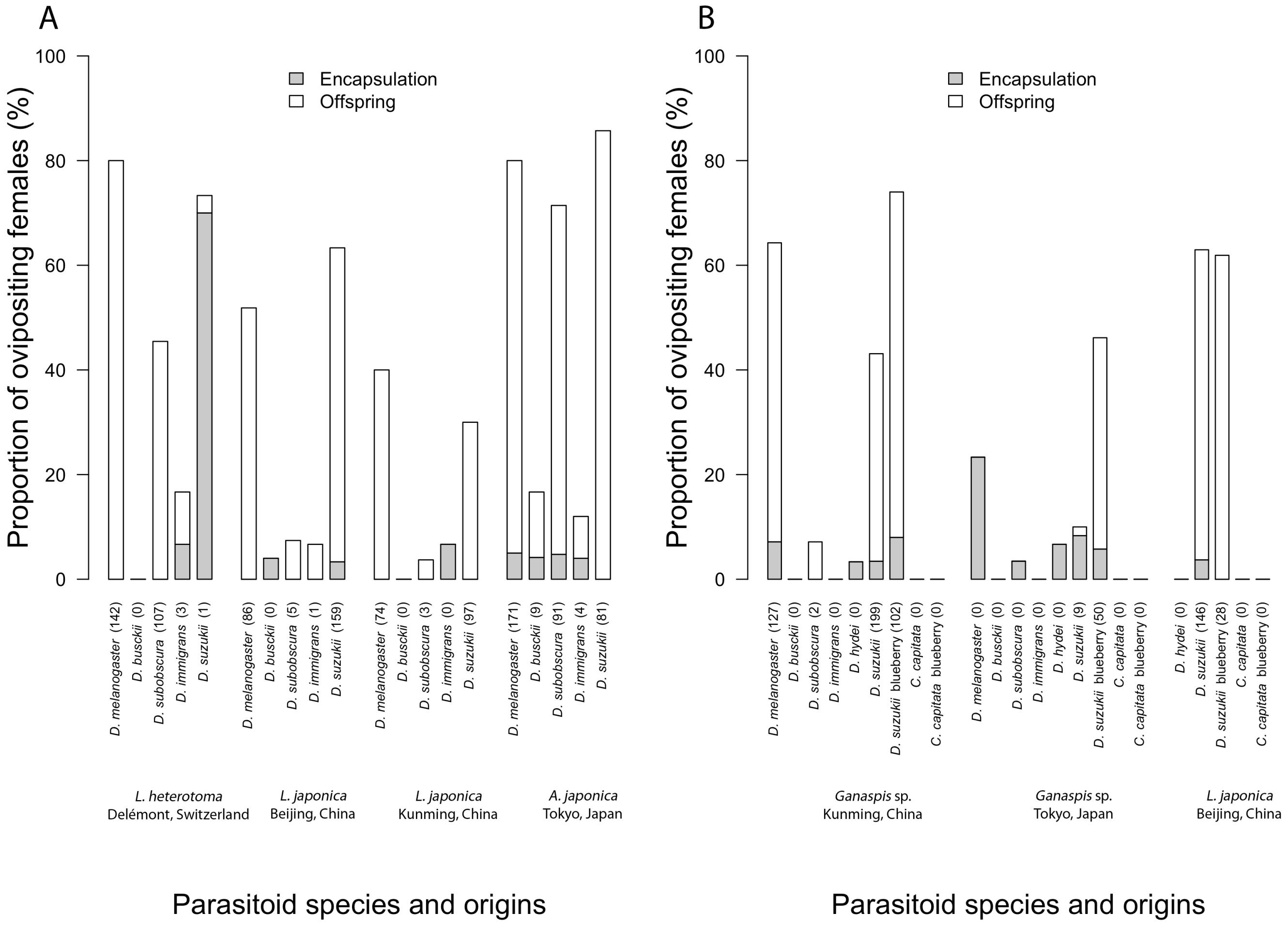


Figure 2. Proportion of ovipositing parasitoid females when exposed to larvae of various hosts in A) CAROLINA$^{©}$ diet and B) blueberries or mixed, blended diet. Oviposition was recorded as successful when either an encapsulated egg or larva was found in the abdomen of the emerged fly or when offspring was produced (in brackets, total number of parasitoid offspring).

DISCUSSION

Among the Asian parasitoids tested, the *Ganaspis* sp. strain from Tokyo showed the highest degree of host specificity. Successful development was observed exclusively in *D. suzukii* in blueberries. Kasuya et al. (2013a) obtained exactly the same results with a population from the same locality (as 'suzukii-specialised' type of *Ganaspis xanthopoda*). They carried out laboratory tests and showed that *Ganaspis* sp. parasitized *D. suzukii* larvae in fresh cherry fruits, but did not parasitize those in a Drosophila artificial diet. In addition, they did not parasitize larvae of the following species: *Drosophila lutescens*, *D. rufa*, *D. auraria*, *D. biauraria* and *D. triauraria* even when these occurred in fresh cherry fruits. However, too few replicates were made on these species to draw firm conclusions regarding their suitability as hosts. Surprisingly, in our study, the Kunming strain of the supposedly similar parasitoid species showed less specificity, as it very successfully parasitized *D. melanogaster* and *D. suzukii* in the blended diet, whereas in earlier trials, we had failed to rear the same strain in an artificial diet without fruits (Girod et al., this thesis Ch. 2). Recent work (Nomano et al., 2017) has shown that the *G. brasiliensis* complex, to which the tested *Ganaspis* sp. belongs, includes several cryptic species with totally different host ranges (see also Kasuya et al., 2013b). It is possible that additional cryptic species or biotypes varying in their specificity occur even within the *G. brasiliensis* group that parasitizes *D. suzukii*. Intraspecific variations in host preference or even host specificity are rather common in parasitoids (Höller et al., 1991; Vazquez et al., 2004; Goldson et al., 2007). It is therefore of upmost importance to improve our understanding of the taxonomy of this group in relation to its specificity before using *Ganaspis* sp. in a biological control programme, in order to choose the most suitable and specific strain. The same *Ganaspis* sp. is the main larval parasitoid of *D. suzukii* in South Korea (Daane et al., 2016), Japan (Kasuya et al., 2013a; Matsuura et al., 2017) and China (Girod et al., this thesis Ch. 1), where it probably also attacks two other fruit-inhabiting drosophilids, *D. pulchrella* and *D. subpulchrella*, which are also the two sister species of *D. suzukii*. These results suggest that the parasitoid associates the presence of the host with its related feeding niche (fresh fruits). Fresh and decaying fruits emit different cues, and it is supposed that, in the evolutionary process, *D. suzukii* has shifted its foraging behaviour towards fresh fruits (Keesey et al., 2015).

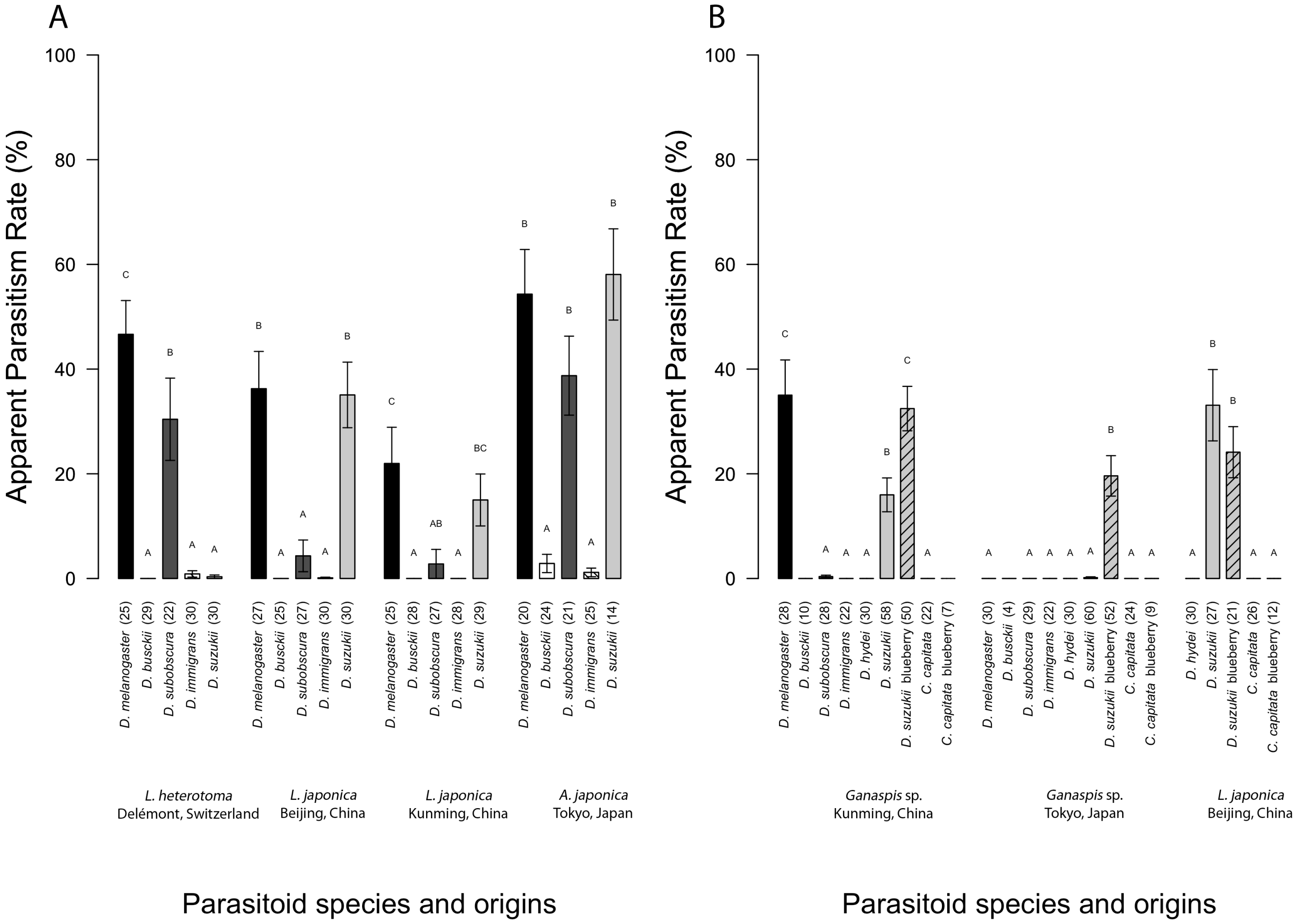


Figure 3. Mean successful parasitism (APR) (±SE) caused by parasitoids exposed to larvae of various hosts in A) CAROLINA$^{©}$ diet and B) blueberries or mixed, blended diet. APR was calculated as the proportion of parasitoid emergence among the total number of insects that emerged (in brackets: number of replicates = females included in the calculation). For each parasitoid and experiment, bars with the same letters indicate non significant differences between treatments (GLM (Tweedie family) Tukey post hoc, p ≤ 0.05). Tests on *D. busckii* in experiment B were not included in the analyses because of the low number of replicates due to the absence of fly offspring in most tubes.

Table 2. Proportion of ovipositing females, outcomes of oviposition, and measured parameters of the parasitism (success and encapsulation) per parasitoid species, origin, host and substrate.

Parasitoid		Host	Substrate	No. of females tested	Outcomes of ovipositing females (%)			Outcomes of parasitism (%)			
Species	Origin				Proportion of Ovipositing Females (POF)	All encapsulated	At least one offspring	Overall Parasitism Rate (OPR)	Apparent Parasitism Rate (APR)	Encapsulation Level (EL)	Encapsulation Rate (ER)
Leptopilina heterotoma	Delémont, Switzerland	*D. melanogaster*	Artificial diet	30	80.00	0.00	80.00	47.56	46.65	0.92	2.91
		D. busckii	Artificial diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. subobscura	Artificial diet	30	45.45	0.00	45.45	30.41	30.41	0.00	0.00
		D. immigrans	Artificial diet	30	16.67	6.67	10.00	1.86	0.87	0.99	56.00
		D. suzukii	Artificial diet	30	73.33	70.00	3.33	29.94	0.33	29.60	99.43
Leptopilina japonica	Beijing, China	*D. melanogaster*	Artificial diet	30	51.85	0.00	51.85	36.26	36.26	0.00	0.00
		D. busckii	Artificial diet	30	4.00	4.00	0.00	0.05	0.00	0.05	100.00
		D. subobscura	Artificial diet	30	7.41	0.00	7.41	4.32	4.32	0.00	0.00
		D. immigrans	Artificial diet	30	6.67	3.33	3.33	0.66	0.12	0.54	75.00
		D. suzukii	Artificial diet	30	63.33	3.33	60.00	35.15	35.07	0.08	5.26
Leptopilina japonica	Kunming, China	*D. melanogaster*	Artificial diet	30	40.00	0.00	40.00	21.97	21.97	0.00	0.00
		D. busckii	Artificial diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. subobscura	Artificial diet	30	3.70	0.00	3.70	2.78	2.78	0.00	0.00
		D. immigrans	Artificial diet	30	7.14	7.14	0.00	0.26	0.00	0.26	100.00
		D. suzukii	Artificial diet	30	31.03	0.00	31.03	15.00	15.00	0.00	0.00
Asobara japonica	Tokyo, Japan	*D. melanogaster*	Artificial diet	30	80.00	5.00	75.00	54.94	54.31	0.63	6.25
		D. busckii	Artificial diet	30	16.67	4.17	12.50	3.12	2.87	0.25	25.00
		D. subobscura	Artificial diet	30	71.43	4.76	66.67	38.85	38.74	0.11	6.67
		D. immigrans	Artificial diet	30	12.00	4.00	8.00	2.26	1.17	1.09	33.33
		D. suzukii	Artificial diet	30	85.71	0.00	85.71	59.27	58.08	1.19	2.08
Ganaspis sp.	Kunming, China	*D. melanogaster*	Blended diet	30	64.29	7.14	57.14	37.49	35.02	2.47	14.78
		D. busckii	Blended diet	60	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. subobscura	Blended diet	30	7.14	0.00	7.14	0.47	0.36	0.11	25.00
		D. immigrans	Blended diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. hydei	Blended diet	30	3.33	3.33	0.00	0.10	0.00	0.10	100.00
		D. suzukii	Blended diet	60	43.10	3.45	39.66	18.61	15.98	2.63	18.34
		D. suzukii	Blueberry	60	74.00	8.00	66.00	36.95	32.46	4.49	15.09
		C. capitata	Blended diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		C. capitata	Blueberry	30	0.00	0.00	0.00	0.00	0.00	0.00	-
Ganaspis sp.	Tokyo, Japan	*D. melanogaster*	Blended diet	30	23.33	23.33	0.00	0.96	0.00	0.96	100.00
		D. busckii	Blended diet	60	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. subobscura	Blended diet	30	3.45	3.45	0.00	0.43	0.00	0.43	100.00
		D. immigrans	Blended diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. hydei	Blended diet	30	6.67	6.67	0.00	0.61	0.00	0.61	100.00
		D. suzukii	Blended diet	60	10.00	8.33	1.67	0.48	0.16	0.32	85.00
		D. suzukii	Blueberry	60	46.15	5.77	40.38	21.20	19.60	1.60	15.63
		C. capitata	Blended diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		C. capitata	Blueberry	30	0.00	0.00	0.00	0.00	0.00	0.00	-
Leptopilina japonica	Beijing, China	*D. hydei*	Blended diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		D. suzukii	Blended diet	30	62.96	3.70	59.26	33.29	33.10	0.19	5.88
		D. suzukii	Blueberry	30	61.90	0.00	61.90	24.13	24.13	0.00	0.00
		C. capitata	Blended diet	30	0.00	0.00	0.00	0.00	0.00	0.00	-
		C. capitata	Blueberry	30	0.00	0.00	0.00	0.00	0.00	0.00	-

In contrast to what was observed for the two *Ganaspis* strains, no difference was found between the two strains of *L. japonica* in terms of their degree of specificity. Both strains happily attacked and developed in *D. melanogaster, D. subobscura* and *D. suzukii* in all substrates but not in the four other hosts, with the exception of one successful development in a *D. immigrans*. *Leptopilina japonica* frequently parasitized *D. suzukii* in Japan (Novkovic et al., 2011; Matsuura et al., 2017), South Korea (Daane et al., 2017) and China, where it probably attacks also *D. pulchrella* and *D. subpulchrella* (Girod et al., this thesis Ch. 1). In Japan, it is also found on *D. biauraria* and *D. rufa* and it has been successfully reared on *D. simulans* in the laboratory (Novkovic et al., 2011). Thus, *L. japonica* is probably not sufficiently specific to be considered for introduction into Europe.

The third Asian parasitoid species tested in this study appeared to be the most polyphagous. *Asobara japonica* attacked all the Drosophilidae proposed in the first experiment and was then excluded from the following tests. This species is already known as a polyphagous parasitoid in Asia, being recorded on more than 25 Drosophilidae species (Nomano et al., 2015). Daane et al. (2016) and Guerrieri et al. (2016) recorded it on *D. suzukii* in South Korea and Mitsui & Kimura (2010), Nomano et al. (2015) and Matsuura et al. (2017) in Japan. Other studies in Europe and North America showed its ability to parasitize *D. suzukii* by affecting haemocyte load, thereby overcoming its cellular immune system (Chabert et al., 2012; Kacsoh & Schlenke, 2012; Poyet et al., 2013). Despite its abundance, *A. japonica* is rarely obtained from *D. suzukii* in Japanese fresh fruits, possibly because of its attraction for hosts in fermenting fruits and decayed mushrooms and plant leaves (Nomano et al., 2015). However, Biondi et al. (2017) showed that, in the laboratory, *A. japonica* females were able to learn exploiting volatiles emitted by fruits infested by *D. suzukii*. Its polyphagy excludes it from the list of potential candidates from introduction.

The European *L. heterotoma* showed a strong interest in *D. suzukii* larvae; however, the vast majority of eggs and larvae were encapsulated, confirming earlier studies (Chabert et al., 2012; Kacsoh & Schlenke, 2012; Poyet et al., 2013, Knoll et al., 2017). Only one *L. heterotoma* progeny was able to overcome the immune response of *D. suzukii* and, although in some cases the rate of encapsulation avoidance may be much higher (*e.g.* 10-30% in Rossi-Stacconi et al. 2015; 2017), parasitism by *L. heterotoma* has not yet been found in the field (Kenis et al., 2016). However, it cannot be ruled out that over time *L. heterotoma* or another native larval parasitoid can become adapted to the new host, as observed in many other cases (Henter & Via, 1995; Urbaneja et al., 2000; Jones et al., 2015).

Implications for biological control and future work

So far, *Ganaspis* sp. appears to be the best candidate for introduction into Europe and other invaded regions. It is the main parasitoid of *D. suzukii* in East Asia (Daane et al., 2016; Matsuura et al., 2017; Girod et al., this thesis Ch. 1), and this study shows that it has the narrowest host range. However, our observations that specificity varies with strains implies that further studies are needed to elucidate mechanisms leading to specificity and to investigate the existence of cryptic species or biotypes showing difference in host location, searching and oviposition behaviour. More geographic strains should be tested on *D. suzukii* in artificial diet and *D. melanogaster,* and genetic studies should be carried out to compare females that succeed and fail to reproduce in diet and in *D. melanogaster*. Multiple-choice tests should also be conducted assessing the preference of *Ganaspis* sp. strains for hosts, substrates and fruits. In addition, more Drosophilidae could be tested with *Ganaspis* sp. however, this study and the preceding one (Girod et al., this thesis Ch. 1,2) also highlighted the difficulty of finding a substrate that is suitable for both *Ganaspis* sp. and the non-target species. *Ganaspis* sp. prefers ovipositing in fruits, and even a diet with blended fruits appeared not suitable for at least one of the two strains tested. In contrast, most Drosophilidae live in decaying plants and fungal material (van Alphen and Janssen 1981), and will not attack fresh or even rotting fruits and, thus, cannot be tested with *Ganaspis* sp. For example, *D. busckii* was successfully reared on a totally artificial diet (Experiment A) but much less so in the same diet mixed with fruits (Experiment B). Besides, showing that *Ganaspis* sp. -or a specific biotype of *Ganaspis* sp.- is specific to larvae in fresh fruits is a strong argument in favour of its probable specificity in regions of introductions where no native Drosophilidae live in fresh fruits. Finally, the taxonomic status of *Ganaspis* sp. should be solved as soon as possible since its taxonomic ambiguity could prevent its use as a biological control agent.

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CHAPTER 4: ORIENTATION OF ASIAN PARASITOIDS OF *DROSOPHILA SUZUKII* TOWARDS THEIR HOST'S ECOLOGICAL NICHE: AN OLFACTOMETER STUDY

In preparation

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ABSTRACT

In this study, the attraction of the Asian parasitoid wasps *Ganaspis* sp. (two strains) and *Leptopilina japonica* (Hymenoptera, Figitidae) towards the invasive Asian spotted wing Drosophila, *D. suzukii,* has been assessed. To better understand the factors impacting host-specificity, olfactometer bioassays have been performed with these two potential biological control agents and compared to those done with the European *Leptopilina heterotoma*. Various odour stimuli corresponding to the quality of the host's substrate (fresh or rotten blueberries or artificial diet) and the host's presence (infested fruit and artificial diet) were investigated as factors influencing attraction in a 4-arm olfactometer.

Clear differences have been observed among the three species. Furthermore, important variations in host location have been noticed between the two *Ganaspis* sp. strains. A Japanese strain of *Ganaspis* sp. showed a higher preference for *D. suzukii* than a Chinese strain, confirming previous host-range studies conducted in the laboratory. The Japanese strain of *Ganaspis* sp. was the only parasitoid to show a strong preference for *D. suzukii* in fresh fruits over artificial diet and decaying fruits. However, all parasitoids were uniformly more attracted to fruits infested with *D. suzukii* than to non-attacked fruits. *Leptopilina japonica* did not show clear preferences between *D. suzukii* in fresh fruit and artificial diet. This suggests that *D. suzukii*'s parasitoids orient themselves towards a combination of volatile compounds emitted by the host plant and cues of their targeted host. The results of this study complements previous olfactometer investigations performed on other *D. suzukii*'s parasitoids like *Asobara japonica*. Taken together, these results will help in the selection of a biological control agent that will be sufficiently specific to be considered for introduction into Europe.

KEY WORDS

Spotted wing Drosophila, host finding behaviour, *Vaccinium corymbosum*, 4-arm olfactometer

INTRODUCTION

Over 6'000 introductions of beneficial natural enemies have been made against insect pests in classical biological control programmes over the last 100 years and the majority of these biological control agents were parasitoids. A good understanding of how these parasitoids locate their target host is a critical step for the evaluation of their specificity and suitability for release in a new environment. *Drosophila suzukii* Matsumura (Diptera, Drosophilidae) is a fruit fly native to Asia that has recently invaded Europe and the Americas (Fraimout et al., 2017). In the invaded areas the pest is largely free of effective natural enemies and competitors, which may explain its abundance on a wide range of wild and cultivated fruits and the important economic impact on fruit production (Lee et al., 2015; Kenis et al., 2016; Mazzi et al., 2017). This has prompted the development of biological control programmes to study exotic parasitoids under quarantine conditions.

Since 2008, when *D. suzukii* was first recorded in Italy, Spain and the United States of America, knowledge of the biology of the pest has been accumulating, in particular regarding its behaviour, physiology and ecology. Its ecological niche differs from all other European and North American Drosophilidae (Keesey et al., 2015). A serrated ovipositor allows the females to lay eggs in ripe fruits as opposed to rotten ones. Studies with fruit extracts show that *D. suzukii* is attracted to ripe fruits and, in particular, to the common fruit volatile isoamyl acetate (Abraham et al., 2015; Revadi et al., 2015). Larval consumption of a ripening fruit leads to accelerated degradation of the fruit, possibly resulting in volatiles that could serve as attractants for natural enemies of the fly. The use of adapted hymenopteran parasitoids as biological control agents in invaded regions could be a solution to limit the impact of the fly on fruits. Using larval parasitoids as biological agents against *Drosophila* spp. may be effective, as they are known to have a considerable impact on the dynamics and regulation of populations of other *Drosophila* spp. (Carton et al., 1986; Poyet et al., 2013; Asplen et al., 2015).

Olfactometer tests have been used for several decades to study the attraction of parasitoids towards their host or host habitat, and to determine the sources of volatile cues that they use in host location. This has also been the case for parasitoids of *Drosophila* spp., in particularly *Leptopilina* spp. and *Asobara* spp. (Vet 1982; Van Alphen et al., 1983; Vet et al., 1983; Vet & Van Opzeeland 1984; Papaj & Vet 1990; De Jong & Kaiser 1991; Cortesero et al., 1993; Couty et al., 1999; Biondi et al., 2017). These studies show that the parasitoids first use volatile cues to locate the host habitat and then forage for specific cues emitted by the host. Different species of parasitoids use different close-range foraging strategies (e.g. ovipositor testing, antennal searching and vibrotaxis) and are also likely to be differentially stimulated by chemical cues (Vet & Alphen 1985).

In this paper, we report the first olfactometer tests conducted with two different genera of parasitoids (*Leptopilina* and *Ganaspis)* from various regions of origin of *D. suzukii*. It is envisioned that a better understanding of the mechanisms of host searching of these potential biological control agents will help to evaluate their specificity, which is key to the success of a classical biological control strategy that avoids any non-target effects.

METHODS

All insect rearing and experiments were carried out in a laboratory and incubators at similar conditions: 22 ± 2°C, 60 ± 10 % RH and a photoperiod of 16:8 h (L:D).

Drosophila suzukii rearing

The original *Drosophila suzukii* colony was obtained from wild fruits collected by S. Fischer (Agroscope Changins, Switzerland) from various sites in Switzerland in 2015. Approximately five hundred adult flies were reared per gauze cage (47.5x47.5x47.5 cm BugDorm-4©) and fed with sugar water provided on dental cotton rolls. Wet cellulose paper was also provided as water source. Two tubes (ø 50x100 mm) containing 10g of commercial artificial fly diet (Formula 4-24 medium©, Carolina Biological SupplyCo., Burlington, NC) with 40mL of 1.43 $g.L^{-1}$ of methyl-4-hydroxylbenzoate and a small amount of yeast to enhance egg laying were placed in each cage as a food source and oviposition substrate. Tubes with *D. suzukii* eggs were changed twice a week and placed in incubators until emergence of adults, which were then randomly distributed among the rearing cages.

Parasitoid species rearing

One European and three Asian strains of parasitoids were used in this study. The European species *Leptopilina heterotoma* Thompson (Hymenoptera, Figitidae) was obtained from a bait trap placed outdoors in Delémont, Switzerland, in the summer of 2015. It was maintained on *D. melanogaster* reared in tubes placed in incubators, by offering the wasps first instar larvae of *D. melanogaster* (for 3-4 days) reared on the fly diet. A drop of honey was added to each tube as a food source. The tubes were checked every second day to remove newly emerged parasitoid adults and maintain the colony.

The following parasitoids were collected in Asia in June 2015 and reared in the quarantine facilities of CABI in Delémont, Switzerland:

(1) *Ganaspis* sp. (Hymenoptera, Figitidae) from *Prunus cerasoides* fruits infested with *D. suzukii*, Kunming, Yunnan, China.
(2) *Ganaspis sp.* from *Prunus serrulata* fruits infested with *D. suzukii*, Tokyo, Japan.
(3) *Leptopilina japonica* Novkovic & Kimura (Hymenoptera, Figitidae) from *Prunus* sp. fruits infested with *D. suzukii*, Beijing, China.

The Figitidae species were identified by Dr. Matthew Buffington (Systematic Entomology Laboratory, USDA ARS, Washington, USA). The *Ganaspis* species were identified as *Ganaspis brasiliensis* Ihering, but recent studies show that the taxonomy of this species should be revised (Buffington & Forshage 2016; Nomano et al., 2017). Therefore, in this study, our samples will be referred as *Ganaspis* sp. The Asian Figitidae strains were kept in rearing boxes (ø 90x50 mm), each containing approximately 50-60 individuals. An Eppendorf tube with a wet cellulose paper was placed in each rearing boxes as a water source. Boxes were closed with a foam plug on which a drop of honey was placed as a food source. Fresh blueberries (*Vaccinium corymbosum*) were placed in each *D. suzukii* rearing cage for 48 hours and then the berries were distributed among the parasitoid rearing boxes for another 48 hours to allow female parasitoids to oviposit in the fly larvae. After parasitoid exposure, fruits were removed and kept in rearing tubes (ø 50x100 mm) with a filter paper at the bottom to absorb leaking fruit juice. The rearing tubes were checked daily for newly emerged adults, which were transferred to new rearing boxes.

Olfactometer tests

A total of three experiments were conducted to test different odour sources to evaluate if the parasitoids were attracted to host habitat volatiles or the host cues, or by a combination of both. The three experiments tested: i) fresh blueberry (same as the ones used for the parasitoid rearing) *vs.* rotten blueberry (10 days old and attacked by fungi); ii) fresh blueberry vs. fresh blueberry attacked by *D. suzukii* and iii) fresh blueberry attacked by *D. suzukii vs. D. suzukii* in artificial diet (same as the one used for the fly rearing) (Table 1.).

Table 1. Experimental design of the three olfactometer bioassays.

Figure #	Wasp species	Origin	Odour sources			
			Arm 1	Arm 2	Arm 3	Arm 4
2	*Leptopilina heterotoma* *Leptopilina japonica* *Ganaspis* sp. *Ganaspis* sp.	Delémont, Switzerland Beijing, China Kunming, China Tokyo, Japan	Fresh blueberry	Empty	Rotten blueberry	Empty
3	*Leptopilina heterotoma* *Leptopilina japonica* *Ganaspis* sp. *Ganaspis* sp.	Delémont, Switzerland Beijing, China Kunming, China Tokyo, Japan	Fresh blueberry	Empty	*D. suzukii* attacked blueberry	Empty
4	*Leptopilina heterotoma* *Leptopilina japonica* *Ganaspis* sp. *Ganaspis* sp.	Delémont, Switzerland Beijing, China Kunming, China Tokyo, Japan	*D. suzukii* attacked blueberry	Empty	*D. suzukii* attacked artificial diet	Empty

The odour sources were tested to evaluate their attraction to parasitoids in a 4-arm olfactometer (two tested odours and two empty arms) (described by D’Alessandro and Turlings, 2005). The olfactometer was placed under a structure that supported 6 neon lights (35W each) and was covered by a white curtain to avoid influence of the laboratory light. The glass olfactometer used was made of a central choosing chamber (ø 6x5 cm), connected to four arms (ø 1.5x5 cm), each connected to a glass elbow (5 cm). The top of the elbow was connected to an insect-trapping bulb (50 ml) and to the bottom part of the bottle containing the odour source (Figure 1.). Cleaned and humidified air entered the odour source bottle at 0.3 l/min (adjusted by a manifold with four flowmeters; Analytical Research System, Gainesville, FL, USA) via Teflon tubing and carried the odour compound through to the central choosing chamber compartment. Wasps were released in groups of ten 2-5 day old, mated and naive females, in the choosing chamber and left for 30 minutes. At the end of the exposure period, the number of females in each arm or in the choosing chamber was counted (Figure 1). Wasps counted in the choosing chamber were annotated as “no choice”. Wasps counted in the remaining two empty arms, which did not have any odour sources, were annotated as “empty arm”. Each group of ten females was tested four times in a row (repetitions) and four replicates for each four parasitoid species and strain were tested (Table. 1.). In order to avoid any biases in the attraction throughout the four repetitions within a tested group of 10 female parasitoids, the whole device was turned by a quarter-turn between repetitions.

Statistical analysis

The attraction between the two tested odours was tested with a generalized linear mixed-effect model (GLMER), using a Poisson family followed by a Tukey post-hoc test (p-value<0.05) for multiple comparisons. The replicates were treated as random factor with nested repetitions. Each model was fitted by maximum quasilikelihood estimation. All models were checked with the 'overdisp' test to estimate the residual deviation of the freedom factor and to take into account possible effects of over-dispersion caused by arm position or wasps affecting each other's responses (Davison & Ricard 2011). Statistical analyses were performed with the R software (version 3.3.3) (R CORE Team, 2017) with the package « Lme 4 » (Bates 2010).

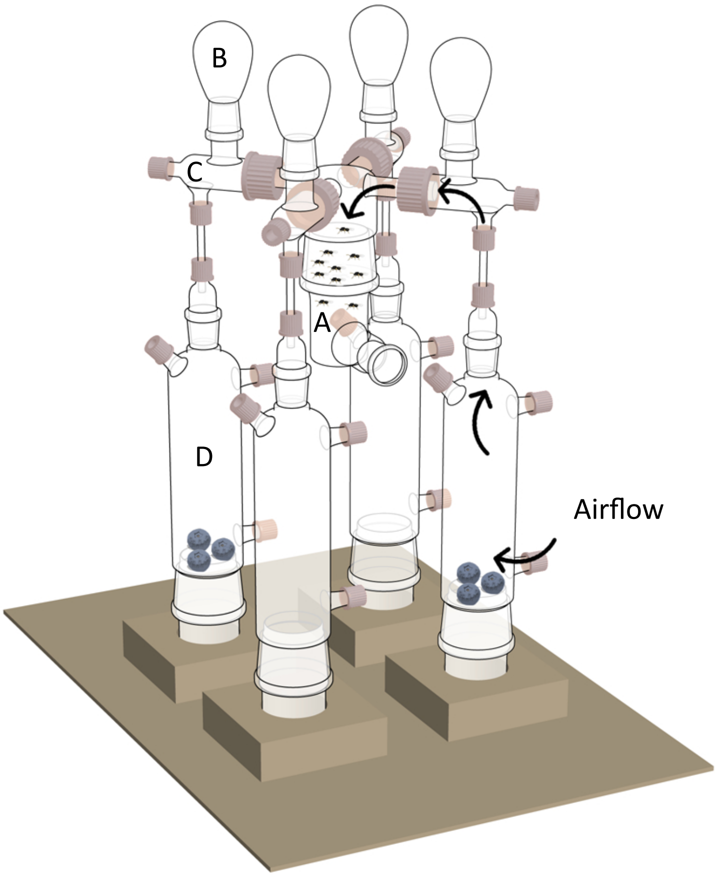

Figure 1. Drawing of a 4-arm olfactometer as used in experiments. A. central choosing chamber (releasing point of the 10 female parasitoids), B. insect collecting bulb, C. elbow and D. odour source bottle. Modified from Thomas Degen (www. thomas-degen.ch)

RESULTS

Fresh blueberry vs. rotten blueberry

The average proportion of females choosing an arm in the olfactometer was low. The lowest proportion was 20% and the highest 41%, with 10% to 25% choosing one of the two odour arms (Figure 2A). For *Ganaspis* sp. Tokyo, none of the females orientated towards the rotten blueberry odour, showing instead a clear preference for the fresh blueberry (Figure 2B). For the other species, the GLMER model showed that neither the interaction "species-odour source" or the "odour source" were significant (χ^2=2.183, p-value=0.336 and χ^2=2.866, p-value=0.091, respectively). Significant variation in the response was led by the "species" factor (χ^2= 11.274, p-value<0,01) (Figure 2B).

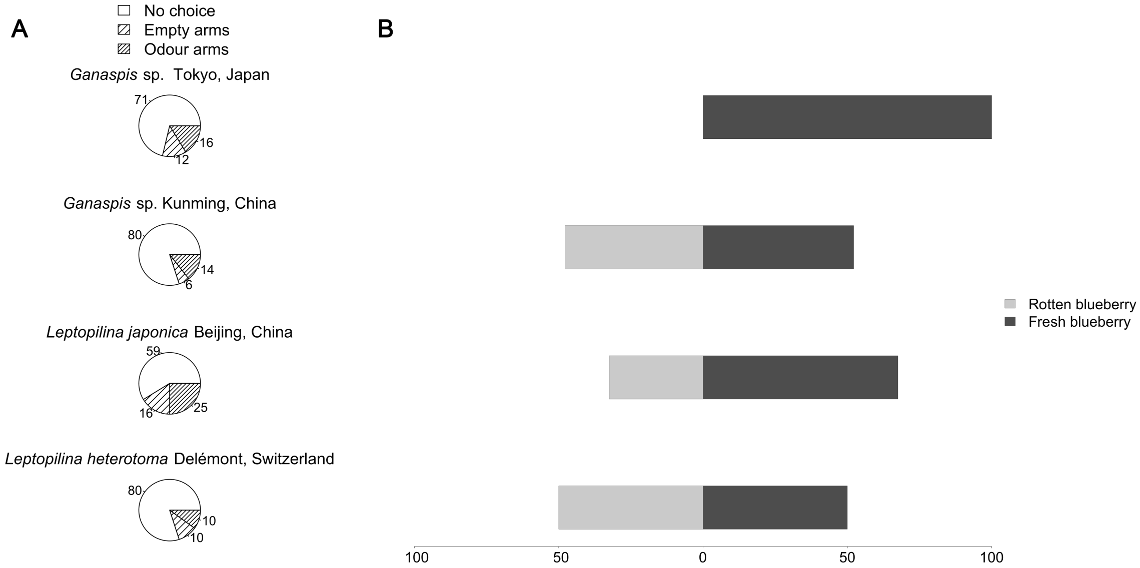

Figure 2. Experiment "fresh blueberry vs. rotten blueberry, A. Proportion of overall responses per parasitoid species in the four-arm olfactometer, No choice: females stayed in the choosing chamber, Empty arms: females chose one of the two no odour source arm, Odour arms: females chose one of the two odour source arm. B. Proportion of responses per parasitoid species (sorted as in A) to odour source volatiles. For each parasitoid species and odour source the same letters indicate no significant differences between treatments (GLMER (Poisson family) Tukey post hoc, p ≤ 0.05). #Data for Ganaspis sp Tokyo were not included in the GLMER model because GLMER in the R software is not able to handle a 0% in one condition, therefore we had to remove that species from the GLMER model of our dataset and test it with a standard Chi-square test, that showed a significant difference between the two arms (χ2= 14.769, p-value<0,001, n=26 parasitoids).

Fresh blueberry vs. fresh blueberry attacked by D. suzukii

The percentage of responding females varied between 26% and 57%. The proportions of female parasitoids that chose an odour arm varied between 18% and 31% (Figure 3A). The model tested showed that the interaction “species-odour source” and the species were not significant (χ^2=0.656, p-value=0.884 and χ^2=7.296, p-value=0.063, respectively) in contrast to the substrate (χ^2=57.419, p-value<0.001). All species preferentially oriented towards the blueberries attacked by *D. suzukii* (Figure 3B).

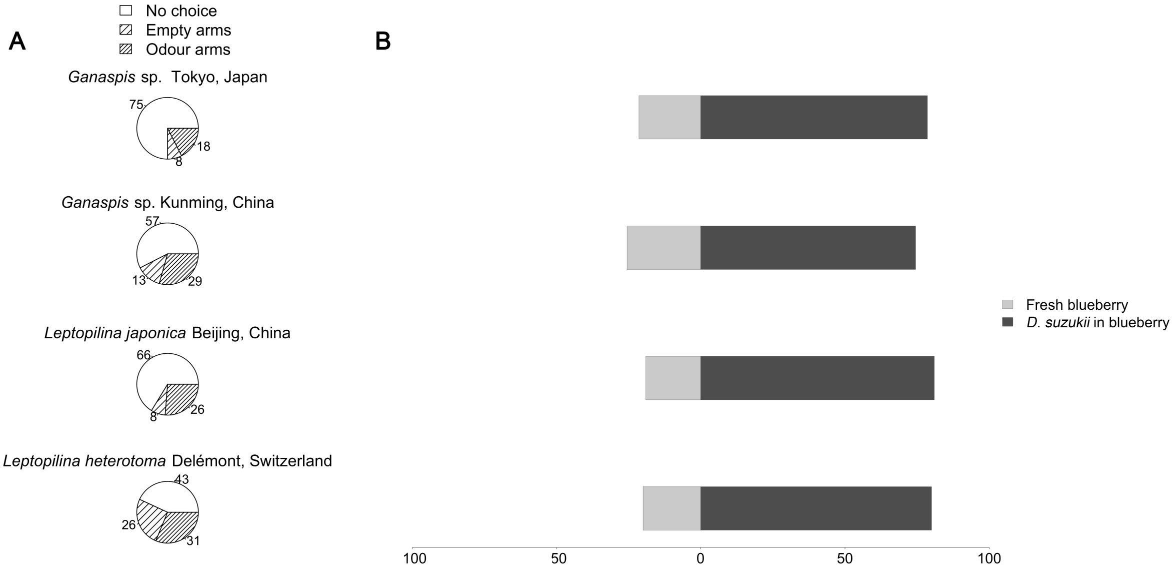

Figure 3. Experiment “fresh blueberry vs. *D. suzukii* attacked fresh blueberry” A. Proportion of overall responses per parasitoid species in the four-arm olfactometer, No choice: females stayed in the choosing chamber, Empty arms: females chose one of the two no odour source arm, Odour arms: females chose one of the two odour source arm. B. Proportion of responses per parasitoid species (sorted as in A) to odour source volatiles. For each parasitoid species and odour source the same letters indicate non significant differences between treatments (GLMER (Poisson family) Tukey post hoc, p ≤ 0.05).

Blueberry attacked by D. suzukii vs. D. suzukii in artificial diet

For this last experiment, the percentage of responding females varied between 20% and 53. The orientation towards odour arms varied between 14% and 27% (Figure 4A).

In the tested model the interaction “species-odour source” was significant (χ^2=37.185, p-value<0.001). *Ganaspis* sp. Kunming and *L. japonica* did not distinguish between the two sources. *Leptopilina heterotoma* was most attracted by artificial diet infested with *D. suzukii* and, conversely, *Ganaspis* sp. Tokyo was found significantly more in the arm with the odour of blueberries attacked by *D. suzukii* (Figure 4B).

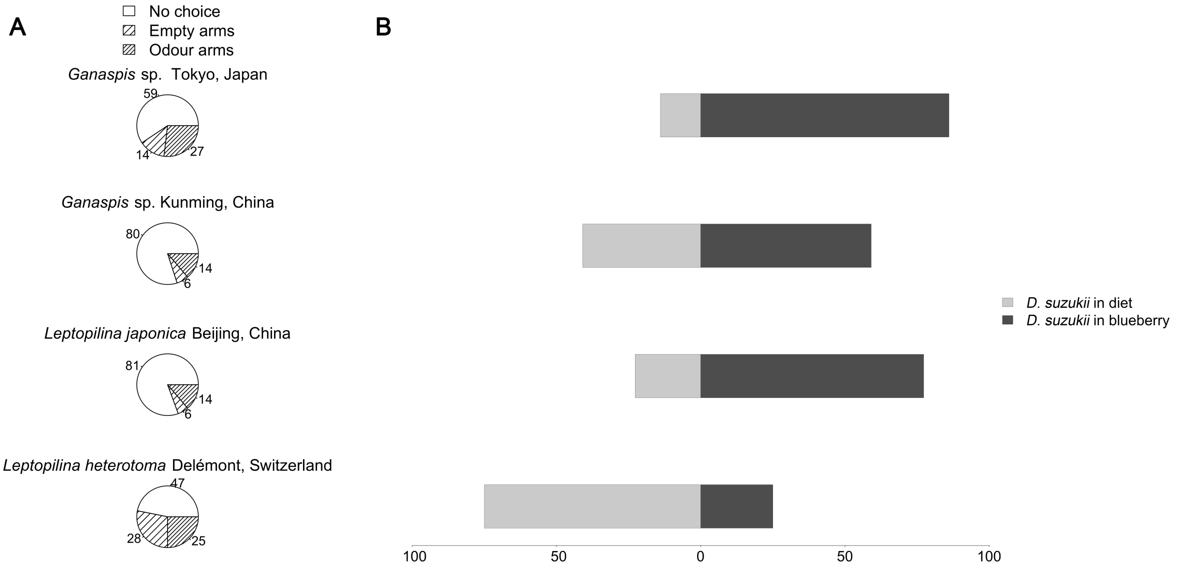

Figure 4. Experiment "*D. suzukii* attacked fresh blueberry vs. *D. suzukii* in artificial diet" See Figure 3 for further explanations.

DISCUSSION

Studying how potential biological control agents are attracted toward their natural hosts in their habitats is increasingly becoming a part of the assessment of classical biological control agents. In the case of *D. suzukii*, one such study was conducted with the Asian parasitoid *Asobara japonica* (Hymenoptera, Braconidae) by Biondi et al., (2017). Their results showed some similarities with the results presented here. Naïve females of *A. japonica* did not show a preference towards the various tested substrates. However, the enforced adult experience with the rearing host medium modified the olfactory preference patterns toward non-natal host fruits. These findings provide evidence of associative learning during the adult stage of *A. japonica*, and demonstrate its plasticity in exploiting the volatiles from various fruits infested by *D. suzukii*. But the preference pattern was modified by enforced adult experience on host fruits. This parasitoid cannot be safely used in biological control programs because of its polyphagy but this study highlighted the plasticity of this parasitoid in exploiting volatiles from various fruits infested by *D. suzukii*.

Our study suggests that *Ganaspis* sp. Tokyo is a promising candidate for future field release to control *D. suzukii* populations, as it shows no interest in foraging in the ecological niche of native European flies – *i.e.* rotten and decaying fruits - and was also not attracted to *D. suzukii* in artificial diet. Yet, when exposed to blueberry odours, it clearly orientated towards fruits infested by *D. suzukii*. Taken all together, these results, as well as results of host range tests (Girod et al., this thesis Ch. 1,2,3) show that *Ganaspis* sp. Tokyo is the most specific parasitoid in terms of host finding and host preference behaviour. But cues emitted by the host habitats had an effect on the searching behaviour of all tested parasitoids. In general, host-parasitoid interactions are influenced by the exploitation of chemical information (Lof et al., 2013). Searching for a host is always a challenge for the parasitoid, especially if the host is buried inside a substrate, for instance a fruit, as it is the case of *D. suzukii* larvae. Long and short distance signals used by the parasitoid are different and complementary. Long distance signals usually come from the host habitat (e.g. host food, host plant volatiles), whereas short-range signals are more directly linked to the host itself (Vet & Dicke, 1992; Geervliet et al., 1994).

Drosophila suzukii's ecological niche varies considerably over the seasons (Diepenbrock et al., 2016). Therefore, further evaluations should test the performance of the *Ganaspis* sp. from Tokyo on a larger variety of host plants, as its host is known from over 150 types of fruits (Lee et al., 2015; Kenis et al., 2016). Similar to what Biondi et al. (2017) found for *A. japonica*, we observed that, when given a choice between the odour of fresh blueberry or *D. suzukii*-infested fresh blueberry, all the tested species were attracted to the latter. In our research we did not determine the specific origin of the attractive volatiles (e.g. pheromones, feces, damaged fruit). The fruit is likely an important source, but Wertheim et al. (2003) showed that, for *L. heterotoma,* aggregation pheromone of the host (in that study *D. melanogaster)* also played a role. The role of such pheromones in the location of *D. suzukii* by parasitoids remains to be investigated, because when given the choice between uninfested fruits or fruits infested with *D. suzukii*, all tested parasitoid species oriented toward attacked blueberries.

In two previous studies (Girod et al., this thesis Ch. 2,3), it proved difficult to find a substrate that allowed *Ganaspis* sp. to develop in non-target species (i.e. *Drosophila* species other than *D. suzukii*). *Ganaspis* sp. clearly prefers ripe fruits, and even a diet with blended fruits appeared unsuitable for at least one of the two tested strains. This suggests that *Ganaspis* sp. requires specific cues to identify the presence of a potential host. This apparent specificity in its host selection behaviour is a positive result for a possible release of *Ganaspis* sp. against *D. suzukii*, as it implies that it will focus on the target pest in fresh fruits and is unlikely to attack other *Drosophila* species living in rotten organic material.

An intriguing observation was that *Ganaspis* sp. from Kunming, which is assumed to belong to the same species as the strain from Tokyo, responded differently. It did not make a distinction between the different host habitat odours. This suggests the presence of cryptic species or biotypes with different levels of specificity. The taxonomy of the *Ganaspis* genus is currently being revised, which is important because ambiguities in the taxonomic status of a biological control agent may prevent its introduction.

It should be noted that we only tested "naïve" wasps, which means that, as adults, they had never encountered any host and were also unfamiliar with the host habitats. An oviposition experience in a particular fruit is likely to increase their responsiveness to the odour that is associated with that experience. Associative learning by parasitoids has been studied in great detail, including with parasitoids of *Drosophila* spp. (Turlings et al., 1993; Vet et al., 1995). It is thought that the flexibility to host-related cues, especially in generalist parasitoids, allows the wasps to optimize their foraging efforts when it is unpredictable in which habitats they may find the most hosts. Learning may also occur during the larval stage and at adult emergence (Turlings et al. 1993). In our experiment, the *L. heterotoma* females had emerged from *D. melanogaster* from artificial diet, in contrast to the Asian parasitoids that had emerged from *D. suzukii* in blueberries. This difference may at least partly explain the differences in preference for a host habitat. However, it would be difficult to compare parasitoid species emerged from a standard habitat since *L. heterotoma* does not develop in *D. suzukii* in fruits whereas artificial diets are unsuitable for *Ganaspis* sp.

Associative learning might facilitate the adaptation of native European parasitoids to eventually alter their behaviour and also find *D. suzukii* in fruits that they would normally not visit. The phenomenon of associative learning could potentially be exploited by "training" wasps with the appropriate odours before they are released.

In conclusion, the olfactometer bioassays confirmed the host range tests (Girod et al., this thesis Ch. 3) and provided another argument suggesting that *Ganaspis* sp. Tokyo is a more promising biological control agent than the European *Leptopilina heterotoma* or the other Asian parasitoids tested in this study. However, further olfactometer experiments should include the behaviour and performance of naïve, as well as experienced females – *i.e.*

that previously parasitized hosts or emerged from various substrates - as associative learning may occur during the development of the parasitoid and the adult stage, which could impact the insects' responses. Finally, it may be useful to identify and test the various compounds emitted by the different substrates in order to determine what specific volatiles are involved in the attraction of the parasitoids. Once it is established which parasitoids have the greatest potential against *D. suzukii* and which foraging cues are most reliable in finding the pest, this information can be used in the selection process of a highly specific parasitoid that can be released in a biological control programme.

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GENERAL DISCUSSION AND CONCLUSION

Worldwide, ecological concerns regarding the impact of invasive pests are increasing. In the context of the recent invasion of *Drosophila suzukii* in Europe*,* an invasive Asian fly, the studies conducted during this thesis evaluated the potential of implementing a classical biological control programme to reduce populations of this fruit insect pest in Europe and other invaded regions. Lots have been done but still, more work is needed to validate these first observations. This would provide an attractive alternative to the current control methods that rely on chemical insecticides or expensive and labour-intensive cultural practices (Haye et al., 2016). However, deliberately introducing an exotic biological agent also involves risks for non-target species, and these are assessed and discussed in this thesis.

Following the invasion of *D. suzukii*, field evaluations were performed to investigate the host-plant range of *D. suzukii* (Lee et al., 2015; Briem et al., 2016; Kenis et al., 2016) and the potential occurrence and impact of local biological control agents in Europe and North America (Chabert et al., 2012; Kacsoh and Schlenke, 2012; Poyet et al., 2013; Knoll et al., 2017; Rossi Stacconi et al., 2015; 2017). It was discovered that *D. suzukii* has an extensive range of crops and wild fruits (> 150 plants) that could serve as alternate hosts to re-infest crops generation after generation. Consequently, sustainable control methods should be developed at landscape level rather than at crop level. It was also shown that generalist pupal parasitoids are able to successfully develop on *D. suzukii* and occasionally attack the invasive fly in the field. In contrast, the more specific larval parasitoids of European and North American *Drosophila* spp. are poorly adapted to this new host. In light of these results, it was decided that surveys for larval parasitoids specialised in *D. suzukii* should be conducted in the native range of *D. suzukii*. These surveys, presented in Chapter 1, were carried out from 2015 to 2017 in 12 provinces in China and 5 Prefectures in Japan. With those led by Daane et al., (2016) in South Korea and some localised studies in Japan (*e.g.* Mitsui and Kimura, 2010; Matsuura et al., 2017), they are the only surveys for parasitoids conducted in the native range of *D. suzukii* to date. This study shows that a complex of at least eight parasitoids attacks *D. suzukii* in East Asia. The Figitid wasp *Ganaspis* sp. was present in every sample from which parasitoids emerged. This species also reached the highest parasitism rates in most regions. In these surveys, only fresh and undamaged fruits were collected and it appeared that *D. pulchrella* and *D. subpulchrella* commonly occurred with *D. suzukii* in our samples. *Ganaspis* sp. also emerged from these fly species, which suggests that it might be specific to *Drosophila* spp. in fresh fruits rather than host specific. This alone would not prevent its introduction in Europe or North America, as native Drosophilidae in these continents are not able to attack fresh and undamaged fruits.

Surveys were conducted in different climatic regions to better understand climatic requirements of Asian parasitoids since climate matching (between the native and introduced areas) is a major factor involved in the success of an introduction (Hoelmer & Kirk, 2005; Haye et al., 2013). Although the highest parasitism rates of *Ganaspis* sp. were observed in sub-tropical climates in Yunnan (China) and Japan, the parasitoid was also found in rather high numbers at temperate sites, including a high elevation site in Central Japan. This is a positive hint that this parasitoid should be able to establish and develop in temperate regions in Europe and North America. However, the ability of *Ganaspis* sp. to survive in different regions in Europe and other invaded ranges should be assessed through climatic modelling (Robertson et al., 2008).

Parasitism rates, as measured in this study, are often incorrect and poor indicators of the impact of parasitoids and the role of parasitism in the population dynamics and natural control of insect pests (Van Driesche, 1983). For example, in our samples, mortality could have affected hosts and parasitoids very differently, and many *D. suzukii* were probably collected at a very early stage, leading to an underestimation of parasitism. At the beginning of this thesis, it was planned to precisely assess the differences in natural enemies and mortality factors between Asia and Europe by carrying out comparative life table studies in China and Switzerland (Bellows et al., 1992). These were made in collaboration with the Yunnan Agricultural University in Yunnan, China, however, they provided disappointing results. In particular, no parasitism has been measured in China despite the fact that collections of naturally infested fruits in the neighbourhood provided parasitoids. Thus, these life tables are not presented in this thesis because they did not provide useful information to further understanding the invasion success of *D. suzukii*, nor for the development of a biological control programme. Nevertheless, the work provided information and recommendations for life table constructions for *D. suzukii* and related pests, and will therefore likely be published for this purpose at a later stage.

Following the Asian surveys, potential candidate parasitoids were selected for further assessments. In particular their specificity, following biological control procedures (Hajek et al., 2016; Heimpel and Mills 2017). Since little was known on the parasitoid biology, baseline data for the assessments of the host specificity of this Asian parasitoid were needed. In Chapter 2, data on the pre-oviposition time of the parasitoids and their development time in the laboratory were collected, as well as the performance of the different species of parasitoids collected in Asia and Europe on two diets (fresh fruits and artificial diet) infested with *D. suzukii*. The main result was that *Ganaspis* sp., *i.e.* the main parasitoid of *D. suzukii* in Asia, could not be successfully reared on *D. suzukii* in artificial diet, accepting only larvae in fruits. This highlights the complexity of finding rearing conditions that satisfy both the requirements of parasitoids and non-target hosts in specificity tests. Our results clearly showed that the type of substrate used heavily influences parasitism success. With this new information and general literature on host range testing (van Lenteren et al., 2006; Mason et al., 2013), experimental protocols were designed to assess the specificity of this Asian parasitoid. In particular, a new diet was developed based on blended fresh fruits providing promising results with *Ganaspis* sp. in preliminary tests.

The host range testing described in Chapter 3 showed that two of the three Asian parasitoids, *Asobara japonica* and *Leptopilina japonica*, were not sufficiently specific to be considered for introduction in Europe. Again, *Ganaspis* sp. provided the most intriguing results, suggesting important variations in specificity between the two tested strains. Both strains were able to develop on *D. suzukii* in fresh fruit, but the Chinese one was also able to develop in blended diet on *D. suzukii* and *D. melanogaster*, whereas the Japanese one laid very few eggs and was barely able to develop in *D. suzukii* in blended diet. These results corroborate Nomano et al. (2017) observations that the *G. brasiliensis* complex, to which the two tested *Ganaspis* sp. strains belong, includes several cryptic species with variation in host range and specificity (Kasuya et al., 2013).

In the host range testing, the goal was to test a large number of potential non-target hosts. Our study started with a group of six Drosophilidae (Subgenus: Sophophora, Dorsilopha and Drosophila) and one Tephritidae. As advised in the literature, hosts were chosen according to their phylogenetic relatedness and sympatry to the target – in this case, *D. suzukii* (Bigler et al., 2006; Kuhlmann et al., 2006). The two sister species of *D. suzukii*, *i.e. D. subpulchrella* and *D. pulchrella*, which also live in fresh fruits in Asia (Takamori et al., 2006), are presently being tested as hosts for *Ganaspis* sp. in China. Ideally, more non-target species should be tested in Europe since there are ca. 50 Drosophila species occurring in Europe (Fauna Europaea 2017). However, the issue of finding a suitable substrate will be even more complex with new host species. Most Drosophilidae live in organic matters, decaying plants, and fungal material (van Alphen and Janssen 1981). These species will not attack fresh or even rotting fruits and new diets satisfying the specific strain of *Ganaspis* sp. and the different drosophilids would have to be found. However, one can also consider that the host habitat of most Drosophilidae is a physical barrier that would prevent any non-target impact by this fresh fruit specialist parasitoid.

In order to better understand the mechanisms leading to specificity, olfactometer bioassays have been performed and presented in Chapter 4, with *Ganaspis* sp., *L. japonica* and the European *L. heterotoma*. Many olfactometer tests have been conducted in the past with European parasitoids of *D. melanogaster* (Van Alphen et al., 1983; Vet & Van Opzeeland 1984; Papaj & Vet 1990; De Jong & Kaiser 1991; Cortesero et al., 1993; Couty et al., 1999), but along with the recent publication of Biondi et al (2017) on *Asobara japonica*, this is the only time that the behaviour of *D. suzukii*'s parasitoids has been reported with this approach. These assays confirmed the previous specificity tests. The Japanese strain of *Ganaspis* sp. was the only parasitoid to show a strong preference for *D. suzukii* in fresh fruits over artificial diet, and for fresh fruits as compared to decaying fruits. However, all parasitoids were equally more attracted to fruits infested with *D. suzukii* than to non-attacked fruits. Clearly identifying which combination of the host and fruit volatiles attracts the parasitoid will help in the selection of a highly specific parasitoid. In addition, once the parasitoid is established, the attractive volatiles could possibly be use to attract the parasitoid in the field (Turlings et al., 1990; Turlings & Wäckers 2004).

To conclude, this thesis highlighted that the larval parasitoid *Ganaspis* sp. shows potential as biological control agent against *D. suzukii* in Europe. The agent may not be able to fully control the pest by itself but combining a classical biological control strategy to the numerous control techniques that have been developed in the past five years (*e.g.* mass trapping, insect proof nets, chemical compounds and more natural molecules), could drastically reduce attacks in the field to an acceptable level. The advantage of classical biological control is that the biological control agent would be able to parasitize the host and lower populations both in crop and non-crop areas, reducing re-infestations of the agricultural fields over the seasons and the different crops. However, before a petition for release can be proposed, the following research should be carried out.

i) Further studies are needed to investigate the existence of cryptic species or biotypes showing difference in host location and searching and oviposition behaviour. In Chapters 3 and 4, only two strains were tested but eight are presently available at CABI and these will be tested on *D. suzukii* and *D. melanogaster* in diet and fresh fruits. At the same time, genetic studies are presently carried out to compare females that succeed or fail to reproduce in diet and in *D. melanogaster*. In addition cross-mating experiments should be conducted to identify potential genetic incompatibilities. It is also important to mention that the ambiguity around the taxonomic status of *Ganaspis* sp. could prevent its use as a biological control agent because, in general, full names are required for biological control petitions and its present full name, *G. brasiliensis*, represents a complex of species, some of which are generalists.

ii) Efforts should be made to elucidate mechanisms leading to specificity. Olfactometers studies described in Chapter 4 should be continued with associated studies to precisely identify volatiles involved in host location. In addition, multiple choice tests should also be carried out with various hosts and substrates, including with the non-specific strains of *Ganaspis* and *L. japonica*, to assess their specificity in choice conditions. In particular, different fruits should be tested since it is important to verify that the *Ganaspis* sp. strain rejecting all diets in laboratory trials will attack all types of fruits in the field.

iii) More Drosophilidae should be considered for host range testing, but the difficulty of testing the specific strain of *Ganaspis* sp. in diet is an issue as in Europe, since no native drosophilids lives in fresh fruits. Another non-native fruit-inhabiting drosophilid, *Zaprionus indianus*, was recently found in Europe (Kremmer et al., 2017) and it would be interesting to test *Ganaspis* sp. on this species. In this particular case, the ability of *Ganaspis* sp. to parasitize *Z. indianus* could be considered as an advantage.

iv) The climatic requirements of *Ganaspis* sp. should be further studied and geographic variations in these requirements should be assessed to select populations that are adapted to the different European climates where *D. suzukii* occurs.

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APPENDIX: CURRENT SWD IPM TACTICS AND THEIR PRACTICAL IMPLEMENTATION IN FRUIT CROPS ACROSS DIFFERENT REGIONS AROUND THE WORLD

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ABSTRACT

After its arrival in 2008, the Spotted Wing Drosophila (SWD), *Drosophila suzukii*, has emerged as a harmful invasive insect pest in North America and Europe. This highly polyphagous pest is a major threat to many economically important fruit crops, but is also known to develop on a wide variety of natural host plants. In Asia, Europe and North America different control measures are applied against SWD, such as chemical, biological, and cultural control. Current controls of SWD rely primarily on the application of insecticides, but cultural management tactics such as sanitation and the use of nets provide a good alternative in some crops. Biological control measures, such as conservation of existing natural enemies in invaded areas, introduction of specialized larval parasitoids from Asia for classical biological control, and the use of indigenous parasitoids for augmentative control are currently being investigated and may become an important management tool in the near future for an area wide control of SWD.

KEY WORDS

Drosophila suzukii, biological control, cultural control, chemical control

AUTHOR CONTRIBUTION STATEMENT

TH, PG, AGSC, XGW, KMD, KAH, CB, JZ and ND wrote the manuscript. All authors edited the manuscript and approved the final version.

KEY MESSAGE

- *Drosophila suzukii*, is a new threat for fruit crop production systems worldwide and new IPM strategies are urgently needed
- We summarized the knowledge and practices currently used for integrated pest management (IPM) of SWD around the world, including chemical, cultural, and biological control
- Effective control of SWD in invaded areas will require an area wide control approach

INTRODUCTION

The Asian Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae) is a new threat for fruit crop production systems worldwide. For decades this insect posed no threat to crop production (Kanzawa 1939), but in 2008 this fly arrived simultaneously in Europe (Italy, Spain) (Calabria et al. 2012; Cini et al. 2014) and North America (Lee et al. 2011), then more recently in South America (Depra et al. 2014). SWD was not recognized as a serious pest before 2010, but recently increasing damage was noticed in Chinese cherry orchards due to prolonged low temperature periods in spring, resulting in delayed maturation of fruits, and an increase in the production area for late maturing cherry varieties (Yang et al. 2011; Wang et al. 2012; Dai 2013; Guo et al. 2014; Liu et al. 2014; Zhang and Gao 2014; Zhang et al. 2015). The colonization of invaded areas in North America and Europe has been largely facilitated by human activities, particularly the movement of infested fruits, climatic conditions similar to the fly's native range (Wiman et al. 2014) and the absence of natural factors regulating SWD populations effectively.

In contrast to the vast majority of *Drosophila* species, which feed on rotting fruits, *D. suzukii* lay its eggs inside ripening fruits, puncturing the fruit's skin with its unique saw-like ovipositor (Atallah et al. 2014). Damage is mainly caused by larval feeding, resulting in the degradation of fruits. In addition, the puncturing of the fruit skin provides a gateway for secondary infections with bacteria and fungi pathogens or additional pests (de Camargo and Phaff 1957; Molina et al. 1974; Louise et al. 1996; Walsh et al. 2011). This highly polyphagous pest is known to develop in many economically important fruit crops, *e.g.* blackberries, blueberries, cherries, peaches, raspberries, strawberries, grapes, bayberries and kiwis (Kanzawa 1939; Bolda et al. 2010; Grassi et al. 2011; Lee at al. 2011; Seljak, 2011; Walsh et al. 2011; Bellamy et al. 2013; Liu et al. 2015). In addition, more than 50 wild host plants have been determined in Europe and the US, providing the pest a large reservoir of alternative hosts throughout the seasons (Baroffio 2015; Lee et al. 2015; Poyet et al. 2015; Kenis et al. 2016). In the Trento district of Italy annul losses in small fruit production were assumed €3.3m per year (De Ros et al. 2013), and in the USA gross revenues for raspberry and strawberry farmers were assumed to decrease by 37% and 20%, respectively (Goodhue et al. 2011). Estimated annual costs to the US fruit production are more than US$ 500m (Bolda et al. 2010).

While new control measures are still being developed in Europe and North America, here we summarize knowledge and practices currently used for integrated pest management (IPM) of SWD around the world, including chemical, cultural, and biological control.

CHEMICAL CONTROL

Evaluations of the efficacy of insecticides for the control of *D. suzukii* have been undertaken in almost all of the major fruit growing regions where it is now distributed. Trials have included laboratory based Petri dish experiments along with field evaluations where experimental plots are treated and sampled for control of the fly (Beers et al. 2011; Bruck et al. 2011; Cuthbertson et al. 2014a). Due to there being a zero tolerance within both fresh and processed berry markets against infested fruit, and with many fruit growing areas experiencing high population numbers, has lead growers across the international fruit growing sector to take a very proactive approach in trying to control *D. suzukii* in order to protect their individual industries (Van Timmeren and Isaacs 2013). Currently, there is limited published information regarding the levels or extent of insecticide resistance in *D. suzukii* populations, but with it having an almost global invasion of fruit producing areas and the only current viable method of control being insecticide-dependent strategies no doubt resistance will become a major problem in the foreseeable future. Much information is known in regards to how *D. melanogaster* has developed resistance to insecticides and the associated problems it has caused (Perry et al. 2008; Remnant et al. 2014; Wan et al. 2014).

The current effective insecticides suggested for managing SWD are principally conventional broad-spectrum products, which are not always compatible with IPM programmes, such as advanced generation pyrethroids and organophosphates (Beers et al. 2011; Haviland and Beers 2012; Van Timmeren and Isaacs 2013). Neonicotinoids have been used to a limited extent in control strategies because they are perceived to be less effective (Bruck et al. 2011), and, if they are used in foliar sprays, are anticipated to have broad-spectrum effects and negative impacts to beneficial arthropods (James 2003; He et al. 2012). The exception regarding broad-spectrum impacts for insecticides effective against SWD are spinosyns (spinosad and spinetoram; Beers et al. 2011; Bruck et al. 2011; Haviland and Beers 2012; Zhang et al. 2015), which, for resistance management, need to be limited in the number of applications made per year on a given crop. As the current effective pesticide options for managing SWD are limited, it is therefore very important to optimise use of the insecticides that are available. Within the United States a total of 18 insecticides are listed for use on blueberry, caneberry, strawberry, grape and stone fruit (Fruit Advisor 2015). These are a mixture of organic and conventional pesticides. Bruck et al. (2011) also screened a wide range of insecticides for efficacy against SWD. In their study several insecticides including pyrethroids (bifenthrin, beta-cyfluthrin, permethrin, zeta-cypermethrin), organophosphates (malathion, diazinon) and spinosyns (spinosad, spinetoram) provided excellent control of adult *D. suzukii* following direct application. Spinetoram and dimethoate have also been screened for efficacy in Italian cherry orchards (Profaizer et al. 2015). Insecticide screening trials by Cuthbertson et al. (2014a) also confirmed the high efficacy of spinosad and chlorantraniliprole against SWD. Several 'coded' products (potentially awaiting EU/UK registration) have also proved highly efficient against various life stages of SWD following both post and pre-dipping blueberry treatments (Cuthbertson et al. 2014a; AGS Cuthbertson unpublished data). Gargani et al. (2013) also undertook berry dipping trials with various organic products; only one product, "Deffort" (Sophora flavescens Aiton, 8%), a fertilizer liquid based on complexing micronutrients enriched with plant extracts with strong anti-stress action displayed any significant direct toxicity.

Cowles et al. (2015) demonstrated that the addition of sucrose as a phagostimulant improved the activity of several insecticides to target SWD adults and as a result increased protection of fruit from infestation. Their study showed an enhancement in activity of several reduced risk insecticides, such as spinosyns, cyantraniliprole and acetamiprid, which provided equivalent or superior protection of blueberry and strawberry fruits when compared with application of conventional insecticides. Potential impacts from using sucrose with insecticides on beneficial non-targets species and pollinators have yet to be determined. Walse et al. (2012) also demonstrated the potential use of postharvest methyl bromide fumigation for treatment of berries prior to shipment. However, since the early 1990s, this fumigant has been known to break down under the influence of strong UV rays, and thus release bromide atoms which deplete the ozone layer (WMO 1995; Dabrowski 2002) and with the phase-out of methyl bromide in industrialised countries in January 2005 (Norman 2005), alternatives to this fumigant are still eagerly sought (Cuthbertson et al. 2013).

CULTURAL CONTROL

Depending on the type of crop, different cultural control methods are currently applied in Asia, Europe and North America. Sanitation is one of the most important cultural control methods to combat SWD around the world (Köppler 2014; Walsh et al. 2011; Tanigoshi et al. 2011; Dreves et al. 2011; Liburd and Iglesias 2013, Shi 2015). During the ripening season, sanitary measures such as clearing ground covering vegetation, removal of dropped and over ripe fruits have been suggested (Lee et al. 2011; Shi 2015). Larvae inside removed fruits have been effectively killed by solarisation, the use of heat to kill insects. Infested fruits can either be placed on the ground in a sunny location and covered with clear plastic sheeting or placed in sun exposed plastic bags for at least two days. Burying infested fruit has been shown to be less effective. Whereas solarisation is effective for berry crops, it is difficult to apply to stone fruits. As viability of SWD eggs is lower under dry, warm conditions (Burrack et al. 2014), cool humid microhabitats should be avoided by pruning to open up the canopy and using wider tree spacing to increase airflow to the canopy and reduce shading. In addition, the use of mulches reducing standing water can further contribute to the reduction of humidity in fruit orchards (Hoashi-Erhardt and Bixby-Brosi 2014). In China, soil tillage in winter is recommended to destroy overwintering habitats of the fly, whereas in spring surrounding ground of cherry trees is supposed to be covered with black plastic fabric, preventing overwintered adults moving up to the tree canopy (Shi and Wang 2015).

Short harvest intervals may further help to reduce the number of infested fruits at harvest. When raspberries that had just matured were collected every two days in Swiss orchards, only little infestation with SWD was noticed, whereas longer harvest intervals lead to higher infestations due to the larger proportion of over mature fruits (C. Baroffio, unpublished data).

Besides sanitary measures, the use of nets covering fruit bearing trees or shrubs is an effective complementary method to physically exclude SWD. Particularly in cherry crops, nets are one of the most important control measures around the world. The recommended mesh size varies between 0.5 x 0.8, 1x1, and 1x 1.6 mm (Grassi and Pallaoro 2012; Gamper 2015; Cormier et al. 2015). Nets with a mesh size of 0.98 mm and 1 x 1.6 mm provided good control of SWD in blueberries in Asia and Europe, respectively (Kawase 2007; Grassi and Pallaoro 2012; Ioriatti et al. 2015). Nets need to be installed before the fruits begin to ripen to prevent any SWD being trapped inside the nets (Caprile et al. 2013). Lure traps placed inside the nets may serve as additional control. Alternatively, bagging cherry clusters in the early fruit stage with white semitransparent paper bags has been recommended in China (Shi and Wang 2015). Furthermore, it is important to control the climate under the nets to avoid infestation with fungi due to increased humidity.

A wide variety of differently shaped and coloured traps containing attractants, usually a mix of apple cider vinegar, red wine and sugar, have been developed primarily for monitoring SWD populations (Landolt et al. 2012; Wei et al. 2012; Lee et al. 2013; Cha et al. 2014; Grassi and Maistri, 2013; Baroffio et al. 2014; Harris et al. 2014, De Los Santos Ramos et al. 2014; Shi 2015; Burrack et al. 2015). However, some traps can also be used for mass trapping SWD. In Switzerland, commercially available ready-to-use traps ("Riga trap") consist of transparent cups filled with 100 ml of attractant (wine, sugar, wine and fruit vinegar), and covered with a lid with five holes (3mm) (for details see: www.becherfalle.ch). Trials conducted in 2014 showed a significant reduction of SWD populations in raspberries (cv Polka) over a period of three weeks, when traps were placed in shady places at fruit height every 2 meters in the perimeter of the crop (density: 200 traps/ha; costs: 155€/ha). Traps were changed every 3 weeks (Baroffio et al. 2015). In Yunnan, China, sweet lure traps made of 600 ml plastic bottles containing variable mixtures of brown sugar, vinegar, wine and water were used to attract and kill flies in pomegranate orchards. Two opposite holes (2cm diameter) were drilled near the bottleneck and the bottom of the trap to allow the flies to enter. Traps were placed in trees every 15 to 20m at a height of 1.5 m and monitored daily for a period of 10 days in early September. The best catches (daily average of 19.7 flies) were achieved using a mixture of brown sugar (50 g) vinegar (50ml), wine (150ml) and water (300ml) (Wu et al. 2012). In the Beijing area, slightly modified traps exposed in natural mountain habitats resulted in catches of 310.3 flies per day during the peak flight period (Zhang et al. unpublished data). Traps used in the Beijing area contained the same lure (250ml), but were made of 1l plastic containers with 30 small holes (0.5 cm diameter). In two independent studies, it was recommended that the optimal height for hanging traps would be 1.5 to 2.0 m or 0.8 to 1.4 m above ground, respectively (Grassi et al. 2009; Guo et al. 2014). Besides lure traps, yellow-green light traps (wavelength 560nm) or frequency trembler grid lamps (1 per 2000 m^2) have been recommended to trap SWD in Chinese bayberry orchards, but no data on the efficiency of these traps have been reported (Liang et al. 2015).

BIOLOGICAL CONTROL

Biological control can be a cost-effective and environmentally safe approach for the management of arthropod pests. Current control programmes for SWD rely primarily on pesticides, and these programmes may be challenged because abundant wild fruits can serve as a reservoir for this highly polyphagous and mobile pest to reinvade managed crops (Lee et al*.* 2015). Natural enemies may also proliferate in both crop and unmanaged habitats, potentially playing a unique role in lessening the fly populations in crop and uncultivated habitats. A previous review by Asplen et al. (2015) discussed the prospects of biological control of SWD using parasitoids, especially introduced Asian parasitoids. Here, we will focus on new developments and the potential implementation of biological control for SWD by means of parasitoids and other biological control agents (predators, nematodes and pathogens).

Parasitoids play an important role in population regulation of *Drosophila* species (Carton et al*.* 1986; Fleury et al. 2004). The majority of studies have focused on some common parasitoids such as *Leptopilina heterotoma, L. boulardi* (Hymenoptera: Figitidae), and *Asobara tabida* (Hymenoptera: Braconidae) that attack *Drosophila* larvae living within fermenting substrates, such as rotting fruits (Prévost 2009). Recently, a number of studies have been undertaken in both the USA and Europe to investigate parasitoid species associated with SWD in its invaded regions (Gabarra et al. 2015; Rossi Stacconi et al*.* 2015; Miller et al. 2015; Wang et al. 2016a) and the suitability of SWD as a host for common Drosophila parasitoids (Chabert et al. 2012; Kacsoh and Schlenke 2012). Two generalist pupal parasitoids, *Trichopria drosophilae* (Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) were found worldwide. Both pupal parasitoids are effective under laboratory conditions; female *P. vindemmiae* and *T. drosophilae* produced a lifetime total of 68.4 and 63.8 offspring on SWD and have an intrinsic rate of increase of 0.14 and 0.12 at 23 °C, respectively (Rossi Stacconi et al*.* 2015; Wang et al.2016a). Augmentative release in greenhouses also showed that *T. drosophilae* was able to successfully parasitize SWD pupae in strawberry (Trottin et al. 2014). In China, *P. vindemmiae* was mass produced on pupae of *Musca domestica* (Diptera: Muscidae) and released for inundative control of *D.*

suzukii in bayberry orchards in the Zhejiang province (Zhou et al. 2014), but with little success. However, larval parasitoids (*e.g.*, *L. heterotoma*, *L. boulardi* and *A. tabida*) that are commonly associated with other *Drosophila* species appear to be largely unable to develop from SWD larvae, presumably due to their strong host immune response against parasitoids (Kacsoh and Schlenke 2012; Poyet et al*.* 2013). A recent field survey reported for the first time the presence of trapped *D. suzukii* adults bearing melanized and encapsulated resident parasitoids in North America (Wang et al. 2016b). In contrast, other larval parasitoids, including *Asobara japonica*, *Ganaspis xanthopoda* (Diapriidae), *Leptopilina japonica* and an undescribed species (*Asobara* sp. TK1) utilize SWD as a host in Japan (Mitsui et al. 2007; Ideo et al*.* 2008; Novkovic et al*.* 2012; Nomano et al. 2015; Kimura and Novkovic 2015). Furthermore, an *Asobara* sp. TK1 and a strain of *G. xanthopoda* were shown to exhibit a high level of specificity for SWD (Nomano et al. 2015; Kasuya et al*.* 2013). Recent explorations in South Korea collected 6 different larval parasitoid species (*A. brevicauda*, *A. japonica*, *A. leveri*, *L. japonica*, *L. formosana*, and *G. brasiliensis*) from SWD from infested wild fruits, and parasitism of SWD by these larval parasitoids was as high as 17% (Daane et al. 2016). *Asobara japonica* was the most widely distributed and abundantly collected species in Japan (Mitsui et al. 2007; Ideo et al*.* 2008; Murata et al. 2009; Mitsui and Kimura 2010) and South Korea (Daane et al. 2016). This larval parasitoid has shown a high rate of successful development from SWD (Kacsoh and Schlenke 2012) and has a high fecundity (117.4 progeny/female) and intrinsic rate of increase (0.22) when parasitizing SWD (A Biondi, XG Wang, and KM Daane unpublished data). It also showed an innate attraction to volatile cues from different infested host fruits (A Biondi et al. unpublished data). *Ganaspis brasiliensis* and *L. japonica* collected from South Korea also readily developed from SWD when tested in the laboratory (Daane et al. 2016). *Leptopilina japonica* has been observed to parasitize larvae of at least three other *Drosophila* species in Japan (Mitsui and Kimura 2010; Novkovic et al. 2012; Kasuya et al. 2013), but virulence also varied with geographically isolated populations (Kimura and Novkovic 2015). If levels of host specificity are considered sufficient, introduction of larval SWD parasitoids native to Asia may add a potentially unique role in regulating SWD populations (Daane et al. 2016).

Predatory bugs, such as species of *Orius* (Anthocoridae), have been observed feeding on SWD in raspberries in the USA (*e.g.*, Walsh et al*.* 2011) and were present in infested fruit samples in Spain (Arnó et al. 2012). Several commercially available *Orius* species have been tested under laboratory conditions. *Orius majusculus* and *O. laevigatus* showed some predatory activity towards SWD larvae but gave no significant suppression of the SWD populations (Cuthbertson et al. 2014b; Malagnini et al. 2014). For example, *O. insidiosus* reduced SWD survival in simple laboratory arenas but not on potted blueberries or bagged blueberry outdoors (Woltz et al. 2015). Other predators such as the beetle *Atheta coriaria* (Staphylinidae) and the bug *Anthocoris nemoralis* (Anthocoridae) also fed on SWD life stages to some extent in laboratory tests (Cuthbertson et al. 2014b; Renkema et al. 2015; Woltz et al. 2015). *Atheta coriaria* did not reduce SWD survival (Woltz et al. 2015), whereas *A. nemoralis* caused 45% mortality of SWD after five days (Cuthbertson et al. 2014b). *Orius laevigatus* (Malagnini et al. 2014) and the predatory mite, *Hypoaspis miles* (Mesostigmata: Laelapidae) (Cuthbertson et al. 2014b), showed no predatory activity on SWD. Although *A. nemoralis* showed potential for suppressing SWD populations within confined arenas, it is unclear if its predatory efficiency would decrease in the open field situation due to field conditions, such as increased difficulty in catching adult SWD (Cuthbertson et al. 2014b). Overall, none of these predators seemed able to control SWD individually, but they would likely contribute to SWD population suppression additively if they were in the SWD ecosystem (Cuthbertson et al. 2014b).

A few commercially available entomopathogenic nematodes have been screened for control of SWD, including: *Steinernema carpocapsae*, *S. feltiae*, *S. kraussei*, and *Heterorhabditis bacteriophora*. All showed low infection rates and were not able to affect SWD survival following infested berry dipping experiments (Cuthbertson et al. 2014a; Woltz et al. 2015). However, upon investigating the same nematodes as potential soil drenches against SWD larvae/pupae, *S. kraussei* was shown to cause approximately 55% pupae mortality, while *H. bacteriophora* provided approximately 95% larval mortality (AGS Cuthbertson, unpublished data). One unidentified nematode species from a South Korean collection of SWD was found to readily attack SWD in laboratory tests (A Biondi, XG Wang, and K Daane, unpublished data). Based on these observations, further screening for more effective nematodes may be warranted and ways to enhance nematode survival in soil under crop plants.

Entomopathogenic fungi have been used successfully to control arthropod pests (Ekesi et al. 2005; Faria and Wraight 2007). The efficacy of several commercially available formulations of entomopathogenic fungi in the genera *Metarhizium*, *Beauveria,* *Lecanicillium, Isaria,* and *Paecilomyces* have been screened against SWD under laboratory conditions (Cuthbertson et al. 2014a; Naranjo-Lázaro et al. 2014; Woltz et al. 2015). Both *L. muscarium* as Mycotal (0.1% solution) and *B. bassiana* as Naturalis (0.3% solution) appear to have no marked impact on fly emergence when dipping SWD-infested fruit into field-rate concentrations of the agents, but direct spray of *B. bassiana* caused 44% adult mortality after seven days (Cuthbertson et al. 2014a). Mycotrol-O, a *B. bassiana*-based bioinsecticide, showed 80% adult mortality ten days after application in strawberries in laboratory cages (Jentsch 2014). Naturalis and another bioinsecticide, Botanigard, which is also based on living spores of *B. bassiana,* showed some suppression of adult SWD (Gargani et al. 2014). The susceptibility of SWD to different strains of *I. fumosorosea* (Pf21, Pf17, Pf15) and of *M. anisopliae* (Ma59) was evaluated through *in vitro* bioassays and the resulting percentages of fly mortality by Pf21, Pf17, Ma59, Pf15 were 85, 60, 57, and 12%, respectively (Naranjo-Lázaro et al. 2014). Woltz et al. (2015) tested *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* as direct sprays on adult SWD and found that only *M. anisopliae* significantly decreased SWD survival. These different results could be due to different strains screened, but the studies suggest that some entomopathogenic fungal strains could be used as biological control agents of SWD. However, there are still obstacles to overcome in the delivery method and lack of persistence of these agents in the field. Entomopathogenic fungi infect their target organisms through the cuticle and one major constraint is bringing the pathogen into contact with the adult fly in the field (Ekesi et al. 2005). Another potential problem is that fungi such as *M. anisopliae* have low residual activity and no effect on SWD fecundity; they did not kill adult flies quickly enough and as a result the next generation of flies began emerging before adult flies that had been treated began to die (Woltz et al. 2015). However, fungi can be easily integrated into existing control strategies, as they may have less effect on natural enemies than on the target pests as compared with conventional insecticides. For example, *M. anisopliae* has been used for the control of tephritid fruit flies in Africa and posed no adverse effect on these flies' parasitoids (Ekesi et al. 2005).

CONCLUSIONS

It is likely that we will see a continuous spread of SWD in the coming years due to increased global trade of fruit crops. The control of SWD in fruit orchards will be particularly challenging because a high number of wild host plants in nearby wood lands, unmanaged private gardens or abandoned orchards provide an enormous refugium for SWD (Lee et al. 2015; Kenis et al. 2016), requiring an area wide control approach.

Current controls of SWD rely primarily on the application of a range of insecticides such as spinosyns, organophosphates, pyrethroids and neonicotinoids. The rapid turnover of SWD generations requires many chemical interventions at the fruit ripening stage (Bruck et al. 2011; Van Timmeren and Isaacs 2013). Whereas insecticides can be effective, they increase the risk of residues in fruits, promote insect resistance, and negatively affect pollinators and natural enemies (Stark and Banks 2003; Desneux et al. 2007). The majority of the screening of chemicals against SWD that has been undertaken has been done so under laboratory conditions. However, laboratory based data generally become more variable when transferred to the field. Thus, product efficacy testing must be tested on a broader scale before strong conclusions or recommendations to the fruit growing industry can be made. The addition of new insecticides for the control of SWD could further be very disruptive to natural enemies already being used in IPM strategies that were developed over a period of time for management of other pests (Roubos et al. 2014). Future management must also emphasize selective use of risk-reduced pesticides (Biondi et al. 2012) to reduce the negative impact on natural enemies.

At small scale productions, chemical control should be coupled with cultural management tactics (Thistlewood et al. 2012). To date sanitation is the most important method to combat SWD. Although costly and time consuming, other control measures can only be effective when the crop is “clean” and SWD reservoirs are reduced as much as possible. The use of nets provides a good alternative to chemical control, especially in cherries, blackberries, raspberries, and blueberries. In berry crops, mass trapping combined with sanitation can be an efficient strategy, however the choice of the attractant is critical, and the control may only work if the traps are at least as attractive as the fruits or used prior to start of fruit ripening.

Generalist natural enemies (including indigenous parasitoids and predators) are all likely to contribute to suppression of SWD populations to some degree within the ecosystems occupied by SWD, although their direct impacts on SWD have not yet been demonstrated in the field. Whereas there is a current lack of effective parasitoids attacking SWD larvae in the invaded regions, these species may exist in Asia and current programs are collecting and screening novel agents. This highlights the importance of conserving all natural enemies, introducing specialized larval parasitoids, and continuing investigations into the possible use of augmentative biological control with indigenous or commercially available biological control agents. Research is currently under way to develop these biological control programmes for SWD in the USA and Europe.

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Curriculum Vitae

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Profile

Research on classical biological control of insect pests. Investigation and sampling of invasive insects in their native and introduced areas. Leading host range laboratory and field experiments with exotic entomophagous insects to assess their potential non-target impact. Maintain large rearing colony of parasitoids and their associated hosts.

Disciplines

Biological control of agricultural insect pests, invasive species, host-parasitoid interactions, ecology, evolution, host specificity and rearing of various insects

Education

December, 6th 2017	Ph.D. defence, University of Neuchâtel Supervisors: Prof. Ted Turlings (UniNE), Dr. Alex Aebi (UniNE), Dr. Marc Kenis (CABI) and Dr. Tim Haye (CABI) Invited members of the committee: Antonio Biondi (University of Catania, Italy), Betty Benrey (UniNE) and Patrick Kehrli (Agroscope)
Dec 2014-Present	University of Neuchâtel & Centre for Agriculture and Bioscience International (CABI) Swiss Center, Delémont, Switzerland Ph.D.'s degree of the Interuniversity Doctoral Program in Organismal Biology
Sept 2012 - June 2014	University of Nice Sophia-Antipolis (UNice), France Master's degree in Biology and Environment: Plant protection, agriculture and environment
Sept 2009 - June 2012	University of Nice Sophia-Antipolis (UNice), France Bachelor's degree in Organisms and Environment Biology
June 2009	Lycée Horticole Vert d'Azur Antibes, France – Scientific baccalaureate

Professional experience

Dec 2014-Present	Ph.D. student, University of Neuchâtel & Centre for Agriculture and Bioscience International (CABI) Switzerland Center, Delémont, Switzerland Supervisors: Prof. Ted Turlings (UniNE), Dr. Alex Aebi (UniNE), Dr. Marc Kenis (CABI) and Dr. Tim Haye (CABI) The main topic of the PhD project I conducted was to study mortality factors and natural enemies (parasitoids) of *Drosophila suzukii* (Diptera: Drosophilidae) in Europe and Asia. The focus of the project was to design and lead host-range assessment and evaluate non-target impacts of Asian parasitoids on fruit flies.

Jan 2014 - June 2014	MSc. student (2nd year), National Institute of Agricultural Research (INRA), Sophia-Antipolis, France Supervisors: Dr. Nicolas Ris (INRA) and Dr. Jean-Luc Gatti (INRA) Experimental study of the potential impact of the introduction of an exotic parasitoid (*Asobara spp.*) against the invasive fruit fly *Drosophila suzukii*.
Feb 2013 - June 2013	MSc. student (1st year), National Institute of Agricultural Research (INRA), Sophia-Antipolis, France Supervisors: Dr. Elodie Vercken (INRA), Dr. Ludovic Mailleret (INRA) and Dr. Thibaut Morel-Journel (INRA) Strategies for introducing organisms in a spatially structured environment: role of propagule pressure and localization of introductions.
Jul 2011 & Jul 2012	Research assistant, National Institute of Agricultural Research (INRA), Sophia-Antipolis, France Supervisors: Prof. Marylène Poirié (INRA) and Dr. Domoinique Colinet (INRA) Evolution and specificity of the host - parasitoid interaction between *Drosophila* and *Leptopilina* sp.. Variability of venom components in immunosuppressive parasitoid wasps.
Supervision experience	Master's thesis: 2, Bachelor's thesis: 3, Research assistants: 4, BSc short internship: 8
Teaching	University of Neuchâtel, Switzerland First year Bachelor's Invertebrate (Mollusca and Arthropoda) laboratory, 24 hours teaching total. Second year Master's Bio-ethnology, An overview of *Drosophila suzukii* an invasive Asian lecture, 4 hours total.
Grants and awards	Travelling grant from the University of Neuchâtel to attend the IEIC5, Kyoto, Japan, October 2017 (1200 CHF) Best student lecture 1st prize from the French Society of Entomology at the Entomophagistes 2017, Lyon, France, May 2017 (300 EUR)
Language skills	French : native speaker, English : fluent (speaking, reading, writing) Spanish : intermediate (speaking, reading), basic (writing)
Digital competences	MS Office Suite, Statistical analysis : R, DNA analysis : Sequencher and MEGA7
Countries worked	France, Switzerland, Japan, and China