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 (Kenis et al., 2016; Lee et al., 2015). 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 increasing resistance of target species and the concern for environmental and human health, alternative methods are needed. 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 Figitidae oviposited more in larvae feeding on blueberry. Ganaspis sp. laid very few eggs in larvae in the artificial diet, suggesting that it may be specialized 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.

KEYWORDS

Asobara, Ganaspis, invasive species, Leptopilina, pre-oviposition period, spotted wing drosophila
short development time of the fly, they have to be applied regularly and can cause severe environmental and health concerns (Bruck et al., 2011; Cuthbertson, Collins, Blackburn, Audsley, & Bell, 2014; Sampson, Werle, Stringer, & Adamczyk, 2017; Smirle, Zuzowski, Ayyanath, Scott, & MacKenzie, 2017; Van Timmeren & Isaacs, 2013). Cultural management such as the use of insect-proof nets, mass trapping and removal or solarization 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. As wild host plants constitute a large reservoir of individuals that can potentially reinfect 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, Bouletreau, Lenteren, & Alphen, 1986; Fleury, Gibert, Ris, & Allemann, 2009). Two generalist pupal parasitoids, *Trichopria drosophilae* Perkins (Hymenoptera, Diapriidae) and *Pachyceropoideus 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, Riudavets, Rodriguez, Pujade-Villar, & Arnó, 2015; Knoll, Ellenbroek, Romeis, & Collatz, 2017; Rossi Stacconi et al., 2015, 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, Allemann, Poyet, Eslin, & Gibert, 2012; Gabarra et al., 2015; Poyet et al., 2013; Rossi Stacconi et al., 2015). The main immune defense observed on *Drosophila* spp. against larval endoparasitoids is encapsulation. This involves cells of the insects' haemolymph that attach to the surface of a parasitoid egg or larva and then melanize 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 (Buffington & Forshage, 2016; Daane et al., 2016; Kasuya, Mitsui, Ideo, Watada, & Kimura, 2013; Mitsui & Kimura, 2010; Nomano, Mitsui, & Kimura, 2015; Novkovic, Mitsui, Suwito, & Kimura, 2011). 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 specialized 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 minimize 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. This 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 whether this success is affected by the host's diet (fresh fruits and artificial diet).

## 2 | MATERIALS AND METHODS

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

### 2.1 | 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 cage nets (BugDorm-45455°) 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 50 × 100 mm) containing 10 g of commercial fly diet (Formula 4–24 medium, Carolina Biological SupplyCo., Burlington, NC) with 40 ml of 1.43 g/L of methyl-4-hydroxybenzoate 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 50 × 100 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 & Forshaw, 2016), 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 bar-coding) 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 90 × 50 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 hr, and then, the berries were distributed among the parasitoid-rearing boxes for another 48 hr to allow female parasitoids to oviposit in the fly larvae. After the exposure, fruits were removed and kept into rearing tubes (Ø 50 × 100 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.

### 2.2 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 hr 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 hr under laboratory conditions. Newly emerged (less than 12 hr old) males and females of Ganaspis sp. and L. japonica were kept together for 24 hr 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 hr to a single blueberry previously exposed to D. suzukii and containing 72-hr-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 hr. 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.

### 2.3 Substrate suitability and performance

A performance experiment based on a 7 × 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. Newly emerged male and female parasitoids (less than 8 hr old) from the rearing colonies were kept together for 3 days in tube to allow mating prior to the experiments. One day prior each test, tubes (Ø 25 × 50 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 25 × 50 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 hr 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 hr. 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-hr 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 squeeving 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 as follows: 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\(_{D,\text{encapsulated}}\)); the number of emerged parasitoids (n\(_{P}\)); the total number of emerged insects.
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*<sub>o</sub>).)

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*<sub>o</sub>) 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, that is 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*, that is 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.

2.4 | Statistical analyses

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.

3 | RESULTS

3.1 | 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, that is, when females were 24–44 hr 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
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34 and 29 days, respectively. For L. japonica and Ganaspis sp., males emerged 3–4 days prior females (Table 2).

### 3.2 Substrate suitability and performance

The POF of A. japonica in the two different substrates was not significantly different ($\chi^2 = 1.181, df = 1, p = .277$). The two L. japonica strains oviposited in both substrates, but significantly more females of the Beijing strain oviposited in blueberries compared to diet ($\chi^2 = 16,484, df = 1, p < .001$). 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 Sheping strains: $\chi^2 = 32,411, df = 1, p < .001$ for both strains; Tokyo strain: $\chi^2 = 16,484, df = 1, p < .0001$). 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 ($\chi^2 = 27,805, df = 1, p < .001$) (Figure 2).

### 3.3 Proportion of ovipositing females

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).

### 3.4 Overall parasitism 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. suzuki, with an average APR less than 2% compared to an average of 36% for the other Asian wasps (Figure 4 and 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

### 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 |
| Lep. japonica Kunming, China  | 0.067 ± 0.06 | 0.667 ± 0.25 | 0.933 ± 0.25 | 0.667 ± 0.21 | 0.733 ± 0.18 | 0.277 ± 0.15 | 0.667 ± 0.34 |
| Lep. japonica Beijing, China  | 0.389 ± 0.14 | 0.389 ± 0.14 | 1.278 ± 0.51 | 0.389 ± 0.21 | 1.667 ± 0.44 | 1.875 ± 0.43 | 1.867 ± 0.45 |
| Lep. japonica Kunming, China  | 0.267 ± 0.14 | 0.267 ± 0.14 | 0.733 ± 0.25 | 0.431 ± 0.19 | 0.833 ± 0.21 | 0.833 ± 0.21 | 1.000 ± 0.30 |
| Lep. japonica Shiping, China  | 0.625 ± 0.34 | 1.188 ± 0.36 | 1.188 ± 0.36 | 1.250 ± 0.39 | 0.500 ± 0.33 | 0.500 ± 0.33 | 1.000 ± 0.33 |
| Lep. japonica Tokyo, Japan   | 0.25 ± 0.16 | 0.25 ± 0.16 | 0.50 ± 0.33 | 0.50 ± 0.33 | 0.625 ± 0.33 | 0.50 ± 0.33 | 1.000 ± 0.33 |
For the two other strains, ER was 100% with no emergence of parasitoids. In blueberry, ER was 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. 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.

### 3.6 Undetermined mortality

Undetermined mortality of immature stages of D. suzukii, that is 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).

4 | 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, Heimpel, Ferns, Harvey, & Kidd, 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 2 days after emergence, but the majority started only after three to 4 days. Accordingly, more offspring was obtained from 3- to 4-day-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 by Burrack, Fernandez, Spivey, and Kraus (2013), who reported high mortality rates in blueberries when densities were 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.

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 specialization 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, Watada, Mitsui, & Kimura, 2008; Kasuya, Mitsui, Aotsuka, & Kimura, 2013). 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 (Gandolfi, Mattiacci, & Dorn, 2003; Turlings, Wäckers, Vet, Lewis, & Tuominson, 1993), 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, Van Lenteren, Boskamp, & Van Dongen-Van Leeuwen, 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 towards 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).

**FIGURE 4** Mean of apparent parasitism rate (APR) (±SE) 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’s post hoc test, letters p < .05 and ***p < .001)
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, for example 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, Driessen, De Haan, & Roodbol, 1987; Mitsui, Van Ackerberg, Nordlander, & Kimura, 2007). To locate host larvae in the substrate, females also rhythmically probe suitable substrates with the ovipositor while walking (Sokolowski & Turlings, 1987; Vet & van Alphen, 1985) 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; Knoll et al., 2017; Poyet et al., 2013; 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, Mitsui, Ideo et al., 2013), 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 (Daane et al., 2016; Guerrieri, Giorgini, Cascone, Carpenito, & van Achterberg, 2016; Ideo et al., 2008; Mitsui & Kimura, 2010; Novković et al., 2011; Wachi, Nomano, Mitsui, Kasuya, & Kimura, 2015), 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.

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**AUTHOR 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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