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EVALUATION OF PARASITISM EFFICACY OF *GANASPIS* CF. *BRASILIENSIS* TO DEVELOP CLASSICAL BIOLOGICAL CONTROL AGAINST *DROSOPHILA SUZUKII*

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RÉSUMÉ
Drosophila suzukii est un ravageur très problématique en culture de fruits rouges. Des travaux préliminaires ont permis d'identifier le parasitoïde larvaire *Ganaspis* cf. *brasiliensis* comme potentiel agent de lutte biologique. L'objectif de cette expérimentation est d'évaluer la capacité du parasitoïde à s'installer en culture et parasiter *D. suzukii*. L'essai est conduit en serre, sur une culture de fraise hors sol, infestée artificiellement par une population de *D. suzukii*. Deux souches du parasitoïde sont étudiées en comparaison d'un témoin sans lâcher de parasitoïde. Les fruits sont récoltés 2 fois par semaine et conservés pendant 3 semaines pour suivre les émergences. Des parasitoïdes ont émergé des fruits récoltés, suggérant la capacité de *G. brasiliensis* à s'installer en conditions semi-naturelles, et à parasiter *D. suzukii*. À l'issue des observations, *G. brasiliensis* a permis de limiter l'accroissement de la population du ravageur de 50 %.

Mots-clés : *Drosophila suzukii*, parasitoïde exotique, *Ganaspis cf. brasilensis*, régulation, lutte biologique classique

ABSTRACT
Drosophila suzukii is a highly problematic pest in soft fruit crops. Preliminary studies have made it possible to identify the larval parasitoid *Ganaspis* cf. *brasiliensis* as potential biological control agent. The aim of this experiment is to evaluate the capacity of the parasitoid to establish into the crop and to measure the parasitism activity. The trial is conducted in greenhouse, on a soilless strawberry crop, artificially infested with a population of *D. suzukii*. Two strains of the parasitoid are studied in comparison to a control without parasitoid release. Fruits are harvested twice a week and stored for 3 weeks to monitor emergence. We observed emergence of adult parasitoids suggesting the ability of *G. brasiliensis* to establish in semi-natural conditions and parasitize *D. suzukii.* At the end of the trial, *G. brasiliensis* has reduced by 50% the increase of the population of *D. suzukii* adults.

Keywords: *Drosophila suzukii*, exotic parasitoid, *Ganaspis cf. brasiliensis*, management, classical biological control

INTRODUCTION

In France, cherries, strawberries, and soft fruits crops are strongly impacted by the presence of the spotted wing drosophila (SWD) *Drosophila suzukii* Matsumara (Diptera: Drosophilidae). This invasive species was introduced in France in 2008 and has since posed significant management issues for growers. Economic losses tied to SWD damages on fruits are tremendous, reaching up to several millions of euros (Walsh *et al*. 2011; Asplen *et al*. 2015). Currently, there is no effective management solution to achieve a satisfying control. Besides, the efficacy of the control by natural enemies in the invaded area is not sufficient (Trottin-Caudal, 2017). Besides, *D. suzukii* reaches very high populations in the environment. *D. suzukii* is extremely polyphagous, attacking cultivated and non-cultivated fruits. More than 30 host plants have been identified (Poyet *et al.* 2015; Weydert *et al.* 2016). Hedgerows surrounding fruit crops, thus constitute a refuge for this species. Thus, the high pressure of the pest is one of the factors that can explain the failure of the protection strategies currently implemented in fruit crops.

Classical biological control is based on the intentional introduction of one or more exotic natural enemies from the native area of the targeted pest, to ensure à long term control of the pest population in the invaded area (Eilenberg *et al*., 2001). Surveys conducted in China and Japan between 2015 and 2017 allowed the collection and identification of several parasitoids of *D. suzukii* (Girod et al, 2018a). Among these natural enemies, the most promising candidate as biological control is the larval parasitoid *Ganaspis* cf. *brasiliensis* (Hymenoptera Figitidae) (Kasuya *et al*. 2013; Girod *et al*. 2018b). Preliminary experiments conducted by INRAE Institut Sophia Agrobiotech (ISA) have allowed selecting two strains of *G. brasiliensis* according to their high host specificity and high parasitism rate (Borowiec *et al.* 2021).

The objective of this experiment is to demonstrate the ability of the parasitoid *G*. *brasiliensis* to establish in a semi-natural habitat and to parasitize *D. suzukii.* A first experiment has been conducted in 2019, with the two strains of the parasitoid previously mentioned. Any establishment have been recorded during this experiment. Hence, in 2020 the experiment has been relaunched, considering previous results from 2019, and technical difficulties to overcome.

MATERIAL AND METHODS

Insect populations

D. suzukii was reared at CTIFL, Centre Opérationnel de Balandran (Bellegarde, France) on an artificial diet, under control conditions (16h day / 8h night; temperature 22°C; relative humidity 60%). This population comes from a laboratory colony provided by INRAE ISA in 2019.

G. brasiliensis is reared at INRAE ISA since 2017. Two strains of the parasitoid were tested in this experiment, called « GT » and « GS6 ». The two strains were collected in Japan, in the Nara area for « GT », and in the Tokyo area for « GS6 ». Ecological specificities differ between the two strains. Indeed, females of the « GT » strain could lay egg in *D. suzukii* larvae reared in artificial diet but only when larvae develop in fresh fruits. This particular behavior makes more difficult to produce adults of the « GT» strain. For this reason, we were not able to test the same number of parasitoids for the 2 strains.

Plant material

Strawberry crop was grown in a soilless system. Greenhouse contains 4 growing rows with 13 slabs by raw, and 5 strawberry plants by slab. Due to COVID-19 sanitary crisis, it was not possible to plant the crop as it was planned. Then, we have to use slabs containing strawberry plants from another experiment. The plants were already producing fruits. We moved the slabs from a plastic tunnel to the greenhouse used for the experiment. Strawberry plants were introduced in the greenhouse on the 19th of June 2020. All the plants were inspected for eventual presence of pests or pathogens before their introduction in the greenhouse. The variety was Clery, planted in January 2020 (week 3). Strawberry plants were contaminated by *Drosophila melanogaster* before their introduction in the greenhouse. The presence of *D. melanogaster* was monitored during all the trial and considered in the analyses of the results.

Evaluation of the efficacy of the parasitoid

The trial was carried out in a confined greenhouse, in an *insect-proof* compartment of 60 m². The trial was arranged in a Fisher bloc design, with 4 repetitions by modalities. Four modalities were tested in the trial:

1 – Control, no release of parasitoid
2 – *G. brasiliensis* strain GS6 repetition 1 (25 females et 8 males)
3 – *G. brasiliensis* strain GS6 repetition 2 (25 females et 8 males)
4 – *G. brasiliensis* strain GT (25 females et 8 males)

Each raw contained the for modalities tested, arranged randomly. Elementary plots consisted in 10 strawberry plants. To avoid any risk of contamination, each elementary plot was covered by an insect-proof net, with a mesh (350µ) preventing the parasitoid to escape, and also the SWD. Elementary plots were infested by *D. suzukii* adults. Infestation consisted in two releases of 8 females and 4 males of *D. suzukii* for each cage, at 2 weeks of interval, on the 25th of June and 8th of July. *D. suzukii* females were one week old and had already laid eggs before the introduction in elementary plots. Adults of the parasitoid *G. brasiliensis* were introduced 24h after the first release of *D. suzukii* (26th of June). Then a second introduction of the parasitoid was realized, 7 days after the first one (7th of July). Adults of the parasitoids were released at the center of the elementary plot. For the two strains of the parasitoid, the total quantity of individual released was 25 females and 8 males, divided into two releases.

Elementary plots were observed 2 times a week, for 4 weeks. For each observation, five mature fruits, presenting typical damage of *D. suzukii* were harvested, and kept in control condition to monitor pest or parasitoid emergence. Considering experimental difficulties experienced in 2019, we used two different systems to monitor emergence of insect adults from the harvested fruits. One system consisted in isolated the strawberry fruit in a paper cup covered by an insect-proof net (mesh 350µ), with paper towel in the bottom to absorb strawberry juice. The other system consisted in a glass tube containing rearing diet for *D. suzukii*, where we placed the SWD larvae extracted from one harvested fruit. Because the second system to monitor emergence was very time consuming, we placed four fruits individually in paper cups, and only the fifth fruit was crashed, and all the larvae extracted were transferred in a glass tube containing rearing diet. This work was done under a magnifier (x20) to be sure to collect all the larvae.

Biological cycle of *G. brasiliensis* is around 3 weeks at 22°C. Then, paper cups and tubes were stored in a climatic chamber, at 22°C ±1°C, for 4weeks, in order to follow the mergences of adults of *D. suzukii* and *G. brasiliensis*. Each cup and tube were identified with the harvest date, elementary plot number and fruit number. For each observation, the number of adults of *D. suzukii* and *G. brasiliensis* emerged was recorded. Then, it is possible to evaluate the ability of the parasitoid to establish in the crop and to measure the relative parasitism rate.

Statistical analyses

Studied variables were the number of SWD emerged, the number of parasitoids emerged and parasitism rate. For each variable, we designed a generalized linear mixt model with the treatment (presence or absence of parasitoids) and the blocs as fixed effects, and the date of observations as random effect. We used ANOVA to compare the effect of the factors treatment and bloc on the studied variables. Then, we completed our analyses by comparing the effect of the treatments on the 3 variables for each date of observation. We used a generalized linear model approach. A *post-hoc* test (Tukey) was used to identify the modalities significantly different. All statistical analyses were carried out using the software R (4.0.2) with the following packages: car, dplyr, emmeans, lme4.

RESULTS

Fruits were harvested for the first time on the 1st of July 2020. Harvest lasted until the 24th of July. After 4 weeks of fruits monitoring for emergence, several adults of *G. brasiliensis* were observed. The first parasitoid emergences were observed from the first harvest on July 1st, and until the end of the trial (Figure 1). Parasitoid emergence was significantly different between the 2 monitoring methods (Anova, $p < 0.0001$). The "tube" method allowed the collection of more individuals than the “paper cup” method. In the cups, adults of *G. brasilienis* have emerged from harvested fruits collected on the 21st of July, with a total of 29 adults of parasitoids emerged. On the other hand, in the “tube” method, we

observed more emergences of adult parasitoids, and emergences occurred sooner. Indeed, the first adult parasitoids emerged from fruits harvested the first week of the trial (July 1st). In total, 51 adults of *G. brasiliens* have merged from the glass tubes (Figure 1).

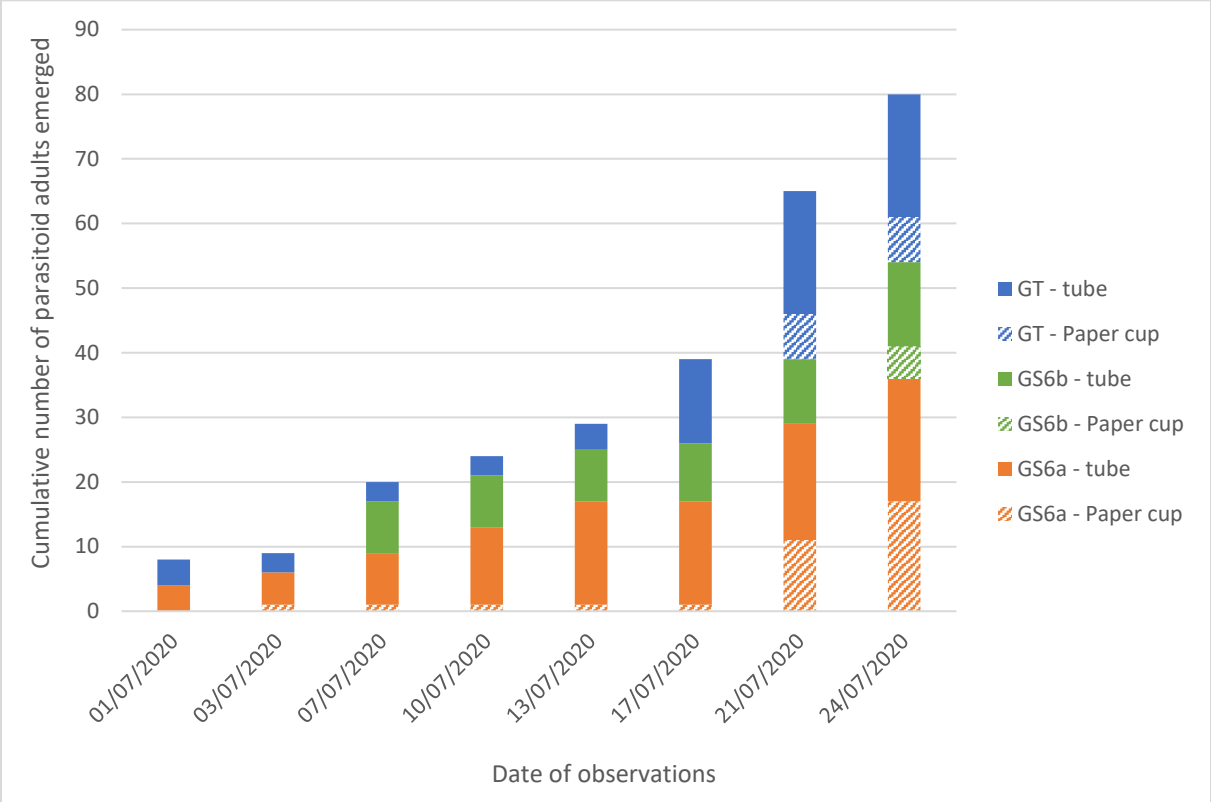

Figure 1 : Cumulative number of emerged adults of *Ganaspis brasiliensis* according to the studied modality

The impact of the emergence monitoring method on the emergence rate of *G. brasiliensis* is reflected in the observed relative parasitism rates. Thus, the relative parasitism rate calculated for the tube method is significantly higher than for the paper cup method (Figure 2) (GLM, Tukey test $p<0.0001$). In the tube method, the GS6 strain showed a parasitism rate ranging from 12% (GS6b) to 16% (GS6a), and the GT strain showed a higher parasitism rate of 18%. However, the measured parasitism rates were not significantly different (GLM, Tukey test $p>0.05$). Then, in the paper cup method, the relative parasitism rate was very low for the two strains, ranging from 0.4% (GS6b) to 5 % (GS6a) for GS6 and solely 0.4% for GT. Hence, the tube method was obviously a better method to estimate emergence rate and parasitism rate of the parasitoid *G. brasiliensis*.

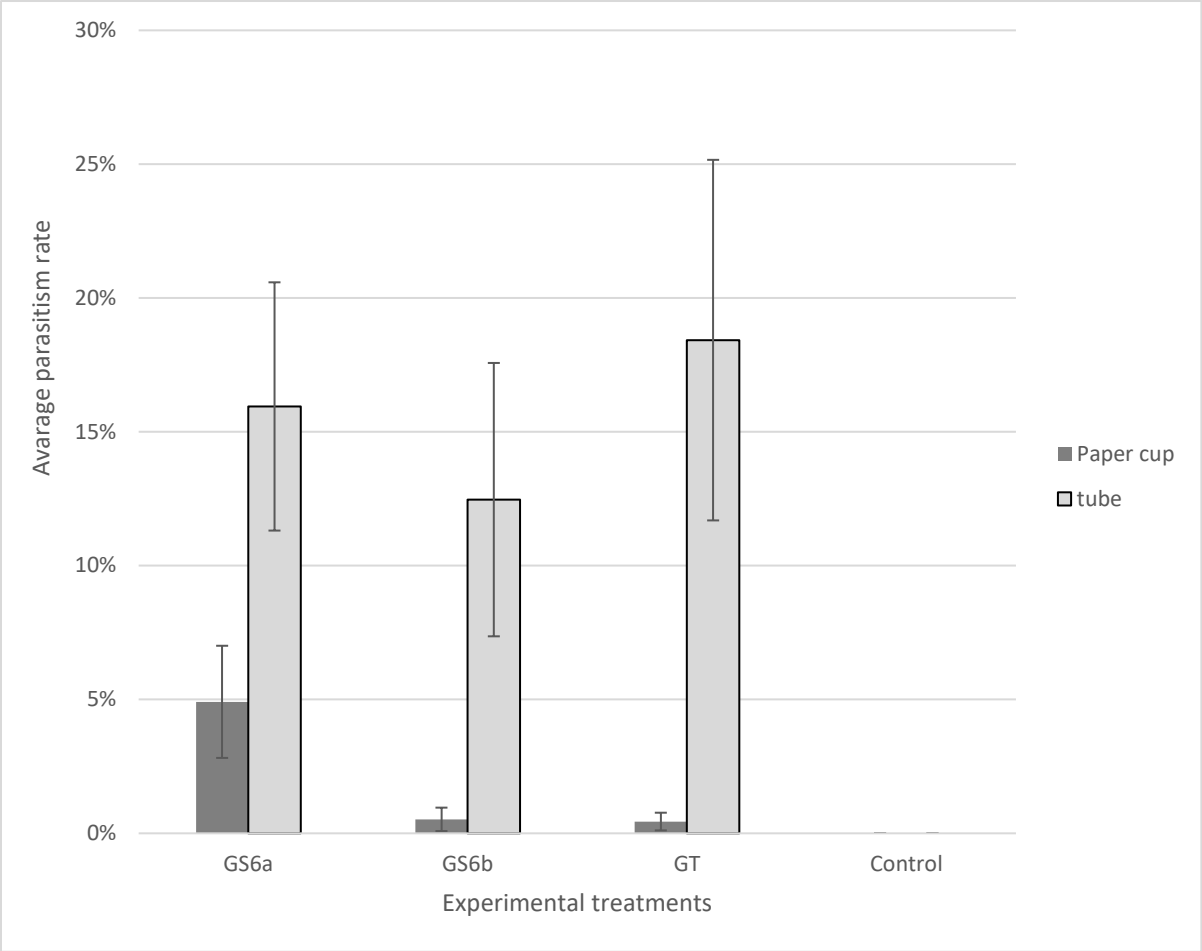

Figure 2 : Average parasitism rate measured for *Ganaspis brasiliensis* as a function of strain and monitoring method for emergence (Goblet vs. tube). Error bars represent the standard error

Artificial contamination with *D. suzukii* was homogeneous among the trial. Fruits infested with *D. suzukii* larvae were observed from the first harvest, and adults have emerged from these fruits. The population of *D. suzukii* increased linearly throughout the trial, in all the modalities studied (Figure 3). Until July 17th, the number of *D. suzukii* adults, and the population dynamic were very similar between the modalities. However, from July 21st, we observed a slowdown in the growth of the *D. suzukii* population in the 3 modalities with parasitoids, while the increase continued in the control. From this date, the population of *D. suzukii* was significantly lower in the modalities with parasitoids compared to the control without release of parasitoids (Tukey test, $p<0.05$). Moreover, the numbers of *D. suzukii* adults counted in the modalities with parasitoids were not significantly different from each other (Tukey test, $p>0.05$). Hence, the 2 strains of the parasitoid studied, GS6 and GT, seemed to have a similar impact on the pest. At the end of the trial, the reduction of the *D. suzukii* population in the modalities with parasitoids varied from 35% for the GT strain to 40% for the GS6 strain (39.4% for GS6a and 40% for GS6b).

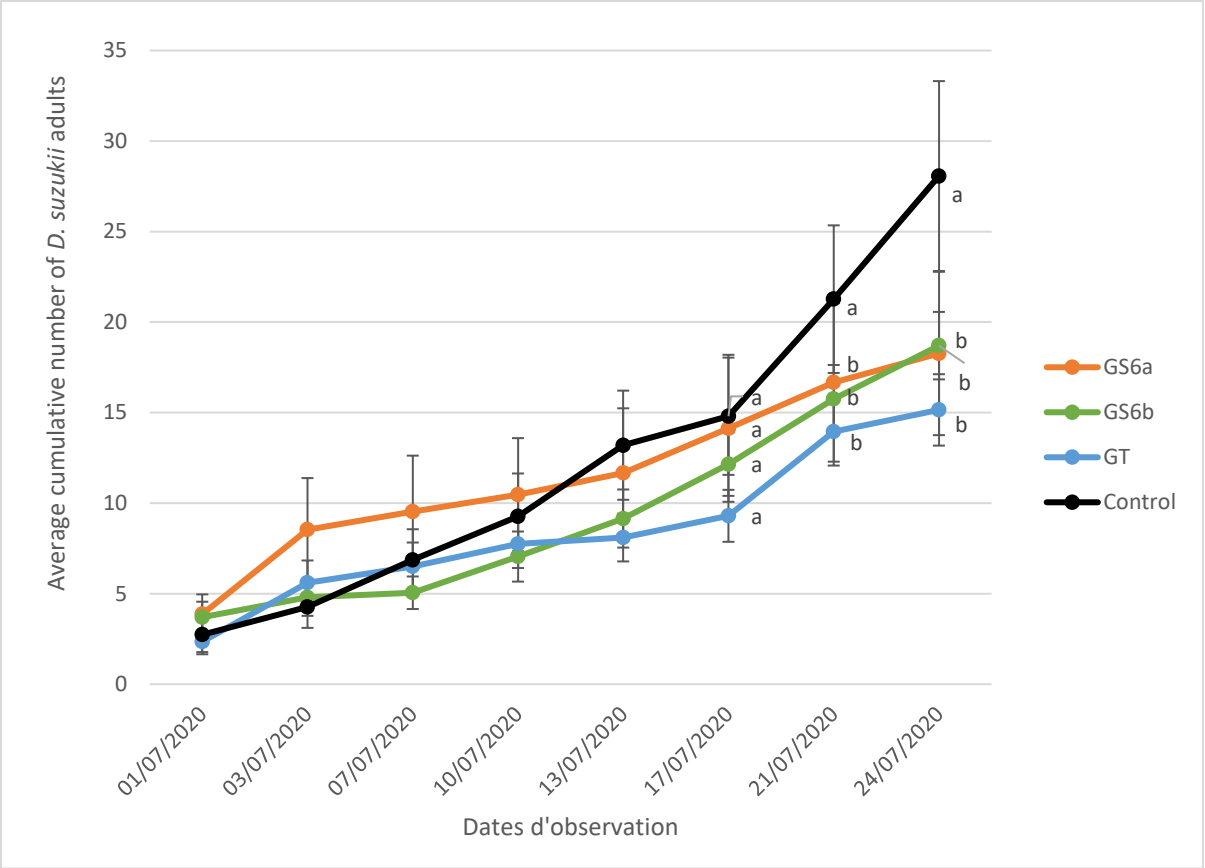

Figure 3 : Average cumulative number of *Drosophila suzukii* adults for the 2 monitoring methods for emergence together (tube and paper cups). The error bars represent the standard error. The letters indicate the statistical groups, the modalities with different letters are significantly different at the 5% threshold (Tukey test).

DISCUSSION

The emergence of *G. brasiliensis* was observed on fruits from the first harvest after the introduction of the parasitoid, which suggested a good acclimation of the parasitoid, in semi-field conditions. Moreover, during the whole trial period, the presence of living *G. brasiliensis* individuals in the cages was noted, reflecting its ability to settle in semi-natural conditions.

The potential for the regulation of *D. suzukii* by *G. brasiliensis* was evaluated by measuring the emergence rate of *D. suzukii* in the presence and absence of the parasitoid. Our results suggested a significant impact of the parasitoid on the population dynamic of *D. suzukii*. Indeed, at the end of the experiment, the population of D. suzukii was reduced by 40% when the parasitoid was introduced in the experimental cages. Furthermore, the two strains studied GS6 and GT seemed to have the same impact on the pest.

In the first experiment conducted in 2019, with *G. brasiliensis* at CTIFL Balandran, the parasitoid was introduced at the dose of 2 individuals/m². Then, no installation and parasitism were observed. For instance, in a previous study on *Trichopria drosphilae*, a pupal parasitoid of *D. suzukii*, the tested doses for introduction were 12 individuals/m² and 20 individual/m² with a good installation of the parasitoid and a maximum parasitism rate of 60% (Trottin-Caudal et al, 2017). In this experiment, *G. brasiliensis* was introduced at the dose of 32 individuals/m², and the experimental plot were covered with an insect-proof net which could favor the installation of the parasitoid.

During the weeks of observation, many Rhizopus-infected fruits were removed from the cages to avoid the spread of the fungus. Most likely, these fruits were infected with *D. suzukii* and the larvae potentially parasitized by *G. brasiliensis*. Removal of the fruit from the cages for prophylactic reasons (mold control) probably reduced the insect populations in the cages, hence the emergence and parasitism rates calculated. Nevertheless, the number of *D. suzukii* individuals was significantly lower

for the GS6 and GT strains compared to the control modality during the two last observations, reflecting the parasitism pressure of *G. brasiliensis*.

However, the pressure exerted by *G. brasiliensis* on the population of *D. suzukii* could be considered as low in this experiment, despite the favorable conditions for the installation of the parasitoid. Few emergences of parasitoids were observed in the experimental cages. And the relative parasitism rate ranged from 12% to 18% which could suggest a limited impact of the parasitoid. However, the numbers of *D. suzukii* adults were 40% lower in the cages with parasitoids. Faced with parasitism, the immune system of *D. suzukii* triggers a strong reaction defense to encapsulate the parasitoid eggs. Although this response is 5 times greater in *D. suzukii* than in other species of this group, it is not a 100% effective. The laid larvae by *G. brasiliensis* can greatly reduce the survival of *D. suzukii* as adults (Kacsoh & Schlenke, 2012). It is possible that the parasitoid parasitized *D. suzukii* larvae without completing its development cycle but inducing mortality of *D. suzukii* in the larval stage.

Regarding the low number of individuals emerging in the paper cups, the experimental set-up for monitoring emergence have had a strong impact on the emergence of the parasitoid. The emergence rate of *G. brasiliensis* observed with the tube method was significantly higher than with the paper cup method. Hence, the experimental set-up with the paper cups has an important limitation, due to the rapid drying of the fruits during the period of emergence monitoring. Indeed, the lack of humidity in the laboratory and the absorbent paper in the cups led to a rapid drying of the strawberries, which induces a high mortality of the larvae. Furthermore, development of mold also impacted the quality of the fruit and the survival of the larvae. These observations could explain the low emergence rate of *G. brasiliensis* individuals during the second and early third week of harvest. Thereafter, the strawberries in the cups were moistened daily. In the tubes, the artificial medium allowed the extracted larvae to complete their development cycle and thus to observe almost all the emergences of *D. suzukii* and *G. brasiliensis*.

As mentioned earlier, we faced a contamination by *D. melanogaster*. Therefore, *G. brasiliensis* was placed in a choice system even if it was not the purpose of the experiment. Nevertheless, we observed the parasitism by *G. brasiliensis* on the two Drosophila species. Indeed, at the pupal stage it is feasible to identify *D. suzukii* from *D. melanogaster,* with the presence of two stalks with small finger-like projections on SWD's pupae. With the presence of both *D. suzukii* and *D. melanogaster* in the cages, the issue of the specificity of *G. brasiliensis* should be considered. While several works demonstrate the high specificity of *G. brasiliensis* for *D. suzukii* (Mitsui & Kimura, 2010; Kacsoh & Schlenke, 2012; Kasuya *et al.* 2013; Girod *et al.* 2018b), the trial conducted at INRA Sophia Antipolis in 2018 showed that out of 944 individuals of *G. brasiliensis* strain GS6, 52% emerged from D. suzukii pupae and 46% from *D. melanogaster* pupae (Ponchon, 2018). *G. brasiliensis* strain GS6 would exhibit a narrower host spectrum with strong parasitism only on *D. suzukii* and *D. melanogaster*, compared to the species tested (*D. busckii*, *D. hydei*, *D. subobscura*). In this trial, all parasitized pupae were observed with a binocular magnifying glass, and only the pupae of *D. suzukii* were parasitized. This observation suggested a good specificity of *G. brasiliensis* for *D. suzukii* in choice condition, but specific experiments are needed to confirm this observation.

CONCLUSION

These preliminary results are encouraging and show the ability of *G. brasiliensis* to settle on strawberry crop and to parasitize *D. suzukii* larvae, under semi-natural conditions. Additional trials will have to be conducted to confirm these observations. These first data will complete those collected by INRAE ISA on efficacy and specificity of *G. brasiliensis*. Then, the next step is the constitution of an authorization dossier to the regulatory administration in France (ANSES), to request the introduction in the environment of the exotic parasitoid *G. brasiliensis*, as a biological control agent against *D. suzukii*. Subject to a positive answer from ANSES, experiments in field conditions could be carried out in the in the Alpes-Maritimes by extension services and INARE ISA. The introduction and establishment of a new biological control agent in the environment could reduce the naturally occurring populations of *D. suzukii*, thus reducing the pressure of the pest on crops; hence, adding a new lever in the control of this pest.

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