Current status of *Drosophila suzukii* classical biological control in Italy

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Abstract

For over a decade, the invasive pest *Drosophila suzukii* (Matsumura) has threatened the soft-skinned fruit production worldwide, causing increased management costs and yield losses. Current integrated pest management (IPM) exploits different control tools but relies mainly on insecticides. The local natural enemy community is mostly composed by generalist species, such as parasitoids attacking fruit fly puparia, unable to control the pest efficiently, in both conservative and augmentative strategies. By contrast, in the native area of *D. suzukii*, sympatric larval parasitoids have co-evolved with the pest and provide a stable control of its population. Foreign explorations and quarantine risk assessment studies for classical biological control programmes have identified different species of parasitoids showing variable levels of specificity. The Japanese G1 lineage of the larval endoparasitoid *Ganaspis brasiliensis* (Ihering) has proved to be much more selective and efficient than other larval parasitoids, including *Leptopilina japonica* Novković & Kimura recently reported in Europe. In this context, a voluntary partnership of Italian researchers coordinated by the Italian National Plant Protection service imported a colony of the G1 lineage of *G. brasiliensis* into Italian quarantine facilities and proposed its release in Italian fields. A three-year working program has been set up in several locations of nine Italian regions/provinces. Field releases of laboratory-reared parasitoids will go along with pre- and post-release sampling of fresh and fallen fruit around the release area, in order to assess the *G. brasiliensis* impact on *D. suzukii* and the potential interactions with other non-target insects. The potential establishment of such an efficient biological control agent would promote the permanent sustainable control of this invasive species thus making IPM less dependent on the use of chemicals, reducing the issues associated with it.

Keywords: *Ganaspis brasiliensis,* host range, invasive species, importation biological control, IPM, parasitoid

1 IMPORTATION BIOLOGICAL CONTROL AGAINST INVASIVE INSECT PESTS

Climate change and anthropic activities, as globalization and growing world trade, are the major source of alien species long-distance movement from their native range to new environments, mostly towards the more economically developed countries. Despite the invasion rate of alien species of mammals, birds and vascular plants is esteemed to decrease over time, as a result of a greater capacity and awareness of their detection at the national entry points, opposite situation is expected for invertebrates (Seebens et al., 2017). Indeed, researchers have already reviewed the incoming and growing invasion rate of exotic arthropods in those countries with highest Human Development Index (HDI). However, the international policies and agreements have not been able to prevent and eradicate the invasive organisms until now (Early et al., 2016).

The introduction of an invasive phytophagous insect in a new habitat certainly alters the local integrated pest management (IPM) strategies in the affected agroecosystem, causing environmental and economic damage. Economic losses of $ 273 million by exotic pests were recently estimated in Italy (Haubrock et al., 2021). In addition, according to the "Enemy Release Hypothesis" (ERH), the lack or the reduced effectiveness of natural enemies in the invaded area promote the successfully establishment of the exotic pest, which will relate with new environmental conditions without a strong selective pressure on it (Keane and Crawley, 2002). The intentional introduction of a co-evolved natural enemy from the same native region of the pest would eventually aid the sustainable management of the invasive species by creating a new ecological balance in the invaded areas (Heimpel and Mills, 2017). This strategy, known as classical biological control, has been adopted worldwide and its success depends on the establishment capacity (as a function of climate match, pest pressure and habitat disturbance) and on the target and non-target potential impact of the imported biocontrol agent in the new habitat (Blackburn et al., 2014). Each classical biological control program requires *ex ante* studies, to identify and assess a potential natural enemy, and *ex post* surveys, to check the effects after repeated releases (Hill and Greathead, 2000; Jarvis et al., 2006). In this context, a global classical biological control program against the invasive pest *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) has been promoted globally with a host-specific Asian parasitoid, following American and European quarantine investigations. Italian national government approved in August 2021 a three-year release and monitoring program of the Asian natural enemy *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae), following a close collaboration between Italian researchers and national institutions and a favourable risk assessment of the parasitic wasp on the national territory.

2 CURRENT STATUS OF THE CLASSICAL BIOLOGICAL CONTROL OF *DROSOPHILA SUZUKII* IN ITALY

2.1 *Drosophila suzukii* and its natural enemies

Drosophila suzukii, commonly known as the spotted-wing drosophila (SWD), is a major invasive pest of soft-skinned fruit native to Eastern Asia and widespread in almost all continents (Asplen et al., 2015; Biondi et al., 2016; Boughdad et al., 2020). In contrast to other non-target drosophilids, which forage and reproduce on fallen and unmarketable fruits, this pest is able to attack fresh fruits close to the harvest through the distinctive females serrated ovipositor. Due to its high spread potential, high fecundity, economic crops damage and wide host range of cultivated and wild plants, *D. suzukii* is considered a global threat and its control is strongly needed in all the infested areas (Asplen et al., 2015). In addition, the pest management gets more challenges considering the SWD opportunistic behavior to fruit dropped on the ground (Walsh et al., 2011) and the surrounding wild vegetation that act as both refugia during unsuitable environmental conditions and source for pest re-infestation in

treated crops (Kenis et al., 2016; Lee et al., 2015). Current pest management techniques rely mainly on non-selective insecticides that negatively affect the whole environmental biotic community and increase production costs (Haye et al., 2016). Other integrated management tools not always succeed in reducing SWD infestation, mostly because of the high pest pressure exerted by *D. suzukii* on crops. Large populations of the pest are built seasonally in the invaded areas due to the lack of efficient biological control agents (BCAs). In Italy, the local biotic community is composed by generalist natural enemies that provide poor predation and parasitism rate on the pest. Additionally, indigenous larval parasitoids do not have the capacity to parasitize *D. suzukii* due to a strong host immune defense, while the pupal ones are generalist and occasionally parasitize this pest (Chabert et al., 2012; Gabarra et al., 2015; Lee et al., 2019). In this context, the commercially available *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) has been employed in augmentative releases in Italy with the aim to decrease early fly infestation in marginal areas before the fruit ripening in the field (Rossi-Stacconi et al., 2018; 2019). However, both conservative and augmentative strategies have proved to be inefficient in the pest control, so a classical biological control (CBC) program was promoted a few years ago looking for sympatric and co-evolved larval parasitoids to introduce in the SWD invaded regions (Guerrieri et al., 2016; Lee et al., 2019). American and Swiss explorations in Japan (Girod et al., 2018a), China (Giorgini et al., 2019) and South Korea (Daane et al., 2016) revealed a broad panel of co-evolved larval parasitoids with different specificity and host range, as braconids in the genus *Asobara* (Guerrieri et al., 2016) and figitids in the genera *Leptopilina* and *Ganaspis* (Daane et al., 2016). Despite the first and only record of the Asian *Leptopilina japonica* Novković & Kimura (Hymenoptera: Figitidae) in Europe (Puppato et al., 2020), many quarantine studies revealed *Ganaspis brasiliensis* as the most specific parasitoid on *D. suzukii*, with the peculiarity of preferring parasitizing the SWD larvae in fresh fruit in the canopy (Girod et al., 2018c). This species would provide a successful pest control in cultivated crops and wild vegetation without affecting the non-target species, so as to be considered the best candidate for CBC program in America and Europe (Biondi et al., 2021; Daane et al., 2021; Giorgini et al., 2019; Girod et al., 2018b; Wang et al., 2020).

2.2 *Ganaspis brasiliensis* importation to Italy and establishment of a national collaborative network

In August 2020, a Japanese strain of *G. brasiliensis* (lineage G1) was imported to the quarantine facility of the Edmund Mach Foundation (EMF; Trento, Italy) from the CABI's Swiss centre located in Delémont (Switzerland). The aim was to apply to the Italian Environmental Protection Authority (EPA) seeking approval to release the parasitoid as a biological control agent (BCA) for the pest *D. suzukii*. By performing a series of experiments under quarantine conditions, researchers at EMF collected data related to the non-target effects *G. brasilensis* might have on endemic or beneficial drosophilids and to the indirect impacts on other species and food web systems (figure 1A,B). In January 2021, a dossier on the benefits and the risks of releasing *G. brasiliensis* in Italy to the environment, market economy, people and communities was produced to support the petition for the parasitoid release (see section 3). Upon completion, the final document was approved and subscribed by several members of the Italian scientific community involved in the study of *D. suzukii* biology, ecology and management. The first draft of the dossier only considered releasing *G. brasiliensis* in the Trentino province (northeastern Italy), one of the areas most damaged by *D. suzukii* in Italy. Eventually, the dossier was extended to include nine provinces and regions located all over Italy (figure 2) and a national collaborative network was established. Such network, named *D. suzukii* Technical Committee, is coordinated by the Council for Agricultural Research and Economics (CREA, Florence, Italy) and includes both scientific experts and representatives of the local phytosanitary services. Since March 2021, the committee has met regularly with the aim to build a platform that underpins a comprehensive national program

of biological control against *D. suzukii*. Specific achievements included i) the obtaining of a *G. brasiliensis* release permit for season 2021 granted by the Italian Ministry of the Ecological Transition, ii) the implementation of a rearing and distribution chain of *G. brasiliensis* for supporting open-field releases at national scale and iii) the coordination of the release and monitoring activities of the different regions/provinces participating in the project.

3 Benefit-risk assessment for the petition to release *Ganaspis brasiliensis* in Italy

In order to promote the classical biological control program on *D. suzukii* in Italy, a deepening document concerning the introduction risk assessment of the parasitic wasp *G. brasiliensis* in Italy was drafted and coordinated by the intense cooperation between EMF researchers and CREA. The dossier follows the guidelines established by the Ministerial Decree of 2 April 2020 "Criteria for the reintroduction and repopulation of native species referred to in Annex D of the Decree of the President of the Republic of 8 September 1997, n 357, and for the introduction of non-native species and population". Specific contents of the dossier relate with i) comprehensive information about the biology and the ecology of the target pest and the selected BCA, ii) data about host-specificity testing performed at the quarantine facility of EMF and already available in literature, iii) environmental and economic impacts of the proposed release, iv) probability of the parasitoid establishment and spread in the proposed release sites, v) release schedule and post-release monitoring program, vi) contingency plan to mitigate potential undesired environmental impacts, vi) detailed map and vegetational characterization of the proposed release sites and vii) list of responsible persons.

3.1 Biological and ecological host range of *Ganaspis brasiliensis*

The exotic *G. brasiliensis* is a koinobiont larval parasitoid widely distributed in South-East Asia, where several field explorations guided by American and European research teams revealed the occurrence of *G. brasiliensis* individuals with different host specificity, following the quarantine laboratory studies. Molecular analysis based on the nucleotide sequences of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene and three nuclear DNA regions (ITS1 and ITS2) of the sampled specimens proved a significant genetic differentiation of *G. brasiliensis* in five lineages (G1-G5), which are morphologically indistinguishable (Nomano et al., 2017). Their different host range and specificity would depend on the geographical distribution in the native areas (Daane et al., 2021). According to the parasitoid ecological specialization assumption, European laboratory research showed that the Japanese G1 lineage was the most host specific to *D. suzukii,* preferring SWD larvae in fresh fruits (Girod et al., 2018b), while American investigations reported a wider host range for the G3 lineage sampled in South Korea, parasitizing *D. suzukii* and other four phylogenetically closer species among a panel of 24 drosophilid hosts (Daane et al., 2021). Few studies were carried out on other lineages, even if they appear to be more generalist and widely distributed than the previous ones (Kimura e Suwito, 2012; Nomano et al., 2017). In summary, *G. brasiliensis* is the most specialized larval parasitoid of *D. suzukii* and the G1 lineage better fits with the classical biological control programmes in all the *D. suzukii* infested areas (Biondi et al., 2021; Daane et al., 2021; Giorgini et al., 2019; Girod et al., 2018b; Wang et al., 2020), considering the lack of negative impact both on non-target hosts and other indigenous natural enemies (Wang et al., 2019).

3.2 Benefits deriving from a successful establishment of *Ganaspis brasiliensis* in Italy

The introduction and establishment of the specialized *G. brasiliensis* represent the most sustainable and potentially successful approach to solve the economic, social and

environmental impact of *D. suzukii* in Italy, as well as in other infested countries. It is clear that the arrival of this pest has negatively impacted the economic management of the affected crop systems through infested nations exclusion from world trade, yield losses and increased production costs. In particular, the latter refer to increased insecticide applications in crops that result both in greater chemical exposure to the whole biotic community and issues related to the maximum residues limits (MRLs) of pesticides in fruits (Tait et al., 2021). The successful biological control service of this Asian natural enemy, which ranges from 17.1 % to more than 70% of efficient parasitism on *D. suzukii* in Asia (Daane et al. 2016; Giorgini et al. 2019; Girod et al. 2018b), will give an additional IPM tool to control SWD infestation in crops and in wild ecosystem, providing indirect economic and environmental benefits by the reduction of chemical applications and environmental pollution. This will result also in social benefits considering that the cherry and small fruits production is constantly growing over the years and represents the main economic resource for several small and medium-size Italian farms, from which depend many people involved in the whole food supply chain (Antonova, 2010; Peano et al., 2017).

3.3 *Ganaspis brasiliensis* field release and monitoring protocols

The 2021 open-field releases could only start in late summer, as the release permit was obtained in mid August. The insectary located at the quarantine facility of FEM provided parasitoids for all other regions/provinces but Sicily and Apulia, which established their own mass-rearing of *G. brasiliensis*. Periodic shipments of adult parasitoids werescheduled according with the productivity of the FEM insectary and the availability of fruit in the field in the different regions. For each shipment, adult parasitoids were aspirated into 50mL vials until each vial contained 100 females and 60 males. Vials had modified cap holding a cotton ring soaked with saturated sugar solution and ventilation hole closed by fine mesh net. Folded paper was placed within each vial to provide a walking support for parasitoids and to decrease mechanical shocks due to manipulation and transport. At the release sites, vials typically were hung on tree branches such that the parasitoids could walk or fly onto the surrounding vegetation (figure 3A). In 2021, a total of 480 adults was released at each site in three consecutive releases (100 females and 60 males per release). Because of the climatic differences between north and south of Italy, open-field releases were conducted in different regions during different times of the fruit seasons. All release sites were intensively monitored before and after the parasitoid release to determine the establishment and spread of *G. brasiliensis*, as well as seasonal patterns of parasitism of *D. suzukii* and other drosophilids by the parasitoid. The monitoring protocol consisted of a systemic sampling of fruits from plants that are known hosts of *D. suzukii* and other drosophilids (figure 3B; Kenis et al. 2016). Fruit was collected directly from plants and from the ground, and from both wild and cultivated hosts. At each site, sampling was conducted within a radius of 200 m from the release point, during a pre-set time of 1 man-hour. Collected fruit was categorized by geographic location, fruit characteristics (plant species, fruit color), habitat (tree vs. ground, commercial crops or non-crop landscape plants) and field management practices (e.g., insecticide applications, cover cropping). At each site, about 10-100 fruits were collected for each category (e.g., on trees or the ground), depending on the availability of fruit. Collected fruit was incubated for at least 35 days at 22°C. Upon appearance, pupae of drosophilids were sorted and placed individually in Eppendorf tubes with a streak of honey-water provided for emerging fly or waps adults. Dead or unemerged puparia were dissected under a microscope to determine the presence or absence of recognizable immature parasitoid cadavers and

pharate fly adults. Parasitism was estimated based on the number of emerged and dissected wasps and flies, while host density was estimated based on the total fly puparia.

CONCLUSIONS

Since the first *D. suzukii* detections in Europe and the USA in 2008, classical biological control has been one of the most considered options for the long-term management of the pest in many countries. Nonetheless, for several years a number of regulatory, social, economic and scientific impediments prevented the implementation of such approach worldwide. Here we described the outlines of the first CBC project against *D. suzukii*, which started in Italy in 2021.

Ganaspis brasiliensis has been released in 36 sites throughout Italy and an intensive monitoring activity has been carried out to produce preliminary data on the parasitoid establishment and dispersal. Although a complete evaluation on the success/failure of a CBC program requires years, such data will provide a baseline for granting authorization for further releases in 2022 and 2023, possibly over an increased number of release sites.

Future perspectives of *D. suzukii* CBC should include its implementation within sustainable IPM programs. For this, different tasks could be developed in order to understand how the action of *G. brasiliensis* can be enhanced in relation to current *D. suzukii* management techniques. For example, the compatibility of modern insecticides and biopesticides (e.g. botanical insecticides) with the parasitoid needs to be characterized in order to evaluate potential sublethal physiological (e.g. reproduction) and behavioral (especially those related to biocontrol, such as host location and parasitism behavior) side effects.

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Figure captions

Figure 1: A) Checking of the *Ganaspis brasiliensis* host-specificity tests at the quarantine facility of the Edmund Mach Foundation. Testing was performed on two Italian strains of *Drosophila suzukii* and on seven other species of endemic drosophilids. B) Adult females of *G. brasiliensis* (lineage G1) parasitizing a *D. suzukii* 1st instar larva within a blueberry.

Figure 2: Map of the Italian regions/provinces involved in the *Drosophila suzukii* biocontrol project in 2021. The number of release sites for each region/province is indicated in brackets. Regions/provinces without rearing facility (light red) received parasitoids from the insectary located at the Edmund Mach Foundation (AP Trento). The star indicates the location of CREA - Council for Agricultural Reseach and Economics, which assumed the role of project coordinator. AP = Autonomous Province.

Figure 3: A) Release of *Ganaspis brasiliensis* in the field. B) Fruit collected at one release site during the post-release monitoring activity.