Current status of Drosophila suzukii classical biological control in Italy

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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 mostly consists of generalist species, mainly parasitoids attacking the puparia of the fruit fly. These antagonists resulted unable to control the pest efficiently, regardless the adoption of conservative or augmentative approaches. 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 programs have identified different species of parasitoids characterized by a variable level 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 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 have been planned. Pre- and post-release samplings of fresh and fallen fruits around the release points will be undertaken to assess the impact of the exotic *G. brasiliensis* on *D. suzukii* and its potential interactions with other non-target insects in the field. The possible establishment of this efficient and specific biological control agent would promote a long-lasting control of this invasive species less dependent on the use of chemicals, reducing the negative effects associated with them.

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

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IPM, parasitoid

IMPORTATION BIOLOGICAL CONTROL AGAINST INVASIVE INSECT PESTS

Climate change, globalization and the growing world trade, are among the main factors behind the long-distance movements of alien species from their native range to new environments, mostly directed towards the more economically developed countries. Whilst the invasion rate of alien species of mammals, birds and vascular plants is estimated to decrease over time, as a result of a greater capacity and awareness of their detection at the national entry points, an opposite situation is expected for invertebrates (Seebens et al., 2017). So far, international policies and agreements have not been able to prevent the arrival or eradicate the invasive organisms (Early et al., 2016).

The introduction of an invasive phytophagous insect in a new habitat alters the local strategies of integrated pest management (IPM) in the affected agroecosystem, resulting in environmental and economic damage. 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 (CBC), has been adopted worldwide and its success depends on: the establishment capacity of the imported biocontrol agent (BCA) as a function of climate match, pest pressure and habitat disturbance; impact on the target species; impact on non-target species in the new habitat (Blackburn et al., 2014). Each classical biological control program requires ex ante studies, to select and characterize 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, following American and European quarantine investigations, a global classical biological control program against the invasive pest *Drosophila suzukii* (Matsumura) (*Diptera: Drosophilidae*) has been promoted in different countries through the release of the host-specific Asian parasitoid *Ganaspis brasiliensis* (Ihering) (*Hymenoptera: Figitidae*). In August 2021, the Italian government approved a three-year release and monitoring program of *G. brasiliensis* against *D. suzukii*, as a result of a close collaboration between Italian researchers and national institutions and a favourable risk assessment of the exotic parasitic wasp undertaken on the national territory.

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

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., 2021). Current pest management techniques mainly rely on non-selective insecticides that negatively affect the whole biocoenosis while increasing the production costs (Haye et al., 2016). Other integrated management tools not always succeed in reducing pest 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 BCAs.

In Italy, the community of autochthonous antagonists is composed mainly by generalist natural enemies that provide poor predation and parasitism rates. Additionally, the success of indigenous larval parasitoids is prevented by a strong host immune defence in *D. suzukii*, while the pupal ones only occasionally attack this pest (Chabert et al., 2012; Gabarra et al., 2015; Lee et al., 2019). In this context, a commercial strain of *Trichopria drosophilae* (Perkins) (*Hymenoptera: Diapriidae*) has been used in augmentative releases in Italy with the aim to reduce the early infestations 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 controlling the pest, therefore a CBC program was promoted a few years ago.

Sympatric and co-evolved larval parasitoids were collected and characterized with the aim of selecting possible candidates to be introduced in the newly invaded regions (Guerrieri

et al., 2016; Lee et al., 2019). American, Italian 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 larval parasitoids with different levels of specificity and host range, including 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 indicated *G. brasiliensis* as the most specific parasitoid of *D. suzukii* with a distinctive preference for larvae developing in fresh fruits within the canopy and almost no effect for non-target species (Girod et al., 2018c). These features made *G. brasilienis* the best candidate for a CBC program to be completed in America and Europe (Girod et al., 2018b; Giorgini et al., 2019; Wang et al., 2020; Biondi et al., 2021; Daane et al., 2021).

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) aiming at obtaining the approval to release the parasitoid as a BCA targeting the pest *D. suzukii*. Under quarantine conditions, researchers at EMF collected data related to the non-target effects *G. brasiliensis* might have on endemic or beneficial drosophilids and to the indirect impacts on other species and food web systems (Figure 1).

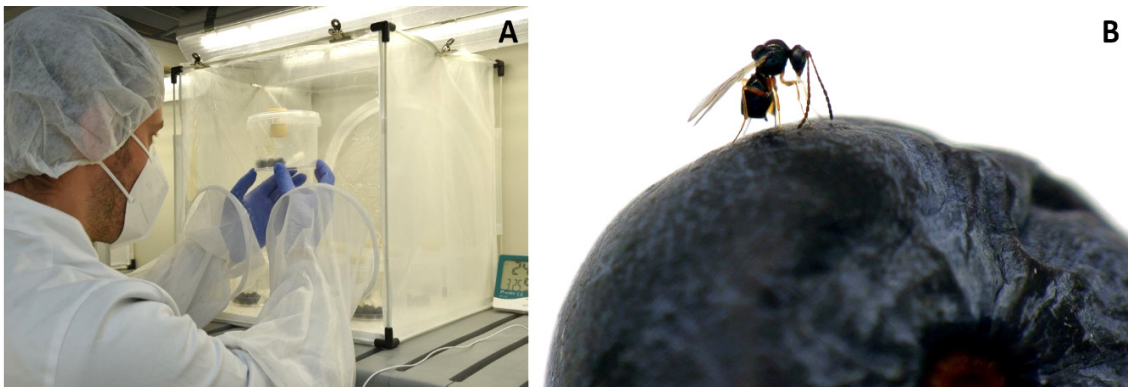

Figure 1. A) Checking of the *Ganaspis brasiliensis* host specificity tests at the quarantine facility of the Edmund Mach Foundation. Testing was performed on two Italian strains of *Drosophila suzukii* and on seven other species of endemic drosophilids; B) Adult female of *G. brasiliensis* (lineage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

In January 2021, a dossier on the benefits and the risks for the environment, the market economy, the people and the communities linked to the release of *G. brasiliensis* in Italy 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 draught of the dossier only considered releasing *G. brasiliensis* in the Autonomous Province of Trento (northern Italy), one of the most damaged areas 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, within the frame of the action of the Ministry of agricultural food and forestry policies and named *D. suzukii* Technical Committee, is coordinated by CREA and includes both scientific experts and representatives of the local plant protection services. Since March 2021, the committee has met regularly with the aim of building a platform that underpins a comprehensive national program of biological control against *D. suzukii*. Specific achievements included i) obtaining a permit for the release

of *G. brasiliensis* during the season 2021 granted by the Italian Ministry of the Ecological Transition, ii) the implementation of a rearing and distribution chain of *G. brasiliensis* to support open-field releases at national scale, and iii) the coordination of the release and monitoring activities in the different regions/provinces participating in the project.

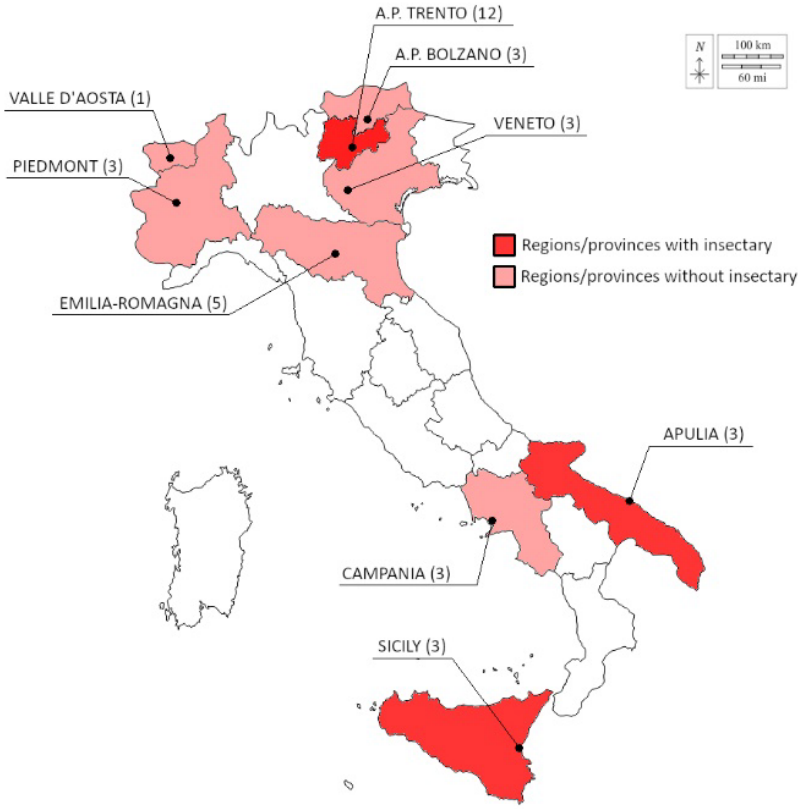

Figure 2. Map of the Italian regions/provinces involved in the *Drosophila suzukii* biocontrol project in 2021. The number of release sites for each regions/province is indicated in brackets. Regions/province without rearing facility (light red) received parasitoids from the insectary located at the Edmund Mach Foundation. AP = Autonomous Province.

BENEFIT-RISK ASSESSMENT FOR THE PETITION TO RELEASE *GANASPIS BRASILIENSIS* IN ITALY

In order to promote the CBC program on *D. suzukii* in Italy, a deepening document concerning the introduction risk assessment of the parasitic wasp *G. brasiliensis* in Italy was draughted and coordinated by an intense cooperation between EMF researchers and CREA. The dossier followed 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, vii) detailed map and characterization of vegetation in the proposed release sites, and viii) list of responsible persons.

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 several strains characterized by different levels of host specificity in quarantine laboratory and field studies. Molecular analysis based on the nucleotide sequences of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene and two nuclear DNA regions (ITS1 and ITS2) of sampled specimens proved a significant genetic differentiation of *G. brasiliensis* in five morphologically indistinguishable lineages (G1-G5) (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 assumption of parasitoid ecological specialization, European laboratories showed that the Japanese G1 lineage was the most specific to *D. suzukii,* preferring to attack 1st and 2nd instar 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 within a panel of 24 drosophilid hosts (Daane et al., 2021). A few studies were carried out on other lineages, even if they appear to be more generalist and widely distributed than the previous ones (Kimura and Suwito, 2012; Nomano et al., 2017). Taken together these results indicated *G. brasiliensis* as the most specialized larval parasitoid of *D. suzukii* and its G1 lineage the one that better fits with the requirements of classical biological control programmes in all regions invaded by *D. suzukii* (Girod et al., 2018b; Giorgini et al., 2019; Wang et al., 2019, 2020; Biondi et al., 2021; Daane et al., 2021).

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 and in other invaded countries. It is clear that the arrival of this pest has negatively affected the economic management of the attacked crop systems through the exclusion from the world trade of products coming from invaded countries, yield losses and increased production costs. In particular, the latter refer to increased insecticide applications in crops, resulting in greater chemical exposure for the whole biocoenosis and issues related to the maximum residues limits (MRLs) of pesticides in fruits (Tait et al., 2021).

The biological control services by *G. brasiliensis*, which ranges from 17.1% to more than 70% of efficient parasitism on *D. suzukii* in Asia (Daane et al., 2016; Girod et al., 2018b; Giorgini et al., 2019), will offer to IPM another tool to control SWD infestation in crops and in wild ecosystems. Fruit quality and environment will both benefit from the consequent reduction of chemical applications. From a social point of view, the success of a biological control program against SWD will favour the productivity and the constant growing of cherry and other small fruits production that represent the main economic resource for several small and medium-size Italian farms involved in the whole food supply chain (Antonova, 2010; Peano et al., 2017).

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 EMF provided parasitoids for all other regions/provinces except Sicily and Apulia, which established their own mass-rearing of *G. brasiliensis*. Periodic shipments of adult parasitoids were scheduled according with the productivity of the EMF insectary and the availability of fruit in the field in the different regions. For each shipment, adult parasitoids were aspirated into 50-mL 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 reduce the mechanical shocks due to manipulation and transport.

At the release sites, vials were hung on tree branches to allow the parasitoids to 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 Italy, open-field releases were undertaken in the provinces/regions during different times of the fruit seasons. All release sites were intensively monitored before and after the parasitoid release to determine the establishment, the spread and the parasitism rate of *G. brasiliensis* on *D. suzukii* and other drosophilids. The monitoring protocol consisted of a systematic sampling of fruits from plants that are recorded hosts of *D. suzukii* and other drosophilids (Figure 3B; Kenis et al., 2016). Fruit was collected from plants and on the ground, on both wild and cultivated plants. At each site, sampling interested a radius of 200 m from the release point, during a pre-set time of 1 man-hour.

Figure 3. A) Release of *Ganaspis brasiliensis* adults in the field; B) Fruit collected at one release site during the post-release monitoring activity (from Edmund Mach Foundation).

Collected fruits were categorized by geographic location, fruit characteristics (plant species, fruit colour), habitat (tree canopy vs. ground, commercial crops or non-crop landscape plants) and field management practices (e.g., insecticide applications, cover cropping, organic). At each site, between 10 and 100 fruits were collected for each category (e.g., on the trees or on the ground), depending on the availability of fruits, and incubated for at least 35 days at 22°C. Upon appearance, pupae of drosophilids were sorted and isolated in 1.5-mL plastic tubes with a streak of honey-water provided for emerging fly or adult wasps. Dead or unemerged puparia were dissected under a microscope to determine the presence or absence of recognizable immature parasitoid cadavers or pharate adult flies. 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, CBC 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 issues 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 a multiple season, such data will provide a baseline for granting authorization for releases in the further years, possibly increasing the number of release sites. Future perspectives of *D. suzukii* CBC should include its implementation within sustainable IPM programs. For this purpose, 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 and specific cropping conditions.

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