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Field Release of *Ganaspis brasiliensis* (Hymenoptera: Figitidae) for Biological Control of Spotted-wing Drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), in the Contiguous United States

Final Environmental Assessment, August 2021

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I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the insect *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae), specifically the G1-lineage of this insect. This organism would be used by the permit applicant for biological control of spotted-wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), in the contiguous United States.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities.

This environmental assessment (EA) was prepared to be consistent with USDA–APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of the parasitoid wasp, *G. brasiliensis,* to control SWD in the contiguous United States. A parasitoid is an insect whose immature stages (larvae and pupae) live as parasites that eventually kill their hosts (typically other insects). This EA considers a "no action" alternative and the potential effects of the proposed action. Notice of this EA was made available in the Federal Register on July 16, 2021 for a 30-day public comment period. Six comments were received on the EA by the close of the comment period. However, all six comments were in support of the proposed action.

The applicant's purpose for releasing *G. brasiliensis* is to reduce the severity of damage to small fruit crops from infestations of SWD in the United States. SWD is native to East Asia (Kanzawa, 1939; Hauser, 2011). It was first detected in California, Spain, and Italy in 2008 and has since established in most fruit growing regions in North America and Europe, as well as some South American countries (Walsh et al., 2011; Emiljanowicz et al., 2014; NAPIS, 2014; Asplen et al., 2015). SWD lays eggs inside ripening fruits, puncturing the fruit's skin with its unique saw-like ovipositor (a tubular organ through which a female insect deposits eggs) (Atallah et al., 2014). Feeding by SWD larvae results in the degradation of fruits, and the puncturing of the fruit skin may also provide a gateway for secondary bacterial and fungal infections (Hamby et al., 2012; Stewart et al., 2014). SWD is highly polyphagous, meaning it is able to develop in many economically important small fruit crops such as blackberries, blueberries, cherries, raspberries, and strawberries, as well more than 100 reported wild host plants (Lee et al., 2011; Lee et al., 2015; Poyet et al., 2015; Kenis et al., 2016; Leach et al., 2019b; Santoiemma et al., 2019). The value of just the five economically important small fruit crops impacted by SWD (blackberries, blueberries, cherries, raspberries, and strawberries) was $4.37 billion annually in 2012 (USDA-NASS, 2013). An effective and host-specific biological control agent for SWD can work to reduce the pest from crops and also from nearby non-crop habitats that act as pest reservoirs for reinvasion of the crop.

Current SWD control programs rely primarily on insecticides that target adult flies. Most of the existing SWD management options (discussed below) are expensive, temporary, have not been effective, and/or include non-target impacts. For these reasons, there is a need to identify and release an effective, host-specific biological control organism against SWD in the contiguous United States that can reduce SWD populations in non-crop habitats. This would reduce the number of SWD that migrate into susceptible crops and would thereby improve the effectiveness of other SWD control tools.

II. Alternatives

This section will explain the two alternatives available to PPBP: no action (no issuance of permits) and issuance of permits for environmental release of the G-1 lineage of *G. brasiliensis* into the contiguous United States. Although APHIS' alternatives are limited to a decision of whether to issue permits for release of *G. brasiliensis*, we describe other methods currently used to control SWD in the United States. Use of these control methods is not an APHIS decision, and their use is likely to continue whether or not PPBP issues permits for environmental release of *G. brasiliensis*.

The PPBP considered a third alternative but will not analyze it further. Under this third alternative, PPBP would issue permits for the field release of the G-1 strain of *G. brasiliensis*. The permits, however, would contain special provisions or requirements concerning release procedures or mitigating measures, such as limited releases of *G. brasiliensis* in the contiguous United States. There are no issues raised indicating that special provisions or requirements are necessary.

A. No Action

Under the no action alternative, the PPBP would not issue permits for the field release of G-1 lineage of *G. brasiliensis* for the control of SWD — the release of this biological control agent would not occur, and current methods to control SWD in the United States will continue. Use of these methods is likely to continue even if PPBP issues permits for release of *G. brasiliensis*. Presently, control of SWD in the United States is limited to chemical and cultural control methods.

Chemical Control

Pyrethroid, organophosphate, neonicotinoid, and spinosyn insecticides can be effective against SWD (Beers et al., 2011; Van Timmeren and Isaacs, 2013).

Cultural Control

Pruning plants to maintain an open canopy, increase sunlight, and reduce humidity will make plantings less attractive to SWD and will improve spray coverage (Cornell University, 2017). Sanitation by eliminating any fruit that has fallen on the ground and any infested fruit remaining on plants can reduce populations of SWD that might infest next year's crops or later-ripening varieties. Placing fine netting over whole plants or canes can keep SWD from attacking fruit on blueberries and other small fruit and possibly on branches of small cherry trees. Early harvest of

fruit as soon as it ripens can be important in reducing exposure of fruit to SWD (Caprile et al., 2011). Chilling berries immediately after harvest to
32º–33ºF will slow or stop the development of SWD larvae and eggs in the fruit.

B. Issue Permits for Environmental Release of *G. brasiliensis*

Under this alternative, PPBP would issue permits for the field release of the G1-lineage of *G. brasiliensis* for the control of SWD in the contiguous United States. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures. The G1-lineage of *G. brasiliensis* from Japan, China, and South Korea is specific to SWD.

1. *Ganaspis brasiliensis* Taxonomic Information

Insect Taxonomy

Order:	Hymenoptera
Family:	Figitidae
Genus:	*Ganaspis*
Species:	*brasiliensis* (Ihering)
Common name:	none

Voucher specimens of *G. brasiliensis* were deposited with the U.S. National Entomological Collection (Washington, D. C.). Additionally, voucher specimens were sent to the Canadian National Collection of Insects, Arachnids and Nematodes (Agriculture and Agri-Food Canada/Agriculture et Agroalimentaire Canada) in Ottawa, Canada, and the Collección Nacional de Insectos (Instituto de Biologia, UNAM) in Mexico.

2. Molecular Analysis of *Ganaspis brasiliensis* lineage

Molecular analysis is a laboratory procedure that involves the study of tissues, cells, and fluids using DNA/RNA analysis techniques for the identification of characteristics at the molecular level. Molecular analyses have been used to differentiate strains of *G. brasiliensis* by sequencing a specific gene (the COI gene) that occurs within the mitochondria of its cells. Mitochondria are organelles within a cell that produce energy for the cell.

Recent work suggests that *G. brasiliensis* can be subdivided into five lineages (Nomano et al., 2017). These include the G1 and G3 lineages reared from SWD in South Korea (Daane et al., 2016); SWD and a similar species *Drosophila pulchrella* in China (Giorgini et al., 2019); and SWD in China and Japan (Girod et al., 2018b; Girod et al., 2018a). Based on host-specificity testing that will be discussed later in this document, the G1-lineage of *G. brasiliensis* appears to be the most specific to SWD.

3. Biology of *Ganaspis brasiliensis*

Ganaspis brasiliensis is a solitary larval parasitoid wasp of SWD. The adult wasp inserts its ovipositor and lays an egg into SWD larvae that are within fruit. *Ganaspis brasiliensis* prefers to attack young first instar SWD larvae. The *G. brasiliensis* egg hatches inside the SWD larva, and over time, eventually consumes the SWD host as it progresses through its prepupal and pupal stages. A single adult male or female emerges from the host puparium (the hardened last larval skin which encloses the pupa). In tests, at 23°C (74°F), *G. brasiliensis* adult females lived 18 days and produced 98 offspring per female.

4. Geographic Range of *Ganaspis brasiliensis*

All lineages of *G. brasiliensis* occur in Asia, but the G1-lineage has been recorded only from East Asia (China, Japan, and South Korea) and not outside of Asia. Historical specimens of *G. brasiliensis* (found in the National Museum of Natural History, Smithsonian Institution, Washington DC, and in the Natural History Museum, Paris, France) were all collected in the Caribbean and in Panama, whereas the newly collected specimens were all collected in Japan, South Korea and China. Recent reexamination confirmed some specimens previously reported as *Ganaspis xanthopoda* or *Ganaspis* sp. in the literature in Indonesia, Malaysia, Thailand, the Philippines, Hawaii, Uganda, Benin, and Brazil are *G. brasiliensis* (Carton et al., 1986; Schilthuizen et al., 1998; Kacsoh and Schlenke, 2012; Kimura and Suwito, 2012; 2015; Buffington and Forshage, 2016; Daane et al., 2016; Nomano et al., 2017; Girod et al., 2018b; Giorgini et al., 2019). *G. brasiliensis* was most likely introduced from Asia to other continents (Buffington and Forshage, 2016; Nomano et al., 2017).

5. Potential Range of *Ganaspis brasiliensis* in North America

A CLIMEX model was developed to predict the potential geographical range of *G. brasiliensis* in North America based on the current known distribution of the G1-lineage in Asia (Figure 1), including geographical coordinates of 37 collection sites where the G1-lineage of *G. brasiliensis* was obtained in China, South Korea and Japan (Kasuya et al., 2013; Daane et al., 2016; Nomano et al., 2017; Giorgini et al., 2019). The model uses weather variables to determine the estimated potential range of *G. brasiliensis* based on those weather variables in the known distribution of the G1-lineage in East Asia.

If released in North America, the G-1 lineage of *G. brasiliensis* would likely establish along western coastal zones and much of the southeastern and east coastal states where SWD is a major concern of small fruit crops. At present, the parasitoid is not considered capable of entering diapause during the winter, but if it does, the predicted range would further expand to include northeast regions. Diapause is a period of suspended development during an insect's life cycle, and is usually triggered by environmental cues, like changes in daylight, temperature, or food availability.

6. Impact of *Ganaspis brasiliensis* on Spotted-wing Drosophila

Girod et al. (2018c) showed *G. brasiliensis* to be one of the most important parasitoids of SWD in Asia, with parasitism levels ranging from 0–75.6 percent.

III. Affected Environment

A. Spotted-wing Drosophila

1. Spotted-wing Drosophila Taxonomic Information

Insect Taxonomy

Order:	Diptera
Family:	Drosophilidae
Genus:	*Drosophila*
Species:	*suzukii* Matsumura
Common name:	Spotted-wing drosophila

SWD can be distinguished from other drosophilids in North America by two key characters: (1) a dark spot on the leading wing edge of males, and (2) a large serrated ovipositor in females. The pest is also identified by its oviposition in and damage to ripening fruit in North America and Europe (Hauser, 2011; Cini et al., 2012; Atallah et al., 2014).

2. Life History of Spotted-wing Drosophila

During the fruit-growing season, SWD population development is largely based on temperature and food availability. Development from egg to adult takes about 7 days at 21.1°C and 13 days at 18.3°C. At constant temperatures, female adult longevity ranged from 2–35 days at 30°C and 10°C, respectively (Tochen et al., 2014). In the United States and Canada, SWD can have 3–9 generations per year (Walsh et al., 2011). During the summer in California, for example, SWD eggs hatched in 1–3 days, larvae matured in 3–13 days, and the pupal period was as short as 4 days (Wang et al., 2016a). Added to the rapid development time is a high reproductive output. Egg-laying can last from 10–65 days, with up to 21 eggs laid per day and each female can deposit up to 600 eggs during her lifetime (Tochen et al., 2014). Eggs are laid in ripening fruits, often with multiple eggs per fruit (Mitsui and Kimura, 2010).

SWD appears to overwinter as adults in temperate regions (Kanzawa, 1939; Dalton et al., 2011; Stockton et al., 2019). Mitsui et al. (2007) reported that SWD collected in autumn were reproductively immature, suggesting a winter reproductive diapause. However, SWD still oviposited during the winter when the temperature was high enough (Kaçar et al., 2016; Leach et al., 2019a). Overwintering adults lived for more than three months when supplied with food, which was often readily available from old or damaged fruit (Kaçar et al., 2016) or tree sap (Kanzawa, 1939; Lee et al., 2011). The low lethal temperatures for SWD were reported as -0.9°C (Kimura, 2004); however, the fly also occurs in colder regions, such as on the northern islands of Japan and in the northern tier of the United States and into Canada where average winter temperatures can fall below 0°C. It is still unclear whether individuals overwinter in warmer refuges (e.g., near heated buildings) or migrate to warmer southern regions to overwinter (Kimura, 2004). In warm regions such as California, SWD pupae (but not eggs or larvae) were shown to slowly develop during the winter and emerge as adults, although there was high mortality (Kaçar et al., 2016).

The termination of overwintering and the onset of dispersal and reproduction may also be temperature dependent. Adult SWD become mobile above 5°C and are most active from 20 to 25°C (Kinjo et al., 2014; Tochen et al., 2014). Little or no reproductive behavior was observed below 10°C or above 30°C, and mated females exposed to temperatures above 31°C laid infertile eggs. After the overwintering period, SWD disperse 100 meters (328 feet) or more in a one-week period to find appropriate food and breeding sources (Klick et al., 2016), and it has been demonstrated that they seasonally migrate several hundred meters in elevation to move from resource-poor to resource-rich or environmentally suitable conditions (Mitsui and Kimura, 2010; Tait et al., 2018).

B. Areas Affected by Spotted-wing Drosophila

1. Native and Worldwide Distribution

SWD is native to East Asia, including China, Japan, and Korea (Asplen et al., 2015), although its precise geographical origin is not known. According to references reported by Hauser (2011) the species may not be native to Japan and could have been introduced into the country at the turn of the century. It has also been reported in other Asian regions, including northern India and Pakistan, Bangladesh, Myanmar, Thailand, and Taiwan. The fly has been found in more than 10 countries in Europe since first being reported in 2008 in Spain and Italy (Cini et al., 2014). Most recently, it was reported in South America; the first record occurred in Brazil in 2013 and it has since been recorded in Uruguay, Argentina, and Chile (Andreazza et al., 2017) (Figure 1A). Habitat suitability models predict that SWD will continue to expand its range (dos Santos et al., 2017) (Figure 1B).

2. Present Distribution in North America

In North America, SWD was first recorded on the island of Hawaii in 1980 and later on other islands in the Hawaiian island archipelago (Hauser, 2011). In the contiguous United States, the fly was first detected in California in 2008 (Bolda et al., 2008) and has since been found in 45 states (in most fruit growing regions) as well as in five provinces in Canada and part of northern Mexico (Asplen et al., 2015). Genetic analyses suggest that it was a Southeast Asian population that invaded the contiguous United States and Europe (Ometto et al., 2013).

3. Spotted-winged Drosophila Hosts

SWD attacks many hosts, being able to develop in many economically important small fruit crops such as blackberries, blueberries, cherries, raspberries, and strawberries, as well more than100 reported wild host plants (Lee et al., 2011; Lee et al., 2015; Poyet et al., 2015; Kenis et al., 2016; Leach et al., 2019b; Santoiemma et al., 2019).

C. Insects Related to Spotted-wing Drosophila and *G. brasiliensis* in the United States

1. Insects Related to Spotted-wing Drosophila

Information regarding insects taxonomically related to SWD is included because closely related insect species have the greatest potential for attack by *G. brasiliensis*.

SWD is a member of the *Drosophila suzukii* subgroup within the *Drosophila melanogaster* species group of the subgenus Sophophora and the genus *Drosophila* (Diptera: Drosophilidae). There are 12 species in the *D. suzukii* subgroup and 180 species in the *D. melanogaster* species group worldwide (Markow and Ogrady, 2006). The majority of *Drosophila* breed on decaying substrates.

Closely related species to SWD occurring in the United States are *D. subpulchrella*, *D. pulchrella*, as well as the related *D. melanogaster* and *D. simulans* (all in the *D. melanogaster* group). Whereas the origin of SWD, *D. subpulchrella,* and *D. pulchrella* is East Asia, *D. melanogaster* and *D. simulans* are thought to be African in origin (Nolte and Schlotterer, 2008). *D. melanogaster* rose to prominence after it was imported into the United States when it was used for genetic studies (Stephenson and Metcalfe, 2013). The value of *D. melanogaster* and *D. simulans* has been primarily in the laboratory, studying both their biology and genetics (Jennings, 2011); outside of the laboratory both species are common nuisance pests.

2. Insects Related to *Ganaspis brasiliensis*

In North America, there are several common parasitoids of *Drosophila* that are in the same family as *G. brasiliensis* (Figitidae) including *Leptopilina boulardi*, *L. heterotoma,* and *Ganaspis* sp. (Hertlein, 1986; Kacsoh and Schlenke, 2012). The G5-lineage of *G. brasiliensis* is present in Hawaii (Nomano et al., 2017). The G5-lineage has been reported to attack SWD, and a Hawaiian population was reared from SWD, but other G5-lineage populations could not successfully develop from SWD (Kacsoh and Schlenke, 2012). The G5-lineage is considered to be a generalist and is not wanted in North America (although it is currently found in central Mexico).

IV. Environmental Consequences

A. No Action

1. Impact of Spotted-wing Drosophila on the Environment

a. Effect of spotted-wing Drosophila on Hosts

SWD has become a devastating pest of soft-skinned fruits including blueberries, blackberries, raspberries, strawberries, and cherries, which have a combined value of over $4.37 billion annually (USDA-NASS, 2013). National crop loss due solely to SWD in the United States was estimated at $718 million annually, and increased costs directly related to management practices are estimated to range from $129 to 172 million annually. Observed crop losses due to SWD in the western United States have been as high as 80 percent (Bolda et al., 2010). Early SWD annual damage in the western United States production areas was estimated at up to $500 million (Goodhue et al., 2011). A more recent estimate of the potential national crop loss was over $1.275 billion (Burrack, 2015). These crop losses are due to a near zero tolerance for SWD-infested fruit for fresh market or whole frozen products. This means that detection of even a single larva in a shipment could lead to complete rejection, motivating growers to be very conservative in their SWD management practices. Insecticide applications are made preventatively (ahead of SWD damage), and as frequently as every 5–10 days from the point at which fruit begins to ripen through to the end of harvest. The risk of crop loss from SWD increases over the course of the growing season due to increasing population pressure. For example, late season sweet cherries may require 11 additional pesticide applications to produce a marketable crop, and late season blueberry, blackberry, and raspberry may require up to 16 pesticide applications.

Insecticide evaluations in blueberry and raspberry indicate a decline in residual performance within 5–10 days, depending on the insecticide, and rainfall further reduces insecticide residual activity (Van Timmeren and Isaacs, 2013). Therefore, even intense insecticide programs have been observed to fail in adverse environmental conditions.

2. Impact from the Use of Other Control Methods

The continued use of chemical and cultural controls at current levels would result if the “no action” alternative is chosen, and may continue even if permits are issued for environmental release of *G. brasiliensis.*

a. Chemical Control

Insecticides can be effective (Beers et al., 2011; Van Timmeren and Isaacs, 2013), but there is a restricted list of acceptable materials and fruit residues present problems for human health (Rodriguez-Saona et al., 2019). Insecticide applications may also negatively impact beneficial organisms (Biondi et al., 2012), and growers have increasingly observed secondary pest outbreaks of scale insects and other pests in Oregon blueberry fields (Lee et al., 2019). More concerning is possible future limitations due to insecticide resistance (Gress and Zalom, 2019).)

b. Cultural Control

Cultural control can be useful in reducing SWD damage, but alone is not effective in eliminating SWD.

These impacts from the use of other control methods may have environmental consequences even with the implementation of the biological control alternative, depending on the efficacy of the G-1 lineage of *G. brasiliensis* to reduce SWD infestations in the contiguous United States.

B. Issue Permits for Environmental Release of *G. brasiliensis*

1. Impact of *G. brasiliensis* on Non-target Insects

a. Scientific Literature

The G1-lineage of *G. brasiliensis* was collected only from SWD or *Drosophila pulchrella* in various locations in South Korea and China, and only from SWD in Japan (Table 1). In China, a mixture of the two lineages of *G. brasiliensis* (G1 and G3) were collected from four different wild fruits infested by SWD or *D. pulchrella*. In Japan, a G1 population was collected from SWD-infested cherries (Kasuya et al., 2013) and is considered a specialist exclusively on SWD (Nomano et al., 2017; Girod et al., 2018a). There is only limited information on naturally occurring hosts for *G. brasiliensis* G2, G4, or G5 lineages. In laboratory tests, G1 attacked SWD and only closely related species and G3 attacked several other tested hosts (Kasuya et al., 2013).

Table 1. Known host records of different lineages of *G. brasiliensis*.

Lineage	Host	Origin	Field or Laboratory Test	Reference
G1	*D. suzukii* (SWD)	South Korea	Field collection	Daane et al., 2016
	D. suzukii (SWD)	South Korea	Laboratory test	Wang et al., 2018
	D. suzukii (SWD)	Japan	Field collection	Kasuya et al., 2013
	D. suzukii (SWD)	Japan	Laboratory test	Girod et al., 2018a
	D. suzukii (SWD), *D. pulchrella*	China	Field collection	Giorgini et al., 2019
	D. suzukii (SWD)	China	Laboratory test	Daane et al., 2016
G2	*D. ficusphila*	Japan	Field collection	Kimura and Suwito, 2012
G3	*D. lutescens*	Japan	Field collection	Kasuya et al., 2013
	D. lutescens, *D. rufa*, *D. auraria*, *D. biauraria* and *D. triauraria*	Japan	Laboratory test	Kasuya et al., 2013
	D. suzukii (SWD), *D. pulchrella,*	China	Laboratory test	Giorgini et al., 2019
	D. suzukii (SWD)	S. Korea	Field collection	Daane et al., 2016
	Drosophila sp. aff. *takahashii*	Malaysia, Indonesia	Field collection	Nomano et al., 2017
G4	*D. eugracilis*	Japan	Laboratory test	Kimura and Suwito, 2012
	Unknown host	Japan, Taiwan	Field collection	Nomano et al., 2017
G5	*D. simulans*	Japan	Laboratory test	Nomano et al., 2017
	D. melanogaster	Hawaii	Laboratory test	Kacsoh and Schlenke, 2012
	D. melanogaster	Uganda	Laboratory test	Kacsoh and Schlenke, 2012

b. Host Specificity Testing

Worldwide, there are over 2,000 described *Drosophila* species whose distributions range from narrowly restricted areas to wide distributions (Markow and Ogrady, 2006). Species may breed exclusively in resources such as fruits, flowers, mushrooms, leaves, tree fluxes, and soil, but most breed on decaying substrates. The family Drosophilidae consists of two subfamilies of Steganinae and Drosophilinae. Steganinae is a small and poorly understood subfamily, while the Drosophilinae consists of eight genera: *Chymomyza*, *Drosophila*, *Hirtodrosophila*, *Liodrosophila*, *Samoaia*, *Scaptodrosophila*, *Scaptomyza* and *Zaprionus*. Among them, *Drosophila* is the largest group with well over 1,500 described species, and the subgenera of *Drosophila* and *Sophophora* account for 90 percent of these species. The genus of *Chymomyza* has about 60 described species, the majority of which are found in the tropics. *Hirtodrosophila* is a cosmopolitan genus (its range extends throughout most of the world), but its highest diversity is concentrated in the tropics. *Liodrosophila* is a small genus of 65 known species from Africa, Asia, and Europe. *Samoaia* is a small genus of seven described species; all are endemic to the islands of Samoa. *Scaptodrosophila* was initially placed in *Drosophila* and about half of the described species are endemic to Australia. *Scaptomyza* is a very poorly studied taxon and several studies strongly suggest placement of *Scaptomyza* in the Hawaiian Drosophilidae endemic to the Hawaiian island archipelago. *Zaprionus* contains about 30 described species including *Z. indianus* (a newly invasive pest and one of the only pest drosophilid species besides SWD in North America).

It would be difficult, if not impossible, to test the majority of non-target species native to North America. However, species that are closely related are more likely to be attacked by the same parasitoid species because they are likely to share characteristics that determine their suitability as hosts. Thus, the researchers representatively selected 24 species from different groups. The 24 selected species represented two subfamilies, 7 genera, 9 subgenera and 20 species groups, with 22 species originally collected from 11 states in the mainland United States, one from American Samoa and one from Japan (Table 2). Except *Gitona americana* (subfamily Steganinae), all other species belong to the subfamily Drosophilinae. The Japanese species *Scaptomyza elmoi* was selected as a surrogate for the Hawaiian Drosophilidae in host specificity studies because of its close relation to those species.

Table 2. Non-target species tested with *G. brasiliensis* (Daane, 2019).

Genus	Subgenus	Group	Species	Habitat
Drosophila	*Sophophora*	*willistoni*	*willistoni*	Fruits
Drosophila	*Sophophora*	*saltans*	*sturtevanti*	Unknown
Drosophila	*Sophophora*	*melanogaster*	*simulans*	Fruits
Drosophila	*Sophophora*	*melanogaster*	*melanogaster*	Fruits and flowers
Drosophila	*Sophophora*	*melanogaster*	*suzukii*	Fruits
Drosophila	*Sophophora*	*obscura*	*persimilis*	Fruits
Drosophila	*Sophophora*	*obscura*	*pseudoobscura*	Infected fruits
Drosophila	*Sophophora*	*obscura*	*subobscura*	Fruits
Drosophila	*Drosophila*	*busckii*	*busckii*	Various niches
Drosophila	*Drosophila*	*repleta*	*hydei*	Decaying plants
Drosophila	*Drosophila*	*virilis*	*montana*	Decaying woody tissue
Drosophila	*Drosophila*	*melanica*	*paramelanica*	Unknown

Genus	Subgenus	Group	Species	Habitat
Drosophila	*Drosophila*	*robusta*	*robusta*	Yeast and bacteria infested saps
Drosophila	*Drosophila*	*immigrans*	*immigrans*	Wood, fruits, and decaying plants
Drosophila	*Drosophila*	*guttifera*	*guttifera*	Unknown
Drosophila	*Drosophila*	*cardini*	*cardini*	Unknown
Drosophila	*Drosophila*	*funebris*	*funebris*	Unknown
Drosophila	*Drosophila*	*tripunctata*	*tripunctata*	Mushroom and fruits
Drosophila	*Drosophila*	*testacea*	*putrida*	Mushroom
Chymomyza		*fuscimana*	*amoena*	Fruits
Scaptodrosophila	*Scaptodrosophila*	*victoria*	*lebanonensis*	Unknown
Hirtodrosophila		*duncani*	*duncani*	Unknown
Samoaia	*Samoaia*	*leonensis*	*leonensis*	Unknown
Scaptomyza			*elmoi*	Unknown
Gitona			*americana*	Fruits

Ganaspis brasiliensis was tested against each of the 24 selected non-target fly species and SWD. For tests of each fly species, twenty 2-day-old fly larvae were placed in a ventilated plastic vial with artificial diet and exposed to one mated female *G. brasiliensis* wasp for 24 hours. Exposed fly larvae were reared until the emergence of flies and wasps. Controls with unexposed fly larvae were also setup under the same conditions until the emergence of adult flies. Each test consisted of 30 replicates (i.e., 30 female wasps were tested for each species) with 10 control replicates. Tests with SWD were conducted under the same conditions using the same batches of parasitoids and served as a positive control for the test of non-target hosts. Some *Drosophila* species larvae are able to defend themselves from parasitoid eggs placed inside their bodies by surrounding the egg with blood cells that eventually form a black capsule surrounding the egg, resulting in death of the immature parasitoid. These capsules are visible in developed adult flies (Chabert et al., 2012). Therefore, all emerged flies were also examined for the presence of a black capsule inside the fly's abdomen to determine the percentage of flies containing black capsules. The number of emerged flies and parasitoids were counted for each replicate.

Summary of host specificity results.

The G1-lineage of *G. brasiliensis* was able to develop mainly from three closely related species, SWD, *D. simulans*, and *D. melanogaster*, which all belong to the *D. melanogaster* species group (Daane, 2019). Although both *D. melanogaster* and *D. simulans* are physiologically suitable hosts for *G. brasiliensis*, these drosophilids typically infest overripe or rotting fruits. In contrast, SWD exploits ripening fruit before they are available to *D. melanogaster* or *D. simulans*. In addition, both are cosmopolitan species and neither is native to the United States.

1. Impact of *G. brasiliensis* on Spotted-wing Drosophila

SWD is generally less of a crop pest in Asia than in the United States. In South Korea, for example, it has never been considered a pest based on the lack of any reported SWD-related damage (Asplen et al., 2015). Thus, the impact of natural enemies in its native range may play an important role in limiting the pest status of SWD, although the actual impact of parasitoids in controlling SWD in Asia has not been evaluated. Based on samples of wild host fruits in South

Korea, percentage parasitism of SWD by larval parasitoids was up to 17.1%. In China, SWD parasitism by the two parasitoids *Leptopilina japonica* and *G. brasiliensis* was over 20 percent in most collected sites but was up to 73.5 percent at one location (Giorgini et al., 2019). Girod et al. (2018c) similarly showed *G. brasiliensis* to be one of the most important parasitoids in Asia, with parasitism levels ranging from 0–75.6 percent. Currently, the estimated SWD parasitism by resident parasitoids in the United States is about 2 percent (Miller et al., 2015). Population model analyses showed that at the 2 percent parasitism level, SWD populations were reduced by approximately 1–2 percent at the end of the growing season. At 15 percent parasitism, SWD populations were reduced by 10–21 percent compared with populations not affected by parasitism (Wiman et al., 2016).

It is expected that *G. brasiliensis* will be able to establish in a wide range of fruit growing regions in the contiguous United States, given its wide distribution in South Korea, Japan, and China (Daane, 2019). The establishment of this introduced larval parasitoid will uniquely contribute to the suppression of SWD, because it attacks fly maggots inside the fruit (while most pesticides target only adult flies and only the two pupal resident parasitoids readily attack SWD). Classical biological control is a potentially useful management strategy for an invasive pest species whenever effective resident natural enemies are lacking in the new distribution range. Biological control is a self-perpetuating control option and the only practical approach for reducing established pest populations in non-crop habitats and natural settings where more intensive management methods are very expensive and/or environmentally undesirable.

2. Impact on Human and Animal Health

Ganaspis brasiliensis is a tiny, stingless wasp. Like all parasitic wasps, the immature stages develop as parasitoids of arthropods where, in this case, feeding of the wasp larva inside the host SWD eventually kills it. This insect poses no risk to humans, livestock, or wildlife.

3. Uncertainties Regarding the Environmental Release of *Ganaspis brasiliensis*

Once a biological control agent such as *G. brasiliensis* is released into the environment and becomes established, there is a possibility it could move from the target insect (SWD) to attack nontarget insects. Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other insect species were to be attacked by *G. brasiliensis*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *G. brasiliensis* generally spread without intervention by man. In principle, therefore, release of this parasitoid at even one site should be considered equivalent to release over the entire area in which potential hosts occur and in which the climate is suitable for reproduction and survival.

In addition, these agents may not be successful in reducing SWD populations in the contiguous United States. Approximately 12 percent of all parasitoid introductions have led to significant sustained control of the target pests, but the majority of introductions have failed to provide control of the pest (Greathead and Greathead, 1992) either because introduction did not lead to establishment or establishment did not lead to control (Lane et al., 1999).

Actual impacts on SWD populations by the G-1 lineage of *G. brasiliensis* will not be known until after release and establishment occurs. Monitoring will be conducted by the permittee to determine the establishment of *G. brasiliensis* (Appendix A). The environmental consequences discussed under the no action alternative may occur even with the implementation of the action alternative, depending on the efficacy of the G-1 lineage of *G. brasiliensis* to reduce SWD in the contiguous United States.

4. Cumulative Impacts

"Cumulative impacts are defined as the impacts on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Release of the G-1 lineage of *G. brasiliensis* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to SWD. Effective biological control from introduced *G. brasiliensis* may not only provide safe, effective, and long-term control of SWD, but the parasitoid may also result in reduced use of insecticides against SWD.

No other agents have been released in the contiguous United States for biological control of SWD; therefore, no competitive interactions between agents are expected. Release of *G. brasiliensis* would not affect the ability of growers to continue to control SWD using current methods.

5. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species, or result in the destruction or adverse modification of critical habitat.

APHIS has determined that, based on the host specificity of the G-1 lineage of *G. brasiliensis,* there will be no effect on any listed species or designated critical habitat in the contiguous United States. In host specificity testing, the G-1 lineage of *G. brasiliensis* only attacked three *Drosophila* species. Although there are federally listed *Drosophila* species that occur in Hawaii, *G. brasiliensis* would not be permitted for release in Hawaii. There are a number of described species of figitids from Hawaii and neighboring islands that are strains that are not specialized on SWD. The G1- lineage of *G. brasiliensis* that is proposed for release in the contiguous United States is a specialist on SWD or on SWD and closely related species, respectively, and pose no risk to native Hawaiian drosophilids. There is greater risk of other, less specialized figitids arriving in Hawaii from Asia, such as the *G. brasiliensis* G5-lineage which is already in Hawaii. There are no federally listed species are known to depend on or use SWD.

V. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects anticipated from the field release of the G-1 lineage of *G. brasileinsis* and its release will not have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, "Protection of Children From Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. There are no circumstances that would trigger the need for special environmental reviews involved in implementing the preferred alternative. Therefore, there are no disproportionate effects on children anticipated because of the field release of the G-1 lineage of *G. brasiliensis*.

EO 13175, "Consultation and Coordination with Indian Tribal Governments", was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications…." Consistent with EO 13175, APHIS will continue to consult and collaborate with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests, in accordance with EO 13175.

VI. Agencies, Organizations, and Individuals Consulted

This EA was prepared and reviewed by personnel from APHIS and University of California, Berkeley. The addresses of participating APHIS units and any applicable cooperators are provided below.

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Appendix A. Release and Post-release monitoring

(From: Daane, 2019)

A. RELEASE

Location of rearing/containment facility and name of person operating the facility
The parasitoid cultures of the G-1 lineage of *G. brasiliensis* that are being considered for release are held at the University of California Berkeley's Insectary & Quarantine Facility. Backup colonies are also being held at the USDA Agricultural Research Service, Beneficial Insects Introduction Research Unit in Newark, Delaware, and the USDA Agricultural Research Service, Western Regional Research Center, Invasive Species and Pollinator in Albany, California.

Timing of the release
Adults of *G. brasiliensis* will be released yearly from April through October, when SWD populations, including larvae, are commonly high (Arnó et al., 2016; Klick et al., 2016; Wang et al., 2016b). Releases will start following release permit issuance by APHIS, and then continue for five years, during which time the parasitoid's impact will be monitored.

Location of initial releases
Quarantine evaluations alone may not adequately describe a natural enemy's field performance (Babendreier et al., 2005; Messing and Wright, 2006). For this reason, field cage trials in controlled crop systems (cherries, blueberries and strawberries) will be conducted the first year at different field stations (e.g., University of California's Kearney Agricultural Research and Extension Centerin Parlier, California), as well as select commercial farms (e.g., Driscoll's and Naturipe Berry Growers, Inc.). These initial cage trials will validate field performance and can be used to facilitate establishment and build colony numbers and vigor. Moreover, releasing in North America's wide-ranging geographic regions where SWD is a pest will help establish field performance under different climates.

Initial open field releases will begin in the second year after permit approval and will be conducted in major cherry, blackberry, strawberry, and blueberry production regions in North America (co-Applicants represent CA, GA, ME, MI, NY, OR, NC and WA). The researchers will also develop regional insectaries to facilitate parasitoid production and release. The regional insectaries will be established with different state agencies (e.g., California Department of Food and Agriculture) for mass-rearing of the G-1 strain of *G. brasiliensis* while improving delivery methodologies.

B. MONITORING

Agent establishment and spread
All release sites will be intensively monitored to determine the establishment and spread of the released parasitoid, as well as seasonal patterns of parasitism of SWD by *G. brasiliensis*. Two different methods will be used to determine if *G. brasiliensis* has established at the sites where

we anticipate releasing parasitoids: (1) sentinel traps baited with SWD-infested fruit and (2) direct sampling of infested fruits.

The permittee will use different host fruits (e.g., cherry, various berries, based on the fruit season) that are infested with SWD as baits for the traps. The fruit will be infested with SWD 1-2 d before they are used for the trapping, so that they contain young SWD larvae. Traps will be placed in a variety of different orchards and unmanaged habitats where host plants of SWD are present, in regions where the parasitoid has been released. Traps will be made of clear plastic containers that have ten 0.8 cm diameter holes punctured in the side for ventilation and to allow flies and parasitoids to enter (but prevent entry by large insects, birds or rodents). Traps will be hung on the host fruit plant by a metal wire that is attached to the container lid. A waxed paper roof structure (a Trécé Pherocon trap cover) will be installed to protect the bait from rain or direct sunlight, as direct sunlight could affect the parasitoids' foraging behavior. A ring of Tanglefoot will be streaked on the wire above the roof as a barrier for foraging ants. Traps will be left in the field for one or two weeks (depending on seasonal temperatures) in order to have different stages of flies available as hosts to maximize the number of potential parasitoid species that are collected. The baited fruit will be collected and processed. Parasitism will also be estimated.

At each of the release sites, field collections of SWD-infested fruits from trees or the ground will be made during the entire fruiting season (from first fruit color change to harvest or after the harvest) if fruit is still available. Collected fruit will be categorized by geographic location, fruit characteristics (plant species, fruit color, brix, firmness, weight), habitat (tree vs. ground, commercial crops or non-crop landscape plants) and field management practices (e.g., insecticide applications, cover cropping) and then processed. About 10–100 fruits will be collected at each site for each category (e.g., on trees or the ground), depending on the availability of fruit.

For field releases, methodologies will be used similar to those we successfully used for the establishment of parasitoids for olive fruit fly control in California (Wang et al., 2011, Daane et al., 2015). The permittee will also continue to study field-released parasitoids to gain a better understanding of biotic and abiotic parameters that may affect field parasitism rates in order to optimize parasitoid release and establishment (Wang et al., 2011). For these studies, cages will be used in different crops and during multiple seasons to encompass biotic and abiotic factors (e.g. crop variety and climatic conditions) that may affect the performance of *G. brasiliensis*. Fruit branches with 10–30 fruits (depending on fruit species) will be isolated in a fine-screened cage, with a cardboard roof to block direct sunlight and a ring of Tanglefoot at the base to block foraging ants and other walking predators.

To precisely estimate parasitism rates, uninfested fruit will be isolated before fruit become susceptible to SWD in the field. Five gravid female SWD will be released into each cage to establish the fly population. These cages will be checked to determine the level of fruit infestation, and adult flies will be removed when each fruit contains 3–5 eggs. Five mated female parasitoids will be released into each cage when first to second instar SWD are available, as these are the preferred host stage for oviposition by this parasitoid species (Sime et al., 2006; Daane et al., 2008). After a 3 to 4 day exposure period, the cages will be removed, the number of live parasitoids (F1) recorded, and the fruit will be taken to the laboratory for emergence of parasitoid offspring (F2) and flies. Collected fruit will be placed in plastic containers (11×11 cm) covered with organdy cloth and fitted with a raised metal grid (2 cm high) on the bottom. The metal grid creates an air space below the fruit that prevents mold formation and allows pre-pupal flies to drop from the fruit and through the grid to the bottom of the container where they can be easily collected (Wang et al., 2011). For each test, fruit will be dissected and any dead or unemerged puparia found will be dissected under a microscope to determine the presence or absence of recognizable immature parasitoid cadavers and pharate adults. Parasitism will be estimated based on the number of emerged and dissected wasps and flies, while host density will be estimated based on the total fly puparia. This design will be used in each trial, with 10 replicates (cages) per trial. The permittee will examine the effects of abiotic or biotic factors (e.g., season, fruit species or variety, fruit stage and size, and host density) on the effectiveness of the parasitoids.

For the open field release, adult female parasitoids will be pre-fed with honey water and then aspirated into vials until each vial contains 20 females and 10 males. A piece of moist tissue paper will be placed on the bottom of the vial to provide water and honey will be streaked on the vial lid. At the release sites, vials typically will be hung on tree branches such that the parasitoids can walk or fly onto the trees. Open releases will be conducted in different regions during different fruit seasons in California and other states. The number of wasps released at each site will depend on the availability of the wasps and site size.

References cited in this appendix are included in VII. References.