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Entomology/ Review

Biological control of fruit flies in Brazil




Abstract – Fruit flies are the main pests of fruit growing in Brazil. They have been managed predominantly with the use of insecticides applied as cover spray and/or as toxic baits. Currently, the trend of management strategies is toward the adoption of methods that cause the lowest environmental impact in large areas. In this context, biological control is an excellent option to be used together with other management strategies, such as sterile insects, because it leaves no residues, does not disturb nontarget pests, and can be permanent if the natural enemy establishes itself in the field. This review paper addresses the current knowledge on the biological control of fruit flies in Brazil, highlighting the great biodiversity of its natural enemies, especially parasitoids, its biology and ecology. The classical biological control programs in Brazil are also reported, from the introduction of *Tetrastichus giffardianus* (Hymenoptera: Eulophidae), in 1937, for the control of *Ceratitidis capitata* (Diptera: Tephritidae), to that of *Fopius arisanus* (Hymenoptera: Braconidae), in 2012, for the control of *Bactrocera carambolae* (Diptera: Tephritidae). Finally, the obtained advances are pointed out, as well as the main bottlenecks and perspectives for the effective use of biological control programs against fruit flies.

Index terms: biological control programs, Braconidae, classical biological control, Diptera, Figitidae, natural biological control.

Controle biológico de moscas-das-frutas no Brasil

Resumo – As moscas-das-frutas são as principais pragas da fruticultura no Brasil. O seu manejo tem sido realizado predominantemente com uso de inseticidas aplicados por cobertura e/ou na forma de isca-tóxica. Atualmente, a tendência das estratégias de manejo está direcionada à adoção de métodos de controle que causem menor impacto ambiental em grandes áreas. Neste contexto, o controle biológico é uma excelente opção para uso em conjunto com outras estratégias de manejo, como insetos estéreis, uma vez que não deixa resíduos, não atinge pragas não alvo e pode ser permanente se o inimigo natural se estabelecer em campo. Este artigo de revisão aborda o conhecimento atual sobre o controle biológico de moscas-das-frutas no Brasil, com destaque para a grande biodiversidade de seus inimigos naturais, especialmente os parasitoides, sua biologia e sua ecologia. Também são relatados os programas de controle biológico clássico no Brasil, desde a introdução de *Tetrastichus giffardianus* (Hymenoptera: Eulophidae), em 1937, para o controle de *Ceratitidis capitata* (Diptera: Tephritidae), até a de *Fopius arisanus* (Hymenoptera: Braconidae), em 2012, para o controle de *Bactrocera carambolae* (Diptera: Tephritidae). Por fim, são destacados os avanços obtidos, bem como os principais gargalos e as perspectivas para uso efetivo de programas de controle biológico contra moscas-das-frutas.

Termos para indexação: programas de controle biológico, Braconidae, controle biológico clássico, Diptera, Figitidae, controle biológico natural.

Beatriz Jordão Paranhos⁽¹⁾ ,
Dori Edson Nava⁽²⁾  and
Aldo Malavasi⁽³⁾ 

⁽¹⁾ Embrapa Semiárido, BR-428, Km 152, Zona Rural, Caixa Postal 23, CEP 56302-970 Petrolina, PE, Brazil.
E-mail: beatriz.paranhos@embrapa.br

⁽²⁾ Embrapa Clima Temperado, BR-392, Km 78, 9º Distrito, Monte Bonito, Caixa Postal 403, CEP 96010-971 Pelotas, RS, Brazil.
E-mail: dori.edson-nava@embrapa.br

⁽³⁾ International Atomic Energy Agency, Department of Nuclear Sciences and Applications, PO Box 100, A-1400 Vienna, Austria.
E-mail: A.Malavasi@iaea.org

 Corresponding author

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Introduction

Biological control measures integrated with the sterile insect technique (SIT) have been widely used in countries with effective fruit fly suppression or eradication programs. Barclay (1987) built a model to estimate pest population control and found that SIT and parasitoids were more efficient when combined: the former is more feasible in low pest populations and the later find their hosts more easily in high pest populations, resulting in a broader pest suppression (Gurr & Kvedaras, 2010). These techniques are being successfully used in Costa Rica (Messing, 1996), southern Mexico (Sivinski et al., 1996), Guatemala (Rendon et al., 2006), and Hawaii (Kaplan, 2008), reducing fruit fly populations there.

In recent years, the use of insecticides – mainly of organophosphates and pyrethroids – has been restricted, and, as a result, fruit fly management in many economically important crops, such as guava (*Psidium guajava* L.) and peach [*Prunus persica* (L.) Batsch], has been jeopardized (Nava & Botton, 2010). An alternative to face the challenges for a sustainable agriculture, is biological control, which can be beneficial due to its synergism with other environmentally friendly methods like SIT and to the consequent decreases in insecticide application, with a lower impact on human health and the environment, besides little or no effect on nontarget species (Gurr & Kvedaras, 2010). In this scenario, biological control must be understood as a suite of ecosystem services (Gurr & Kvedaras, 2010).

In natural environments, biological control can keep pests low in a population; Murillo et al. (2015), for example, found that 88% of *Anastrepha obliqua* larvae infesting yellow mombin (*Spondias mombin* L.) were parasitized. However, in large fruit crop areas, there are restrictions that limit the capacity of parasitoids to keep fruit fly populations below desirable levels (Bateman, 1972; Gilstrap & Heart, 1987; Wharton, 1989). Among these restrictions, stand out the lower intrinsic growth rate (“r”) of the parasitoid, compared with its fruit fly hosts (Vargas et al., 2002). The presence of even one tephritid fruit fly species can cause significant economic losses, and it is not possible to control these pest populations with just a single method. In mango (*Mangifera indica* L.), these losses, for instance, can surpass 48% in Benin, Africa, regardless of the control method applied (Vayssières et al., 2009). It should be

noted that production losses vary with fruit host and fruit fly species.

In the last decade, with the growing restrictions on the chemical control of fruit crops, awareness of food security has also increased. Therefore, phytosanitary and quality regulations have become more restrictive for export to first world countries, causing a greater burden to exporters and negatively affecting the exported volumes (Melo et al., 2014). In this context, biological control should be more valued and its use more intensified.

In spite of the increase in researches on biological control, in general, it still represents less than 1% of all control methods used in agriculture, which total around US\$30 billion (Griffiths et al., 2008). In the case of Brazil, based on the classical biological control activities already carried out and on the information available on native parasitoids, the development of applied biological control programs, including parasitoid mass production and inundative releases, could be successful, especially when combined with other control methods under integrated pest management (IPM) and area-wide concepts. However, a thorough knowledge of the biology of the parasitoids, including their potential as a control method, is needed in order to implement a suitable management strategy.

The taxonomic families and species of wasps that are considered parasitoids vary according to the fruit fly and host fruit species found in an area. The large diversity of native fruit species and fruit fly species of the genus *Anastrepha* is associated with a large number of parasitoid species (Canal & Zucchi, 2000). This review paper presents an overview of the biological control, both natural and applied, of fruit flies in Brazil.

Native parasitoids

In Brazil, fruit flies are attacked by many parasitoid species of the families Braconidae, Figitidae, Pteromalidae, and Diapriidae. The first two families have a larger number of described species and are characterized as larvae-pupae koinobiont endoparasites (Canal & Zucchi, 2000; Ovruski et al., 2000). The species of the families Pteromalidae and Diapriidae are idiobiont parasites of pupae; however, the former are ectoparasites and the later are endoparasites.

Currently, there are 25 species recorded in Brazil (Table 1), although it is likely that this number is much

Table 1. Records of parasitoid species attacking fruit flies (Tephritoidea) in Brazil, including host relationships.

Family	Subfamily	Species	Fruit fly host species	Reference	Instar and development period	Feeder type	Parasitism mode
Braconidae	Alysiinae	<i>Asobara anastrephae</i> (Muesebeck)	<i>Anastrepha</i> sp., <i>Anastrepha antunesi</i> , <i>Anastrepha atrigona</i> , <i>Anastrepha bahiensis</i> , <i>Anastrepha coronilli</i> , <i>Anastrepha fractura</i> , <i>Anastrepha obliqua</i> , <i>Anastrepha striata</i> , and <i>Anastrepha zenildae</i>	Canal & Zucchi (2000); Costa (2005); Silva et al. (2007b); Deus et al. (2010); Dutra et al. (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Alysiinae	<i>Idiasta delicata</i> (Papp)	<i>Anastrepha</i> sp.	Costa (2005)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Doryctobracon areolatus</i> (Szépligeti)	<i>Anastrepha antunesi</i> , <i>Anastrepha amita</i> , <i>Anastrepha atrigona</i> , <i>Anastrepha bahiensis</i> , <i>Anastrepha bistrigata</i> , <i>Anastrepha coronilli</i> , <i>Anastrepha distincta</i> , <i>Anastrepha fraterculus</i> , <i>Anastrepha fractura</i> , <i>Anastrepha leptozona</i> , <i>Anastrepha manihoti</i> , <i>Anastrepha obliqua</i> , <i>Anastrepha pseudanomala</i> , <i>Anastrepha psedoparallela</i> , <i>Anastrepha serpentina</i> , <i>Anastrepha sororcula</i> , <i>Anastrepha striata</i> , <i>Anastrepha rheediae</i> , <i>Anastrepha turpiniae</i> , <i>Anastrepha zenildae</i> , <i>Ceratitis capitata</i> , <i>Rhagoletotrypeta pastranai</i> , and <i>Neosilba</i> sp.	Leonel Jr. et al. (1995); Canal & Zucchi (2000); Costa (2005); Pereira (2009); Deus et al. (2010); Jesus et al. (2010); Marsaro Júnior et al. (2010); Nicacio et al. (2011); Garcia & Ricalde (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Doryctobracon brasiliensis</i> (Szépligeti)	<i>Anastrepha amita</i> , <i>Anastrepha fraterculus</i> , <i>Anastrepha fractura</i> , <i>Anastrepha serpentina</i> , <i>Anastrepha sororcula</i> , and <i>Rhagoletotrypeta pastranai</i>	Canal & Zucchi (2000); Dutra et al. (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Doryctobracon crawfordi</i> (Viereck)	<i>Anastrepha coronilli</i>	Deus et al. (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Doryctobracon fluminensis</i> (Lima, 1938)	<i>Anastrepha fraterculus</i> , <i>Anastrepha montei</i> , <i>Anastrepha parallela</i> , <i>Anastrepha pickeli</i> , <i>Anastrepha pseudoparallela</i> , and <i>Hexachaeta eximia</i>	Canal & Zucchi (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Microcrasis lonchaeae</i> (Lima)	<i>Rhagoletotrypeta pastranai</i> and <i>Neosilba pendula</i>	Canal & Zucchi (2000); Garcia & Ricalde (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Opius bellus</i> Gahan	<i>Anastrepha antunesi</i> , <i>Anastrepha atrigona</i> , <i>Anastrepha coronilli</i> , <i>Anastrepha distincta</i> , <i>Anastrepha fraterculus</i> , <i>Anastrepha fractura</i> , <i>Anastrepha hastata</i> , <i>Anastrepha leptozona</i> , <i>Anastrepha manihoti</i> , <i>Anastrepha montei</i> , <i>Anastrepha obliqua</i> , <i>Anastrepha pastranai</i> , <i>Anastrepha serpentina</i> , <i>Anastrepha sororcula</i> , <i>Anastrepha striata</i> , <i>Anastrepha turpiniae</i> , <i>Rhagoletis ferruginea</i> , and <i>Ceratitis capitata</i>	Canal et al. (1994); Leonel Jr. et al. (1995); Canal & Zucchi (2000); Silva & Ronchi-Teles (2000); Jesus et al. (2008); Marsaro Júnior et al. (2010); Pereira et al. (2010); Dutra et al. (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Opius itatiayensis</i> Lima	<i>Tomoplagia</i> sp.	Canal & Zucchi (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Opius</i> sp.	<i>Anastrepha</i> sp., <i>Anastrepha distincta</i> , <i>Anastrepha leptozona</i> , and <i>Anastrepha obliqua</i>	Canal & Zucchi (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Braconidae	Opiinae	<i>Opius tomoplagiae</i> Lima	<i>Tomoplagia rudolphi</i>	Canal & Zucchi (2000)	Larva-pupa	Endoparasitoid	Koinobiont

Continued...

Table 1. Continued...

Family	Subfamily	Species	Fruit fly host species	Reference	Instar and development period	Feeder type	Parasitism mode
Braconidae	Opiinae	<i>Utetes anastrephae</i> (Viereck)	<i>Anastrepha amita</i> , <i>Anastrepha bahiensis</i> , <i>Anastrepha coronilli</i> , <i>Anastrepha fraterculus</i> , <i>Anastrepha manihoti</i> , <i>Anastrepha obliqua</i> , <i>Anastrepha sororcula</i> , <i>Anastrepha striata</i> , <i>Anastrepha turpiniae</i> , <i>Anastrepha zenildae</i> , <i>Rhagoletotrypeta pastranai</i> , and <i>Ceratitis capitata</i>	Canal et al. (1995); Canal & Zucchi (2000); Nicácio et al. (2011); Dutra et al. (2013); Costa (2005)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Aganaspis nordlanderi</i> (Wharton)	<i>Anastrepha bahiensis</i> , <i>Anastrepha coronilli</i> , <i>Anastrepha striata</i> , and <i>Neosilba</i> sp.	Guimarães et al. (2000); Garcia & Ricalde (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Aganaspis pelleranoi</i> (Brèthes)	<i>Anastrepha amita</i> , <i>Anastrepha bahiensis</i> , <i>Anastrepha coronilli</i> , <i>Anastrepha fraterculus</i> , <i>Anastrepha serpentina</i> , <i>Anastrepha striata</i> , <i>Ceratitis capitata</i> , <i>Neosilba</i> sp., <i>Neosilba pendula</i> , and <i>Neosilba perezii</i>	Guimarães et al. (2000); Dutra et al. (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Lopheucoila anastrephae</i> (Rhower)	<i>Anastrepha amita</i> , <i>Anastrepha pseudoparallela</i> , <i>Neosilba</i> spp., and <i>Lonchaea</i> sp.	Guimarães et al. (2000); Nicácio et al. (2011); Garcia & Ricalde (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Odontosema anastrephae</i> Borgmeier	<i>Anastrepha fraterculus</i> and <i>Anastrepha pseudoparallela</i>	Guimarães et al. (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Odontosema albinerve</i> Keiffer	<i>Anastrepha serpentina</i>	Fernandes et al. (2013)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Tropideucoila weldi</i> Costa Lima	<i>Neosilba pendula</i>	Guimarães et al. (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Tropideucoila rufipes</i> Ashmead		Guimarães et al. (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Trybliographa</i> sp.	<i>Anastrepha</i> spp. and <i>Neosilba</i> spp.	Guimarães et al. (2000)	Larva-pupa	Endoparasitoid	Koinobiont
Figitidae	Eucoilinae	<i>Trybliographainfuscata</i> Diaz, Gallardo & Uchôa	<i>Anastrepha</i> spp. and <i>Neosilba</i> spp.	Souza-Filho et al. (2009)	Larva-pupa	Endoparasitoid	Koinobiont
Pteromalidae	Pteromalinae	<i>Pachycrepoides vindemmiae</i> (Rondani)	<i>Anastrepha fraterculus</i>	Salles (1996)	Pupa	Ectoparasitoid	Idiobiont
Pteromalidae	Spalangiinae	<i>Spalangia endius</i> Walker	<i>Anastrepha</i> spp.	Aguiar-Menezes et al. (2003)	Pupa	Ectoparasitoid	Idiobiont
Diapriidae	Diapriinae	<i>Coptera haywardi</i> Loiacono	<i>Anastrepha</i> spp.	Aguiar-Menezes et al. (2003)	Pupa	Endoparasitoid	Idiobiont
Diapriidae	Diapriinae	<i>Trichopria anastrephae</i> Lima	<i>Anastrepha</i> sp. and <i>Anastrepha fraterculus</i>	Garcia & Corseuil (2004)	Pupa	Endoparasitoid	Idiobiont

higher since many wasp species remain undescribed. Therefore, this number could be considerably increased if additional works on the occurrence and distribution of these species were to be carried out in regions not yet studied. In addition, the development of new strategies and methodologies for collecting wasps – mainly in the egg and pupal stages – could also contribute to identify species that are still unknown. Among the braconids, *Doryctobracon areolatus* (Szépligeti) is the most abundant and frequent parasitoid attacking many fruit fly species in different fruit hosts, being recorded

in almost every Brazilian state (Canal et al., 1995; Leonel Jr et al., 1995, 1996; Matrangolo et al., 1998; Canal & Zucchi, 2000; Aguiar-Menezes et al., 2001; Uchôa-Fernandes et al., 2003; Silva & Silva, 2007; Marinho et al., 2009; Silva et al., 2010b; Nicácio et al., 2011; Nunes et al., 2012). The species *Doryctobracon brasiliensis* (Szépligeti) and *Opius bellus* (Gahan) also are common, but their geographic distribution is reduced (Salles, 1996; Nunes et al., 2012). For the figitids, the most common and widespread species is *Aganaspis pelleranoi* (Brèthes) (Hymenoptera:

Figitidae) (Guimarães et al., 1999; Garcia & Corseuil, 2004; Nunes et al., 2012).

In the country, most of the studies on native wasps focus on Braconidae and Figitidae, specifically on their: occurrence, distribution, and faunistic analysis (Leonel Jr., 1991; Canal et al., 1994, 1995; Leonel Jr. et al., 1995, 1996; Salles, 1996; Aguiar-Menezes & Menezes, 1996, 1997, 2001; Matrangolo et al., 1998; Guimarães et al., 1999; Aguiar-Menezes et al., 2001, 2003; Araujo & Zucchi, 2002; Garcia & Corseuil, 2004; Guimarães et al., 2004; Souza-Filho et al., 2007, 2009; Silva & Silva, 2007; Marinho et al., 2009; Silva et al., 2010b; Bittencourt et al., 2011; Nicacio et al., 2011; Nunes et al., 2012); behavior (Guimarães & Zucchi, 2004; Silva et al., 2007a); and taxonomy (Canal & Zucchi, 2000; Guimarães et al., 2000). Although there is a great amount of information on the distribution and occurrence of parasitoids for some Brazilian regions, information is still scarce for others. The major bottleneck regarding the lack of knowledge is related to the few studies on the ecology and potential for parasitism of native parasitoids, both which affect the implementation of applied biological control programs.

Aganaspis pelleranoi (Figure 1 A) is the best well-known native species in terms of its biology (Ovruski, 1994; Guimarães et al., 2000). A laboratory colony was established by Gonçalves et al. (2013, 2014, 2016) based on bioecology. These studies showed that the wasp prefers to attack third-instar larvae of *Anastrepha fraterculus* (Wiedemann) due to their best emergence rate.

Another important wasp species, *D. areolatus* (Figure 1 B), is often found, at a high frequency, in

many areas and hosts around Brazil. Biological studies on this species have shown that it prefers second-instar larvae, although it can also attack third-instar ones (Nunes et al., 2011). Murillo et al. (2015) observed that *D. areolatus* parasitizes eggs and young larvae of *A. obliqua*. Its development on *A. fraterculus* takes approximately 25 days, at 25°C, and adult longevity is 10 to 16 days (Salles, 2000). The species is distributed from the south of the United States to South America (Wharton & Marsh, 1978; Wharton & Gilstrap, 1983), infesting a large number of fruit fly species in many host fruits, most likely due to the large ovipositor of its female (Sivinski, 1991). In some areas, its frequency is around 70% of all wasps collected (Leonel Jr. et al., 1995). Therefore, due to its morphology, *D. areolatus* is one of most promising species to be used in large biological control programs against native fruit fly species such as *A. fraterculus*. In this case, it is critical to have a colony well adapted to laboratory conditions. Unfortunately, to date, this has not been very successful. Efforts have been made in Tapachula, Mexico, with limited results (Cancino et al., 2010). Recently, in Brazil, some colonies of this species were established in a laboratory setting using naked *A. fraterculus* larvae (Nunes et al., 2011; Gonçalves et al., 2016).

Other native wasp species have been reared under laboratory conditions, such as *O. bellus*, *D. brasiliensis* (Figure 1 C), and *A. pelleranoi*, allowing studies on their biology, behavior, and interspecific competitiveness (Nunes et al., 2011; Gonçalves et al., 2013, 2014; Poncio et al., 2016). The obtained results are very promising for future applied work.



Figure 1. Fruit fly parasitoids native to Brazil: A, female of *Aganaspis pelleranoi*; B, female of *Doryctobracon areolatus*; and C, female of *Doryctobracon brasiliensis*. Photos A, B, and C by: Paulo Lanzetta.

Parasitoids introduced into Brazil

The first fruit fly parasitoid introduced, in 1937, into Brazil was the larval-pupal gregarious koinobiont endoparasitoid, *Tetrastichus giffardianus* Silvestri (Hymenoptera: Eulophidae) (Figure 2 A). A low number of adults were released in citrus (*Citrus* spp.) orchards, in the state of São Paulo, to help control the Mediterranean fly (medfly), *Ceratitidis capitata* (Wiedemann) (Fonseca & Autuori, 1940). There have been no records of the species in this region since its first release, but, after 60 years, it was recovered in Northeastern Brazil, 2,000 km away, from *C. capitata* puparia collected in almond (*Prunus dulcis* D.A. Webb), cherry [*Prunus avium* (L.) L.], and plum (*Prunus* spp.) orchards (Costa et al., 2005), where no previous releases or introductions had been reported.

The koinobiont endoparasite of larva-pupa, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) (Figure 2 B), native to the Indo-Philippines and recovered in *Bactrocera* species, was introduced into Brazil in 1994 (Walder et al., 1995) and has been easily reared in many laboratories on larvae of the tephritid fruit flies, *C. capitata* and *A. fraterculus*. When the host is *A. fraterculus*, the wasps emerge with an ovipositor 2–4 mm longer and a higher percentage of female progeny; both of these traits are highly desirable for a mass-rearing and release operation (Paranhos et al., 2008). In Brazil, inoculative releases of this species were made in a peach orchard in the municipality of Piracicaba, in the state of São Paulo, in 1996 (Walder, 2002), and in Recôncavo Bahiano, in the state of Bahia, in 1995 (Carvalho, 2005). In succeeding years, there was a release of 3.5 million wasps in a 25-ha citrus orchard in Itapetinga, also in

the state of São Paulo, and of 200,000 wasps in coffee (*Coffea* spp.) and citrus orchards in the municipality of Cosmópolis, in the same state, where 19.8% parasitism was achieved (Walder, 2002). In all areas where the wasps were released, there were successive records of its establishment in the field, with no negative impact on the native parasitoid species (Alvarenga et al., 2005; Carvalho, 2005). In Southern Brazil, after small releases, the wasps partially controlled *A. fraterculus* infestation in guabiroba (*Campomanesia* spp., Myrtaceae), but did not become established there, probably due to the colder winters than in other Brazilian regions (Sugayama, 2000).

Later, millions of *D. longicaudata* wasps were released in Oiapoque, in the state of Amapá, Northern Brazil, in a tentative program to control the carambola (*Averrhoa carambola* L.) fruit fly, *Bactrocera carambolae* (Drew & Hancock), a quarantined species that had been introduced into that area in 1996 (Carvalho & Nascimento, 2000).

Diachasmimorpha longicaudata is by far the most studied wasp species due to its good adaptation to the laboratory environment, easy rearing procedures under artificial conditions, intense foraging behavior on fallen fruit in nature, and high capacity of females to exploit available resources (Purcell et al., 1994; Sivinski et al., 1998). It is also a generalist species that attacks a large number of tephritids, including *Anastrepha*, *Ceratitidis*, and *Bactrocera* species.

The major constraint against widely using larval parasitoids is fruit size, since the female ovipositor might not reach larvae located deeper in larger fruit (Sivinski, 1991). In this regard, the koinobiont egg parasitoid, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) (Figure 2 C), would have a competitive



Figure 2. Exotic fruit fly parasitoids currently used in Brazil: A, female of *Tetrastichus giffardianus*; B, female of *Diachasmimorpha longicaudata*; and C, female of *Fopius arisanus*. Photos A, B, and C by: Valmir Antonio Costa, Sônia Poncio, and Paulo Lanzetta, respectively.

advantage in relation to the larval parasitoid, considering most tephritids lay their eggs under the peel (Wang et al., 2003).

In Brazil, there is no known report of any parasitoid of fruit fly eggs, except *D. areolatus*, which shows ability in parasitizing eggs and larvae (Murillo et al., 2015). For this reason and focusing on the control of *B. carambolae* in the state of Amapá, Embrapa Amapá imported the species *F. arisanus* from the colony established in November 2012 at the laboratory of Agricultural Research Service of United States Department of Agriculture, located in Hilo, Hawaii. After the normal quarantine at Embrapa Meio-Ambiente, in April 2013, sub-colonies were sent to laboratories at Embrapa Semiárido, which sent samples to Moscamed Brasil and to Embrapa Clima Temperado, and, then, in 2015, to Embrapa Amapá, as an irradiated host (*C. capitata*).

Many successful studies are currently underway with this species. The main topics discussed include: rearing procedures, such as the use of irradiated medfly eggs; host-female density; egg host age; exposition time; and oviposition substrate. There are also behavioral studies on interspecific competition with native species and parasitism efficacy in different fruits cultivated in Brazil.

Bioecology of fruit fly parasitoids

The mechanical factors that most affect parasitism rate are: peel thickness, pulp thickness, and size of ovipositor. According to Aluja et al. (1990), 94% of the wasps infesting mango were *D. areolatus* (Figure 1 C), which have a long ovipositor and can reach fruit fly larvae inside the pulp. The species of wasps with the shortest to longest ovipositors are: *Opius* spp., *Utetes anastrephae*, *D. areolatus*, and *D. longicaudata* (Leonel Jr., 1991; Matrangolo et al., 1998).

To enhance biological control, semiochemicals have an important role in all trophic levels. Synomones are released by plants and attract pollinating insects and parasitoids (Elzen et al., 1984). Larval parasitoids, such as *D. longicaudata*, use the odors of a complex mixture of fruit and host larvae compounds to locate infested fruit (Carrasco et al., 2005), and, after landing, actively search for the fruit fly larvae inside the fruit following the vibration caused by their continuous feeding (Greany et al., 1977; Messing & Jang, 1992). In general, the eggs of the parasitoids hatch before the host larvae

pupate and their development is completed during the fruit fly pupal stage (Lawrence, 1981). At the end of the cycle, instead of a fruit fly, a wasp emerges.

The success of the introduction of a new exotic species is influenced by many factors, and the final results also vary significantly. This is why, in the colonization process, one species introduced into a new area can be better adapted than another for no clear reason. For a successful introduction, it is important to have previous knowledge of the diversity, interspecific competition, habitat, hour of activity, searching behavior, interaction among species of fruit flies, and impact on nontarget species (Sivinski et al., 1996).

Paranhos et al. (2013a) studied the interspecific competition between the exotic species *D. longicaudata* and the native *D. areolatus* and *U. anastrephae*. In the internal competition, inside the fruit fly larvae, *U. anastrephae* and *D. longicaudata* were similar but better than *D. areolatus*; however, in the external competition, before laying eggs, *D. areolatus* showed the advantage of having a larger ovipositor and, consequently, of reaching more easily larvae inside larger fruits. Additionally, it was observed that females of *D. areolatus* inserted their ovipositor in green fruits, suggesting that the species could parasitize larvae at different stages and, therefore, be more competitive (Carvalho, 2005). Furthermore, even in orchards with inundative and inoculative releases of the exotic species *D. longicaudata*, the native species *D. areolatus* was the most common in many fruits such as Surinam cherry (*Eugenia uniflora* L.), guava, carambola, and mango (Matrangolo et al., 1998).

The parasitism rate varies significantly according to the fruit fly host species, the fruit infested by the tephritid, and the wasp species. In Malaysia, where *F. arisanus* is native, the natural parasitism of *B. carambolae* larvae collected in the field can reach 75% (Vijayseraran, 1984). However, in nature, parasitism is usually from 1 to 10%. In Australia, Costa Rica, and Fuji, where *F. arisanus* and *D. longicaudata* were introduced, the former species is more abundant than the later (Wang et al., 2003).

In Hawaii, a classical biological control program against fruit flies, *Bactrocera cucurbitae*, *C. capitata*, and *Bactrocera dorsalis* were the most successful worldwide (Wharton, 1989), and the introduced parasitoid species *D. longicaudata*, *F. arisanus*, and *T. giffardianus* were established as the most common parasitoids in the Hawaiian islands (Bokonon-Ganta

et al., 2007). However, in Argentina, the two exotic species *T. giffardianus* and *F. arisanus*, which were introduced, respectively, in 1947 and 1967, did not establish themselves in the country (Ovruski & Schliserman, 2012).

Among the fruit fly parasitoids, *F. arisanus* has a competitive advantage because it attacks eggs laid under the fruit skin, which are easier to reach than larvae deep in the pulp; therefore, theoretically, it could out-perform other species. Moreover, studies carried out in the São Francisco River Valley showed that the species has good efficacy on guava- and mango-hosting medflies (Coelho, 2017).

Other fruit fly biological control agents and Sterile Insect Technique (SIT)

In the São Francisco Valley, an important fruit-growing area in Northeastern Brazil, the medfly accounts for more than 99% of all captured species of tephritid fruit flies (Haji et al., 2005). In the medfly rearing facility established in the region, SIT and augmentative biological control are the main techniques applied. Studies have shown that the use of parasitoids and sterile flies could increase the effectiveness in controlling medflies (Rendon et al., 2006) and that the parasitoids *Diachasmimorpha kraussii* and *F. arisanus* contributed more to the decrease in fly emergence.

The United States was the first country to apply IPM to control different species of fruit flies, such as *B. cucurbitae*, *C. capitata*, and *B. dorsalis*, in the Hawaiian Islands. For this, the country adopts SIT, cultural practices, bait-spray applications, sanitation, and parasitoids (Klungness et al., 2005). In Argentina, medfly control has also been successful through different area-wide actions such as SIT, bait spray with selective insecticides, cultural practices, and quarantine systems (Guillén & Sánchez, 2007). The results of this program have shown the eradication of *C. capitata* in some areas and the low prevalence of this pest population in others (Guillén & Sánchez, 2007). Recently, the country has added augmentative biological control with *D. longicaudata* to its management strategies (Ovruski & Schliserman, 2012).

Entomopathogens

The most promising entomophagous agents for fruit fly control are fungi and nematodes. Studies with

some strains of *Metarhizium anisopliae* (Metsch.) and *Beauveria bassiana* (Bals), as a biological control method, have shown a great potential for their use against *A. fraterculus* (Carneiro & Salles, 1994; Destéfano et al., 2005) and *C. capitata* (Mochi et al., 2006; Almeida et al., 2007; Souza, 2010). In Northern Brasil, Silva et al. (2016) found a great mortality of *B. carambolae* immatures when using *M. anisopliae*. In Guatemala, Flores et al. (2013) observed that sterile medfly males inoculated with conidia of *B. bassiana* can be a feasible way to spread entomophagous agents, in the field, through horizontal transmission to wild males in lekking behavior and to wild females in mating or attempted mating, without negative impacts on nontarget species.

Concerning nematodes, it was found that the virulence of the genus *Steinernema* and *Heterorhabditis* to *C. capitata* was between 32.5 to 87.5% (Rohde, 2007). The species *Heterorhabditis indica* IBCB-n5, at doses of 1 and 10 JI per centimeter, caused 66–99% mortality of pre-pupae stage *C. capitata* (Silva et al., 2010a). In Spain, Laborda et al. (2003) reported medfly larvae mortality over 90%, when using the commercial product Biorend C (*Steinernema* spp. + Chitosan), without pathogenicity to pupae. In *A. fraterculus*, *H. bacteriophora* RS88 was more virulent to the larval stage (Barbosa-Negrisoni et al., 2009); however, it also showed certain efficacy to control pupae, as did *S. riobrave* RS59.

Concluding remarks

To achieve the best control of fruit flies in Brazil, a national program within an integrated pest management (IPM) framework and an area-wide system is needed. It should focus on the three most critical regions of the country: Northeast, against *Ceratitis capitata*; South, against *Anastrepha fraterculus*, where the largest commercial fruit orchards are located; and North, to keep the *Bactrocera carambolae* population low, with phytosanitary barriers to avoid its escape to other states where fruit orchards are grown in a large scale.

In this case, monitoring is essential because it provides information about the density and location of fruit flies; therefore, it should be carried out in all these regions by using traps and attractants specific to each species. Based on the obtained data, different control methods should be planned in the IPM

program, according to fruit fly species and availability of tools, including cultural practices to keep orchards clean of rotten and ripe nonmarked fruits, bait-spray applications, and biological control with parasitoids and fungi, as well as other methods effective in controlling these pestiferous fruit fly species. Finally, when the fruit fly population is low enough (fly per trap per day below 1), sterile insects, if available, should be released in an area-wide system.

Biological control by itself is not enough to keep fruit fly infestation as low as required. However, it is an additional environmentally friendly tool to ensure the reduction of conventional insecticide applications, aiming for sustainable agriculture. The biological agents used should be chosen according to the fruit fly species, the plant host, and the region of Brazil where they will be applied, observing environmental conditions. It should be pointed out that studies are necessary to decide on the type of biological control application: conservative, classic, or augmentative. When possible, at least one agent for each stage of the fruit fly life cycle should be used to avoid, as much as possible, the emergence of adult fruit flies.

In Southern Brazil, besides *Diachasmimorpha longicaudata*, *Doryctobracon areolatus* could be one of the native species with the greatest potential to be used in a biological control program against *A. fraterculus* as it is present in all environments and in large quantities. In regions where *C. capitata* is predominant, as the San Francisco Valley, native parasitoids should not be the main agent, although they parasitize this species. Finally, in the North, in the case of *B. carambolae*, which is not parasitized by native species, the strategy must be using exotic species such as *Fopius arisanus* and *D. longicaudata*.

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