Chapter 7
Augmentative Biological Control as a Workable Strategy Within an Area-Wide Integrated Fruit Fly Management Approach: Case Studies from Mexico and Argentina

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Abstract Augmentative release of parasitoids is a suitable strategy to control fruit fly populations. Parasitoid release is relevant because it may be used strategically to integrate with other control methods. Augmentative Biological Control (ABC) aims to release high densities of mass produced into an area to suppress the population growth rate of the target fruit fly species. In Latin America, there are several examples of ABC against *Ceratitis capitata* (Wiedemann) and against species of the Neotropical genus *Anastrepha* Schiner. The introduced parasitoid *Diachasmimorpha longicaudata* (Ashmead) is the main species used in ABC programs. Successful outcomes have been achieved in Mexico and central-western Argentina by *D. longicaudata* mass releases. In "BioPlanta San Juan," Argentina, *D. longicaudata* is mass reared on larvae of *C. capitata* Vienna-8 (*tsl*) strain. This parasitoid is released in irrigated semiarid fruit-growing valleys. On the contrary to Mexico, *D. longicaudata* is mass produced on larvae of *Anastrepha ludens* (Loew) at the biofactory of the Fruit Fly National Program to release it over larger subtropical areas. Post-release data have shown higher values than 50% of host mortality. This chapter provides particular information on the use of biological control, within the area-wide

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program in environmentally distinguishable regions. Advantages, social problems, and their relation with costs are also discussed.

Keywords *Ceratitis capitata* · *Anastrepha* spp. · Fruit fly pests · Parasitoid augmentative release · Parasitoid mass rearing · Eco-friendly pest control method

7.1 Introduction

Tephritid fruit flies biological control in Latin America began with the attempt to achieve a permanent establishment of different species of exotic natural enemies in various countries of South and Central America and the Caribbean Islands between the 1930s and 1980s. These programs were essentially based on the use of hymenopterous parasitoids mainly from Asia, Africa, and Australia that had first been established in Hawaii. Consequently, tephritid pest control programs initially involved a Classical Biological Control approach aiming at effective long-term fruit fly control, being Hawaii the source of supply parasitoid species for Latin America (Ovruski et al. 2000; Garcia et al. 2020). The ambitious initial planning focused on controlling populations of the exotic and highly invasive *Ceratitis capitata* (Wiedemann) established in Central and South America, as well as various pest species from the Neotropical genus *Anastrepha* Schiner, which were scattered throughout all of Latin America. Although changes in the taxonomic nomenclature of many species over the years make it difficult to know the precise number of parasitoid species released in Latin American countries, a recently updated review by Garcia et al. (2020) listed 18 species. The distribution of those parasitoid species in 17 Latin countries was previously reviewed and reported by Clausen (1956), Gilstrap and Hart (1987), Wharton (1989), Sivinski (1996), Purcell (1998), Ovruski et al. (2000), Garcia et al. (2017), and most recently by Garcia et al. (2020). Results of parasitoid release first series for establishment purposes did not substantially reduce fruit fly pest populations (Wharton, 1989). However, a relevant issue was that two introduced larval parasitoid species, the eulophid *Aceratoneuromyia indica* (Silvestri), which was originally collected in India (Clausen 1978), and the braconid *Diachasmimorpha longicaudata* (Ashmead), native from Southeast Asian (Wharton and Gilstrap 1983), were successfully established and are currently widely distributed in Latin America (Garcia et al. 2020). The Asian-native braconid egg-pupal parasitoid *Fopius arisanus* (Sonan) was so far recorded as established on *C. capitata* only from Costa Rica, where it was introduced in Hawaii in 1955.

In the early 1990s, Knipling's theoretical models published on suppression/eradication of insect pest populations by eco-friendly augmentation methods triggered a breakthrough in the applied use of pest control methods using living organisms (Knipling 1992). As Knipling saw it, augmentative release strategies, either parasitoids or sterile insects, were to be implemented against overall pest populations in areas large enough to deeply reduce the pest insect movement factor (Knipling 1992, 1998, 1999). Based on the degree of selectivity of different control methods for reducing insect pest populations, releasing specific parasitoids is a highly selective strategy (Knipling 1979, 1992, 1998). The suppression of fruit fly pest populations by parasitoid augmentative release activities (Augmentative Biological Control = ABC) is closely linked to complementary techniques of pest reduction with a capacity for eradication, such as the Sterile Insect Technique (SIT), among others (Knipling 1992). Consequently, biological control has become an indispensable supplement to any area-wide integrated fruit fly pest management (AW-IFFPM) program (Hendrichs et al. 2007; Montoya et al. 2007). Based on the principles of beneficial insect augmentation as published by Knipling (1992), the first augmentative release of a parasitoid against a pest fruit fly was carried out in Hawaii. The braconid *Diachasmimorpha tryoni* (Cameron) was mass released, achieving a significant reduction of *C. capitata* populations in coffee-growing areas on Maui Island (Wong et al. 1991). Hawaii's experience spurred fruit fly control programs in Latin America to develop and promote the ABC using parasitoids. Thus, some pilot programs were first established in Costa Rica, Guatemala, and Mexico between the 1980s and 1990s to assess the effectiveness of augmentative releases of mass-reared parasitoids against tephritid pest populations. Those programs were then expanded to other Central and South American countries.

Almost all parasitoid augmentative release programs against pest fruit fly species in Latin America have relied considerably on the introduced *D. longicaudata*. This is primarily because it is easy to mass rear; therefore, efficient mass-production procedures have been implemented in biofactories in various countries, such as Argentina, Brazil, Costa Rica, Guatemala, and Mexico (Sivinski et al. 1996, 2000a, b; Ovruski et al. 2003; Cancino and Montoya 2008; Morera-Montoya et al. 2019; Suárez et al. 2019).

The handiness of *D. longicaudata* mass rearing has been a determining factor in establishing fruit fly ABC programs in different Latin American countries. The importance and development of fruit fly ABC involving Latin America have been well documented by Gingrich (1993), Sivinski (1996, 2013), Messing (1996), Malavasi (1996), Purcell (1998), Ovruski et al. (2000), Bokonon-Ganta et al. (2007), Garcia and Ricalde (2013), Garcia et al. (2020), and Dias et al. (2018).

Although many of the first programs were temporary and/or ineffective, with parasitism rates below 10% (Ovruski et al. 2000; Garcia et al. 2020), two remain active today: the biological control of economically important species of the *Anastrepha* genus in different regions of Mexico, and the biological control of *C. capitata* in the fruit-growing valleys from the province of San Juan, central-western Argentina.

The continuity of both Mexican and Argentinean fruit fly biological control programs for more than 10 years has yielded a wealth of experience in handling *D. longicaudata* that is relevant to highlight and analyze. It is noteworthy that both have different pest target species in very different environments, but they share an augmentative approach using inundative releases, which involve the same introduced parasitoid species, *D. longicaudata*. The strategy of both ABC programs is focused on quick control of the target fruit fly over a short-term period through constant and systematic releases of the parasitoid by flooding the pest-affected area. Therefore, in this chapter, both *D. longicaudata* augmentative release programs are described in-depth and their procedures and current outcomes are analyzed from a general perspective.

7.2 Augmentative Biological Control (ABC): An Eco-Friendly Strategy for Fruit Fly Suppression

The augmented parasitoid release approach for pest control is based on releasing mass-reared individuals into a particular area in numbers far over those naturally occurring, in such a way that the target area is flooded with released control agents (Hajek 2004; van Lenteren 2012). This eco-friendly method is usually applied when the indigenous natural enemies are absent or provide ineffective pest control (Hajek 2004).

The ABC strategy is highly suitable to overcome the limitations of classical biological control when used against tephritid fruit fly pests (Sivinski 2013). These difficulties mainly concern the following issues: (1) higher fecundity and dispersal ability of fruit flies than their parasitoids, (2) low levels of biodiversity in agroecosystems which lead to a lack of shelter, limited food sources, and absence of environments for multiplication of natural enemies, and (3) the mismatch between the presence of the fruit fly pests and their natural enemies (Montoya et al. 2012). It is also noteworthy that the effectiveness of ABC is primarily achieved in isolated eco- and agroecosystems that limit the spread of both the fruit fly pest and parasitoids, or in areas large enough to achieve adequate control by preventing fly movement between environments (Montoya et al. 2000a, 2012, 2017; Sivinski 2013; Montoya and Toledo 2020). However, there are some environments with particular characteristics where ABC may be highly effective against fruit fly pests, for instance: (1) farming land where no phytosanitary measures against pests are implemented but a high density and variety of host plants are available, (2) organic fruit-growing areas, (3) lands that are fly pest reservoirs found on the edge of high-yielding cultivated areas and are often difficult to grow crops on, and (4) inaccessible wilderness areas.

Experience over the last four decades revealed that the success of ABC against fruit fly pests using hymenopteran parasitoids depends on a wide range of biological variables, mainly related to the insect itself and the environment in which it is released. All facets involved in host foraging, as well as host range breadth, must be taken into account when using parasitoids within the ABC strategy (Sivinski and Aluja 2012). From an operational point of view, the choice of the parasitoid best suited to control a specific fruit fly species in a particular environment plays a crucial role in successful control (Sivinski 2013). The environment should enable parasitoids to disperse and to live for an adequate period (usually a week) and develop an efficient searching capacity. Other essential tools are as follows: (1) estimation of the parasitoid release rate, (2) post-release field monitoring to assess the effectiveness of the released agent in controlling the target pest, and (3) standardization of mass-rearing quality control parameters (Wong and Ramadan 1992; Messing et al. 1993; Purcell et al. 1994; Cancino and Montoya 2004; Sivinski and Aluja 2012; Cancino et al. 2002, 2020).

The ABC is a valuable strategy within AW-IFFM programs, particularly in combination with the SIT to achieve a synergistic effect against the pest (Barclay 1987; Knipling 1992, 1998, 1999; Sivinski 2013; Gurr and Kvedaras 2010). Research on this issue was performed in Hawaii (Wong et al. 1992; Purcell 1998; Vargas et al. 2004), Costa Rica (Messing 1996), Mexico (Orozco et al. 2004), and Guatemala (Sivinski et al. 1996; Rendon et al. 2006) and has yielded encouraging results.

7.3 Selection Criteria of *Diachasmimorpha longicaudata* for Fruit Fly ABC in Latin America

The Asian-native *D. longicaudata* (Hymenoptera: Braconidae, Opiinae) is one of the most widely used parasitoid species in the tephritid fruit fly biological control programs (Wharton 1989; Vargas et al. 2012; Garcia et al. 2020). It is a generalist, larval–pupal, koinobiont, and solitary endoparasitoid of fruit flies. It can successfully develop into larvae of at least 34 fruit flies species mainly belonging to *Bactrocer*a, *Anastrepha,* and *Ceratitis* genera (Wharton and Gilstrap 1983; Chinajariyawong et al. 2000; Garcia et al. 2020), all of which are kept hidden inside the fruit until they are ready to pupate (Segura et al. 2007). Females of this exotic parasitoid forage host larvae both at the soil level in fallen fruit and in fruit still hanging on the tree canopy and always oviposit into a host by drilling through the fruit pericarp from the exterior (Sivinski and Aluja 2003). Volatiles directly emanating from both host larva and host habitat undoubtedly play a relevant role in short-range host location by *D. longicaudata* females (Greany et al. 1977; Messing and Jang 1992; Eben et al. 2000; Stuhl et al. 2011; Segura et al. 2012, 2016; Buonocore Biancheri et al. 2019). In addition, both visual cues (Leyva et al. 1991; Messing and Jang 1992) and the influence of associative learning on preference patterns in size and color are highly relevant during host-habitat searching by *D. longicaudata* (Segura et al. 2007).

Aspects of *D. longicaudata* biology in both conditions, namely, experimental lab rearing (Greany et al. 1976; Ashley and Chambers 1979; Lawrence et al. 1978; Lawrence 1981,1988; Duan and Messing 2000; Viscarret et al. 2006; Paranhos et al. 2008; van Nieuwenhove et al. 2012; van Nieuwenhove and Ovruski 2011; Suárez et al. 2012; Buonocore Biancheri et al. 2022a) and mass rearing using irradiated hosts (Wong and Ramadan 1992; Purcell et al. 1994; Cancino and Yoc 1994; Montoya et al. 2000b; González et al. 2007; Cancino et al. 2009a, b, c, 2010; Suárez et al. 2019, 2020) have shown both high reproductive capacity and adult emergence rate.

The biological and ecological features of *D. longicaudata* encouraged its selection as a biocontrol agent for ABC programs in Latin America. These include its adaptability to different environments into which it has been introduced (López et al. 1999; Sivinski et al. 2000a; Carvalho 2005; Montoya et al. 2007, 2017; Suárez et al. 2014; Cancino et al. 2019; Buonocore Biancheri et al. 2022b), and its readily adaptable to different fruit fly species of economic importance, such as *C. capitata*, *Anastrepha fraterculus* (Wiedemann), *A. ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), *A. striata* Schiner, *A. suspensa* (Loew), and *A. sororcula* (Zucchi) (Ovruski et al. 2000; Montoya et al. 2007). In addition, *D. longicaudata* host-finding ability among several patches with different host densities (Núñez-Campero et al. 2016) in a wide variety of fruit species at canopy and soil levels (García-Medel et al. 2007) is another trait regarded as a criterion for its choice. Research on host density choice (Núñez-Campero et al. 2016) proved that *D. longicaudata* females achieve the highest attack level at a 1:30 parasitoid:host ratio, which would indicate a significant parasitism rate for a biocontrol agent. This finding is useful for optimizing the number of parasitoid individuals for augmentative release in the field. Interestingly, studies on self-superparasitism in *D. longicaudata* found that this biological phenomenon is an adaptive parasitism strategy under particular conditions (Montoya et al. 2000b), and middling levels of superparasitism in host larvae lead to a female-biased sex ratio and do not affect life-history traits of parasitoid offspring (González et al. 2007, 2010; Montoya et al. 2012). This strategy is another valuable feature explaining the success of *D. longicaudata* as a biocontrol agent (Altafini et al. 2013).

The development and enhancement of methods for *D. longicaudata* mass production in biofactories, which involve host radiation techniques under rearing quality control standards, were decisive for the use of this exotic parasitoid species in ABC programs in several Latin American countries (Montoya et al. 2000a, 2007, 2011; Cancino and Montoya 2008; Hendrichs et al. 2009; Lopez et al. 2009; Cancino et al. 2012, 2020; Costa et al. (2016); Dias et al. 2018; Garcia et al. 2020; Suárez et al. 2019, 2020). This is mainly because host radiation simplifies the handling of mass-reared parasitoids, significantly improves sanitation, and eases the movement of parasitoids across borders (Cancino et al. 2012; Sivinski 2013). Noteworthy improvements in packaging and release processes, which lead to a consistent distribution and optimal field performance of parasitoid individuals under an area-wide approach, have also strongly contributed to the use of *D. longicaudata* as a biocontrol agent in Mexico (Montoya et al. 2012; Cancino et al. 2017).

7.4 ABC in Mexican and Argentinean Fruit Fly Management Programs

The implementation of parasitoid augmentative releases requires both mass production and large-scale management of biological material, which includes the host species and the natural enemy. Therefore, the establishment of biofactories to achieve mass production of living organisms is a fundamental step. However, this must be framed in a complex logistical context coordinated by an overall program covering all issues related to an AW-IFFPM.

In line with the above, the Mexican National Programme against Fruit Flies began in 1992 under the Mexican government's supervision and support to achieve low and free tephritid fruit fly pest prevalence areas. The aim of the program envisages the suppression or eradication of fruit-growing areas in Mexico of the four economically and quarantine important fruit fly species from the Neotropical-native *Anastrepha* genus, such as *A. ludens*, *A. obliqua*, *A. striata*, and *A. serpentina* in mango, citrus, guava, and Sapotaceae crops (Montoya et al. 2007). This technique is an important part of integrated fruit fly management in Mexico.

Application of parasitoids adopting the concept of ABC started in 1994 when an original process was established through which about 50 million fly puparia parasitized by *D. longicaudata* have been weekly released. The Moscafrut facility, located in Metapa de Dominguez in the southeastern state of Chiapas, Mexico, every week sends parasitized puparia to different places throughout the country. Puparia are sent by plane using hypoxia to avoid the reduction of adult emergence by physiological damage (Cancino and López-Arriaga 2015). These puparia are packed for the pre-release period in regional centers and applied to the parasitoids according to the designed program.

The releases are performed to reduce the wild fruit fly populations surrounding the commercial orchards (Cancino et al. 2020). This supposes to reduce the capacity of the pest population to invade the commercial orchards. The strategy is applied in wide commercial areas covered with mango, oranges, and guavas that are primarily grown for export and other fruits with local interest (zapotaceous, citric, etc.). The use of ABC in combination with other integrated management strategies, such as SIT, cultural, mechanical, and legal control, allows for achieving low prevalence or fruit fly-free zones more quickly, which facilitates fruit marketing (Montoya et al. 2007; Cancino et al. 2020).

The San Juan Fruit Fly Control and Eradication Programme (ProCEM-San Juan, Spanish acronym) was initially established in the 1970s and is currently supported by both the Government of the Province of San Juan and the San Juan Foreign Trade Chamber. The province of San Juan is located in the Argentinean fruit-growing region locally known as "Cuyo." In this dry central-western fruit-producing region, mainly stone and pome fruits, olives, figs, and a wide variety of grapes are cultivated in isolated irrigated areas known as oases. The ProCEM-San Juan is under the supervision of the National Agri-Food and Animal Health and Quality Service (SENASA, Spanish acronym) through the National Fruit Fly Control and Eradication

Programme (PROCEM, Spanish acronym). In the Cuyo region, the only economically important tephritid species is *C. capitata*, which negatively affects fresh fruit production, marketing, and export (Guillén and Sánchez 2007; Alós et al. 2014). In this region, *C. capitata* mainly inhabits urban areas, commercial high-yielding crops, and rural farms with fruit orchards (Guillén and Sánchez 2007). *Ceratitis capitata* populations are currently managed by the ProCEM-San Juan through an AW-IFFPM approach based on a combination of different techniques, such as the SIT, cultural, physical, and biological control, air and ground chemical and bait spray application, and a quarantine system protection which involves phytosanitary barriers located at entry areas to the province (Díaz et al. 2008; Alós et al. 2014; Suárez et al. 2019). The ABC was incorporated into the activities of ProCEM-San Juan through the mass production of *D. longicaudata* at the San Juan Medfly and Parasitoids Mass Rearing Biofactory, locally known as "BioPlanta San Juan" (from now on: San Juan Biofactory), in early 2010. This is the first biofactory in Argentina to rear *D. longicaudata* under a large-scale scheme, which currently reaches 350,000 adults per week (Suárez et al. 2019, 2020). The San Juan Biofactory is a unit of the Directorate of Plant, Animal, and Food Health belonging to the Secretariat of Agriculture, Livestock, and Agroindustry from the Production and Economic Development Ministry of the Government of the Province of San Juan. Due to the artificial irrigation (oases) systems used in the province of San Juan, environmental conditions become propitious to test the effectiveness of *D. longicaudata* on *C. capitata* through open-field. This aim of the ProCEM-San Juan involves a renewed effort to encourage the broad use of biological control within a framework of environment-friendly strategies combination, such as the complementary use of the SIT, mass trapping, and the ABC, to suppress *C. capitata* populations.

7.5 *Diachasmimorpha longicaudata* Mass-Rearing Procedures in Mexico and Argentina

The successful use of the ABC strategy is linked to a set of inter-coordinated steps. The first is the establishment of mass rearing of the natural enemy species selected for release in the field. These mass-produced individuals at reasonable costs must be highly competitive and effective once released against the target pest. The quality control care in *D. longicaudata* mass-production begins with host larval rearing.

The mass production of *D. longicaudata* in Mexico is based on the use of *A. ludens* larva. Almost ten million irradiated larvae are exposed to parasitization daily. The host exposition is divided into three sessions of 4–5 million larvae exposed to a colony of approximately 1,152,000 female adults, a relationship of 2 larvae/female. The adults are maintained in metallic cages (30 × 30 × 40 cm) with a density of 4000 insects. An initial period of 26° (4 days) and a posterior change to 21 °C (2 days) permit adequate emergence of males and females (more than 60% with a sex ratio of 2♀:1♂). For parasitoid reproduction, 5- to 10-day-old females are used. Due to the similar times of emergence with flies and parasitoids required to use irradiated hosts (Cancino et al. 2009a, 2012). Doses of 45 Gy (gamma rays 4.60/min) are applied to 14 million host larvae daily, which avoids the risk of flies' emergence in big quantities (Cancino et al. 2002; Cancino and Montoya 2008). Another advantage of the use of radiation is the improvement of the production process sanitation (Cancino et al. 2009a, 2012).

Recently, 60% of adult emergence is normally obtained; however, 50% is more consistent. The emergence is the main parameter to analyze the quality of mass production. Although in discussion the weight of larvae is the basic parameter to preserve a high emergence, a weight of 24.06 ± 1.4 mg in the larva of *A. ludens* has been used as the most important requirement; little knowledge there is about other parameters with influences on the parasitoid quality. Another important parameter in mass production is the offspring sex ratio; this is biased toward females, which is considered the result of the size of the host and superparasitism (Montoya et al. 2011). Older larvae are bigger and produce more females; however, more than one egg by the larva host develops superparasitism, which produces an intraspecific competitively from which the progeny has a sex ratio biased females (Montoya et al. 2011). This is an important point in the massive production of *D. longicaudata* developed in *A. ludens* larva as host (Gonzalez et al. 2010; Montoya et al. 2011).

Much work has currently been done to reduce the cost of the larval diet. About 70% of the cost of production in *D. longicaudata* corresponds to ingredients and larval diet elaboration. Reduction in the cost of larval diet implicates a direct reduction in the cost of parasitoid production. However, many changes in the parasitoid mass production process have been important to achieve more efficient production and improve the quality of adult parasitoids (Montoya et al. 2011; Cancino et al. 2012; López et al. 2021). Fifty million pupae of parasitoid *D. longicaudata* are weekly sent to the centers of packing distributed in fruit production regions along México. An average of about 50% of emergence is common, and the survivor, flight, and fecundity capacity are maintained into the tolerance levels, concluding that the quality is good.

Diachasmimorpha longicaudata mass rearing at the San Juan Biofactory takes place in the Parasitoid Rearing Laboratory which houses a 7 × 5 × 3.5-m room for adult production under a lighting condition of 9000 lm/m^2 provided by twenty 40 W-fluorescent light tubes evenly distributed on the roof. Adults of *D. longicaudata* are reared on *C. capitata* third-instar larvae of Vienna-8 temperature-sensitive lethal (*tsl*) genetic sexing strain without inversion (from now on: $Dl_{(Cc\text{-}tsl)}$). These are irradiated at 90 Gy to avoid the adult flies' emergence from non-parasitized *C. capitata* puparia when parasitoids are released in the field (Suárez et al. 2019). Once a day, irradiated $Dl_{(Cc\text{-}tsl)}$ larvae are placed into 10 × 1-cm synthetic nylon mesh-covered dishes. Each device loads ca. 1000 naked host larvae. Eight of these dishes are placed on top of a 60 × 60 × 30-cm rectangular iron-framed, mesh-covered parasitoid adult-multiplying cage. Each adult-rearing cage has a capacity of 220 pairs per 100 cm^2. Adult parasitoids are provided with honey and water ad libitum and kept at 25 ± 1 °C, 65 ± 5% RH, and 12:12 h L:D. After the exposure period, oviposition devices are removed from cages and parasitized host larvae are transferred to

Table 7.1 Production and quality parameters of *Diachasmimorpha longicaudata* in mass-rearing conditions, using two different species of host

Parameter	*Anastrepha ludens*	*Ceratitis capitata* Viena 8 strain
Weight of larva host (mg)	24.06 ± 2.0	10.2 ± 0.3
% mortality of parasitized host	0.62 ± 0.1	25.8 ± 1.0
Weight of pupa (mg)	12.4 ± 1.5	7.3 ± 0.5
% adult emergence	63.7 ± 6.3	53.3 ± 2.0
Sex ratio (female/male)	2.4 ± 0.4	1.2 ± 0.2
Female survivor (days)	5.8 ± 0.2[a]	31.5 ± 2.4[b]
Flight capacity (% of fliers)	80.1 ± 7.8	–
Fecundity (sons/female/day)	3.2 ± 1.8	2.2 ± 1.1

[a] Recorded without water and food

[b] Recorded including water and food (honey)

45 × 30 × 6-cm rectangular white plastic, mesh-covered trays containing sterilized poplar shavings as the pupation medium on the bottom. These trays are kept in a 4 × 4 × 3-m Puparium Training room at 26 °C and 70% RH in darkness. Puparia are sifted to separate them from the substrate and transferred to 20 × 10-cm cylindrical plastic cups, which are incorporated into clean parasitoid-rearing cages. Batches of host puparia with fly emergence percentages <90% are discarded and not used for parasitoid rearing. Before radiation, and hence to the parasitoid exposure, the quality of *C. capitata* Vienna-8 *is* larvae is determined by an average weight of 1000 larvae samples per batch. Batches of third-instar host larvae of 10 mg average weight (±0.5 SE) are selected for parasitoid rearing. About three liters of naked host larvae, that is, no artificial rearing medium, are placed into hard plastic flasks (3000 ml capacity) to be radiated. The radiation of larvae is performed in an IMO-1 mobile Gamacell irradiator with a Co-60 source of γ radiation, located in the Biofactory San Juan, and belongs to the National Atomic Energy Commission (Argentina). The dose is applied at a rate of 23.6 Gy/min under oxygen-free (parameters of production and mass rearing of both mass rearing are summarized in Table 7.1).

7.6 Parasitoid Packing and Release

Pre-release packaging of *D. longicaudata* requires another mass-handling process where indoor environmental conditions for keeping parasitized puparia and adult parasitoids densities per each releasing container are determining factors in achieving high emergence percentages of copulated, well-fed, and healthy females. This procedure is essential for synovigenic females, such as *D. longicaudata*, because females require a feeding period for egg maturation. In addition, the best suggestion in biological control is to release mated females (Pratissoli et al. 2009).

The National Mexican Programme has adopted the terrestrial releases of parasitoids. The cylindrical plastic container locally called "Arturito" is used for the distribution of parasitoids in the field (Montoya et al. 2012). The "Arturito" is a container of 30 L (30 × 30 cm, high × diameter) with two mesh-covered side windows and with a lid at the top. In each container until 10,000, 14 day-old parasitized puparia are introduced. Puparia in containers are maintained for 4 days at 26 °C to cover their more rapid emergence. In the next 2 days, the temperature is reduced to 21 °C, during the emergence continues and the copula is often. On the seventh day, the copulated females are ready for release (Montoya et al. 2012). These containers are carried by car and distributed in wild vegetation near commercial orchards. Releases are mainly located in areas with a high density of host fruits of economically important *Anastrepha* species (Montoya et al. 2017). An average of 1500 parasitoids (2♀:1♂) per ha are released every week during the fruit infestation season (Cancino et al. 2017).

The ProCEM-San Juan has recently adopted a packaging and terrestrial release technology for *D. longicaudata* similar to that used by the Mexican program, which involves 20-L modified circular polypropylene containers called among technicians as "Argentinean Arturitos" (from now on: AA). Parasitoids are packaged as parasitized puparia. Each AA holds 400 cm^3 of parasitized puparia, which is equivalent to a density of 24,000 *C. capitata* Vienna-8 *tsl* puparia. This density of parasitized puparia produces about 11,000 adult *D. longicaudata* with an offspring sex ratio close to 50% females. The AAs are stored for a week in the Parasitoid Packaging room at 25–26 °C, 56% RH, and 12:12 h. Release occurs on the 7th day of adult parasitoid emergence. At this time, all females were born, fed, and copulated. The AAs are transported to the release area by an open-top van.

Early *D. longicaudata* releases in San Juan, which were carried out between 2009 and 2015, were made using the same paper bags that were used for sterile flies released by ProCEM-San Juan (Suárez et al. 2014; Sánchez et al. 2016). In those early parasitoid release campaigns, parasitized *C. capitata* Vienna-8 *tsl* puparia were packed in 17 × 49-cm (width × height) bags of 45-g sulfite paper with a narrow strip of tissue paper filled with icing sugar as food. Each bag had a density of 1300 puparia. The bags were closed at the top with six staples. Bagged parasitized puparia were placed inside plastic crates with a 20-bags capacity. Then, crates were deposited in a dark packing room at 25 ± 1 °C and 70 ± 5% RH for 5 days, until both males and females emerged. The average parasitoid emergence was ca. 40%, equivalent to about 520 individuals per bag, and a female:male sex ratio of ca. 1:1. All release bags were taken to target areas in an air-conditioned vehicle on the sixth day of adult emergence (Sánchez et al. 2016). However, as also occurred in other parasitoid release programs using paper bags, parasitoids tore the paper with their jaws, which, if undetected, could lead to adult escape, which reduced the number of individuals required for effective control in the field. For that reason, a more efficient packaging and release systems, such as the design and use of the device "AA" aforementioned, was sought.

7.7 Results of *Diachasmimorpha longicaudata* Augmentative Releases in Mexico and Argentina

Augmentative releases of *D. longicaudata* in fruit production regions in Mexico have consistently reported parasitism between 40 and 50%, in many cases with significantly high values (Montoya et al. 2000a, 2017; Isiordia-Aquino et al. 2017; Cancino et al. 2020). Due to the high diversity of fruits present in subtropical and tropical environments, percentages of parasitism are variable, which is mainly related to hosting fruit sizes (Montoya et al. 2000a, 2017; Cancino et al. 2020). Although it has not been strongly established, other factors such as attraction to semiochemicals, fly species, and physical characteristics of the fruit (color, texture, etc.) could also have a relationship (Sivinski 1991; Carrasco et al. 2005). The environmental conditions in which *D. longicaudata* can optimize its effectiveness as a control agent for populations of Tephritidae flies are also unknown. Although there is no sufficient evidence to ensure the effectiveness of *D. longicaudata* in a wide range of environmental conditions, the parasitism in the release areas with the presence of larvae has been very consistent.

The release of an exotic introduced parasitoid into a new environment is the case of *D. longicaudata*. This is a new association with the larva of *Anastrepha* spp., in which *D. longicaudata* enface competition with the native species of parasitoids (Sivinski et al. 2000b; Miranda et al. 2015). However, the percentages of parasitism of *D. longicaudata* have not affected the levels of parasitism of native parasitoids (Montoya et al. 2017). Percentages of parasitism from native parasitoids are usually less than 5% (López et al. 1999; Sivinski et al. 2000b), and most host larvae are "free spaces" that are used by *D. longicaudata* (Murphy et al. 2014). This is a very important advantage in the use of *D. longicaudata* as a biocontrol agent in competitive tropical or subtropical environments (Miranda et al. 2015; Murillo et al. 2019).

The Mexican program is essentially operational; therefore, it is difficult to analyze systematically parasitism data. However, a review of the historical data in Tecpan, Guerrero State, *D. longicaudata* mass releases in wild areas made it possible to stop the movement of the *A. obliqua* population between natural and commercial mango orchard areas, which involved 10,000 ha. Parasitoid releases were done in the surrounding area covered with the native tropical plum *Spondias mombin* (L.), which is the preferred host for *A. obliqua* in the region. *Anastrepha obliqua* populations were suppressed in the wild vegetation, which reduced the pest's ability to spread to cultivated areas. This is considered the main reason because the commercial area in Tecpan maintains the status of a fruit fly low prevalence area (Cancino et al. 2020). In the Soconusco region (Chiapas State), the parasitism percentage varies between 30 and 40%. These results are along all year, due to the constant infestation of fruits by diverse species of *Anastrepha* species. This region has high humidity (60–80% HR), and the temperature is an average above 20 °C. The main fruit production corresponds to mango for exportation and other fruits at a low level (zapotaceas, tropical plumps, oranges, etc.) for the national or local market. According to evaluations, the augmentative releases of *D. longicaudata* have contributed notably to the fruit fly suppression in other fruit production regions of Mexico (Montoya et al. 2000a; Isiordia-Aquino et al. 2017; Cancino et al. 2020). Currently this is an important technique in the integrated pest management of fruit flies in Mexico.

The earliest *D. longicaudata* releases in the province of San Juan were primarily aimed at assessing the effectiveness of mass-reared parasitoid females to find and successfully parasitize wild *C. capitata* larvae in different host fruit species once released (Suárez et al. 2014). The relevance of these releases was that they were carried out under semi-arid environmental conditions in six ecologically isolated fruit-growing valleys of San Juan. The releasing sites were located in both urban and rural areas of Santa Lucía (31°32′26″ SL 68°29′52″ WL), Zonda (31°32′51″ SL 68°43′50″ WL), Pocito (31°39′17″ SL 68°34′46″ WL), Caucete (31°39′10″ SL 68°16′49″ WL), Rivadavia (31°31′52″ SL 68°35′42″ WL), and Ullum (31°27′44″ SL 68°44′09″ WL) valleys. Terrestrial releases were used to disperse 40,000 adult parasitoids per target site through 2009. Parasitoids were ground-released in sulfite bags like the one used for TIE at places in which no insecticides were regularly applied, and where there was a wide diversity of *C. capitata* host plants, such as backyards and small orchards. Individuals of *D. longicaudata* were recovered from six *C. capitata*-infested fruit species, such as *Citrus reticulata* Blanco (tangerine), and *C. sinensis* (L.) Osbeck (sweet orange) (Rutaceae), *Diospyros kaki* L. (persimmon) (Ebenaceae), *Ficus carica* L. (fig) (Moraceae), *Rosa canina* (Bastard) Rapin (Rose) (Rosaceae), and *Vitis vinifera* L. (grape) (Vitaceae). Likewise, parasitoids were collected in five release sites, such as Pocito, Zonda, Santa Lucía, Caucete, and Rivadavia. The highest mean parasitism percentages of *C. capitata* were found in both fig and rose, 1–21% and 4–17%, respectively, which varied according to the month and the collecting site. Parasitism in persimmon, grape, tangerine, and sweet orange was 11, 8, 4, and 3–4%, respectively. These preliminary releases provided valuable data on the performance of the exotic parasitoid *D. longicaudata* in a semi-desert region with environmental conditions completely different from the insect's native site (tropics). Release areas involved oases in which host plants are gathered under artificial irrigation. Data showed that *D. longicaudata* females mass reared on *C. capitata* Vienna-8 *tsl* larvae can efficiently find and parasitize wild *C. capitata* larvae in fruits of different physical characteristics from February to June, which covered summer and autumn seasons.

Based on this information, a pilot test of weekly parasitoid augmentative releases was exclusively designed to assess the *D. longicaudata* effectiveness on the *C capitata* population in a particular cropped area with high pest infestation levels. In this regard, parasitoid releases were carried out over fig crops in southern San Juan during the postharvest stage and over a period of nine consecutive weeks, between March and May 2012 (Sánchez et al. 2016). Releases were made in two experimental plots of 23.400 m^2 (ca. 2.3 ha) each, while another two plots of the same size were used as controls. Each plot included 522 commercial fig trees. Plots were isolated from fly inflows from surrounding areas by dry traps containing both an attractant and an insecticide. A density of one trap per 200 m^2 was used to isolate plots. Parasitoids were released by ground through TIE sulfite bags every week by using a system transect. About 5200 parasitoids were released per experimental plot each week, which is 2261 parasitoids/ha. Artificial devices were fabricated to hold non-infested figs and expose them to natural infestation by the oviposition of wild *C. capitata* females, which led to the larval development into the fruit. Therefore, host larvae were naturally exposed to *D. longicaudata* females. Those plastic devices were hung from branches on the inner middle portion of selected fig trees at 1.5 m above soil level. Each device had three figs. Thirty devices, which involve 90 figs, were scattered throughout each plot, 3 days before each release date. Results showed an interesting performance of *D. longicaudata* as a biocontrol agent, as pest mortality by a direct effect of parasitoid releases ranged from 16% to 75%. These findings revealed *D. longicaudata* as a promising natural enemy for *C. capitata* control in San Juan, as well as in other similar fruit-growing regions of Argentina. Other *D. longicaudata* release trials similar to the one described above were carried out from 2017 to 2018 in commercial multi-fruit farms close to the urban area of south-central San Juan province. Results were encouraging to support ABC against *C. capitata*, as average parasitism rates were close to 40% (Fernando Murúa, pers. commun). At present, terrestrial releases of *D. longicaudata* are carried out in urban areas using the AA devices described above (Results of parasitism in México and Argentina from different fruits and fly species are reported in Table 7.2).

7.8 Conclusions

Currently, *D. longicaudata* represents the best option for the biological control of fruit fly pests in America. This exotic species meets all requirements for operational mass production and release activities. First, mass rearing generates a high percentage of parasitoid emergence with good adult quality; second, packaging procedures allow the release of adult parasitoids without losing their quality. The results achieved by these two programs described in this chapter clearly show that *D. longicaudata* can be used as an important biocontrol agent. Two relevant highlights can be gleaned from the information provided in this chapter: (a) the high versatility of *D. longicaudata* in adapting to different environments and (b) the adequacy of mass-rearing procedures, product quality, and release packaging in maintaining the total quality concept (Leppla and Ashley 1989; Leppla and Fisher 2009). Regarding these points, there are much data about the performance of *D. longicaudata* in different environments (Sivinski et al. 1996, 2000a; Montoya et al. 2000a; Sánchez et al. 2016; Montoya et al. 2017; Cancino et al. 2019). However, the effectiveness of *D. longicaudata* according to the environmental conditions has been analyzed in a few studies (Sánchez et al. 2016; Harbi et al. 2018a, b). In this chapter, we demonstrate that the effectiveness of *D. longicaudata* in controlling different species of pest fruit flies in ecologically contrasting environments is high. The province of San Juan, Argentina, has a typical dry environment opposite to most of the tropical Mexican conditions where *D. longicaudata* releases were performed. An efficient host searching capacity in these two environments is reflected by high parasitism

Table 7.2 Parasitism ranges of different fruits in areas with application of augmentative releases of *Diachasmimorpha longicaudata* in México and San Juan, Argentina

Common name	Scientific name	% de parasitism	References
Mexico			
Ataulfo mango	*Mangifera indica*	5–50%	Montoya et al. (2000a), Cancino et al. (2020)
Creole mango	*Mangifera indica*	20–50%	Montoya et al. (2000a), Cancino et al. (2020)
Grape fruit	*Citrus grandis*	10–40%	Isiordia-Aquino et al. (2017), Cancino et al. (2020)
Guava	*Psidium guajava*	17–60%	Montoya et al. (2000a), Isiordia-Aquino et al. (2017), Cancino et al. (2020)
Hog plum	*Spondias mombin*	30–60%	Montoya et al. (2000a), Cancino et al. (2020)
Myrtle	*Luma apiculata*	50–70%	Isiordia-Aquino et al. (2017)
Sour orange	*Citrus auraintium*	8–60%	Montoya et al. (2000a), Isordia-Aquino et al. (2017), Cancino et al. (2020)
Sweet orange	*Citrus sinensis*	20–60%	Isordia-Aquino et al. (2017), Cancino et al. (2020)
Tangerine	*Citrus reticulata*	30–50%	Cancino et al. (2020)
San Juan, Argentina			
Fig	*Ficus carica*	1–21%	Suárez et al. (2014)
Grape	*Vitis vinífera*	8%	Suárez et al. (2014)
Rose	*Rosa canina*	4–17%	Suárez et al. (2014)
Persimmon	*Diospyros kaki*	11%	Suárez et al. (2014)
Sweet orange	*Citrus sinensis*	3–4%	Suárez et al. (2014)
Tangerine	*Citrus reticulata*	4%	Suárez et al. (2014)

rates after releases. This involves parasitoid females found the host, oviposit it, and developing successfully. However, periodical releases are needed to preserve high parasitism percentages during the pest fly population increase. In both scenarios, with different pest species and environments, augmentative releases involve an effective strategy for target population suppression (Montoya et al. 2000a; Sánchez et al. 2016).

The most frequent query for including biological control in the framework of fruit fly control programs is the costs related to the production and release of the natural enemy. The answer requires a complete analysis including different variables, which are out of the scope of this work (Paine et al. 2015). However, the contribution of biological control in integrated fruit fly management is unquestionable. Reducing fruit fly populations in a regional program is a priority, and chemical control is a very effective tool (Matthews et al. 2014). However, chemical control can be a technique with negative effects on the environment and with a very low appreciation for people. The costs of these negative impacts are difficult to quantify because they involve different variables and usually occur over the long term (Sanchez-Bayo 2021; Mendez-Espinoza et al. 2006). On the other hand, biological control is defined as an eco-friendly technique with a low environmental impact, and it has a very good reputation for a high level of social acceptance.

Different improvements are needed for cost reduction and to increase parasitoid effectiveness. It is very important to develop low-cost diets to produce hosts with special nutritive requirements for parasitoids. In addition, standardization of quality parameters to provide indicators of adult effectiveness, improvement of adult packaging logistics, adequate parasitoid release rates per area, and timing of releases are priorities to be addressed by future studies. The use of new technology as the "chilling insect technique" for aerial releases is in advance (Cancino et al. 2020).

Releases of *D. longicaudata* are nowadays a fundamental tool in the framework of an AW-IFFPM performed by the Mexico and San Juan fruit fly programs. It is worth noting that both programs do not yet have a cost analysis for the use of ABC, but this method has received significant social acceptance as an environmentally and human health-friendly strategy.

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Contents

Part I General Aspects of Fruit Fly Management

Part II Management of Fruit Flies in the Countries of the Americas

Flávio Roberto Mello Garcia *Editor*

Management of Fruit Flies in the Americas

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