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Rearing *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) on Mediterranean Fruit Fly and its Introduction into Senegal against Oriental Fruit Fly (Diptera: Tephritidae)

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Abstract. Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (aka *B. invadens* Drew, Tsuruta, and White), a serious pest of tropical fruits, particularly mango, was first reported in Africa in 2003 and quickly spread to over 27 countries. In the parasitoid introduction reported herein, *Fopius arisanus* (Sonan) was reared on and shipped to Senegal inside pupae of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), which is endemic to Africa, rather than its usual *B. dorsalis* host, because *B. invadens* was still treated as a separate species from *B. dorsalis* in 2012, and to avoid the risk of fly escape from unparasitized pupae in the shipment. From 2013 to 2014, 14 shipments, totaling approximately 246,000 *F. arisanus*, were sent from Hilo, HI, USA to Dakar, Senegal and released in 12 mango and orange orchards in the Casamance region of southern Senegal. Parasitoids were emerged from pupae, processed and small scale rearing done on locally available *B. dorsalis* for subsequent releases. Limited numbers of *F. arisanus* had also been released in 2012 from cultures maintained in Cotonou, Benin, by IITA under the PADERCA project, but parasitism was relatively low. During 2013 and 2014 parasitism rate in mango fruits has steadily increased to 20–25%. Based on this technique, a similar approach has been used for introduction of *F. arisanus* against carambola fruit fly, *Bactrocera carambolae* Drew & Hancock, into Brazil.

Key words: fruit flies, *Ceratitis capitata*, *Bactrocera dorsalis*, natural enemies, biological control, rearing, colonization

Oriental fruit fly, *Bactrocera dorsalis* (Hendel), the most damaging pest Dacine fruit fly species in the world (Vargas et al. 2015), is distributed throughout tropical and subtropical Asia and was accidentally introduced and quickly became widespread in many of the Pacific Islands, including Hawaii and Tahiti (White and Elson-Harris 1992, Clarke et al. 2005, Vargas et al. 2015). Two closely related species in the *B. dorsalis* complex have become established on large continents: *B. carambolae* Drew and Hancock, the carambola fruit fly, in Suriname (South America) in 1975, and *B. dorsalis* (until recently referred to as *B. invadens* Drew, Tsuruta, and White) in Kenya (Africa) in or before 2003 (Drew et al. 2005, Rousse et al. 2005), where it quickly spread to over 27 countries (Ekesi and Billah 2007).

From the original splitting of *B. dorsalis* into four distinct species (Drew and Hancock 1994) and the later description of *B. invadens* Drew, Tsuruta and White (Drew et al. 2005), Drew and Romig (2013) more recently declared *B. philippinensis* Drew and Hancock a junior synonym of *B. papayae* Drew and Hancock, which was in turn, along with *B. invadens*, declared a synonym of *B. dorsalis*, while *B. carambolae* remained a valid species (Schutze et al. 2015). With the recent spread of *Bactrocera* spp. into new areas of the world and failed eradication attempts, there has been renewed interest in biological control for sustainable economic suppression of these destructive and invasive fruit pests.

Soon after the introduction of *B. dorsalis* into Hawaii in 1945 (Fullaway 1947), 32 species of parasitoids were imported and released from Asia, two of which became the most effective natural control agents (Bess et al. 1961, Clausen et al. 1965). *Diachasmimorpha longicaudata* (Ashmead) (Braconidae) increased rapidly following its 1948 release, but lost its dominant position to *Fopius arisanus* (Sonan) after 1950 (Sonan) (van den Bosch and Haramoto 1953, Ramadan et al. 1992). This latter species dramatically reduced infestation of fruit through a high level of parasitism (65-70%), and has since remained the dominant parasitoid species, owing to its ability to target host flies at the egg stage (Haramoto and Bess 1970, Vargas et al. 1993). The United States Department of Agriculture (USDA), Agricultural Research Service (ARS) Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center (DKI-USPBARC) has been active in promoting biological control of *Bactrocera* spp, using classical, augmentative, conservation, and IPM approaches, and by disseminating parasitoids to other parts of the world to target not only *Bactrocera* but also *Ceratitis* and *Anastrepha* spp. (Vargas et al., 2012a, Groth et al. 2016). Of these, the introductions and post-release surveys in French Polynesia are particularly well documented (Vargas et al. 2007, 2012b, Leblanc et al. 2013) and provided important information that facilitated subsequent introduction into continental areas of South America and Africa.

Mango (*Mangifera indica* L.) provides essential nutrition and a valuable source of income in many West African countries, but significant damage is caused by four fruit fly species: *B. dorsalis*, *Ceratitis cosyra* (Walker), mango fruit fly, *C. silvestri* Bezzi and *C. quinaria* (Bezzi) (Vayssières et al. 2015). Species of *Ceratitis*, native to that region, are most common during the dry season with the main pest species, *C. cosyra*, reaching a peak at the end of the dry season, whereas the introduced *B. dorsalis* is scarce during the dry season, but populations increase rapidly from the end of April to reach a peak at the end of June, during the rainy season, causing considerable damage to mid and late season fruits (Vayssières et al. 2008). Commercial loss of mangos, based on the proportion of fruits with at least one observed oviposition puncture, is estimated to increase from less than 25% in early April to greater than 75% in July (Vayssières et al. 2008).

Preliminary releases of 5,000 specimens of *F. arisanus* from cultures maintained in the International Institute of Tropical Agriculture (IITA Benin) were made at three mango orchards in Senegal in June 2012, and suppression of *B. dorsalis* was reported in these orchards, compared to control orchards (Ndiaye et al. 2015). Additional shipments of *F. arisanus* from Hawaii were introduced in 2013 and 2014, with 2 years of results from field monitoring reported herein. In the present study we report on rearing of *F. arisanus* on *C. capitata*, in comparison to the usual rearing protocol on *B. dorsalis*, and its introduction and establishment in the Casamance region of Senegal. The reason for shipping *F. arisanus* in *C. capitata* pupae was precautionary, to avoid increasing genetic diversity through mating of Hawaiian *B. dorsalis*, of East Asian origin, with African flies of Indian subcontinent origin, and because *B. dorsalis* and *B. invadens* were in 2013, and are still by some authors (Drew and Romig 2016), regarded as separate species.

Materials and Methods

Parasitoid rearing and releases. *Fopius arisanus* were initially obtained from a colony reared for over 200 generations on *B. dorsalis* at the DKI-USPBARC facility in Hilo, HI. The colony for Senegal, established in September 2012 and maintained through January 2013, was a mixture of the strain maintained on *B. dorsalis* eggs and a small number of parasitoids obtained from *C. capitata* eggs. After January 2013, a separate colony of *F. arisanus*, reared exclusively from *C. capitata* and destined for releases in Senegal, was maintained at the DKI-USPBARC facility. Rearing methods for *F. arisanus* on *B. dorsalis*, outlined by Manoukis et al. (2011), were similar on *C. capitata* except that *C. capitata* eggs were exposed to parasitoids instead of *B. dorsalis* eggs. Fourteen shipments of *F. arisanus* inside *C. capitata* pupae were sent by airplane to Senegal between February 2013 and October 2014. Parasitoids were transferred from the Dakar International Airport to the Ziguinchor DPV Laboratory (Casamance), where a small laboratory was established to emerge parasitoids and evaluate fly infestations and parasitism in field-collected fruit samples. In preparation for field releases, parasitoids were allowed to emerge from pupae placed inside cubical cages (26 x 26 x 26 cm), with approximately 2,000 wasps held inside each cage until release. Wasps were provided with creamy textured honey (Bradshaws, Sioux Falls, IA) and water. Dead parasitoids inside cages were counted after 4 days to estimate the number of wasps released into the field. During releases, cubical cages were generally placed under host trees and opened gently, allowing parasitoids to disperse to nearby ripe host fruits. In addition to releases of imported shipments, a colony of *B. dorsalis* was established in the laboratory to rear wasps for further augmentative releases, following the methodology of Harris et al. (1991). Percent parasitism by *F. arisanus* (expressed as the number of adult *F. arisanus* / number of adult *B. dorsalis* + *F. arisanus* emerged from field-collected fruits) was calculated for the years 2012–2014 to document the rate of parasitism after establishment. To assess the possible impact of *F. arisanus* on fruit infestation, fruit fly (*B. dorsalis*) emergence per kg of fruit was compared for 2012 to 2014.

Study sites, where parasitoids were released, were located in the Casamance region in southern Senegal, and consisted of 12 low-elevation (13–24 m asl) orchards that contained mango (*Mangifera indica* L.) (see Ndiaye et al. 2015) and orange (*Citrus sinensis* (L.) Osbeck) (Figure 1). These were suitable hosts for *B. dorsalis* and *F. arisanus* and could be collected consistently in relatively large numbers for evaluations.

Ripe fruits were collected from trees and the ground between 2012 and 2014, monthly between May and August for mango, and in January and October for orange. Details on numbers of collections and fruits sampled are included in Figure 2 footnote. Sample handling procedures were described previously in Vargas et al. (2007). Briefly, fruits were weighed and placed in batches on wire metal screen (43 x 28 x 6 cm) inside plastic holding boxes (50 x 32 x 15 cm) that contained

1.5 cm of moist sand. After 3 weeks of incubation, the sand was sifted weekly to extract pupae, that were transferred to smaller plastic containers and held until emergence of flies or parasitoids, that were counted and recorded. Fruit and recovered pupae were held in a room maintained at 22 ± 5°C, ambient relative humidity (40-90%), and a photoperiod of 12:12 (L:D) hours.

Statistical analysis. We compared parasitization by strains of *F. arisanus* reared on *C. capitata* and *B. dorsalis*. A generalized, linear mixed model was used to fit the proportions, total parasites/total pupae using the logit link for the binomial (SAS Institute 2013). The fixed effects were host, year and the interaction. The random effects were dates within years and host x dates within years.

Results

In the rearing laboratory in Hawaii, *F. arisanus* parasitism on *C. capitata* consistently increased over time and eventually compared favorably to those reared on *B. dorsalis*, its native host (Table 1). Quality control parameters for the parasitoids shipped are summarized in Table 2. Fourteen shipments were sent to Senegal, and emerging parasitoids released at 12 selected sites in the Casamance region (Table 3, Figure 1). Mean (± SEM) numbers of *B. dorsalis* per kg of host fruit and mean (± SEM) percent parasitism by *F. arisanus* on samples collected at the 12 sites, from May 2012 to October 2014, are summarized in Figure 2. For mango, parasitism steadily increased from May 2012 to August 2014, while parasitism in orange consistently remained low. Fly emergence per kg fruit, on the other hand, was not decreased with increase of parasitism on mango. There was a trend for slightly higher parasitism mean (± SEM) in coastal (23.0 ± 2.9) than inland farms (19.3 ± 4.7), though the difference was not significant.

Discussion

To understand how results from this study might be useful in planning future biological control releases in other areas of Senegal and other West African countries, it may be helpful to revisit efforts to control *B. dorsalis* using *F. arisanus* in French Polynesia (Leblanc et al. 2013). Subsequent to its 1996 invasion and unsuccessful eradication attempt, ten shipments of parasitoids from Hawaii (500,000 insects) were released between 2002 and 2004. Within 3 years, *F. arisanus* was established throughout Tahiti and nearby Moorea Islands, so abundant in infested guava that parasitoids recovered from field-collected fruits were used for its release and establishment to control *B. dorsalis* throughout the territory (Vargas et al. 2007, Leblanc et al. 2013). By 2009 mean parasitism on flies infesting guava, Polynesian chestnut (*Inocarpus fagifer* (Parkinson) Forsberg) and tropical almond (*Terminalia catappa* L.), which fruits are available throughout the year, was 64.8% on Tahiti Island, and emergence of *B. dorsalis* per kg of fruit had declined by 92.3%, compared to the 2002 pre-release levels (Vargas et al. 2012b). Noteworthy, parasitism on mango, available only for a few months of the year, was not as high (24.9%) (Vargas et al. 2012b). A second braconid parasitoid, *Diachasmimorpha longicaudata*, introduced in 2007–08, became equally widespread, though parasitism rates have not been higher than 10% (Vargas et al. 2012b). Establishment and impact of *F. arisanus* in French Polynesia is the most successful example of classical biological control of fruit flies in the Pacific outside of Hawaii.

Recent experience in French Polynesia and data presented here for Africa support *F. arisanus* as a prime biological control candidate to suppress *B. dorsalis* in other African countries and *B. carambolae* in South America. Although originally im-

Table 1. Percent recovery (mean and 95% confidence intervals) of *F. arisanus* parasitoids from colonies reared on *C. capitata* and *B. dorsalis* in Hawaii from 2013 to 2014, during the same period as the releases.

Year	Species	Mean % parasitoid emergence	Lower limit	Upper limit
2013	*C. capitata*	17.94	16.01	19.87
2013	*B. dorsalis*	49.32	46.13	52.51
2014	*C. capitata*	46.81	42.57	51.04
2014	*B. dorsalis*	43.48	39.30	47.65

Year (df = 1, 23.87, $F = 14.67$, $P = 0.0008$), species (df = 1, 23.87, $F = 20.22$, $P = 0.0002$), and year*species (df = 1, 23.87, $F = 29.03$, $P < 0.0001$) Proc Glimmix (SAS 2013).

Table 2. Quality control parameters (mean ± SEM) for *F. arisanus* reared on *C. capitata* in Hawaii and shipped to the Casamance area of Senegal during 2013 and 2014.

Year	No. samples	Pupae weight (g)	Viable pupae (%)	Flies (%)	Parasitoids (%)	Parasitoid sex ratio (F/M)	No. pupae per g
2013	9	1,000	74 ± 8	61 ± 7	13 ± 3	1.10 ± 0.20	146 ± 6
2014	5	625	84 ± 1	40 ± 3	44 ± 4	1.36 ± 0.05	169 ± 3

Table 3. Number of *F. arisanus* shipments and total number released at 12 release sites in the Casamance area of Senegal, obtained from Benin and released by DPV (2012), and from Hawaii (2013 and 2014).[1]

Year	No. shipments	No. pupae shipped	No. releases	Estimated no. released
2012	5	55,260	5	25,000
2013	9	145,750	11	160,000
2014	5	105,813	6	86,000

[1]Release data based on records maintained by Crop Protection Directorate (DPV) Km 15 Rufisque Road, P.O.Box 20054 Thiaroye, Dakar, Senegal. Shipment data during 2013 and 2014 based on data maintained at the USDA-ARS DKI Pacific Basin Agricultural Research Center, Hilo, HI 96743, USA.

ported into Hawaii to target *B. dorsalis*, it does parasitize *C. capitata* (Harris et al. 1991), of which it has also become the predominant parasitoid in such crops as coffee (*Coffea arabica* L.) (Vargas et al. 2001). Likewise, we anticipate increased mortality on *C. capitata* in areas where mixed populations of both pest flies occur in Africa. The effect on *C. cosyra* and other Ceratitinae is unknown and should be studied. Unique to this project was using *F. arisanus*, normally reared on its native *B. dorsalis* host, and rearing it on a less preferred host, *C. capitata*, for releases against *B. dorsalis*. This technique has also been used for introductions of *F. arisanus* from Hawaii to Brazil against *B. carambolae*, and possible adaptation to the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Groth

No.	Locality	Parasitism
1	Diana Kabar	21.7 %
2	Koubanac	25.9 %
3	Diouloulou	23.4 %
4	Kataba	26.6 %
5	Boutoute	11.6 %
6	Tenghory	17.9 %
7	Niaguis	18.0 %
8	Djibelor	24.1 %
9	Bourofaye Diola	19.9 %
10	Brin	24.2 %
11	Diakene Diola	21.2 %
12	Boucotte Djinbering	19.2 %

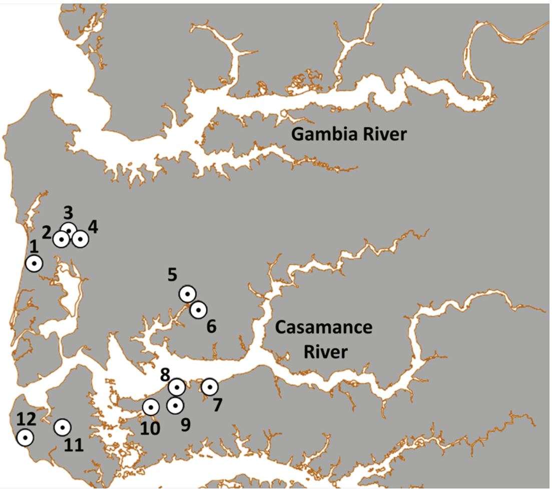

Figure 1. Twelve mango and orange sampling sites in the Casamance region of Senegal, with percent parasitism at each site in 2014.

et al. 2016). Initially, parasitization of *C. capitata* in the laboratory colony was very low, but increased from 18 to 47% over a 2-year period (Table 1), similar to the increase from 10 to 67% over 25 generations reported by Harris et al. (2007). How long the effects of conditioning *F. arisanus* on *C. capitata* in the laboratory lasts in the field, as far as parasitizing specific hosts (e.g. *C. capitata* or *B. dorsalis*), and whether level of parasitization and parasitoid abundance is determined by fruit fly species and their population dynamics, are not known and merit further study.

Many of the tropical hosts infested by *B. dorsalis* and parasitized by *F. arisanus* in French Polynesia and Hawaii are also common in tropical Africa and South America. *Fopius arisanus* may be reared in the laboratory or easily recovered from heavily infested fruits for transport and establishment in different areas or countries in tropical Africa. However, releasing *F. arisanus* in a continental setting brings some new questions and challenges, especially with ensuring that enough wasps are released for the population to become self-sustaining throughout the year, even when fruiting host material, such as mango, is scarce. Studies in the Casamance from 2012 through 2014 suggest only modest levels of parasitism (20–25%), at least within the first three years of its establishment, and slightly higher parasitism in coastal areas. Nonetheless, we would expect an increase in the size of the area inhabited by *F. arisanus* over time, and possibly an increase in percent parasitism, as observed on Tahiti and other islands in French Polynesia, which can be accelerated by releasing the parasitoids at multiple locations over larger areas. Currently, parasitoid releases from additional shipments from Hawaii are being made in northern areas of Senegal, where mango cultivation is more extensive and climate much dryer than the Casamance area. For future releases, we caution that while biological control observed in small orchards in the Casamance area appears sustainable, it may be important to test whether augmentative releases could improve levels of field parasitism and significantly decrease damage to fruits during certain

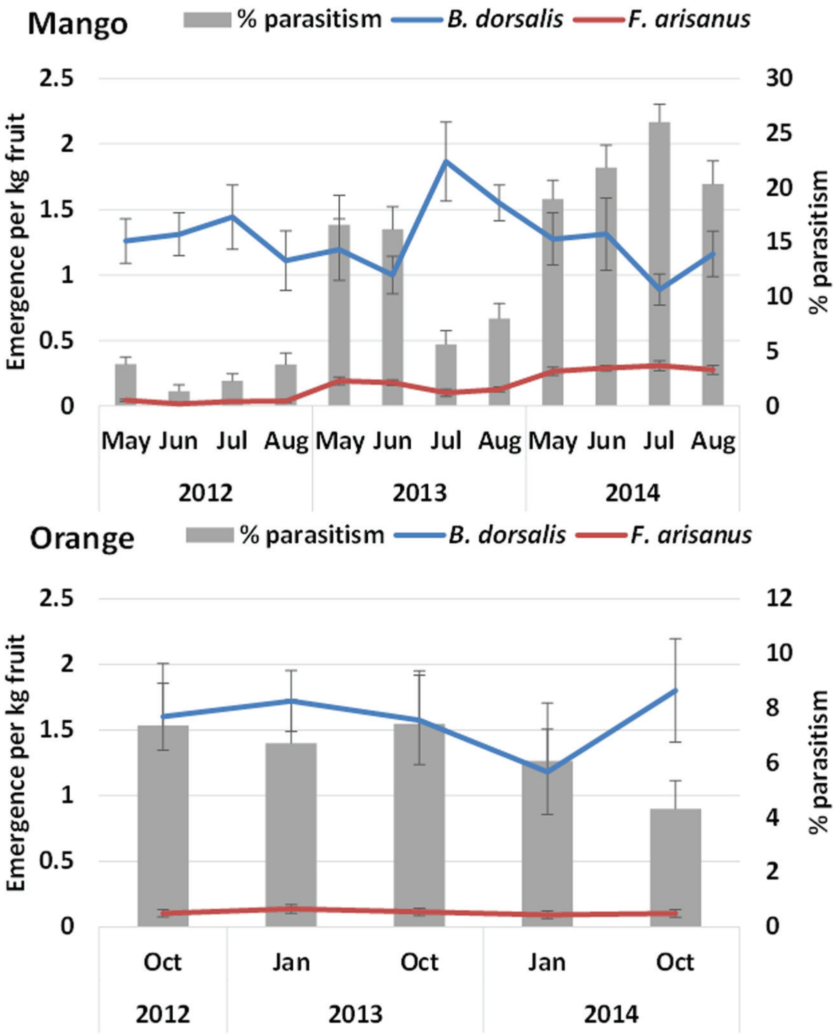

Figure 2. Mean (± SEM) numbers of *B. dorsalis* per kg and mean (± SEM) percent parasitism by *F. arisanus* from samples of mango and orange sampled from twelve locations between May 2012 and August 2014*.

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*Number and weight of fruits sampled were: For mango: 2012: 451 fruits (345.2 kg) in May, 466 fruits (372.0 kg) in June, 413 fruits (318.6 kg) in July, 472 fruits (366.3 kg) in August. 2013: 491 fruits (322.8 kg) in May, 534 fruits (367.6 kg) in June, 475 fruits (311.9 kg) in July, 563 fruits (406.7 kg) in August. 2014: 437 fruits (356.7 kg) in May, 428 fruits (322.3 kg) in June, 427 fruits (364.9 kg) in July, 432 fruits (359.0 kg) in August. For orange: 2012: 264 fruits (101.7 kg) in October. 2013: 242 fruits (105.4 kg) in January, 265 fruits (109.7 kg) in October. 2014: 231 fruits (100.3 kg) in January, 211 fruits (90.3 kg) in October 2014.

times of the year in larger orchards, when parasitoids are not abundant. For example, *D. longicaudata* is mass reared and released in mango orchards to reduce damage by *Anastrepha* spp. in Mexico (Montoya et al. 2000).

In conclusion, due to the modest levels of parasitism by *F. arisanus* in the present study, other available IPM tools such as crop sanitation, reduced risk protein baits, and male annihilation should also be integrated with parasitoids into effective IPM programs for management of fruit flies in mango orchards (Vargas et al. 2001, 2015; Vayssières et al. 2009). Because Africa now has a similar complex of fruit fly species attacking tropical fruits and vegetables to those found in Hawaii, the Hawaii Fruit Fly Area-Wide Pest Management program, recommending the 1-2-3 approach (1 = sanitation, 2 = protein bait, and 3 = male lures) has particular relevance to Africa for sustainable control of fruit flies, especially the *Bactrocera* species (Vargas et al. 2016).

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