A review on the Tephritid fruit flies of economic interest in Cuba: species, plant hosts, surveillance methods and management program implementation

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Abstract

Background: The presence of several Tephritid species in Cuba required of special surveillance methods to determine the free-pest zone or at least to determine the exact species inhabiting the island and the economical repercussion that could affect to the export market. Our previous studies of surveillance, monitoring and training methods set up the protocols for an area-wide fruit fly management irrespectively of the fruit species. In this work, we upgraded the surveillance of *Anastrepha* species in export commodities and in other crops.

Methods: Several commodities (fruit varieties) were sampled including not host fruits. A deep surveillance of citrus was also included. Collected infested fruits were retrieved to the laboratory to allow larva development to identify emerged adults to species level. Monitoring traps were also used in citrus plots to confirm the *Anastrepha suspensa*-free status of this commodity.

Results: Monitoring traps allowed to confirm the presence of different *Anastrepha* species in Cuba namely *A. suspensa*, *A. soroana, A. obliqua*, *A. ocresia*, *A. insulae* and *A. interrupta,* and also *Toxotrypana curvicauda*. An aditional species, belonging to *Anastrepha* genus, *A.* sp., was also recorded, not matching any described species. Fruit fly major population peak was found to fit with ripening season of stone fruits and other non-citrus fruits. Following the surveillance of tephritid larva´ infested fruits, five new host species were confirmed for *Anastrepha suspensa*: sapodilla, rose apple, cocoplum, custard apple, and gac fruit. And two new ones for *Anastrepha obliqua*: the cocoplum and yellow mombin. Citrus surveillance was clear, no tephritid fruit fly was found in any *Citrus* species in all along Cuba Island.

Conclusions: The absence of fruit flies in citrus commodities reveals the success of the implemented management program including surveillance, monitoring and personnel training, putting into value the area-wide Cuban fruit fly management program for *Anastrepha* species.

Keywords: Anastrepha spp., host status, monitoring systems.

Introduction

The Cuban citrus industry experienced a decrease of production in the early 90's, comparable with that experienced in the early 60's. However, early in this new century (S. XXI), the production has recovered its yield. With more than 109,000 hectares devoted to fruit cultivation, only 20% are dedicated to citrus whereas more than 24% are devoted to mango groves, which highlighted the Cuba ability to open its fruit market. The Cuban citrus and fruit industry operates within Fruit Trees Company Group (GEF), the Ministry of Agriculture, several small companies and the Tropical Fruit Trees Research Institute (IIFT) that provide scientific and technical support for all Cuban fruit culture (FAO, 2003).

Nowadays, Cuba is working to develop the country's fruit demand in a sustainable way in order to cover both internal demand (population and tourist facilities) and exports markets but not only focused in Citrus (http://www.atcitrus.com/english/15460) also covering other fruits trees (Fig. 1). Within this fame, the IIFT had developed special methodologies to control pest insects that would affect this new age of fruit production. More precisely, the Ecology joint unit formed by IIFT and IES (Ecology and Systems Institute), had developed a laboratory for fruit fly species and their hosts identification. These two research units are also responsible of surveillance, monitoring and training methods to set up protocols for an area-wide fruit fly management (Borges-Soto et al., 2011, 2015). These protocols cover one of the key insect pests, the Tephritidae fruit flies. This group of flies encompasses one of the most destructive world-wide distributed pest species, grouped in three main genera, *Anastrepha, Bactrocera* and *Ceratitis,* many of them not present in the Cuba Island.

In Cuba it had been identified ~30 species of tephritids, belonging to 15 genera (in alphabetical order: *Acinia, Acrotaenia, Anastrepha, Blephanroneura, Dioxyna, Dyseuresta, Euaresta, Euarestoides, Hexachaeta, Tetreuaresta, Tomoplagia, Toxotrypana, Trupanea* and *Xanthaciura*), with many of them without reference to their host plant (Fernandez et al., 1997; Rodriguez Velásquez et al., 2001; Ovruski et al., 2005). This list of species was obtained mainly from the Zoological Collection repository at the IES, meaning that in most cases the description is based on the type or holotype specimen archived, not from captured material, for which its real status of presence and the estimation of their economic impact on crops production in the Cuba Island still requires further research. The geographical localization of Cuba Island, its climatic situation with increase of strong climatic events (i.e. hurricanes, tropical storms, etc.) and the market opening along the establishment of new cultivars could benefit the arrival and establishment of new species from the surrounding countries. As an example of highly pestiferous Tephritidae species found in the Cuba island' neighbors we can found members of *Bactrocera* and *Ceratitis* genus; *Bactrocera correcta* is regularly trapped in Florida, and even *Ceratitis capitata* which often invades also Florida induce the establishment of SIT and other eradication methods with a great economic impact on citrus growers (Thomas et al., 2010). Thus, the presence of any of these species in Cuba will represent a negative point in their export market, by the quarantine measures imposed by other countries in which species are not present (i.e. USA, Mexico, among others), and a

negative impact in the fruit gross production by the direct damage that these species exerts to the production.

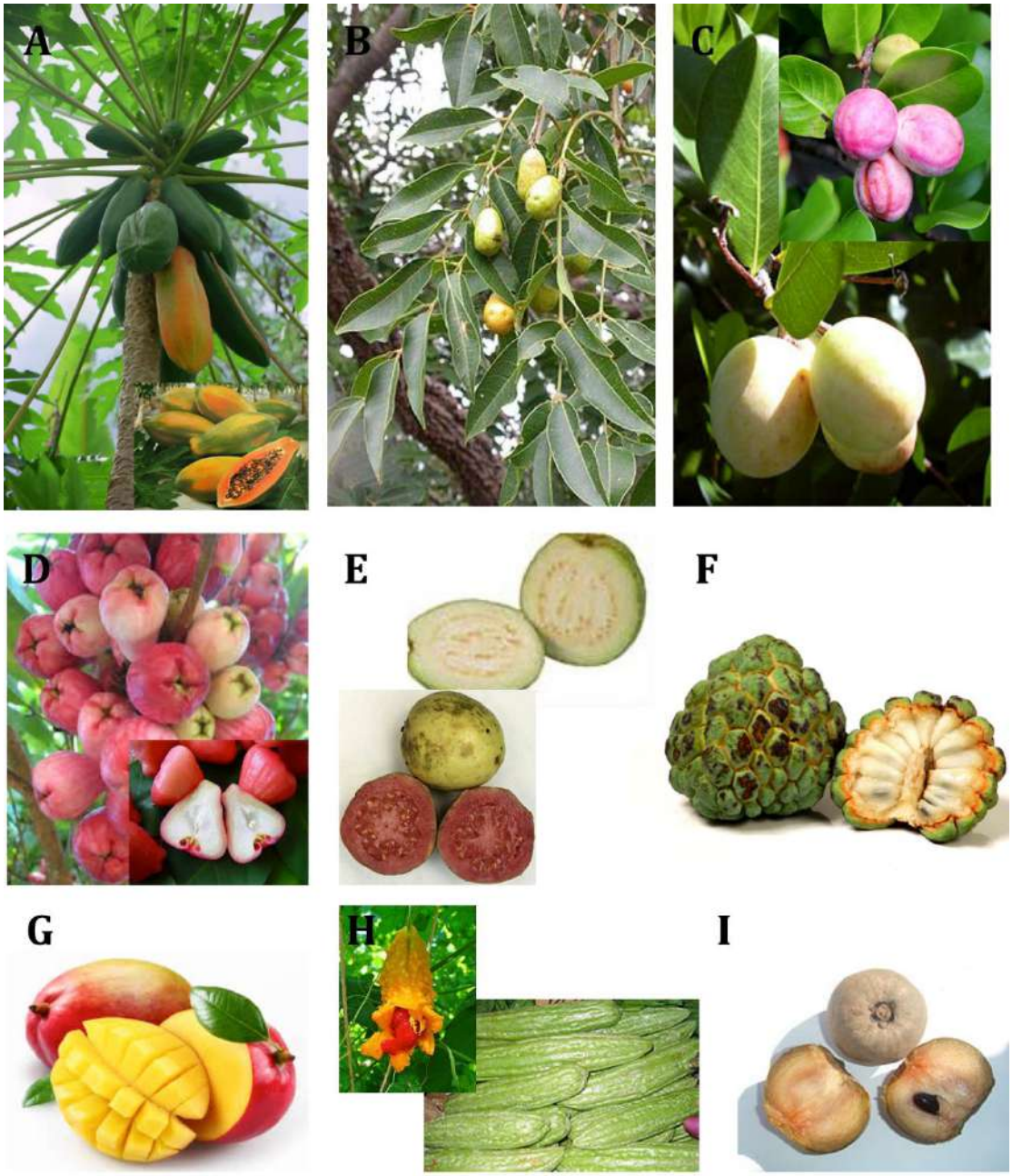

Fig. 1. Some of the fruits, other than Citrus, in production in Cuba, that are susceptible of being attacked by Tephritidae fruit flies. A. Papaya, tree and open fruit; B. Jobo, Yellow monbin or Hog plum; C. Icaco or Cocoplum (*Chrysobalanus icaco* Lin.); D. Pomarrosa or Rose apple (*Syzygium jambos* L.); E. Guava or Guayaba; F. Mamón or Custard Apple (*Anona reticulata* L.); G. Mango; H. Cundeamor or Gac fruit (*Momordica cochinchinensis*), one ripe open fruit in the plant and some market size; and I. Níspero or Sapodilla (*Manilkara zapotilla* (Jacq.) Gilly)). Pictures were taken mostly from Wikipedia.

In this context, it was advisable to continue the work developed during the past decade by the Entomology-Ecology unit of IIFT (Borges-Soto et al., 2011). Of special interest were the

developed training methods to be spread among Cooperatives for Agricultural production staff members and growers. But, also, the highlighted and supported the surveillance trap-net around all the citrus production areas, and the monitoring system for other fruit crops (Borges-Soto et al., 2015). It is within this last point on which lies the main objective of the present work: a review on the establishment and pursuing of surveillance methods focused mainly, but not restricted to the *Anastrepha* genus, including the determination of host species sensitive to these fruit flies, and with some review on the detection and identification of natural enemies.

Material and Methods

Study sites

Four Citrus companies were selected: Ceiba (Artemisa), Victoria de Girón (Jagüey Grande, Matanzas), Arimao (Cienfuegos), and Ciego de Avila, located along Cuba island (Fig. 2).

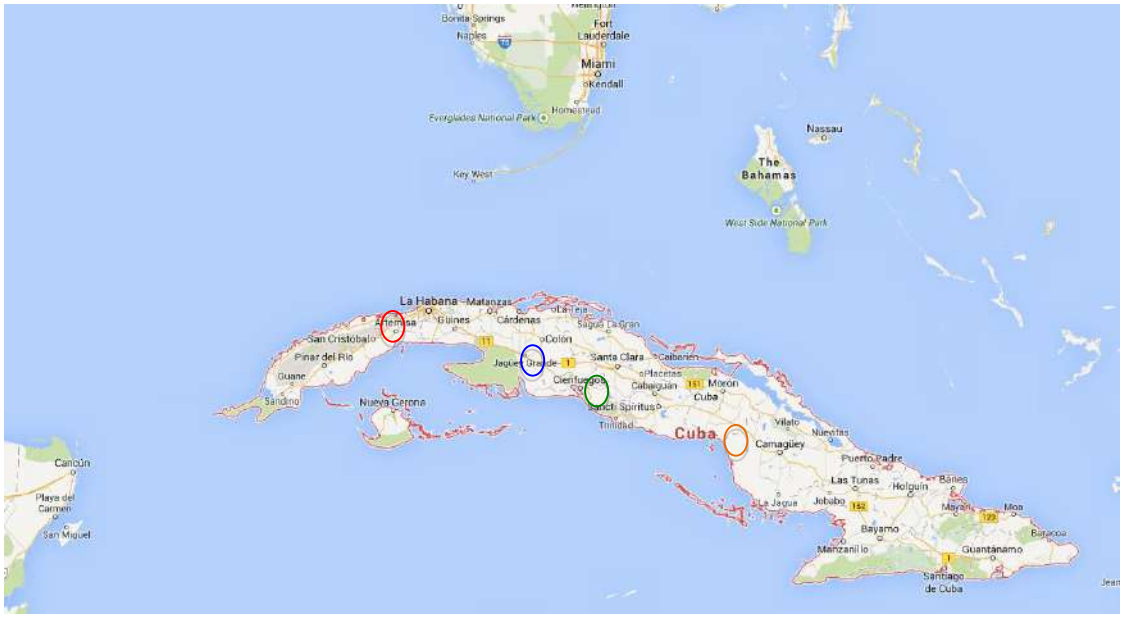

Fig. 2. Location of four selected Citrus companies in Cuba: Ceiba (Artemisa) in red; Victoria de Girón (Jagüey Grande, Matanzas) in blue; Arimao (Cienfuegos) in green; and Ciego de Ávila in orange. Picture from Google maps.

The 'Empresa Cítricos Ceiba' (Caimito) has 9.2 ha of production, distributed into 92 plots of unknown area including houses, farms and other buildings, roads and 'no-crop areas' with some wild plants (https://www.ecured.cu/Empresa_C%C3%ADtricos_Ceiba_(Caimito)). Cultivars include oranges, lemons, grapefruit, guava, mango, papaya (also known as 'fruta bomba') and several vegetables (tomatoes, sweet peppers, cucumbers, among others for local markets and own consumption). Noticeable is one of the selected plots for study in which citrus and guava are alternated (i.e. one row of citrus trees and one row of guava trees).

The 'Empresa Cítricos Jagüey Grande-Victoria de Girón', actually has 25,459 ha for crop production (https://www.ecured.cu/Jag%C3%BCey_Grande_(Jag%C3%BCey_Grande)) of several citrus species, mango, guava, papaya, aguacate and vegetables.

The 'Empresa Citricos Arimao' moved from the ~ 2,000 ha to less than ~1,200 ha (http://www.opciones.cu/cuba/2013-02-15/empresa-citricos-arimao-una-organizacion-que-fructifica/). In this enterprise, production includes orange, lemon, grapefruit, mango, guava, papaya and vegetables.

The 'Empresa Ceballos-Ciego de Ávila' with more than 8,000 ha, include orange, lemon, grapefruit, mango, guava, papaya, pineapple, tomatoes and other vegetables, such as potatoes and other roots, and sugarcane as principal crops (http://www.invasor.cu/economia/7871-abanderada-de-la-eficiencia-empresa-agroindustrial-de-ciego-de-avila).

All the companies include not only the citrus or other fruits plots, but also houses, farms, the roads, and non-crop areas were some alternative hosts can be found.

At all locations, monitoring systems were set up to determine Tephritidae fruit flies presence, population fluctuation, host fruits preference and natural enemies' presence. Monitoring was performed as described previously (Borges-Soto et al., 2010; 2015), which consisted on trapping and fruit survey as explained below.

Trapping systems

Three kinds of traps were used: Mc Phail, Rebell and Jackson. Trimedlure (targeting *C. capitata* males) was used to lure some of the traps in all the study plots. Traps contained also food attractants, to target all other Tephritidae species females, either Torula yeast or sugar cane molasses. Different concentrations of food attractants were tested in one of the study sites. Baited traps were located at different points within the Citrus enterprises; other surrounded fruits crops were surveyed too (Table 1). Mc Phail traps were serviced on a weekly basis whereas Rebell and Jackson were serviced on a quarterly basis as determined previously (Borgues-Soto et al., 2011). At each revision time, trap content was transferred to 70% ethanol vials, labeled and retrieved to the IIFT laboratory for species identification. Traps were surveyed from 2010 to 2013.

All *Anastrepha* species and parasitoids were separated and stored in amber glass vials with 70% ethanol for further processing. Specimens were first isolated from the whole trap capture and identified under binocular microscope with the aid of the corresponding taxonomic keys.

Infestation level was determined using FTD formula (Colling Sanchez, 1994):

FTD= Total no. flies / (Traps/ha)* sampling period (in days)

Category FTD

Null 0.00

Low <0.01

Medium 0.01-0.08

High >0.08

Table 1. Monitoring traps used at each Citrus enterprise, fruit culture type or location, total trap number per culture (per hectare), and bait/lure used in each type.

Culture type or location	Trap type	Total[1]	Bait[2]
(E.C.) Ceiba, Artemisa			
Citrus	Rebell	12	Torula yeast
	Jackson		Trimedlure
Mango, Papaya, Guava	Rebell	9	Torula yeast
	Jackson		Trimedlure
	Mc Phail		
(E.C.) Jagüey Grande-Victoria de Girón'			
Citrus	Rebell	nd	Torula yeast
	Jackson		Trimedlure
Mango, Papaya, Guava	Rebell	nd	Torula yeast
	Jackson		Trimedlure
	Mc Phail		
(E.C.) Arimao, Cienfuegos			
Citrus	Mc Phail	3	Torula yeast
			Trimedlure
Mango, Papaya, Guava	Mc Phail	4	Torula yeast
			Trimedlure
(E.C.) Ciego de Ávila			
Citrus	Jackson	3	Trimedlure
Guava	Rebell	2	Trimedlure
	Mc Phail	1	Torula yeast
Plum	Mc Phail	1	Torula yeast
Mango	Mc Phail	1	Torula yeast
	Rebell	1	Trimedlure
Papaya	Jackson	1	Torula yeast
		1	Trimedlure
Postharvest Plant	Rebell	1	Trimedlure
	Mc Phail	1	Torula yeast
Neighboard	Jackson	1	Trimedlure

[1]Total number of traps, each cover at least one hectare of cultivar; [2]Food baits were either Torula yeast or sugar cane molasses depending on availability; nd, not determined.

Fruit collection and inspection system

In all four Citrus companies, citrus fruits and other deciduous cultivated fruits (mango and guava mainly) were collected before (at color changing) and at ripening (after complete color change) stages. In this study, also non cultivated fruits (alternative hosts) present in the vicinity of the Citrus companies or found between Citrus and other cultivars (mango, guava, papaya) were also collected (ripe fallen fruits were excluded).

Citrus fruits (mainly Navel oranges and grapefruits) were inspected for oviposition scars under binocular in place at pre-ripening and at laboratory at ripening stage (all at pre-harvest time). At

least 10 trees with 10 fruits per tree, per site and sampling date were randomly selected for oviposition scars inspection.

All the fruits with clear oviposition damage or other kind of damage due to insect feeding were labeled and retrieved to the laboratory. At laboratory, fruits were dissected to inspect for Tephritidae larva and natural enemies (like entomopathogenic nematodes) presence. After determination of larva presence, these fruits were stored to allow development into adult for complete species identification, including identification of parasitoids. Emerged adults (fruit fly or parasitoid) were preserved in amber crystal vials with 70% ethanol for further studies.

All fruits were packaged following Cuba's rules 70-11 to avoid fruit fly or other pests spread among Citrus Enterprises.

Results and Discussion

Trapping

In overall, monitoring traps allowed confirming the presence of different *Anastrepha* species, previously reported to be found in Cuba: *A. suspensa*, *A. soroana*, *A. obliqua*, *A. ocresia*, *A. insulae* and *A. interrupta*. These traps also recorded an unnamed one, here identified as *A.* sp., not matching any morphological description in the used keys, nor in the past reports of species lists (Fernandez et al., 1997; Rodríguez Velásquez et al., 2001). Several voucher specimens of this unnamed species *A.* sp. have been deposited at the IES institute collection for further research. Another species was identified as *Toxotrypana curvicauda*, however this species was found only in traps from papaya plots.

From the results, we observed that from the three types of traps used, Mc Phail was the best in capturing fruit flies, followed by the Rebell type (data not shown). Of the two food attractants used, Torula yeast or sugar cane molasses, the Torula yeast was the best for *Anastrepha* species even at the lowest concentration used (Fig. 3).

Fruit fly major population peak was found to fit with ripening season of stone fruits and other non-citrus fruits, which lies around August month, also fitting the raining season (Fig. 4). Despite this high number at ripening time, overall FTD was of 0.0054 *Anastrepha* sp. (pooled fly species per trap and day along the three seasons tested) indicating a low prevalence of flies in the Citrus enterprises.

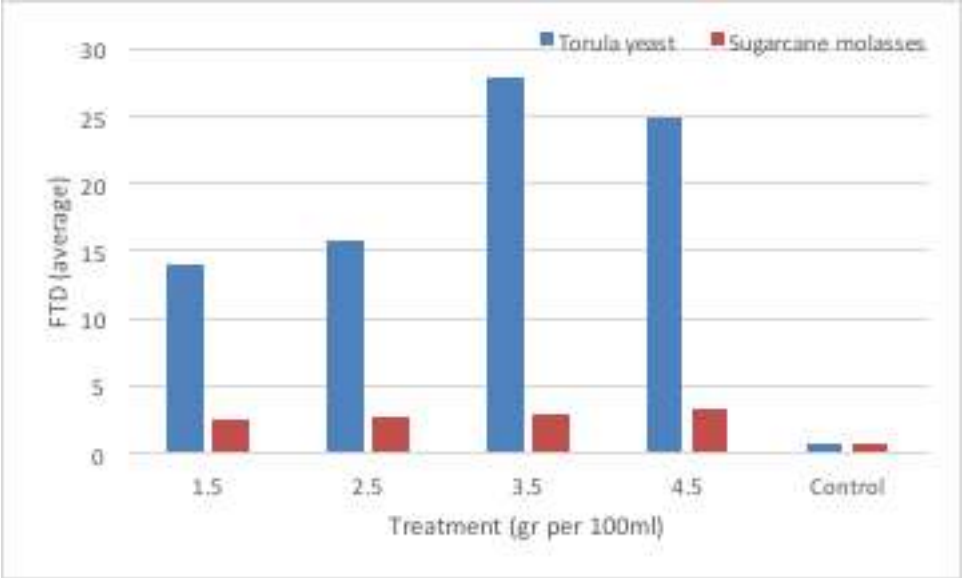

Fig. 3. Average *Anastrepha suspensa* captures (as FTD) in Mc Phail traps baited with Torula yeast (blue) or with sugarcane molasses (red) at different concentrations. Traps were located in one of the guava plots at 'E.C. Jagüey Grande-Victoria de Girón'. Control traps contained 51 cc of hydrolysate yeast used elsewhere as control bait.

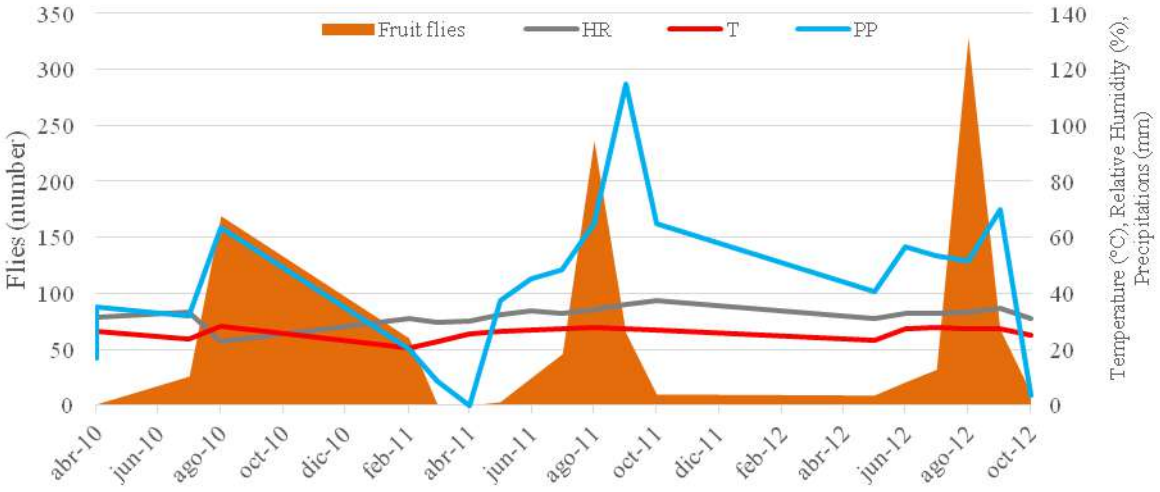

Fig. 4. Population dynamics and total captures of *Anastrepha* spp. During three seasons in four sites in Cuba, with relation to environmental conditions (average temperature (Celsius degrees; in red), relative humidity (percentage; in dark grey) and precipitation (average mm raining; in light blue).

Traps baited with Trimedlure as lure were setup according to a National program for the surveillance of arrival of *C. capitata* to Cuba from neighboring countries mediated by strong climatic events (i.e. hurricanes). None of the traps reported any medfly specimen, as in the previous decade, indicating that the surveyed areas are free from medfly, fulfilling one of the quarantine measures required from citrus importing countries (i.e. USA, Canada). This result can be extended to the whole country, as the study plots covered the most important citrus production areas of the country.

Fruit surveillance

In the citrus fruits surveillance, Tephritidae fruit flies were obtained from near 1,000 fruits collected in any of the four companies evaluated, despite the species captured in traps in the four locations.

Among the commercial fruits, guava and mango showed the highest larval presence values. Other deciduous or stone fruits collected with oviposition symptoms were retrieved to the laboratory for further processing, as host species determination, fruit fly species identification and natural enemies' detection after complete development of recovered larvae.

As an example, the abundance of *Anastrepha suspensa* larvae in Jagüey Grande followed the ripening status of host fruits. The highest peak for *Spondias mombin* (or Jobo) was on July, for plum in June and for guava in September (Fig. 5).

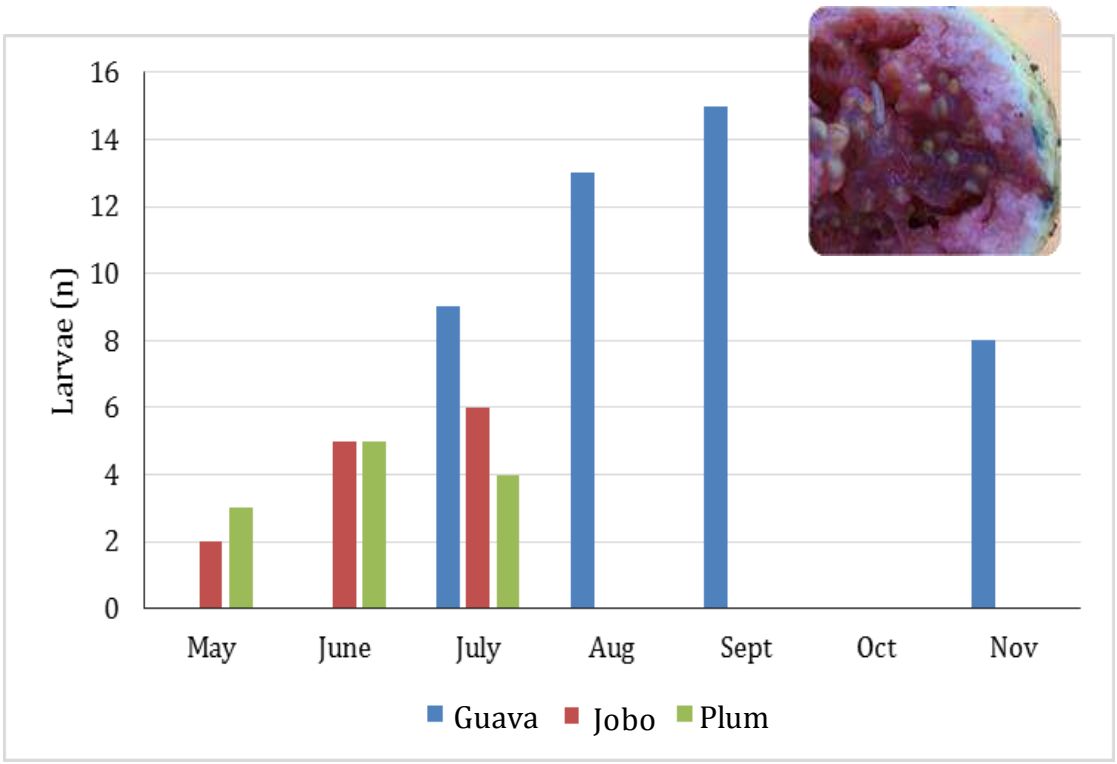

Fig. 5. Fluctuation of larvae number (average larvae per fruit) of *Anastrepha suspensa* in several fruits (guava in blue, jobo in red, and plum in green) along the year in Jagüey Grande. A red guava infested fruit is shown to notice the damage produced by the *A. suspensa* larva.

At Ceiba the most affected fruits were guava with a clear population peak around June and another in September (Fig. 6). In this enterprise, the *A. suspensa* population reached approximately 0.68 larvae/fruit at the highest point. However, in this enterprise, *A. obliqua* was detected co-infesting with *A. suspensa* (Fig. 6), but at lower rate (0.04 *A. obliqua* larvae per fruit).

Following the surveillance of Tephritidae larva-infested fruits, five 'new' host species were recorded for *Anastrepha suspensa*: sapodilla (or níspero in Cuban; *Manilkara zapotilla* (Jacq.) Gilly), rose apple (or pomarrosa; *Syzygium jambos* L.), cocoplum (or icaco; *Chrysobalanus icaco* Lin.), custard apple (or mamón; *Anona reticulata* L.), and gac fruit (or cundeamor; *Momordica cochinchinensis* Spreng.). And two new ones for *Anastrepha obliqua*: the cocoplum (Icaco) and yellow mombin or hog plum (jobo fruit in Cuba; *Spondias monbin* L.) (Table 2). Even if this list is considered here new host species, this is due to the consideration of the available literature affecting fruit samples from Cuba.

In previous works were listed the observed Tephritid species and their associated plant hosts, among it appeared *A. suspensa* with sapodilla, rose apple, cocoplum and yellow mombin as host plants (Fernández et al., 1997; Rodríguez Velásquez et al., 2001). But, as stated by the own authors, the distribution of *Anastrepha* species in the Cuba island is unknown and many of the hosts plants assigned to each species is those that appear at the collection data, meaning that the authors didn't performed a deep study on *Anastrepha* species host plants available in Cuba. For this reason, this work summarizes a first step in the identification of commercial and alternative hosts for the economic important *A. suspensa* species.

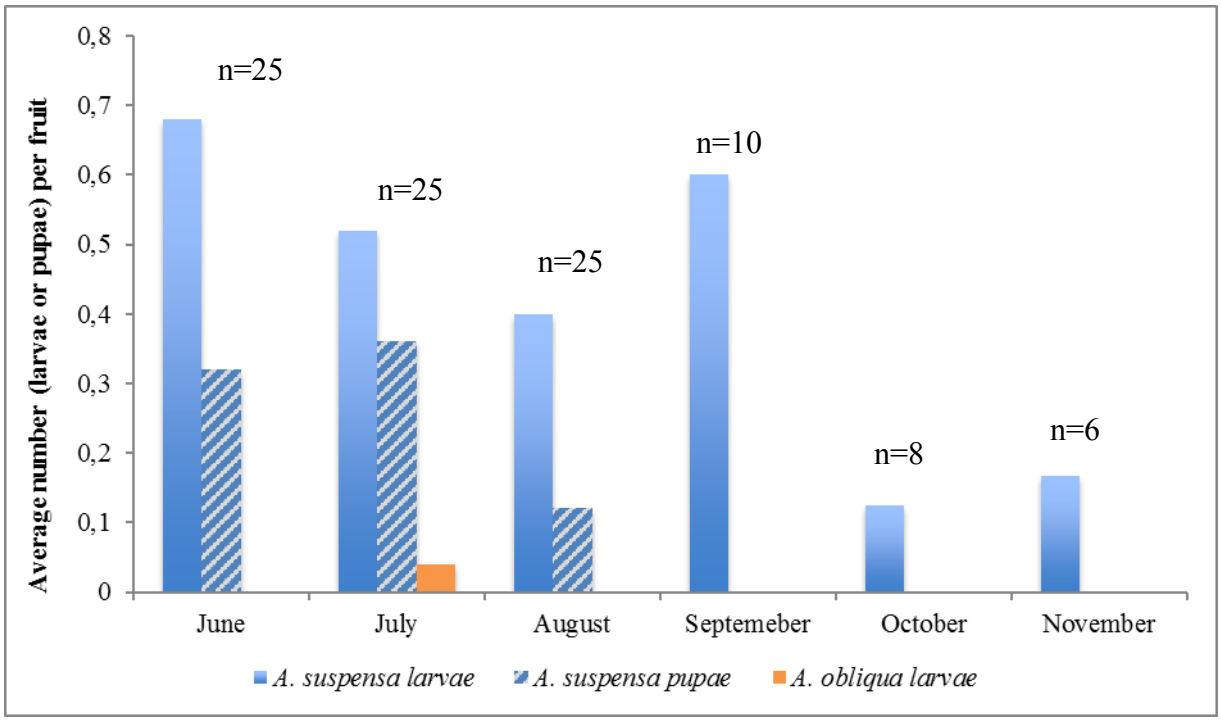

Fig. 6. Average larvae (solid colors) and pupae (striped bars) number per guava fruit along the year in Ceiba (Matanzas, Cuba). The number of fruits collected in each month is indicated above the bars as n.

Table 2. Fruit flies detected in evaluation area, with indication of species name, fruit host scientific name and common name. Pictures of the representative fruit fly species type are presented below each name (pictures obtained from http://paroffit.org).

Fruit fly species	**Fruit host (scientific name)**	**Fruit host (common name)**
Anastrepha suspensa 	*Manilkara zapota* G *	Níspero or Sapodilla
	*Momordica charantia**	Cundeamor or Gac fruit
	*Eugenia gamboa L**	Rose apple or Pomarrosa
	Chrysobalanus icaco Lin *	Icaco or Cocoplum
	Mangifera indica	Mango
	Psidium guajava. L	Guayaba or Guava
	*Anona reticulata L**	Mamón or Custard Apple
Anastrepha obliqua 	*Chrysobalanus icaco Lin**	Icaco or Cocoplum
	*Spondias monbin L**	Jobo or Yellow monbin, or Hog plum
	Mangifera indica	Mango
	Chrysophyllum caimito. L	Caimito, Golden leaf tree fruit, star apple
	*Spondias monbin L**	Jobo
Anastrepha ocresia 	*Manilkara zapotilla* (Jacq. Gilly)	Níspero or Sapodilla
Toxotrypana curvicauda 	*Carica papaya*	Papaya

Control agents: entomopathogen nematodes and parasitoids

As it is known, biocontrol agents can be found in agro-ecosystems if a sustainable and environmentally friendly techniques are applied, enhancing and allowing a self-control by spreading into the ecosystem. During the past years, in Citrus enterprises, IPM has been introduced for control tephritid fruit flies and other pests while keeping chemical pesticides at low levels. These low pesticides application had allowed the establishment of biological control of tephritid species. It was found the braconid species *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae), which seems to contribute greatly to biological control of *Anastrepha* species (Fig. 7). This species has been detected at low numbers (n=12 in 2011; n=11 in 2012; and n= 12 in 2013) in mango and Guava cultivars infested by *Anastrepha suspensa* and/or *A. obliqua;* its large developmental time didn't allow an exact assignment of host.

Utetes anastrephae has a wide distribution in temperate areas of continental America (http://entnemdept.ufl.edu/creatures/beneficial/wasps/utetes_anastrephae.htm). This species is thought to be a complex of closely related species with color-variable body, small subtle differences in ovipositor, thorax drawings or host species preferences that deserve further studies (Wharton & Yoder, 2014). This species has been cited exerting different percentages of parasitism in other countries, reaching a maximum parasitism rate of 66.7% against *A. obliqua* when infesting *Spondias mombin* (López et al., 1999), where it was considered a native parasitoid (Silva et al., 2010; Ovruski & Schliserman, 2012; Garcia & Ricalde, 2013). López et al., (1999) also found *U. anastrephae* in guava fruits infested by *Anastrepha* species at 0.1% parasitism rate, whereas these authors did not found any in infested mangoes.

Due to the low detection numbers, we don´t know if the species found in this work is native of Cuba or has reach it as specimens travelling with hurricanes or tropical storms. Further research with the aid of molecular markers would be necessary to determine this last point as some morphological variations have been detected, along by continuing the surveillance in infested fruits.

Fig. 7. *Utetes anastrephae* (Viereck) specimens in dorsal (left) and lateral (right) view.

In addition to this parasitoid, an entomopathogenic nematode, *Heterorhabditis indica,* has also been found infesting *Anastrepha suspensa* larvae (Fig.8), as also previously reported

(Borgues-Soto et al., 2011). Some assays with this nematode showed a control of *Anastrepha suspensa* larvae by inducing 76.7-86.6% larva mortality, but further research should be done to establish a rearing colony of this nematode, along with the determination of best suited test protocols and field release. This mortality rate is similar to those obtained in laboratory with other entomopathogens like the fungus *Metarhizium anisopliae* (Quesada-Moraga et al., 2008; Dimbi et al., 2013) or *Beauveria bassiana* (Dimbi et al., 2003).

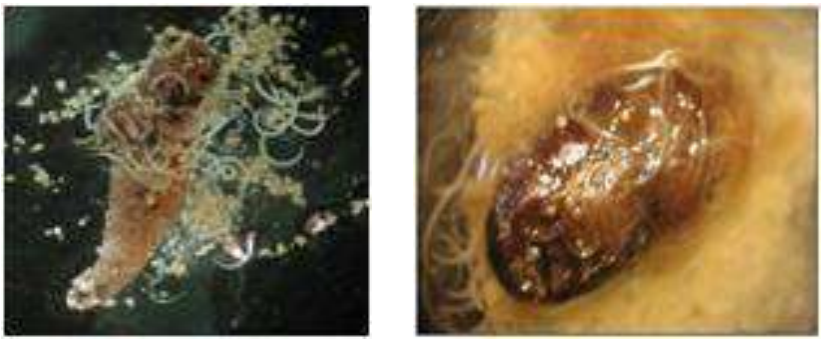

Fig. 8. *Heterorhabditis indica* specimens in larva (left) and pupae (right) of *Anastrepha suspensa* (picture from M. Gomez, M. Montes, M. Borges, D. Hernandez and J.L. Rodriguez; 2013).

As explained before, biological control is an environmental friendly technique that seems enhanced by the actual procedure for the Tephritidae fruit flies control program in Cuba. However, this point deserves further research to improve the presence of parasitoids and other natural enemies in Citrus agro-ecosystems with inter-cropping systems.

Conclusion

The key result of this work is the determination of complete absence of *Ceratitis capitata* and other *Anastrepha* species infesting Cuban citrus fruits, despite the registered captures of *Anastrepha* spp. Adults in baited traps. None of the collected citrus fruits presented oviposition scars nor larva tunnels, even if in their vicinity other plant host species (like icaco, jobo, cundeamor, pomarrosa or níspero) and inter-cropping systems (mango, guava or plum) exists and presents infestation mainly by *Anastrepha* species.

These results highlight the success of the implemented management program in citrus which include surveillance, monitoring and personnel training, putting into value the area-wide fruit fly management program in Cuba for the remaining fruit species. Moreover, it seems that the inter-cropping system developed by the Citrus enterprises, along the presence of alternative *Anastrepha* spp. plant hosts serves as push-pull system protecting Citrus species (Cook et al., 2007; Aluja & Rull, 2009), system that deserves further research to enhance the presence of natural enemies.

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