Interspecific competition between egg parasitoids: Native *Fopius caudatus* and exotic *Fopius arisanus*, in *Ceratitis cosyra*

Miriam Frida Karlsson[a,b,*], Espérantos O. de Souza[c,d], Pascal M. Ayelo[b,c,1],
Joseph Ayomidé Zannou[b,c], Gbelidji Souvenir Blaise Mègnigbèto[e], Aimé H. Bokonon-Ganta[c,d]

[a] *Department of Plant Protection Biology, Swedish University of Agricultural Sciences (SLU), SE-230 53 Alnarp, Sweden*
[b] *International Institute of Tropical Agriculture (IITA), 08 BP 0932 Tri Postal, Cotonou, Benin*
[c] *Department of Crop Production, Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC), 03 BP 2819 Cotonou, Benin*
[d] *Entomology Laboratory, Department of Plant Production, National Plant Protection and Quarantine Service (DPV), Porto-Novo, Benin*
[e] *International Chair in Mathematical Physics and Applications (ICMPA) (CIPMA-Chaire UNESCO), University of Abomey-Calavi (UAC), 03 BP 2819 Cotonou, Benin*

GRAPHICAL ABSTRACT
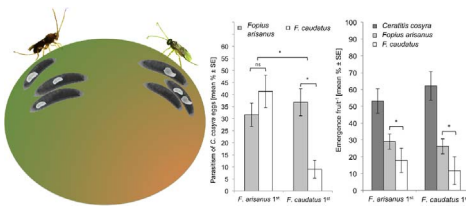
ARTICLE INFO

ABSTRACT

Tephritidae fruit flies are parasitized by Braconidae wasps, of which some are used in biological control programs. Releases of exotic parasitoids might affect, or be affected by, native species exploiting a common host. We studied interspecific competition between the native African parasitoid *Fopius caudatus* (Szépligeti) and the exotic Asian parasitoid *Fopius arisanus* (Sonan) (both Hymenoptera: Braconidae) in African native *Ceratitis cosyra* Walker (Diptera: Tephritidae). Wasp behaviour on infested fruits and dissection of fruit fly eggs assessed acceptance, oviposition performance and suitability for egg development, parasitism, and effect of parasitism by heterospecific species. While *F. arisanus* outcompeted *F. caudatus* in parasitization of relatively younger eggs, chased away *F. caudatus*, and oviposited more, *F. caudatus* survived better than *F. arisanus*. *Fopius caudatus* parasitized more in 2-day-old *C. cosyra* eggs than in 1 or 3-day-old egg. More oviposition by *F. caudatus* was found in fruits previously exposed to *F. arisanus*, coinciding with presence of more developed host eggs, while such difference was not observed for *F. arisanus*. *Fopius caudatus* was an inferior competitor than *F. arisanus* in *C. cosyra*-infested African mango *Irvingia gabonensis* (Irvingiaceae).

1. Introduction

Interspecific competition among parasitoids might affect the population dynamics of insects (Xu et al., 2013). Competitive interactions can occur between parasitoid species exploiting the same host either as extrinsic competition between adults exterior the host substrate or as intrinsic competition between parasitoids developing within the same host (Cusumano et al., 2012). Both extrinsic and intrinsic interspecific

competition mechanisms are known phenomenon in Braconidae wasps, parasitizing Tephritidae species (Cancino et al., 2014; Murillo et al., 2016; Paranhos et al., 2013). Interspecific competition in a natural enemy guild, might be a step in the process towards coexistence between species in the same niche (Feng et al., 2015), or cause partial or total displacement (Reitz and Trumble, 2002). Competition between different species might also cause species niche separation, as niche partition is not only an effect of resource-based competition (Jeffries and Lawton, 1984). Competitive interactions between species will only occur in spatial and temporal co-occurrence and when their niches overlap (Kaplan and Denno, 2007). A greater abiotic and biotic environmental complexity might allow for separate niches and enhance coexistence in an area (Costamagna and Landis, 2004). The establishment of released parasitoids in biological control programs is affected by competition between parasitoids and may affect the outcome of the effort (Cabello et al., 2011; Wang et al., 2008). Impact evaluation of the exotic species on the native ecosystem before the release, is therefore essential (Alyokhin and Messing, 2003). Studies of competition dynamics among parasitoid species that share, or are expected to share, the same host species and/or habitat are important for evaluating their efficiency (Bogran et al., 2002; Hoffmeister, 1992). The potential impact on the environment and non-target species of a released biocontrol agent in a biological control program should not be detrimental. An introduced parasitoid species should not outcompete or drive other native or exotic parasitoid species into extinction and should not interfere with natural enemies that are already partially effective. Although competition between parasitoid species might reduce the survival of, and the parasitism by, the released parasitoid in a biological control program (García-Medel et al., 2007), competitive interactions among parasitoids might not necessarily affect host population suppression (Bogran et al., 2002). The coexistence by a guild of parasitoids exploiting a common host can cause complementary parasitism impact (Kroder and Messing, 2010), or be less efficient in suppressing the pest, as their coexistence may have disruptive effects upon each other (Collier et al., 2002; Rosenheim et al., 1995). The parasitoid guild of *Anastrepha suspense* (Loew) (Diptera: Tephritidae) in Florida consists of native *Utetes anastrephae* (Viereck), released *Doryctobracon areolatus* (Szepligeti), and recently released *Diachasmimorpha longicaudata* (Ashmead) (all Hymenoptera: Braconidae) and their coexistence is affected by intrinsic competition where *D. areolatus* is an inferior competitor, widely displaced to the margins of its host range (Paranhos et al., 2013).

Augmentative releases of parasitoids are relatively well established management methods of Tephritidae pest fruit flies (Montoya et al., 2000; Vargas et al., 2007). A number of solitary Braconidae parasitoid species are specialized and exclusively survive in some fruit flies belonging to the Tephritidae family (Quicke, 2015). In order to contribute to the control of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) that invaded Africa in 2003 (Drew et al., 2005) and became a devastating pest in fruit crops such as mango (*Mangifera indica* L. (Anacardiaceae)) (Vayssières et al., 2009), the exotic parasitoid *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) has recently been introduced in several regions in the continent (Gnanvossou et al., 2016; Ndiaye et al., 2015). *Fopius arisanus* is a solitary egg-pupal endoparasitoid, indigenous to the Indo-Australian region (Wharton, 1989) capable of parasitizing and developing in at least twenty Tephritidae fruit fly species, particularly from the genus *Bactrocera* and *Ceratitis* (Chinajariyawong et al., 2000; Quimio and Walter, 2001; Rousse et al., 2005). It has been used successfully to reduce Tephritidae population through augmentative releases programs (Vargas et al., 2012). Where releases of *F. arisanus* have been made in Benin, Kenya, Mozambique, and Senegal, the closely related species *Fopius caudatus* (Szépligeti) (Hymenoptera: Braconidae) is present (Foba et al., 2012; Ndiaye et al., 2015; Steck et al., 1986; Vayssières et al., 2011; Wharton et al., 2000). *Fopius caudatus* is the most common indigenous parasitoid (70%) in Tephritidae infested mango, cashew (*Anacardium occidentale* L. (Anacardiaceae)), pepper

(*Capsicum annuum* L. (Solanaceae)), guava (*Psidium guajava* L. (Myrtaceae)), African peach (*Sarcocephalus latifolius* (Smith) Bruce (Rubiaceae)), shea (*Vitellaria paradoxa* Gaertn (Sapotaceae)), and African custard-apple (*Annona senegalensis* Pers. (Annonaceae)) in Benin and Senegal (Ndiaye et al., 2015; Vayssières et al., 2011). *Fopius caudatus* parasitism approximates 25% in Tephritidae infested grafted mango (Vayssières et al., 2011). The highest level of parasitism is observed in fruits infested with *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) (Ndiaye et al., 2015). *Ceratitis cosyra* is together with *B. dorsalis* the main pest insects in mango in Africa (Salum et al., 2013). Export mango consignments to Europe are most commonly intercepted due to the fruit flies *C. cosyra* and *B. dorsalis* (Steck, 2015).

Fopius arisanus is known to suppress larval parasitoid species such as *D. longicaudata* and *Fopius vandenboschi* (Fullaway) (Hymenoptera: Braconidae) by intrinsic competition based on both mechanical and physiological inhibitions (Van den Bosch and Haramoto, 1953; Wang et al., 2003). Competition superiority of *F. arisanus* is hence documented for parasitization occurring in different developmental stages of the host. Influence of interspecific competition between egg-parasitoids of the genus *Fopius* remains sparsely documented. One study with two egg-parasitoids *F. arisanus* and *Fopius ceratitivorus* (Wharton) (Hymenoptera: Braconidae) demonstrated that the outcome of the intrinsic competition depends on which of the species occupies the host first (Bokonon-Ganta et al., 2005). *Fopius arisanus* and *F. caudatus* parasite eggs of some common Tephritidae fruit flies such as *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), *C. cosyra*, and *B. dorsalis*. Thus, the potential for competition between these parasitoid species is possible. The interaction between them is a new association since *F. arisanus* is an Asiatic species, whereas *F. caudatus* originates from Africa. They would consequently, not have evolved niche divergence to avoid competition with each other yet.

The two wasp species, *F. caudatus* and *F. arisanus* are solitary egg parasitoids (Wharton and Gilstrap, 1983; Wharton, 1999) belonging to the subfamily Opiinae. When several solitary braconid species parasite a tephritid fruit fly, only one parasitoid will develop and emerge (Montoya et al., 2012). Ability to avoid super-parasitism or multiparasitism is therefore a key biological parameter for solitary parasitoids in insect pest management. Since the two parasitoid species *F. arisanus* and *F. caudatus* may share the same host in the same environment, it is important to evaluate the potential competition effects between them. The aim of our research was to assess the interspecific competition between the two *Fopius* species in *C. cosyra,* a host fly that is shared by both wasps. We studied their behaviour, the suitability of host egg developmental stages, the extrinsic competition and partially the intrinsic competition as resource competition within the host eggs.

2. Materials and methods

2.1. Fruit fly

The established colonies of *C. cosyra* originated mainly from emerging adults from a mixture of mango, African peach and African custard apple collected from the field. *Ceratitis cosyra* used for the assays were reared on African mango (*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (Irvingiaceae)), and papaya (*Carica papaya* L. (Caricaceae)). The flies were provided water and 1:3 mixture of enzymatic hydrolysed yeast (CAS: 100684–36-4, Affymetrix, Santa Clara, CA, USA) and un-bleached sugar.

Rearing of *C. cosyra* was done both in the Entomology Laboratory at the Department of Plant Production at the National Plant Protection and Quarantine Service (DPV), and at the International Institute of Tropical Agriculture (IITA), in Benin. At DPV, the laboratory conditions were $25 \pm 2\,°C$, $85 \pm 5\%$ RH, and 12:12 L:D photoperiod. Colonies of *C. cosyra* in IITA were kept in a semi-outdoor rearing facility with $27 \pm 3\,°C$, $80 \pm 10\%$ RH, 12:12 L:D photoperiod. Mature (¾ ripening, fruits being yellowish/greenish) African mangos were offered to

cohorts of the flies for 72 h. Fruits were incubated individually in plastic bowls, standing on a layer of sterilized sand serving as pupation medium. Pupae were collected at 10 days post-incubation in Petri dishes, which were placed in Plexiglas cages (30 × 30 × 30 cm) for adult emergence. The fruit fly populations have been kept in rearing for nearly 30 generations, with multiple additions of field-collected individuals.

2.2. Parasitoids

Fopius caudatus were obtained from *C. cosyra*-infested African peaches collected in the department of Borgou, Benin. Parasitoids were field-collected before and during the time of the experiments, and maintained in rearing for approximately five generations. Ripe *I. gabonensis* fruits were exposed to 50 pairs of either *C. cosyra* or *C. capitata* for 24 h in laboratory cages (25 × 25 × 25 cm) and thereafter exposed to *F. caudatus* for parasitization during 48 h. A complementary diet (200–300 g) of papaya was added during incubation to the infested *I. gabonensis* when necessary. Rearing of *F. caudatus* was done both at DPV and at IITA-Benin under similar laboratory conditions; 26 ± 1 °C, 75 ± 5% RH and 12:12 L:D photoperiod. The parasitoids were observed being more active to mate and to oviposit outdoor than in laboratory. Rearing cages with male and female adults were therefore taken outdoor during the day (9AM-5PM), and placed in the shade of a tree (5m tall) with a relatively dense canopy where the ambient conditions were 26.8 ± 6 °C, 88 ± 8% RH. The average wind speed was 4.8 km/h and average solar radiation above tree was 13 MJ m^{-2} day^{-1}.

Fopius arisanus were obtained from the Biocontrol unit rearing of IITA-Benin, where they were maintained at 26 ± 1 °C, 70 ± 5 RH and a 12:12 L:D photoperiod. They were reared on *B. dorsalis* for about 20 generations with papaya as the main larval food substrate. Ripe papaya fruits were exposed to 50 pairs of *B. dorsalis* for 4 h in Plexiglas cages (20 × 20 × 20 cm) and directly thereafter exposed to 50 pairs of *F. arisanus* for parasitization during 48 h. A complementary diet of 200 g of papaya was added to the infested papaya during incubation.

Pupae were separately collected from both parasitoid species at 10 days post-incubation, and then placed in small plastic jars (10 cm diam. × 5 cm high) covered with fine mesh screen (1.4 mm gauge size) allowing the emerging parasitoids to escape into the Plexiglas cage while the emerging fruit flies were kept inside the plastic jars. The newly emerged male and female parasitoids were kept together in the cage where drops of pure honey and water were provided. Placing the cage exposed to daylight by a window stimulated mating behaviour.

2.3. Bioassays

The assays 1, 3 and 5 were conducted at the Entomology laboratory in DPV. The laboratory conditions were as described in Section 2.1. The other three assays (2, 4 and 6) were conducted at the research station IITA-Benin. Natural infestations of test fruits were done by introducing one ripe *I. gabonensis* fruit per Plexiglas cage (15 × 15 × 15 cm) and exposing it to 50 pairs of *C. cosyra* for 4 h. Artificial infestation with *C. cosyra* eggs in *I. gabonensis* was made only for the assay 2. All experiments were conducted with mated female parasitoids, 8–15 days old, with two days of oviposition experience on *C. cosyra*-infested *I. gabonensis*. Incubation of fruits in the assays followed the same procedure as in the *C. cosyra* rearing, hence fruits were incubated individually and ten days post-incubation, pupae were collected and counted and emergence of flies and wasps were recorded. The tests were conducted in cages that were left outdoor (except during rainy days), amid a tree shade, where the ambient conditions varied between 26.8 ± 6 °C, and 88 ± 8% RH.

2.3.1. Host age effect on wasp oviposition performance and survival

Assay 1. *Fopius caudatus* and *F. arisanus* survival in young *C. cosyra* eggs

Ceratitis cosyra-infested *I. gabonensis* fruits were immediately after infestation presented to five individuals of either *F. caudatus* or *F. arisanus* for 4 h. Hence, the wasps had separately access to 0–8 h-old *C. cosyra* eggs. Parasitoids were left in a cage (15 × 15 × 15 cm) and were provided with water and pure honey. Infested and parasitoid-exposed fruits were placed directly into incubation. Emergence of *F. caudatus*, *F. arisanus,* and *C. cosyra* from *I. gabonensis* were recorded. The assay was repeated six times per parasitoid species. Additional six replicates were conducted with *F. arisanus*. Twenty *F. arisanus* were presented to one *C. cosyra*-infested fruit for 4 h in a cage (15 × 15 × 15 cm). The additional replicates were complemented with dissection of 30 *C. cosyra* eggs per fruit, to confirm parasitism. Remaining fruit part was placed into incubation.

Assay 2. *Fopius caudatus* and *F. arisanus* behaviour on fruits infested with *C. cosyra* eggs of different ages

Test fruits were artificially infested. Papaya domes were introduced into a cage with 50 pairs of *C. cosyra* for 4 h to obtain *C. cosyra* eggs. The domes were made by dividing the papayas in half, removing the pulp, and perforating the surface of the fruits (depth 0.4 cm) with entomological pins (diam. 0.1 cm). Eggs were collected from the domes and 25 *C. cosyra* eggs (five eggs per puncture) were inoculated in a piece of *I. gabonensis* with a size of 2.5 cm in diameter and 0.5 cm thick. Artificially infested test fruit pieces were used for behavioural assays after 24, 48, and 72 h post-oviposition in domes. One fruit piece covered the base of a small transparent plastic jar (2.5 cm in diam. × 3 cm high). The jar was used as a test arena and was covered with a lid with mesh (1 mm gauge size). Three parasitoids (either *F. arisanus* or *F. caudatus*) were placed in the jar for 8 h (8AM-4PM). Observations of behaviour were done during two minutes at 30 min intervals. The behavioural events resting, walking, palping with antennae, probing with ovipositor, and ovipositing were recorded. In all the bio-assays, distinction between behavioural events, specifically probing and ovipositing, was based on previous description (Wang and Messing, 2003). Thirty test jars were prepared for each host egg age, per parasitoid species. Dissections of all exposed-eggs were done to calculate the parasitism by *F. arisanus* and *F. caudatus*. Eggs were singly dissected in fine drop of water under a stereomicroscope (Olympus XZS10) at a magnification of maximum 100×.

2.3.2. Extrinsic and intrinsic competition between wasp species during simultaneous exposure to host eggs

Assay 3. *Fopius caudatus* and *F. arisanus* competition during simultaneous exposure to young host eggs

Ceratitis cosyra-infested *I. gabonensis* fruits were placed in cages (15 × 15 × 15 cm) and exposed to five individuals of each parasitoid species, *F. caudatus* and *F. arisanus* simultaneously. The parasitoids were left in Plexiglas cages for 6 h and had hence access to 0 to10-h-old *C. cosyra* eggs. The probing, and ovipositing behaviour, were observed for one minute, every 15 min during the first hour. At each observation, all parasitoids were collectively observed and we recorded the number of times each behavioural activity was performed. Fruits were incubated individually and the numbers of *F. caudatus*, *F. arisanus,* and *C. cosyra* that emerged from *I. gabonensis* were recorded. The assay was repeated six times.

Assay 4. Ratio-effect of competition between wasp species during simultaneous exposure of host eggs

Pieces of infested *I. gabonensis* fruit were cut to cover the bottom of transparent plastic jars (8 cm diam. × 5 cm high) and covered with a meshed lid. *Ceratitis cosyra* eggs were approximately 40–55-h-old during the assay. The jars served as the testing arenas and one jar corresponded to one replicate. Four different ratios between *F. caudatus* and *F. arisanus* were used as treatments. The numbers of the two parasitoid species tested were 1:1, 2:1, 4:1, and 1:2 for *F. caudatus* and *F. arisanus* respectively. The parasitoids in each treatment were offered the same infested fruit piece at the same time. Parasitoids of each species were introduced in the jar and left to oviposit for 24 h. One drop

of honey was provided to the parasitoids. Hourly observations of the behavioural events resting, palping, probing, ovipositing and confrontation were done for two minutes per jar. The number of individuals per parasitoid species that performed each of the behavioural activities was recorded at each observation time. To examine the parasitism and superparasitism rates, tiny pieces were cut from the exposed fruit pieces and 10 eggs/replicate were dissected under stereomicroscope as described in Section 2.3.1, assay 2. The remaining parts of the fruit pieces were incubated. The number of emerged fruit flies and parasitoids were recorded after pupae collection. Seven jars were prepared for each treatment.

2.3.2. Behavioural and survival effect of subsequent heterospecific parasitization

Assay 5. *Fopius arisanus*-parasitization effect on *F. caudatus* oviposition choice

One-half of each *C. cosyra*-infested *I. gabonensis* fruit was presented to five *F. arisanus* during 6 h (12PM-6PM) in a Plexiglas cage (15 × 15 × 15 cm). The fruits were thereafter removed from the cage of *F. arisanus* and placed in empty Plexiglas cages during the night (14 h). The following day five *F. caudatus* were tested in a two-choice assay where the treatments were one *C. cosyra*-infested fruit (control) and one *C. cosyra*-infested fruit previously exposed to *F. arisanus*. *Fopius caudatus* were presented in the assay to 24–30-h-old *C. cosyra* eggs during 6 h. The parasitoid behavioural activities probing, and ovipositing were observed as described in Section 2.3.1, assay 2. Fruits were incubated, pupa collected, and emergence recorded. Emergence was compared between the previously *F. arisanus*-parasitized and the nonparasitized fruits to determine *F. caudatus* choice of oviposition after intercalated heterospecific parasitization. The assay was repeated six times.

Assay 6. 14-h intercalated heterospecific parasitization effect on behaviour and survival of *F. caudatus* and *F. arisanus*

Infested fruits were 20 h post-infestation presented to either ten *F. caudatus* or to ten *F. arisanus* for parasitization in Plexiglas cages (15 × 15 × 15 cm) during 10 h (8AM-6PM). The parasitoids had hence access to 24–34-h-old eggs. The parasitoid behavioural activities such as attraction (landing on fruit), resting, walking, palping with antenna, probing with ovipositor, ovipositing, and leaving were observed during two minutes, every hour for ten hours. The fruits were removed from the cages of the parasitoids and placed in empty Plexiglas cages during the night (14 h; 6PM-8AM). Thereafter, previously exposed-fruits were introduced to the heterospecific parasitoids for 10 h (8AM-6PM), when *C. cosyra* eggs were 48–58-h-old. Observations of parasitoid behaviour were conducted again as on the previous day. Dissections of 20 eggs (some became larvae) per fruit were done to calculate the parasitism rate by *F. arisanus* and *F. caudatus*. Eggs were singly dissected as described in Section 2.3.1., assay 2, and the *Fopius* species were identified based on the egg shape and its developmental stage (egg or larvae). Preliminary observations had shown that by 10–12 h post-parasitization, eggs of *F. caudatus* and *F. arisanus* were not hatched yet and were clearly differentiable. Parasitoid larvae that were found during dissection were hence attributed to the parasitoid species that was offered *C. cosyra* eggs at the first instant. Fruits with the remaining eggs were incubated individually. Emergences from collected pupae were monitored and numbers of *F. caudatus*, *F. arisanus*, and *C. cosyra* emerging from *I. gabonensis* were recorded. The assay was repeated 12 times per treatment for the behavioural observation and seven replicates were used for the dissection of eggs.

2.4. Statistical analysis

Observed behavioural activities such as attraction, resting, walking, probing and ovipositing were analysed with a generalized linear model (glm) with a Poisson distribution and Log link function in the assays 1, 3, 5 and 6. In all the bioassays, data at each observation interval from all replicates were considered independently to calculate the mean number of parasitoids performing each behavioural activity. Analysis of behaviour in relation to egg suitability tests (assay 2) was done with a vector generalized linear model (vglm) with a multinomial distribution. In the assay with four ratios of *F. arisanus* and *F. caudatus* (assay 4), behavioural activities were analysed with a glm with binominal responses. The behavioural data were classified into number of cases (not number of individuals), where one of the species (as a group or alone) performed more (was the winner) behavioural activities than the other species. Hence, the response of *F. arisanus* was compared with that of *F. caudatus* and was thereafter separated into > 0 and ≤ 0 response differences between the parasitoid species. The numbers of collected pupae and emerged fruit flies from the different treatments were analysed using a negative binominal glm. Analysis of emergence of the parasitoids was done with a glm, with Poisson distribution. The nonparametric Wilcoxon test was used to analyse the emergence of flies (assay 1).

3. Results

3.1. Host age effect on wasp oviposition performance and survival

Assay 1

Fruits infested with young *C. cosyra* eggs and parasitized by five *F. caudatus* females yielded emergence of 1.16 ± 0.44 *F. caudatus* and 61.0 ± 7.26 *C. cosyra* fruit^{-1}. When five *F. arisanus* had parasitized fruits infested with young *C. cosyra* eggs, no emergence of the parasitoid was recorded, yet 64.83 ± 9.99 *C. cosyra* emerged fruit^{-1}.

From the additional replicates, only a total of two (2) (0.33 ± 0.23 fruit^{-1}) *F. arisanus* emerged from infested fruits, exposed to twenty *F. arisanus*. Dissection of the fruit fly eggs showed a parasitism rate of $46.66 \pm 2.35\%$. The number of pupa per fruit was 25.16 ± 6.48, of which $77.79 \pm 5.30\%$ eclosed, and mostly flies emerged (97.98%).

Assay 2

Adult oviposition performance in eggs of different ages showed that *F. caudatus* were more active i.e. performed more walking, palping, probing and oviposited more on the 48-h-old eggs, compared to 24-h and 72-h-old eggs. Dissection revealed parasitism ($2.93 \pm 2.0\%$) by *F. caudatus* only in 48-h-old eggs. *Fopius arisanus* probing and ovipositing were higher on the 24-h and 48-h-old eggs compared to 72-h-old eggs while there was no difference in resting, walking, and palping between the egg ages (Fig. 1).

3.2. Extrinsic and intrinsic competition between wasp species during simultaneous exposure to host eggs

Assay 3

Observation of behavioural events during simultaneous exposure of fruits infested with *C. cosyra* eggs to *F. caudatus* and *F. arisanus* showed that *F. arisanus* always displaced *F. caudatus* when confrontations occurred. *Fopius arisanus* as well as *F. caudatus* were observed inserting their ovipositor in the fruits and there were no difference neither in probing ($z = 1.40$, $P = .162$) between *F. arisanus* (12.17 ± 1.42) and *F. caudatus* (9.50 ± 1.26), nor in oviposition ($z = 1.148$, $P = .251$) between *F. arisanus* (5.83 ± 0.98) and *F. caudatus* (4.33 ± 0.85). No *F. arisanus* emerged, while 0.83 ± 0.48 *F. caudatus* emerged per fruit. From the test fruits 67.0 ± 8.32 *C. cosyra* emerged.

Assay 4

The different ratios of *F. arisanus* and *F. caudatus* influenced the number of individuals that were walking, palping, probing, and ovipositing (Fig. 2). Walking by *F. arisanus* was nearly always observed in higher number of cases than for *F. caudatus*. Cases where *F. caudatus* did more palping and probing than *F. arisanus,* were found when they were in equal number (1:1) or when *F. caudatus* were in great numerical superiority (1:4). *Fopius arisanus* did more probing in the ratio 2:1 (Fig. 2). Oviposition behaviour by *F. caudatus* was only observed in
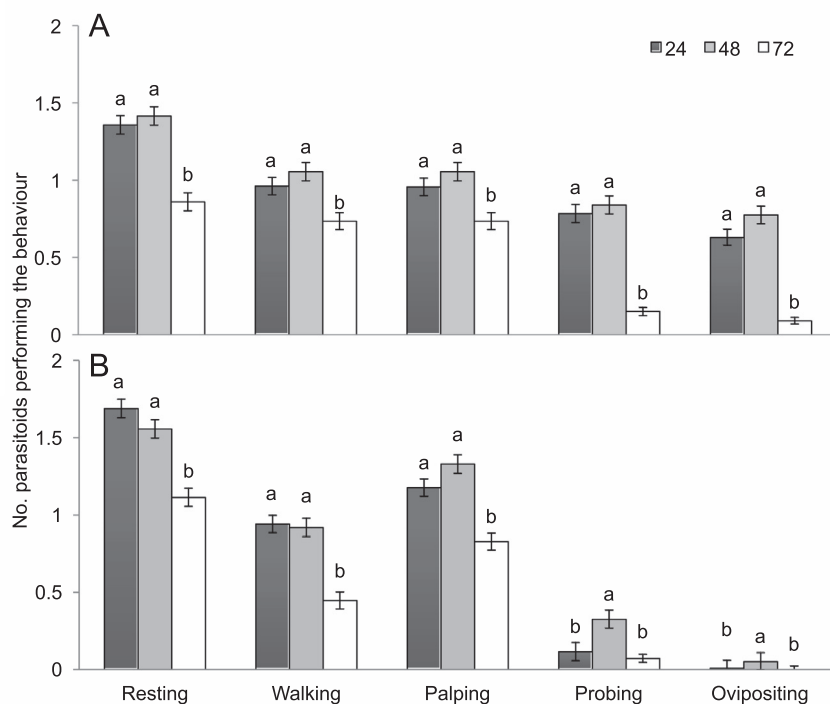
Fig. 1. Behavioural activities by A) *Fopius arisanus* and B) *F. caudatus,* on artificially *C. cosyra*-infested African mango *Irvingia gabonensis*. *Ceratitis cosyra* eggs were presented to the parasitoids 24 h, 48 h and 72 h post oviposition. Bars with same lower case letters above are not significantly different at P < .05.

treatments where *F. caudatus* were in numerical superiority (2:1, 4:1) yet it was always *F. arisanus* that oviposited more than *F. caudatus*, even when in four times numeric disadvantage (Fig. 2). Confronting behaviour occurred between *F. caudatus* and *F. arisanus* in the ratios 4:1 and 1:2, where *F. arisanus* chased away *F. caudatus*. Parasitism differed between the ratios and was highest when *F. arisanus* were in numerical superiority (1:2) (Table 1). Emergence from the different treatments was low and only *F. arisanus* emerged (Table 1).

3.3. Behavioural and survival effect of subsequent heterospecific parasitization

Assay 5

Observed *Fopius caudatus* attraction towards previously parasitized fruit did not differ from attraction towards fruit only infested with *C. cosyra* (control). Almost 50% of *F. caudatus* were situated on the fruits and this attraction to the test fruits declined (z = 2.468, P = .0136) during the first hour; 15, 30, 45 and 60 min, after introduction of *F. caudatus*. Oviposition was low and did not decline with time and did not differ between fruit previously exposed and that non-exposed to heterospecific parasitoid species (Table 2). Number of pupae and emerged fruit flies did not differ between the two choices. No *F. arisanus* emerged from the fruits, while *F. caudatus* emerged from both treatments and the emergence was not different between fruits previously parasitized and fruit only infested with *C. cosyra* (Table 2).

Assay 6

The parasitism by *F. arisanus* and *F. caudatus* in *C. cosyra* was not only related to previous parasitism by heterospecific species but also related to the developmental time of the host egg. Both factors can have influenced the behaviour performed by the two parasitoid species on the fruit. Hereafter we named the treatments according to both order and age; 1st/24 h and 2nd/48 h.

There was a general behavioural difference between the two parasitoid species. *Fopius arisanus* was more attracted to the infested fruits (z = 10.61, P < .0001), did more probing (P < .0001), and ovipositing (P < .0001), than *F. caudatus*. *Fopius caudatus* left the fruit more often than *F. arisanus* (z = 3.674, P = .0002). In both order/age treatments, there was a difference between species, while it was slightly

more pronounced at the first order/age (Table 3). Influence from the factor order/age resulted in a lower attraction (z = 2.434, P = .0149) and oviposition (z = 3.519, P = .0004) at 1st/24 h treatment than at 2nd/48 h. The difference was more pronounced for *F. caudatus* than for *F. arisanus*. Only palping differed for *F. arisanus* between 24 h and 48 h (1.94 ± 0.13 and 1.54 ± 0.11 respectively, z = 2.343, P = .019). *Fopius caudatus* did however act differently depending on the egg ages, since attraction (2.38 ± 0.14 and 3.31 ± 0.17, z = 4.228, P < .0001), probing (1.26 ± 0.10 and 1.79 ± 0.12, z = 3.328, P = .0009) and ovipositing (0.73 ± 0.08 and 1.59 ± 0.12, z = 6.015, P < .0001) were higher in the older egg, one day after parasitism by *F. arisanus* than in the first order/age treatment. Probing was the most common behavioural activity that *F. arisanus* performed while most of *F. caudatus* performed either probing or palping (Table 3).

The order/age in which *F. arisanus* and *F. caudatus* parasitized *C. cosyra* influenced also the total parasitism rate. When *F. arisanus* has parasitized first, and *F. caudatus* after, the percentage of parasitized fruit fly eggs was higher (63.23 ± 5.0%) than when *F. caudatus* had parasitized first, and *F. arisanus* after (41.94 ± 5.3%) (z = 11.48 P < .0001). Parasitism by *F. arisanus* did not differ between the two treatments (1st/24 h and 2nd/48 h) (z = 0.776, P = .438) while parasitism by *F. caudatus* differed between treatments with a higher parasitism on the second day (z = 5.151, P < .0001), when *C. cosyra* eggs were 48-h-old after parasitism by *F. arisanus*. Parasitism rate was higher for *F. arisanus* than *F. caudatus* at 24 h (z = 2.056, P = .0398) but there was no difference between the two parasitoid species at 48 h (z = 1.407, P = .160) (Table 4).

Superparasitism was found in about 15% of the dissected eggs and larvae (46/320) and a total of 226 *Fopius* sp. were identified in the 320 examined *C. cosyra*. The superparasitism differed between the two treatments, with a higher superparasitism when *F. arisanus* was parasitizing first, than when *F. caudatus* was first (27.10 ± 4.78%, 2.58 ± 2.02% respectively, χ^2 = 22.418, df = 1, P < .0001). Absolute parasitism, taking into account all the eggs laid including the superparasitism, was higher for *F. arisanus* than *F. caudatus* (37.10 ± 5.94% and 35.81 ± 9.76% respectively, χ^2 = 11.733, df = 1, P = .0006).

Parasitoid emergences ranged between 12 and 29% of emerged fruit
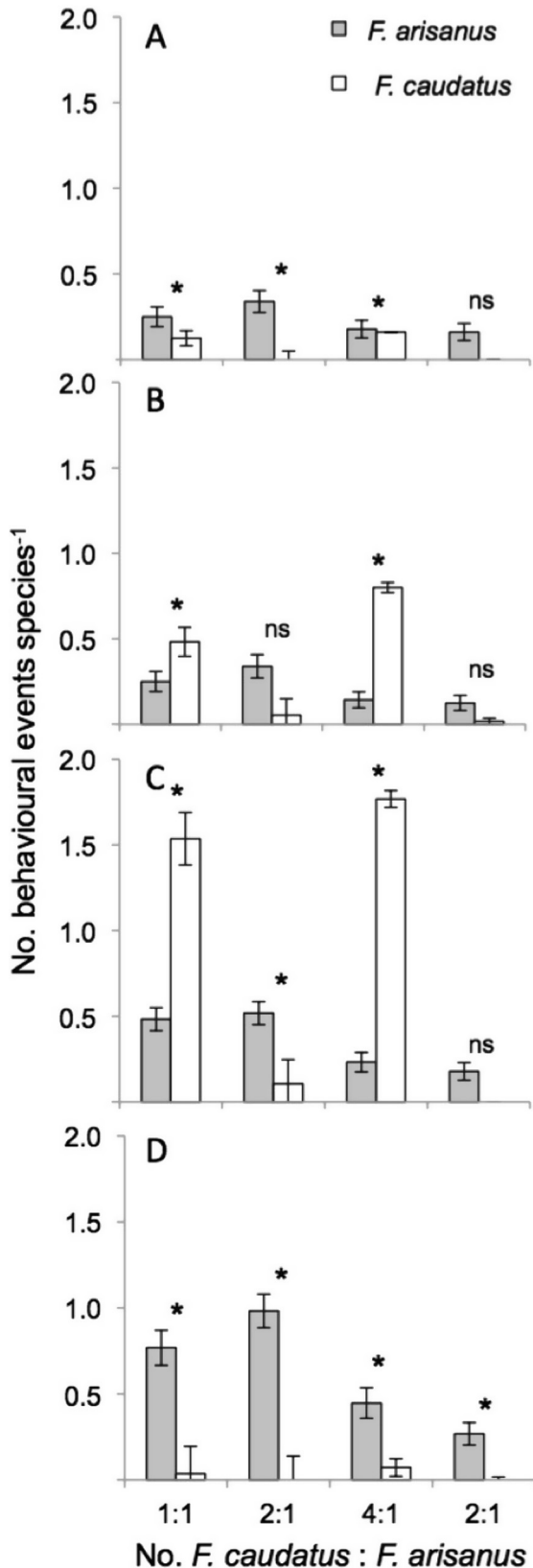
Fig. 2. Influence of four different ratios; 1:1, 2:1, 4:1 and 1:2 between *F. caudatus* and *F. arisanus* respectively on their A) walking B) palping C) probing D) ovipositing in *C. cosyra*-artificially-infested *I. gabonensis*. * above bars indicate significant difference at P < .05 between *F. caudatus* and *F. arisanus*.

fly (Fig. 3). Emergence of *F. arisanus* per fruit was higher than for *F. caudatus* (z = 6.021, P < .0001). The total emergence of parasitoids and fruit fly was higher when *F. arisanus* parasitized before *F. caudatus* (62.25 $fruit^{-1}$) than when *F. caudatus* parasitized before *F. arisanus* (47.42 $fruit^{-1}$) (z = 6.965, P < .0001). Emergence did not differ between the two treatments neither for *F. arisanus* (z = 1.93, P = .0536) nor for *F. caudatus* (z = 0.899, P = .369) (Fig. 3). Comparison of parasitoid emergence in relation to the numbers of parasitized eggs revealed a difference between *F. arisanus* and *F. caudatus* with a higher emergence rate of *F. caudatus* compared to *F. arisanus* (χ^2 = 8.3132, df = 1, P = .0040).

4. Discussion

Fopius caudatus was an inferior competitor compared with *F. arisanus* on the host *C. cosyra* in African mango. *Fopius arisanus* females laid a higher number of eggs compared to *F. caudatus*, regardless of host egg age. When an equal number of *F. arisanus* and of *F. caudatus* were simultaneously released on an infested fruit or when *F. arisanus* was in numerical superiority, *F. caudatus* were not able to parasitize the fruit fly eggs.

4.1. Effect of extrinsic competition between parasitoid species

Extrinsic competition by adult parasitoids was observed on the surface of infested fruits, where *F. arisanus* with physical aggression displace *F. caudatus*. We furthermore witnessed a higher oviposition foraging activity by *F. arisanus* than by *F. caudatus,* resulting in *F. arisanus* achieving the highest parasitism rate. *Fopius caudatus* was not able to oviposit while being in numerical inferiority or in same number as *F. arisanus* and it was only when being in 4x numerical superiority over *F. arisanus* that *F. caudatus* showed a more frequent probing behaviour compared to the competing species and managed to perform oviposition. Confrontations occurred when *F. arisanus* were in numerical superiority resulting in displacement of *F. caudatus*. The presence of a competitor might change a parasitoid behaviour, based on the negative effects suffered through previous interaction (Feng et al., 2015). This behavioural change might result in spreading the risk of interspecific competition by locating in scattered, less profitable, poorly exploited hosts (Aluja et al., 2013; Jeffries and Lawton, 1984; Paranhos et al., 2013) or persuading defence and/or aggressive behaviour by remaining/selecting the most beneficial host, even though there is a risk for competition (Bell, 1990; Mohamad et al., 2014). An inferior access to hosts or lesser capacity to locate an oviposition site than an adjacent competitor, is of disadvantage in an extrinsic competition (Cusumano et al., 2012). One destructive effect for the weaker species in a competition might be competitive displacement, either from a host, or from a habitat (Duyck et al., 2004; Eitam et al., 2004; Rwomushana et al., 2009). Introduced Braconidae wasp *D. longicaudata* was dominated by *F. vandenboschi* then successively nearly replaced by *F. arisanus* in the management of Tephritidae fruit flies in Hawaii (Van den Bosch and Haramoto, 1953; Vargas et al., 2012). Our results show differences between *F. arisanus* and *F. caudatus* in host location activity, parasitization capacity, and displacement power, which are properties for competitive displacement (Harcourt, 1990). The smaller body size of *F. caudatus* than *F. arisanus* (Fischer, 1929; Rousse et al., 2006), together with e.g. reproductive capacity, host fruit choice, and pest availability will further shape their interaction, and the interaction with other Tephritidae pests and their parasitoids (Reitz and Trumble, 2002).

4.2. Effect of intrinsic competition between parasitoids

We observed a slight difference between *F. caudatus* and *F. arisanus* in their emerging capability from *C. cosyra* eggs. While parasitism was higher for *F. arisanus,* emergence rate did not reflect the same difference pattern. We do however not assert that the higher survival of *F.*

Table 1

Influence on parasitism and emergence of four different ratios between the parasitoid species *F. caudatus* and *F. arisanus*.

Ratio *F. caudatus:F. arisanus*	Parasitism by both *F. caudatus* + *F. arisanus* [% ± SE]	Emergence *F. arisanus F. caudatus* [No. ± SE]	
1:1	0.14 ± 0.14 b	0.71 ± 0.71	0
2:1	0c	0	0
4:1	0.29 ± 0.20b	0.43 ± 0.43	0
1:2	1.29 ± 0.60a	0.86 ± 0.59	0

Table 2

Fopius caudatus choice between *C. cosyra*-infested fruit (control) and *C. cosyra*-infested fruit previously exposed to *F. arisanus* plus development of the insects.

	Control	Previously parasitized by *F. arisanus*	Z	P
Attraction to infested fruit[a]	2.37 ± 0.43	2.83 ± 0.47	0.182	.856
Oviposition in infested fruit[a]	0.92 ± 0.27	0.92 ± 0.27	0.103	.902
Number of pupae fruit^{-1}	61.50 ± 4.68	59.67 ± 4.58	0.28	.780
Number of emerged *C. cosyra* fruit^{-1}	54.00 ± 3.93	51.00 ± 3.78	0.55	.582
Number of emerged *F. caudatus* fruit^{-1}	0.83 ± 0.37	0.50 ± 0.29	0.699	.484

[a] Mean No. (± SE) *F. caudatus* observed fruit^{-1} observation^{-1}.

caudatus compared to *F. arisanus* is owing to intrinsic competition i.e. physiological suppression or asphyxiation by the dominant parasitoid. Further studies are required to conclude if the co-parasitization does affect the survival of the heterospecific wasp. Intrinsic competition between *F. arisanus* and larval parasitoids out-competes the larval parasitoids through physiological suppression, killing heterospecific eggs within a few days (Sime et al., 2008; Wang et al., 2008). Intrinsic competition between *F. arisanus* and the egg-parasitoids *F. ceratitivorus* showed that the chance of winning depends on which species occupies the host first (Bokonon-Ganta et al., 2005), yet the emergence of *F. arisanus* is higher than for *F. ceratitivorus* in 24−h-old *C. capitata* eggs and *F. arisanus* is considered a superior intrinsic competitor (Kroder and Messing, 2010).

4.3. Capacity of interspecific discrimination between parasitoid species

Both wasps were as likely to oviposit into fruits previously exposed to their respective heterospecific. We found however more superparasitized hosts, i.e. parasitized more than once by female(s) of the same species, and multiparasitized eggs, i.e. parasitized by females of both species, when *F. caudatus* parasitized after *F. arisanus* than when *F. arisanus* parasitized after *F. caudatus*. Super- and multi parasitism by *F.*

caudatus reached approximately 7% and for *F. arisanus* not more than 2% which might mean that *F. arisanus* capacity to avoid previously parasitized eggs is higher than for *F. caudatus*. The capacity to avoid multiparasitism is not common between parasitoid species while intraspecific discrimination is more important (Cusumano et al., 2012). It is likely that encounters of hosts already parasitized by conspecifics are a more common event than encounters of heterospecific parasitoid species. Thus, the selection pressure to evolve interspecific discrimination is weak even if there are some cases in which it occurs between egg parasitoid species (Agboka et al., 2002; Cusumano et al., 2016) and between Braconidae species parasitizing tephritid larvae (Aluja et al., 2013). Assays that more explicitly compare superparasitism with multiparasitism for both parasitoid species could probably further help to understand their capacity to distinguish previously parasitized eggs.

4.4. Effect of egg age on parasitoid performance and survival

Fopius caudatus and *F. arisanus* parasitization and survival were differently affected by the developmental stage of *C. cosyra* egg/larvae. While 48-h-old eggs stimulated highest probing and ovipositing by *F. caudatus*, there was no difference between *F. arisanus* ovipositing in 24 or 48-h-old eggs. Probing, ovipositing and parasitism by *F. arisanus* were higher in the 24 h and 48-h-old eggs than in 72-h-old *C. cosyra* eggs/larvae. Previous comparison of parasitism as emergence of *F. arisanus* from 24 h and 48-h-old *C. capitata* eggs, revealed that the 24-h-aged eggs yielded more parasitoids than the 48-h-old eggs (Kroder and Messing, 2010), while we did not observe this difference in *C. cosyra*. The developmental stage of *C. cosyra* eggs did nevertheless affect parasitoid emergence, and more so for *F. arisanus*. Relative emergence (emerging progeny/parasitizing females) from young *C. cosyra* eggs was higher for *F. caudatus* than for *F. arisanus*. When parasitizing very young, i.e. < 6-h-old, *C. cosyra* eggs, we found extremely few emerging *F. arisanus*, while emergence of *F. caudatus* adults was much more common. This superior capability of *F. caudatus* to develop in younger eggs was one verified biological advantage that *F. caudatus* had over *F. arisanus*.

Synchronization between development of parasitoid and its host was recorded from the egg-pupal parasitoid *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) in *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) (Murillo et al., 2015), and might be important for egg-pupal parasitoids. Most egg parasitoids lay their eggs in the pregastrula stage of the embryo, which will protect them from the immunological response of the host (Strand and Pech, 1995). Nevertheless, if the fly embryo has not been formed at the time of parasitoid larval hatching, there is a risk that the larva dies. Hence, the development of the host egg influences parasitoid survival, especially in the case of tephritid host species with a long embryonic developmental time such as *Anastrepha ludens* (Loew) and *A. serpentina* (Wiedermann) (Diptera: Tephritidae) (Zenil et al., 2004); *Anastrepha* spp. require 3–4

Table 3

Comparison of observed behavioural activities between *F. arisanus* and *F. caudatus* at intercalated heterospecific parasitization.

Observed behaviour	1st/24 h			2nd/48 h		
	F. arisanus [No. parasitoids fruit^{-1}]	*F. caudatus*	P	*F. arisanus* [No. parasitoids fruit^{-1}]	*F. caudatus*	P
Attraction[a]	4.78 ± 0.2	2.38 ± 0.14	< .0001	4.73 ± 0.2	3.31 ± 0.17	< .0001
Resting	1.78 ± 0.12c	1.41 ± 0.11a	.0218	1.68 ± 0.12c	1.29 ± 0.10b	.0131
Walking	0.11 ± 0.03e	0.15 ± 0.04d	.3710	0.06 ± 0.02e	0.13 ± 0.03d	.0959
Palping	1.94 ± 0.13c	1.41 ± 0.11a	.0015	1.54 ± 0.11c	1.67 ± 012a	.4450
Probing	3.02 ± 0.16a	1.26 ± 0.10a	< .0001	3.02 ± 0.16a	1.79 ± 0.12a	< .0001
Ovipositing	2.33 ± 0.14b	0.73 ± 0.08b	< .0001	2.32 ± 0.14b	1.59 ± 0.12ab	< .0001
Leaving	0.16 ± 0.04d	0.37 ± 0.06c	.0022	0.26 ± 0.05d	0.42 ± 0.06d	.0288

Mean (± SE) in the same column compare the behaviours per species and numbers followed by the same letter are not significantly different at P < 0.05. [a]Attraction is the sum of the parasitoids on the fruit and not considered in the test among behavioural activities on the fruit.

Table 4
Comparison of parasitism by *F. arisanus* and *F. caudatus* after dissection of *C. cosyra* eggs.

	Parasitism [%]		Superparasitized eggs [No. (%)]		Mutiparasitized eggs [No. (%)]	
	F. arisanus	*F. caudatus*	*F. arisanus*	*F. caudatus*	*F. arisanus*	*F. caudatus*
1st/24 h	36.77 ± 5.57a (a)	9.03 ± 3.69b (b)	7 (2.19)	3 (0.94)	–	–
2nd/48 h	31.61 ± 4.80a (a)	41.29 ± 6.73a (a)	0 (0)	20 (6.25)	2 (0.63)	24 (7.5)

Means (± SE) in the same column followed by the same lower case letters or means in the same row followed by the same letter in parenthesis are not significantly different at P < .05.
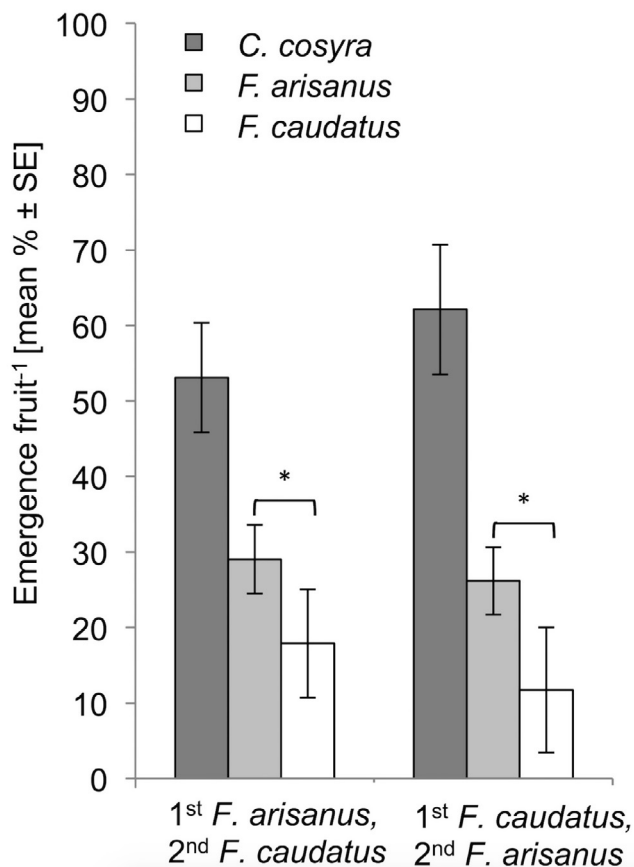
Fig. 3. Emergence of *C. cosyra*, *F. arisanus* and *F. caudatus* as percentage of total emergence per *I. gabonensis* fruit. * above bars indicate significant difference at P < .05 between *F. caudatus* and *F. arisanus*.

days of incubation at 26–28 °C for embryo development. Advanced embryonic development of older eggs compared to younger eggs has been used to explain a higher emergence of *F. arisanus* from 48-h-old *C. capitata* eggs compared to younger ages, and to explain emergence of *F. arisanus* from 72-h-old *A. ludens* and *A. serpentina* eggs compared to no emerged parasitoids from two *Anastrepha* species exposed as < 4-h-old eggs (Zenil et al., 2004). The developmental time of *C. cosyra* is relatively long, compared e.g. to *B. dorsalis* (Ayelo et al., 2017; Salum et al., 2013). We might assume that by the time *F. arisanus* eggs eclosed, the embryo in *C. cosyra* eggs had not yet had formed sufficiently to allow *F. arisanus* development, yet this is still to be confirmed. The oviposition window for egg parasitization is only two days since *F. arisanus* and *F. caudatus* rarely parasitize larvae and we observed 100% *C. cosyra* eggs after 48 h while at 72 h, 98% of the eggs had hatched into first instar larvae. The window length nevertheless changes with fruit fly and with fruit, as the time for development of Tephritidae eggs differs depending on fruit substrate (Hintenou et al., 2016). In our study we used *C. cosyra* infesting the fruit *I. gabonensis* and other flies and fruits might offer appropriate developmental stage during shorter or longer time.

4.5. Host and fruit suitability

Both *F. arisanus* and *F. caudatus* parasitize *C. cosyra* under laboratory conditions, (Mohamed et al., 2010); this study). Whether *F. arisanus* parasitize *C. cosyra* in the field is to our knowledge sparsely documented. *Fopius caudatus* has been reared exclusively from Tephritidae flies in the Ceratitidine tribe (Steck et al., 1986; Wharton et al., 2000) but information about its capability to parasitize other species is lacking. *Ceratitis cosyra* and *B. dorsalis* share some host fruits, e.g. both species infest mango and African mango (Gnanvossou et al., 2016; Rwomushana et al., 2009). *Fopius arisanus* searching for an appropriate oviposition site is primarily determined by fruit species and only thereafter by Tephritidae fruit fly (Ayelo et al., 2017). *Fopius arisanus* has an oviposition preference for *B. dorsalis* over *C. cosyra* but does however parasitize and emerge from both species (Mohamed et al., 2010). After releases, *F. arisanus* recovery was greatest from African mango in Benin (Gnanvossou et al., 2016), and from mango, *Citrus* spp., guava, etc. in Senegal (Ndiaye et al., 2015). Both *F. arisanus* and *F. caudatus* have been reared from e.g. mango and *Citrus* spp (Foba et al., 2012; Ndiaye et al., 2015; Rousse et al., 2007; Vayssières et al., 2011), making it likely that *F. arisanus* and *F. caudatus* will search for host fly on the same fruit. Data about field competition is still lacking and this study is to our knowledge the first brief evaluation of the effect of the recent introduction of the exotic *F. arisanus* on the endemic parasitoid fauna in Africa.

4.6. Conclusion

There is not always one winner in interspecific competition and one species might not be better or worse competitor (Cabello et al., 2011). *Diachasmimorpha areolatus* is a better extrinsic competitor and locate host patches better while *D. longicaudata* is superior in exploiting these patches i.e. intrinsic competition (Eitam et al., 2004). The result of the interspecific competition after release of *F. arisanus* in an environment where *F. caudatus* is present might cause a reduction of parasitism by the native species in some fruits due to the higher capacity by *F. arisanus* to oviposit and chase away the competitor. We do not expect *F. caudatus* to reduce the chance for *F. arisanus* to develop and establish in the field. Their interaction might depend on availability of fruits, fruit flies' and parasitoids preferences and oviposition performance. The competition consequence might be observed if the population of *F. arisanus* will be greater than the native *Fopius* and on specific grafted mango varieties, where parasitism by *F. caudatus* has been observed to be higher, than in e.g. non-grafted mango (Vayssières et al., 2011). The level of *B. dorsalis* and *C. cosyra* infestation and the parasitoids' different capacity to survive in the flies will further shape their population levels. There is possibility that two parasitoid species searching for oviposition sites in a shared fruit-diverse habitat, might co-exist. Capacity to parasitize in different stages of the host in numerous host plant species might be central in creation of a stable niche division. More studies, preferably in the field, upon host choice, fruit choice, population size, and host suitability will further clarify the tritrophic interaction between parasitoid species, Tephritidae fruit flies, and fruits.

Acknowledgments

References

Agboka, K., Schulthess, F., Chabi-Olaye, A., Labo, I., Gounou, S., Smith, H., 2002. Self-, intra-, and interspecific host discrimination in *Telenomus busseolae* Gahan and *T. isis* Polaszek (Hymenoptera: Scelionidae), sympatric egg parasitoids of the African cereal stem borer *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). J. Insect Behav. 15, 1–12 doi:10.1023/A:1014471826914.

Aluja, M., Ovruski, S.M., Sivinski, J., Córdova-García, G., Schliserman, P., Nuñez-Campero, S.R., Ordano, M., 2013. Inter-specific competition and competition-free space in the tephritid parasitoids *Utetes anastrephae* and *Doryctobracon areolatus* (Hymenoptera: Braconidae: Opiinae). Ecol. Entomol. 38, 485–496. http://dx.doi.org/10.1111/een.12039.

Alyokhin, A.V., Messing, R.H., 2003. Parasitism of Hawaiian non-frugivorous fruit flies (Diptera: Tephritidae) by an exotic parasitoid, *Eurytoma tephritidis* Fullaway (Hymenoptera: Eurytomidae). Proc. Hawaiian Entomol. Soc. 36, 29–37.

Ayelo, P.M., Sinzogan, A.C., Bokonon-Ganta, A.H., Karlsson, M.F., 2017. Host species and vegetable fruit suitability and preference by the parasitoid wasp *Fopius arisanus*. Entomol. Exp. Appl. 163, 70–81. http://dx.doi.org/10.1111/eea.12561.

Bell, W., 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35, 447–467. http://dx.doi.org/10.1146/annurev.ento.35.1.447.

Bogran, C.E., Heinz, K.M., Ciomperlik, M.A., 2002. Interspecific competition among Insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology 83, 653–668.

Bokonon-Ganta, A.H., Ramadan, M.M., Wang, X.G., Messing, R.H., 2005. Biological performance and potential of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg-larval parasitoid of tephritid fruit flies newly imported to Hawaii. Biol. Control 33, 238–247. http://dx.doi.org/10.1016/j.biocontrol.2005.02.015.

Cabello, T., Gámez, M., Torres, A., Garay, J., 2011. Possible effects of inter-specific competition on the coexistence of two parasitoid species: *Trichogramma brassicae* Bezdenko and *Chelonus oculator* (F.) (Hymenoptera: Trichogrammatidae, Braconidae). Community Ecol. 12, 78–88. http://dx.doi.org/10.1556/ComEc.12.2011.1.10.

Cancino, J., Montoya, P., Barrera, J.F., Aluja, M., Liedo, P., 2014. Parasitism by Coptera haywardi and *Diachasmimorpha longicaudata* on *Anastrepha* flies with different fruits under laboratory and field cage conditions. BioControl 59, 287–295. http://dx.doi.org/10.1007/s10526-014-9571-1.

Chinajariyawong, A., Clarke, A.R., Jirasurat, M., Kritsaneepiboon, S., Lahey, H.A., Vijaysegaran, S., Walter, G.H., 2000. Survey of opiine parasitoids of fruit flies (Diptera: Tephritidae) in Thailand and Malaysia. Raffles Bull. Zool. 48, 71–101.

Collier, T.R., Kelly, S., Hunter, M.S., 2002. Egg size, intrinsic competition, and lethal interference in the parasitoids *Encarsia pergandiella* and *E. formosa*. Biol. Control 23, 254–261.

Costamagna, A.C., Landis, D.A., 2004. Effect of food resources on adult *Glyptapanteles militaris* and *Meteorus communis* (Hymenoptera: Braconidae), parasitoids of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). Environ. Entomol. 33, 128–137. http://dx.doi.org/10.1603/0046-225X-33.2.128.

Cusumano, A., Peri, E., Bradleigh Vinson, S., Colazza, S., 2012. Interspecific extrinsic and intrinsic competitive interactions in egg parasitoids. BioControl 57, 719–734. http://dx.doi.org/10.1007/s10526-012-9451-5.

Cusumano, A., Peri, E., Colazza, S., 2016. Interspecific competition/facilitation among insect parasitoids. Curr. Opin. Insect Sci. 14, 12–16. http://dx.doi.org/10.1016/j.cois.2015.11.006.

Drew, R.A.I., Tsuruta, K., White, I.M., 2005. A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. African Entomol. 13, 149–154.

Duyck, P.-F., David, P., Quilici, S., 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). Ecol. Entomol. 29, 511–520.

Eitam, A., Sivinski, J., Holler, T., Aluja, M., 2004. Biogeography of braconid parasitoids of the caribbean fruit fly (Diptera: Tephritidae) in Florida. Ann. Entomol. Soc. Am. 97, 928–939. http://dx.doi.org/10.1603/0013-8746(2004) 097[0928:BOBPOT]2.0.CO;2.

Feng, Y., Wratten, S., Sandhu, H., Keller, M., 2015. Interspecific competition between two generalist parasitoids that attack the leafroller *Epiphyas postvittana* (Lepidoptera: Tortricidae). Bull. Entomol. Res. 10, 1–8. http://dx.doi.org/10.1017/S0007485314000923.

Fischer, V.M., 1929. Newe Braconiden-parasiten von schädlichen insekten (Hymenoptera). Bull. Entomol. Res. 20, 297–312.

Foba, C.N., Afreh-Nuamah, K., Billah, M.K., Obeng-Ofori, D., 2012. Species composition of fruit flies (Diptera: Tephritidae) in the citrus museum at the Agricultural Research Centre (ARC), Kade, Ghana. Int. J. Trop. Insect Sci. 32, 12–23. http://dx.doi.org/10.1017/S1742758412000045.

García-Medel, D., Sivinski, J., Díaz-Fleischer, F., Ramirez-Romero, R., Aluja, M., 2007. Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as single- or multiple-species cohorts in field cages: influence of fruit location and host density. Biol. Control 43, 12–22. http://dx.doi.org/10.1016/j.biocontrol.2007.06.008.

Gnanvossou, D., Hanna, R., Bokonon-Ganta, A.H., Ekesi, S., Mohamed, S.A., 2016. Release, establishment and spread of the natural enemy Fopius arisanus (Hymenoptera: Braconidae) for control of the invasive oriental fruit fly Bactrocera dorsalis (Diptera: Tephritidae) in Benin, West Africa. In: Ekesi, S., Mohamed, S.A., Meyer, M. De (Eds.), Fruit Fly Research and Development in Africa - towards a Sustainable Management Strategy to Improve Horticulture. pp. 575–600.

Harcourt, D., 1990. Displacement of *Bathyplectes curculionis* (Thoms.) (Hymenoptera: Ichneumonidae) by *Bathyplectes anurus* (Thoms.) in eastern Ontario [Canada] populations of the alfalfa weevil, *Hypera postica* Gyll. (Coleoptera: Curculionidae). Can. Entomol. 122, 641–646.

Hintenou, M.V., De Souza, E.O., Bokonon-Ganta, A.H., Bossou, I.G., Zannou, E.T., 2016. Étude comparative du développement de *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) sur *Irvingia gabonensis* et *Musa* sp. Int. J. Biol. Chem. Sci. 10, 1590–1601 org/10.4314/ijbcs.v10i4.12.

Hoffmeister, T., 1992. Factors determining the structure and diversity of parasitoid complexes in tephritid fruit flies. Oecologia 89, 288–297.

Jeffries, M.J., Lawton, J.H., 1984. Enemy free space and the structure of ecological communities. Biol. J. Linn. Soc. 23, 269–286. http://dx.doi.org/10.1111/j.1095-8312.1984.tb00145.x.

Kaplan, I., Denno, R.F., 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecol. Lett. 10, 977–994. http://dx.doi.org/10.1111/j.1461-0248.2007.01093.x.

Kroder, S., Messing, R.H., 2010. A new parasitoid from Kenya, *Fopius ceratitivorus*, complements the extant parasitoid guild attacking Mediterranean fruit fly in Hawaii. Biol. Control 53, 223–229. http://dx.doi.org/10.1016/j.biocontrol.2010.01.003.

Mohamad, R., Wajnberg, E., Monge, J., Goubault, M., 2014. The effect of direct interspecific competition on patch exploitation strategies in parasitoid wasps. Oecologia 177, 305–315. http://dx.doi.org/10.1007/s00442-014-3124-2.

Mohamed, S.A., Ekesi, S., Hanna, R., 2010. Old and new host-parasitoid associations: parasitism of the invasive fruit fly Bactrocera invadens (Diptera: Tephritidae) and five African fruit fly species by Fopius arisanus, an Asian opiine parasitoid. Biocontrol Sci. Technol. 20, 183–196. http://dx.doi.org/10.1080/09583150903447794.

Montoya, P., Liedo, P., Benrey, B., Cancino, J., Barrera, J.F., Sivinski, J., Aluja, M., 2000. Biological control of *Anastrepha* spp. (Diptera: Tephritidae) in mango orchards through augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 18, 216–224.

Montoya, P., Pérez-Lachaud, G., Liedo, P., 2012. Superparasitism in the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) and the implications for mass rearing and augmentative release. Insects 3, 900–911. http://dx.doi.org/10.3390/insects3040900.

Murillo, F.D., Cabrera-mireles, H., Barrera, J.F., 2015. *Doryctobracon areolatus* (Hymenoptera, Braconidae) a parasitoid of early developmental stages of *Anastrepha obliqua* (Diptera, Tephritidae). J. Hymenopt. Res. 46, 91–105. http://dx.doi.org/10.3897/JHR.46.5586.

Murillo, F.D., Liedo, P., Nieto-López, M.G., Cabrera-Mireles, H., Barrera, J.F., Montoya, P., 2016. First instar larvae morphology of Opiinae (Hymenoptera: Braconidae) parasitoids of *Anastrepha* (Diptera: Tephritidae) fruit flies. Implications for interspecific competition. Arthropod Struct. Dev. 45, 294–300. http://dx.doi.org/10.1016/j.asd.2016.01.003.

Ndiaye, O., Ndiaye, S., Djiba, S., Ba, C.T., Vaughan, L., Rey, J.-Y., Vayssières, J.-F., 2015. Preliminary surveys after release of the fruit fly parasitoid Fopius arisanus Sonan (Hymenoptera Braconidae) in mango production systems in Casamance (Senegal). Fruits 70, 1–9. http://dx.doi.org/10.1051/fruits/2015001.

Paranhos, B.J., Sivinski, J., Stuhl, C., Holler, T., Aluja, M., 2013. Intrinsic competition and competitor-free-space influence the coexistence of parasitoids (Hymenoptera: Braconidae: Opiinae) of neotropical Tephritidae (Diptera). Environ. Entomol. 42, 717–723. http://dx.doi.org/10.1603/EN12282.

Quicke, D.L.J., 2015. The Braconid and Ichneumonid Parasitoid wasp: Biology, Systematics. Evolution and Ecology, John Wiley Sons, Ltd, pp. 685.

Quimio, G.M., Walter, G.H., 2001. Host preference and host suitability in an egg-pupal fruit fly parasitoid, Fopius arisanus (Sonan) (Hym., Braconidae). J. Appl. Entomol. 125, 135–140. http://dx.doi.org/10.1046/j.1439-0418.2001.00514.x.

Reitz, S.R., Trumble, J.T., 2002. Competitive displacement among insects and arachnids. Annu. Rev. Entomol. 47, 435–465.

Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J., Jaffe, B.A., 1995. Intraguild predation among biological control agents: theory and evidence. Biol. Control 5, 303–335.

Rousse, P., Chiroleu, F., Veslot, J., Quilici, S., 2007. The host- and microhabitat olfactory location by Fopius arisanus suggests a broad potential host range. Physiol. Entomol. 32, 313–321. http://dx.doi.org/10.1111/j.1365-3032.2007.00575.x.

Rousse, P., Gourdon, F., Quilici, S., 2006. Host specificity of the egg pupal parasitoid *Fopius arisanus* (Hymenoptera: Braconidae) in La Réunion. Biol. Control 37, 284–290. http://dx.doi.org/10.1016/j.biocontrol.2005.12.008.

Rousse, P., Harris, E.J., Quilici, S., 2005. *Fopius arisanus*, an egg-pupal parasitoid of Tephritidae. Overview Biocontrol News Inf. 26, 59–69.

Rwomushana, I., Ekesi, S., Ogol, C.K.P.O., Gordon, I., 2009. Mechanisms contributing to the competitive success of the invasive fruit fly Bactrocera invadens over the indigenous mango fruit fly, *Ceratitis cosyra*: the role of temperature and resource preemption. Entomol. Exp. Appl. 133, 27–37. http://dx.doi.org/10.1111/j.1570-7458.2009.00897.x.

Salum, J.K., Mwatawala, M.W., Kusolwa, P.M., Meyer, M.D., 2013. Demographic

parameters of the two main fruit fly (Diptera: Tephritidae) species attacking mango in Central Tanzania. J. Appl. Entomol. 2003, 441–448. http://dx.doi.org/10.1111/jen.12044.

Sime, K.R., Daane, K.M., Wang, X.G., Johnson, M.W., Messing, R.H., 2008. Evaluation of *Fopius arisanus* as a biological control agent for the olive fruit fly in California. Agric. For. Entomol. 10, 423–431. http://dx.doi.org/10.1111/j.1461-9563.2008.00401.x.

Steck, G.J., 2015. Mango Fruit Fly, Ceratitis cosyra (Walker) (Insecta: Diptera: Tephritidae), Entomology and Nematology Department. UF/IFAS Extension, University of Florida, EENY286, pp. 1–3.

Steck, G.J., Gilstrap, F.E., Wharton, R.A., Hart, W.G., 1986. Braconid parasitoids of Tephritidae (Diptera) ingfesting coffee and other fruits in West-Central Africa. Entomophaga 31, 59–67.

Strand, M.R., Pech, L.L., 1995. Immunological basis for compatibility in parasitoid-host relationships. Annu. Rev. Entomol. 40, 31–56.

Van den Bosch, R., Haramoto, F.H., 1953. Competition among parasites of the oriental fruit fly. Proc. Hawaiian Entomol. Soc. 15, 201–206.

Vargas, R.I., Leblanc, L., Harris, E.J., Manoukis, N.C., 2012. Regional suppression of *Bactrocera* fruit flies (Diptera: Tephritidae) in the pacific through biological control and prospects for future introductions into other areas of the world. Insects 3, 727–742. http://dx.doi.org/10.3390/insects3030727.

Vargas, R.I., Leblanc, L., Putoa, R., Eitam, A., 2007. Impact of introduction of *Bactrocera dorsalis* (Diptera: Tephritidae) and classical biological control releases of *Fopius arisanus* (Hymenoptera: Braconidae) on economically important fruit flies in French Polynesia. J. Econ. Entomol. 100, 670–679.

Vayssières, J.-F., Korie, S., Ayegnon, D., 2009. Correlation of fruit fly (Diptera Tephritidae) infestation of major mango cultivars in Borgou (Benin) with abiotic and biotic factors and assessment of damage. Crop Prot. 28, 477–488. http://dx.doi.org/10.1016/j.cropro.2009.01.010.

Vayssières, J.-F., Wharton, R., Adandonon, A., Sinzogan, A., 2011. Preliminary inventory of parasitoids associated with fruit flies in mangoes, guavas, cashew pepper and wild fruit crops in Benin. BioControl 56, 35–43. http://dx.doi.org/10.1007/s10526-010-9313-y.

Wang, X., Messing, R.H., 2003. Foraging behavior and patch time allocation by *Fopius arisanus* (Hymenoptera: Braconidae), an egg-larval parasitoid of tephritid fruit flies. J. Insect Behav. 16, 593–612. http://dx.doi.org/10.1023/B:JOIR.0000007698.01714.56.

Wang, X.-G., Messing, R.H., Bautista, R.C., 2003. Competitive superiority of early acting Species: A case study of opiine fruit fly parasitoids. Biocontrol Sci. Technol. 13, 391–402. http://dx.doi.org/10.1080/0958315031000104514.

Wang, X.G., Bokonon-Ganta, A.H., Messing, R.H., 2008. Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: Effect of co-evolutionary history on competitive superiority. Biol. Control 44, 312–320. http://dx.doi.org/10.1016/j.biocontrol.2007.10.012.

Wharton, R., Trostlel, M., Messing, R., Copeland, R.S., Kimani-Njogu, S.W., Lux, S., Overholt, W.A., Mohamed, S., Sivinski, J., Trostle, M.K., Messing, R.H., Copeland, R.S., Kimani-Njogu, S.W., Lux, S., Overholt, W.A., Mohamed, S., Sivinski, J., 2000. Parasitoids of medfly, *Ceratitis capitata* and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. Bull. Entomol. Res. 90, 517–526.

Wharton, R.A., 1999. A review of the old world genus *Fopius*, with description of two new species reared. J. Hymenopt. Res. 8, 48–64.

Wharton, R.A., 1989. Classical biological control of fruit-infesting Tephritidae. In: Robinson, A.S., Harper, G. (Eds.), World Crop Pests. Fruit Flies, Their Biology, Natural Enemies and Control. Elsevier, pp. 303–313.

Wharton, R.A., Gilstrap, F.E., 1983. Key and status of opiine braconid (Hymenoptera) parasitoids used in biological control of *Ceratitis capitata* and *Dacus*, s.l. (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 76, 721–742.

Xu, H.Y., Yang, N.W., Wan, F.H., 2013. Competitive interactions between parasitoids provide new insight into host suppression. PLoS One 8, 16–21. http://dx.doi.org/10.1371/journal.pone.0082003.

Zenil, M., Liedo, P., Williams, T., Valle, J., Cancino, J., Montoya, P., 2004. Reproductive biology of *Fopius arisanus* (Hymenoptera: Braconidae) on *Ceratitis capitata* and *Anastrepha* spp. (Diptera: Tephritidae). Biol. Control 29, 169–178. http://dx.doi.org/10.1016/S1049-9644(03)00140-3.