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# Coexistence between two fruit fly species is supported by the different strength of intra- and interspecific competition

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**Abstract.** 1. Unravelling the strength and modes of interspecific interactions between resident and introduced species is necessary in order to understand the basis of their coexistence or the displacement of the former by the latter. In Argentina, the indigenous Tephritidae fly *Anastrepha fraterculus* overlaps its distribution and host fruit with the introduced species *Ceratitis capitata*.

2. This study focused on the relative strength of intra- and interspecific competition during the larval stage as a potential factor supporting coexistence. Classical competition experiments (addition and substitution) were conducted between larvae of the two species reared in artificial larval diet. The study evaluated whether a temporal separation between oviposition events affects the outcome of the competition.

3. When both species started to consume the resource at the same time, *A. fraterculus* experienced a negative effect in larval survival, pupal weight and duration of larval stage, while for *C. capitata*, pupal weight decreased. When *A. fraterculus* started feeding 1 day earlier than *C. capitata*, the negative effects became milder, and when the temporal separation increased, these effects were reversed. Substitution experiments showed an increase in pupal weight when larvae had to share the resource with heterospecific larvae, and showed negative effects suffered for both species when they shared the resource with conspecific individuals.

4. These results suggest that intraspecific competition is stronger than interspecific competition, and a differential oviposition preference could generate an asynchrony of these species in nature. Such mechanisms could favour coexistence between *A. fraterculus* and *C. capitata* in an environment previously occupied only by the former.

**Key words.** Competitive advantage, interspecific larval competition, invasive species, niche overlap, stable coexistence, Tephritidae.

## Introduction

The coexistence of species that interact competitively has long interested ecologists, but the processes enabling two competitors to coexist are not completely understood (Hutchinson, 1959, 1961; Miller, 1969; Grubb, 1977; Werner, 1979; Brown,

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1981; Ricklefs & Schluter, 1993; Brown *et al.*, 1995). The simplest model of interspecific competition that explains the stable coexistence of two species, the Lotka–Volterra model (Lotka, 1925; Volterra, 1926), states that coexistence is possible when intraspecific competition is stronger than interspecific competition. An alternative non-exclusive explanation of coexistence between competitor species was proposed by Tilman (1994) on the basis of MacArthur and Wilson (1967) concepts on the trade-off between competitive skill and colonisation. Tilman

(1994) proposed that coexistence of species is favoured by environmental disturbance if it empties (at least partially) the niche, or even generates new niches. In this sense, disturbance would allow a trade-off between competition and colonisation among species. Interspecific competition derived from situations in which a new species is introduced is not uncommon (Fitt, 1989; Duyck *et al.*, 2004, 2006). Biological invasions can lead to the displacement of the indigenous species, or, alternatively, to a stable coexistence of the involved species under tolerable levels of competition (Human & Gordon, 1996; Juliano, 1998; Amarasakare, 2002; Braks *et al.*, 2004; Duyck *et al.*, 2007; Perdureau *et al.*, 2011; Deus *et al.*, 2016).

The Tephritidae family comprises several species that have invaded new areas posing important economic problems to fruit or vegetable crops in tropical and subtropical areas worldwide (Duyck *et al.*, 2007) and thus became good candidates for competition studies. Most of these species are polyphagous and infest a wide range of host plants (White & Elson-Harris, 1992). For some systems, it has been found that coexistence between Tephritidae species can be allowed by niche partitioning via host range (Duyck *et al.*, 2008). However, there are many systems where it is common to find more than one species infesting the same fruit (Pavan, 1978; Malavasi *et al.*, 1980), creating the needed conditions for the establishment of interspecific competitive interactions among larvae (Prokopy *et al.*, 1984). According to the conventional niche theory, the primary determinant of competition is resource overlapping (or niche overlap), providing opportunities for competitive responses (the ability of a species to withstand competition exerted by other species) and competitive effects (the negative effects of one species on another one) (Goldberg & Barton, 1992). In the case of fruit flies, the impact of competition will depend on both the total density of larvae within a particular fruit and the relative abundance of each species. This can be asymmetrical between the two species (Aluja & Liendo, 1993), a phenomenon that has also been described in host–parasitoid systems (Tuda, 1996). Moreover, the effect of these two factors on the competing individuals will depend on the quality of the fruit in terms of the number of larvae that are able to complete their development (i.e. the carrying capacity of the fruit), which varies amongst host species and phenological stages.

Aside from the nutritional requirements of each species, the strength of competition and its outcome could also depend on differences between competing species in their developmental times (Mishima, 1964). For instance, larvae that develop faster will have access to more resources than will those that take longer to complete their development (Qureshi *et al.*, 1987; Blanckenhorn, 1999; Krijger *et al.*, 2001; Duyck *et al.*, 2006). Likewise, the chronology in which larvae hatch could affect the outcome of competitive interactions. This occurs when the competing species differ in the phenological stage of the fruit in which oviposition takes place (Fitt, 1989). Dukas *et al.* (2001) found that larval and pupal parameters in *C. capitata* showed a significantly larger variance when clutches were laid on successive days than when they were laid on the same days, suggesting that older larvae have a competitive advantage over younger ones. Rwomushana *et al.* (2009) also showed that an asynchrony of infestation of 3 days is

enough for *Ceratitis cosyra* Walker to increase its competitive advantage over *Bactrocera invadens* (now *Bactrocera dorsalis* Hendel) Drew, Tsuruta & White, an invasive species with which *C. cosyra* cannot compete when oviposition is synchronic.

*Anastrepha fraterculus* (Wiedemann) is a polyphagous Tephritidae fruit fly species native to Argentina that infests about 80 fruit species from different plant families, including Myrtaceae, Rosaceae and, less frequently, Rutaceae (Norrbom, 2004). *Ceratitis capitata* (Wiedemann) is native to Africa and was first detected in Argentina in 1900 (Vergani, 1956). This species is also highly polyphagous, attacking nearly 350 fruit species from a diverse range of families, such as Myrtaceae, Rosaceae, Rutaceae, and Solanaceae (Liquidó *et al.*, 1991). In Argentina, *A. fraterculus* and *C. capitata* widely overlap their distribution and host ranges (Ovruski *et al.*, 2003; Segura *et al.*, 2006; Oroño *et al.*, 2008; Devescovi *et al.*, 2015). Liendo *et al.* (2016) found differences in the way in which these species responded to intraspecific competition and evidenced a different pattern of resource distribution among conspecific larvae. While *C. capitata* larvae facing a shortage of nutritional resources develop a strategy of resource distribution similar to that described as scramble competition (also called exploitation competition), *A. fraterculus* larvae showed a response similar to that described as contest competition (also called interference competition) (Liendo *et al.*, 2016). Invasive species tend to have higher ranks than previously established species in the hierarchy for one or both forms of competition (scramble and contest) (Duyck *et al.*, 2006). In order to outcompete the resident species, invaders should possess combinations of life-history traits that increase their competitive ability, even at the expense of lower colonisation ability (Duyck *et al.*, 2007).

In this study, we evaluated the effect of the interaction of *A. fraterculus* and *C. capitata* on the development of the immature stages and compared the response of both species under different scenarios of competition. To this end, we conducted addition and substitution experiments transferring newly hatched larvae of both species into a container with a fixed amount of larval diet. *Anastrepha fraterculus* has longer developmental times than *C. capitata*, and it prefers unripe fruit for oviposition, whereas *C. capitata* prefers ripe fruit over unripe fruit (Malavasi *et al.*, 1983; Joachim-Bravo *et al.*, 2001). Therefore, we put particular emphasis on evaluating the extent to which a temporal separation between the two species in the timing of egg hatch affects the outcome of larval competition. We discuss our findings in the context of the successful colonisation of a new habitat by *C. capitata* and the establishment of a stable coexistence with the native *A. fraterculus*.

## Material and methods

### Insects

All experiments were conducted with insects from laboratory colonies because there is no evidence that adaptation to laboratory condition affects intra- or interspecific patterns of competition in tephritids (Duyck *et al.*, 2007; Liendo *et al.*, 2016). The *A. fraterculus* colony was derived from the experimental colony

kept at Estación Experimental Agroindustrial Obispo Colombres (EEAOC, Tucumán, Argentina), which was originally established with pupae recovered from infested guavas (*Psidium guajava* L.) at Horco-Molle (Tucumán), in 1997 (Jaldo, 2001). Pupae from Tucumán were shipped in 2007 to the Instituto de Genética 'E. A. Favret' (IGEAF-INTA Castelar, Buenos Aires, Argentina) and were used to establish an experimental rearing following the same procedures as in EEAOC (Jaldo *et al.*, 2001; Vera *et al.*, 2007). The *Ceratitidis capitata* colony was established at IGEAF in 1994 with pupae from an experimental rearing kept at ISCAMEN (Mendoza, Argentina), originally obtained from infested peaches (*Prunus persica* L.). Rearing methods for *C. capitata* followed Terán (1977).

#### Experimental procedure

In order to perform larval competition experiments, we followed the methodology of Duyck *et al.* (2006), who transferred newly hatched larvae (rather than eggs, as in the majority of previous studies) into containers with a fixed amount of artificial larval diet. To do so, eggs were collected from the laboratory colony and were then bubbled for 24 h (with the aid of an aquarium air pump). After that, 0.3 ml of eggs were transferred to a 15 cm × 20 cm × 3 cm (length × width × depth) tray containing a layer of agar (20%, 2.5 cm thick) and then kept under controlled conditions (temperature 25 °C ± 1 °C, RH 70 ± 10%) until hatching. Agar provided appropriate humidity conditions for embryos to develop and also kept larvae alive until they were given access to nutritional resources.

Newly eclosed (< 24 h old) larvae were carefully transferred into a plastic container (hereafter referred to as patch) with a fine brush under a stereoscopic microscope (10×, SZ 30 Olympus, Tokio, Japan) (Duyck *et al.*, 2006). Each patch consisted of a cylindrical plastic cup (diameter 2.8 cm, height 1 cm) that contained 3.65 g of artificial diet. Larval diet was composed of brewer's yeast, wheat germ, sugar, citric acid and agar (Salles, 1995). Sodium benzoate and methylparaben were added as preservatives. This diet is regularly used in the artificial rearing of *A. fraterculus* and it was chosen for the current experiments because previous studies showed that *C. capitata* is able to successfully develop in this diet as well as *A. fraterculus* (Liendo *et al.*, 2016). After larvae were transferred, each patch was placed inside a larger plastic container, on top of a layer of vermiculite that acted as pupation substrate for larvae. On the sixth day, vermiculite was sifted, and pupae were collected and individually weighed in a precision scale (0.1 mg, APX-200; Denver Instrument, Bohemia, New York). In all cases, the date of pupation was recorded. This procedure continued on a daily basis until there were no more larvae in the patch. This way, pupae were collected within 24 h after they exited the patch.

Each pupa was placed individually in a plastic tube (1.5 ml) which had been previously perforated to allow aeration. The tubes with the pupae were kept under controlled conditions (temperature 25 °C ± 1 °C, RH 70 ± 10%). For tubes coming from patches with two competing species, the species was determined upon adult emergence and recorded together with the day of emergence. Non-emerged pupae were determined under

microscope (10×, SZ 30 Olympus, Japan) by the morphology of the anterior spiracles (Steyskal, 1977).

For each patch and species, a number of variables known to be affected by larval competition were measured. These included: larval survival (number of recovered pupae/number of larvae initially transferred × 100), pupal weight, duration of the larval stage (time elapsed between the day larvae were transferred and the day pupae were collected), and pupal survival (number of emerged adult/number of collected pupae × 100).

#### Experiment 1: evidencing interspecific larval competition

Classical addition experiments (Mather & Caligari, 1981) were carried out to evaluate whether larval development is affected when larvae are forced to share the resources with a potential competitor species and, if so, which variables responded to competition. To this end, larvae of the two species were simultaneously transferred to the same patch at the carrying capacity described for this system (patches containing 3.65 g of artificial diet; Liendo *et al.*, 2016). The carrying capacity was considered to be the threshold density above which larvae begin to exhibit adverse effects as a result of sharing the resource with conspecific larvae. For the artificial patch used in this work, Liendo *et al.* (2016) determined that the carrying capacity was 50 larvae per patch for *A. fraterculus*, and 40 larvae per patch for *C. capitata*. Therefore, in this experiment 50 newly hatched larvae of *A. fraterculus* and 40 newly hatched larvae of *C. capitata* were transferred to a single patch. Two additional treatments were performed: (i) 50 larvae of *A. fraterculus* per patch; (ii) 40 larvae of *C. capitata* per patch. Seventeen replicates (patches) were performed per treatment.

#### Experiment 2: comparing intraspecific and interspecific larval competition effects

In this experiment, we compared the effects of competing with heterospecifics and conspecifics larvae. Substitution experiments (Mather & Caligari, 1981) were carried out by setting each species' carrying capacity (50 larvae per patch for *A. fraterculus* and 40 larvae per patch for *C. capitata*) and changing the proportion of larvae of the two species that were transferred to the patch. In the case of *C. capitata*, four treatments were carried out: (i) 100% *C. capitata*: 40 *C. capitata* larvae per patch; (ii) 75% *C. capitata*: 10 *C. capitata* larvae were replaced by *A. fraterculus* larvae; (iii) 50% *C. capitata*: 20 *C. capitata* larvae were replaced by *A. fraterculus* larvae; (iv) 25% *C. capitata*: 30 *C. capitata* larvae were replaced by *A. fraterculus* larvae. Likewise, to compare the effect of conspecific and heterospecific larval competition on *A. fraterculus* larval development, four treatments were carried out: (i) 100% *A. fraterculus*: 50 *A. fraterculus* larvae per patch; (ii) 75% *A. fraterculus*: 12 *A. fraterculus* larvae were replaced by *C. capitata* larvae; (iii) 50% *A. fraterculus*: 25 larvae *A. fraterculus* larvae were replaced by *C. capitata* larvae; (iv) 25% *A. fraterculus*: 37 *A. fraterculus* larvae were replaced by *C. capitata* larvae. Seventeen replicates were performed per treatment. As there is a density-independent mortality of about 30% of the transferred larvae for *A. fraterculus*

only (Liendo *et al.*, 2016), the number of larvae transferred of this species was corrected with this percentage. This procedure ensured that the final number of larvae within the patch was as stable as possible when *A. fraterculus* larvae were used to replace *C. capitata* larvae.

#### *Experiment 3: effect of temporal advantage of clutches*

In accordance with the suggested oviposition preference of *A. fraterculus* for unripe fruit, we evaluated whether larvae of this species are able to escape the adverse effects of competition when there is a temporal separation between oviposition events that occur in the same patch. To this end, newly hatched larvae of *A. fraterculus* were transferred to each patch and newly hatched larvae of *C. capitata* were transferred on the same day or later in asynchrony of 1, 2, 3 or 4 days (simulating an infestation). As in the addition experiment, the number of larvae that were transferred in the patch corresponded to the carrying capacity for each species. Additionally, larvae of each species were transferred at their respective carrying capacity as a control. Twelve replicates were performed per treatment.

#### *Data analysis*

The effect of larval density on the recorded variables was analysed by building specific models for each dependent variable. For pupal weight and duration of the larval stage we used general linear mixed models, with treatment (number of larvae transferred) as the fixed factor and replicate as the random factor. For larval survival and pupal survival there is only one value per replicate, so linear models were used, with treatment (number of larvae transferred) as the fixed factor and no random component. We worked on the assumption that, for any given dataset, there are several valid models that can explain the data and, of those that fit best, the simplest one should be chosen. One criterion to determine model fit is the Akaike information criterion (AIC); the lower the AIC values, the better the model fits the data (McCullagh & Nelder, 1983). Here we used the AIC-based approach to find ways to cope with some heteroscedasticity in the data. The lowest AIC value was returned by the models using the 'varident' option (a different variance for each group). For all variables, multiple comparisons were performed using Fisher's least significant difference (LSD) ( $\alpha = 0.05$ ). All the analyses were conducted using INFOSTAT, 2014 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

## **Results**

#### *Experiment 1: evidencing interspecific larval competition*

*Anastrepha fraterculus* larval survival decreased drastically when larvae shared the resource with *C. capitata* ( $F_{1,32} = 346.81$ ,  $P < 0.001$ ) (Fig. 1a). In the case of *C. capitata* this variable was not statistically different among treatments ( $F_{1,32} = 0.02$ ,  $P = 0.879$ ) (Fig. 1a).

For both species, pupal weight was significantly different between treatments (*A. fraterculus*,  $F_{1,664} = 439.6$ ,  $P < 0.001$ ; *C. capitata*,  $F_{1,1239} = 170.224$ ,  $P < 0.001$ ). In both cases, the weight decreased when the larvae shared the resource with the competing species (Fig. 1b).

The duration of the larval stage increased for *A. fraterculus* when they shared the resource with *C. capitata* ( $F_{1,664} = 58.72$ ,  $P < 0.001$ ) (Fig. 1c). However, this variable was not statistically different among treatments for *C. capitata* ( $F_{1,1239} = 7.85$ ,  $P = 0.052$ ) (Fig. 1c).

Pupal survival was not affected in *A. fraterculus* ( $F_{1,32} = 2.26$ ,  $P = 0.142$ ) (Fig. 1d). In the case of *C. capitata*, the pupal survival decreased significantly when the resource was shared with *A. fraterculus* ( $F_{1,32} = 5.24$ ,  $P = 0.028$ ) (Fig. 1d).

#### *Experiment 2: comparing intraspecific and interspecific larval competition effects*

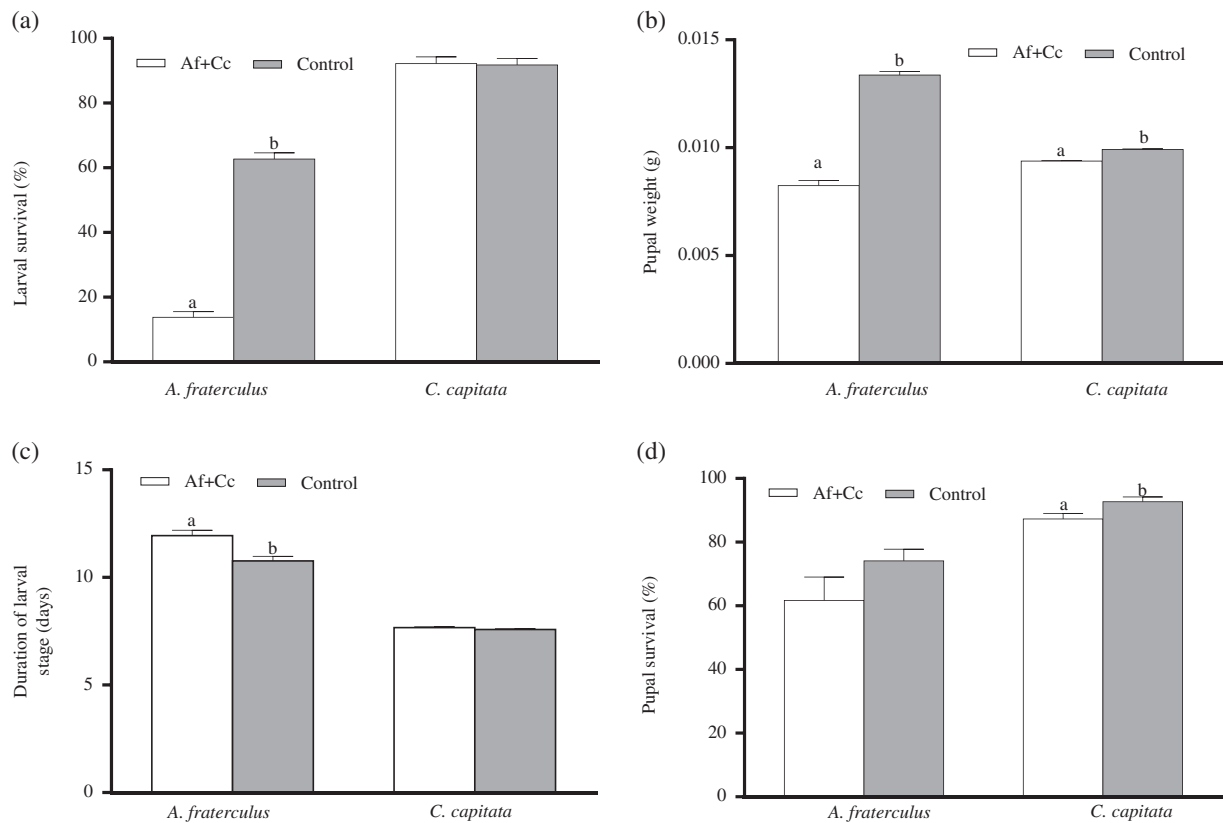
Larval survival was not affected in *A. fraterculus* ( $F_{3,65} = 0.35$ ,  $P = 0.789$ ), whereas it decreased in *C. capitata* larvae when 75% of the larvae were replaced by the competing species ( $F_{3,65} = 4.26$ ;  $P = 0.008$ ) (Fig. 2a).

For both species, pupal weight significantly increased with the increase in relative density of the competing species (*A. fraterculus*,  $F_{3,1630} = 36.96$ ,  $P < 0.001$ ; *C. capitata*,  $F_{3,1561} = 9.35$ ,  $P < 0.001$ ). In the case of *A. fraterculus*, the four treatments differed from each other, with a significant increase in pupal weight as the proportion of heterospecific larvae increased (Fig. 2b). In *C. capitata*, the comparison between treatments showed differences between the treatment of 100% *C. capitata* and the treatments of 75%, 50% and 25% *C. capitata*, whereas the treatment of 75% *C. capitata* showed differences with the treatment of 25% *C. capitata* (Fig. 2b).

The duration of the larval stage was not statistically different among densities for both species (*A. fraterculus*,  $F_{3,1630} = 4.24$ ,  $P = 0.06$ ; *C. capitata*,  $F_{3,1561} = 1.70$ ,  $P = 0.165$ ) (Fig. 2c). Likewise, pupal survival was not affected (*A. fraterculus*,  $F_{3,65} = 0.56$ ,  $P = 0.642$ ; *C. capitata*,  $F_{3,65} = 1.93$ ,  $P = 0.132$ ) (Fig. 2d).

#### *Experiment 3: effect of temporal advantage of clutches*

For both species, larval survival showed statistically significant differences among treatments (*A. fraterculus*,  $F_{5,65} = 22.77$ ,  $P < 0.001$ ; *C. capitata*,  $F_{5,65} = 121.13$ ,  $P < 0.001$ ). In the case of *A. fraterculus*, when the temporal gap between clutches increased, larval survival also increased. The comparison among treatments allowed detection of three different groups: (i) day 0 and day 1, with values lower than the control; (ii) day 2 and day 3, with intermediate values, similar to the control; and (iii) day 4, with values of pupal survival significantly higher than the control (Fig. 3a). Conversely, for *C. capitata*, larval survival decreased when the temporal separation between clutches increased, and the comparison among treatments showed differences with all treatments except for the control and day 0, and day 2 with day 3 (Fig. 3b).



**Fig. 1.** Evidencing interspecific larval competition on life-history traits of *Anastrepha fraterculus* and *Ceratitis capitata* (Experiment 1): (a) larval survival; (b) pupal weight; (c) duration of larval stage; (d) pupal survival. Error bars denote SE (calculated over the average value for each replicate for each treatment). For each species, bars superscripted with different letter differ significantly by Fisher's least significant difference ( $P = 0.05$ ). Af + Cc, *A. fraterculus* and *C. capitata* together in the same patch; control, only one species in the patch (*A. fraterculus* or *C. capitata*).

Pupal weight differed significantly for both species under study (*A. fraterculus*,  $F_{5,1336} = 52.58$ ,  $P < 0.001$ ; *C. capitata*,  $F_{5,1723} = 191.39$ ,  $P < 0.001$ ). For *A. fraterculus*, pupal weight increased as the temporal separation increased and the different treatments were grouped as follows: (i) day 0 and day 1; (ii) day 2, day 3, and day 4; (iii) control (Fig. 3c). In contrast to this, the weight of *C. capitata* pupae decreased as the temporal separation in favour of *A. fraterculus* increased and five groups were identified: (i) day 0; (ii) day 1; (iii) day 2 and day 3; (iv) day 4; and (v) control (Fig. 3d).

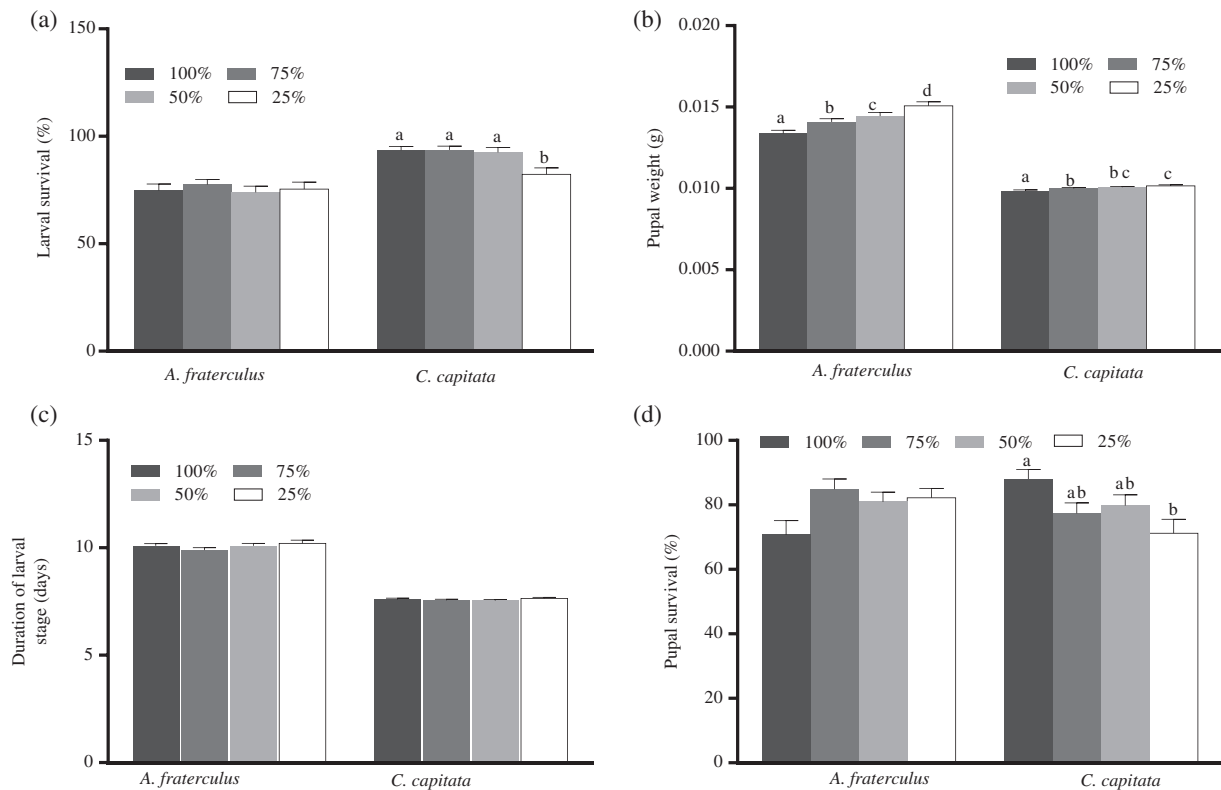
For both species, the duration of the larval stage increased when the temporal separation increased (*A. fraterculus*,  $F_{5,1292} = 39.25$ ,  $P < 0.001$ ; *C. capitata*,  $F_{5,1724} = 64.68$ ,  $P < 0.001$ ). For *A. fraterculus*, the different treatments were grouped as follows: (i) day 0 and day 4; (ii) control; (iii) day 3; and (iv) day 1 and day 2 (Fig. 3e). For *C. capitata*, the comparison between treatments allowed the treatments to be grouped as follows: (i) day 0; (ii) day 1 and day 2; (iii) day 3 and control; and (iv) day 4 (Fig. 3f).

Pupal survival was significantly affected by the temporal gap between clutches for both species (*A. fraterculus*,  $F_{5,29} = 3.14$ ,  $P = 0.021$ ; *C. capitata*,  $F_{5,29} = 3.72$ ,  $P = 0.010$ ). For *A. fraterculus*, treatment day 0 differed significantly from the other treatments, as it showed a higher value than the rest

(Fig. 3g). For *C. capitata*, the only treatment that differed from the rest was day 4, showing lower values of survival (Fig. 3h). This variable was not measured in all replicates because we found anomalous records in adult emergence due to a failure in the incubator where the pupae were kept.

## Discussion

*Anastrepha fraterculus* and *C. capitata* are frequently found sharing the same host (Pavan, 1978; Malavasi *et al.*, 1980; Devescovi *et al.*, 2015), establishing the required conditions for two species to establish a competitive interaction. In this study, interspecific competition between these two species was assessed during the larval stage under controlled laboratory conditions. Besides larval and pupal survival, which has obvious effects on fitness, presence of the larvae of the other species has been shown to affect other parameters that may indirectly affect the fitness of the flies. An increase in density negatively affected the pupal weight, which, in most insects, is correlated with the size of the adults and their overall reproductive success (Quiring & McNeil, 1984; Averill & Prokopy, 1987; Liedo *et al.*, 1992; Honěk, 1993; Allen & Hunt, 2001; Dukas *et al.*, 2001; Nufio & Papaj, 2004; Navarro-Campos *et al.*,



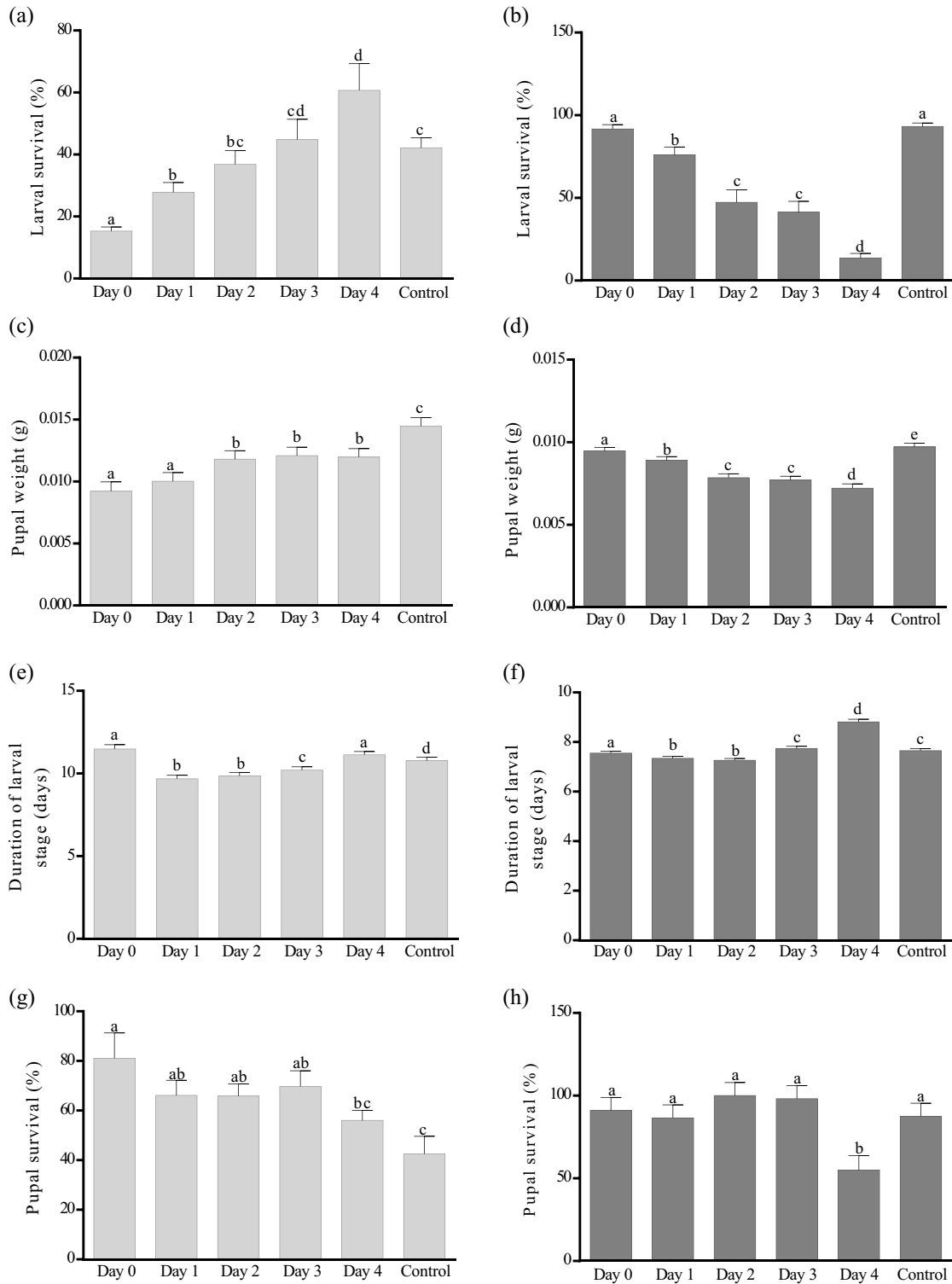
**Fig. 2.** Comparing intraspecific and interspecific larval competition effects on life-history traits of *Anastrepha fraterculus* and *Ceratitis capitata* (Experiment 2): (a) larval survival; (b) pupal weight; (c) duration of larval stage; (d) pupal survival. Error bars denote SE (calculated over the average value for each replicate for each treatment). For each species, bars superscripted with same letter do not differ significantly by Fisher's least significant difference ( $P = 0.05$ ). 100% indicates only one species in the patch; 75% indicates 75% of one species and 25% of the other species; 50% indicates 50% of one species and 50% of the other species; 25% indicates 25% of one species and 75% of the other species.

2011), although there are some exceptions (Honěk, 1993). In addition, the two species responded in a different way. This indicates that the species adopt different strategies when they face a situation of interspecific competition for resources. This valuable information about the competitive ability of each species may contribute to an understanding of the coexistence of *A. fraterculus* and *C. capitata* in an environment previously occupied only by the former.

*Ceratitis capitata*, native to Africa, has reached a worldwide distribution over the past century (Gilstrap & Hart, 1987). It is therefore generally accepted that the invasive potential of this species is very high. For *A. fraterculus* there is no record of invasions to new habitats. In this scenario, we expect a competitive interaction between larvae of *A. fraterculus* and larvae of *C. capitata*. Addition experiments indicate that *A. fraterculus* suffered a negative effect in parameters such as larval survival, pupal weight and duration of larval stage. For *C. capitata*, only a small decrease in pupal weight and pupal survival was observed, which may be due to a density-dependent effect and is not necessarily affected by the identity of the competitor species (Liendo *et al.*, 2016). Therefore, our experiments suggest that the species with a higher invasive potential is also a stronger competitor. Other studies have also shown that invasive species might have a superior competing ability compared with indigenous species

(Juliano, 1998; Byers, 2000; Vila & Weiner, 2004). Duyck *et al.* (2006) found that *Bactrocera zonata* (Saunders), a species with high invasive potential, had better competitive skills than those of *Ceratitis catotirii* Guérin-Ménéville, a native species in La Réunion. These authors suggested that the ability to resist interspecific competition by the resident species is the limiting factor for Tephritidae invasion.

In the three experiments, a marked difference between the two species was observed in the duration of the larval stage. *Anastrepha fraterculus* larvae needed approximately an additional 2 days (compared with *C. capitata*) in order to reach the pupal stage. This difference could favour *C. capitata* and partially explain its competitive advantage. This phenomenon was so stable that it was not necessary to perform Experiment 3 giving *C. capitata* larvae a temporal advantage. Previous studies in other Tephritidae found a competitive advantage for older over younger larvae (Averill & Prokopy, 1987; Dukas *et al.*, 2001). However, these differences in developmental times (which, in our case, would favour *C. capitata*) could be balanced by the oviposition preference of the females. Previous studies suggested that *A. fraterculus* oviposits into the fruit at a stage of maturity less advanced than that of *C. capitata* (Malavasi *et al.*, 1983; Joachim-Bravo *et al.*, 2001). This preference would confer the larvae of *A. fraterculus* a temporary advantage and the



**Fig. 3.** Effect of temporal spacing of clutches on life-history traits of *Anastrepha fraterculus* (left) and *Ceratitis capitata* (right) (Experiment 3): (a, b) larval survival; (c, d) pupal weight; (e, f) duration of larval stage; (g, h) pupal survival. Error bars denote SE (calculated over the average value for each replicate for each treatment). For each species, bars superscripted with same letter do not differ significantly by Fisher's least significant difference ( $P = 0.05$ ). Day 0, larvae of the two species transferred the same day; day 1, larvae of *C. capitata* transferred 1 day after larvae of *A. fraterculus*; day 2, larvae of *C. capitata* transferred 2 days after larvae of *A. fraterculus*; day 3, larvae of *C. capitata* transferred 3 days after larvae of *A. fraterculus*; day 4, larvae of *C. capitata* transferred 4 days after larvae of *A. fraterculus*; controls, larvae of one species only.



results of co-infestation could be other than those evidenced in the addition experiments. Accordingly, an initial advantage of 2 days for *A. fraterculus* improved its chances of survival and also allowed pupae to gain weight at the expense of *C. capitata* which subsequently starts facing developmental constraints. Nevertheless, *A. fraterculus* also suffers an effect of competition despite this advantage, as the pupal weight was lower and the duration of the larval stage was greater with respect to its control treatment. We highlight here two interesting results from Experiment 3. Firstly, larval survival of *A. fraterculus* was higher when there was a 4-day gap between clutches than in the control. A possible explanation could be that the late presence of *C. capitata* larvae encourages a differential use of the nutrients by *A. fraterculus*, in a way that a higher number of its larvae are able to develop to pupae even when faced with less favourable conditions. Secondly, pupal survival of *A. fraterculus* was higher when both species were transferred on the same day than in the control. This can be explained by the fact that, under highly competitive conditions, whilst there were fewer larvae, they were in better shape to complete their development. The mechanisms underlying these interactions require further study.

Lotka (1925) and Volterra (1926) proposed that stable coexistence between two species that compete for the same resource could be achieved if intraspecific competition is stronger than interspecific competition. Substitution experiments allow both effects to be compared and thus constitute a valid experimental approach for testing this hypothesis (Keddy, 2001; Duyck *et al.*, 2006). Our results show that in both species, pupal weight increased when they had to share the resource with heterospecific larvae. Furthermore, in *C. capitata* there was also a decrease in larval mortality and pupal survival. The negative effects suffered by larvae of *A. fraterculus* and *C. capitata* under competitive conditions are greater when they share the resource with individuals of the same species than when they develop with larvae of the competing species. This suggests that intraspecific competition is stronger than interspecific competition, at least when the experiments are performed around the threshold densities. Under the Lotka–Volterra model, the relative strength of intra- and interspecific competition between these two coexisting fruit fly species might have helped to stabilise the community after the invasion of *C. capitata*, allowing both species to coexist. This phenomenon has been observed in other systems and is considered as a facilitator of the coexistence of species with overlapping resources (Connell, 1983). However, the density of larvae in the patch probably plays a fundamental role in allowing for the stabilisation of both species' populations; when infestation exceeds the loading capacity of the fruit, one species excludes the other, at least when both species have infested the fruit at the same time. To our knowledge, the present study constitutes the first evidence of a differential effect of intra- and interspecific competition in the Tephritidae family. Nonetheless, the fact that the experiments were carried out under laboratory conditions and using laboratory colonies limits the extent of our conclusions. Extensive sampling of mono- and co-infested fruit will surely shed some light on the relative strength of intra- and interspecific competition under field conditions in natural populations.

Duyck *et al.* (2004) identified two dominant ways in which pairs of Tephritidae species interact competitively. The first one is hierarchical competition, in which one species always dominates and excludes the other. This phenomenon has been observed in Australia, where *C. capitata* was displaced by *Bactrocera tryoni* (Froggatt) (DeBach, 1966), and in Hawaii where *C. capitata* was displaced by *B. dorsalis* (Duyck *et al.*, 2004). The second one is stable coexistence based on differences in niche requirements. Rwomushana *et al.* (2009) found that *Bactrocera* species tend to have superior competitive ability compared with three *Ceratitidis* species over a range of temperatures and infestation asynchrony. Nonetheless, this study performed only addition experiments, and therefore it is not possible to evaluate the relative contribution of differences between intra- and interspecific competition on species coexistence. Substitution experiments allow a comparison of the effects of these two types of competition (Inouye, 2001) and in our case provided evidence suggesting that intraspecific competition is stronger than interspecific competition. Our study showed that a temporal difference gives an advantage to *A. fraterculus*. A differential oviposition preference (in terms of the degree of ripeness of the fruit) could favour such asynchrony within the fruit and thus promote the coexistence of these two species in nature, even when *C. capitata* has a shorter developmental period and presumably higher competitive ability. In all, our work provides experimental results that contribute to understanding the way in which these two species interact, although these studies should be validated and complemented with data from natural populations. Such information is relevant not only to understand competitive interactions, but also because it contributes to designing management strategies after the introduction of a pest species into new habitats.

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