

Fruit infestation patterns by *Anastrepha fraterculus* and *Ceratitis capitata* reveal that cross-recognition does not lead to complete avoidance of interspecific competition in nature

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- Abstract**
- 1 The avoidance of parasitized or infested hosts, which is a common phenomenon in parasitic wasps and phytophagous insects, may act both intra- and interspecifically. Most studies on chemically-mediated avoidance of interspecific competition in insects have been conducted at the individual level. The role of this behaviour on the spatial distribution of offspring of sympatric species with overlapping host ranges has been overlooked.
 - 2 In the present study, two analytical approaches were used to investigate the co-infestation patterns of the fruit flies *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), aiming to unravel the importance of cross-species infestation recognition in nature.
 - 3 Guava fruit were sampled in an area of coexistence of these two fruit flies and individually categorized as non-infested, infested by one of the species or infested by both species. The frequency of each type of fruit was compared with the frequency distributions expected under two models: an independent oviposition model and a competition avoidance model. As an alternative approach, co-occurrence patterns were evaluated using null models.
 - 4 The results showed that avoidance of competition could be occurring in nature, although only in a few cases in which infestation levels are moderate. The two approaches revealed that the spatial scale has significant impact on the resulting co-occurrence patterns, such that opposite behaviours towards infested fruit are inferred at the largest (mainly aggregated oviposition pattern) versus the smallest scale (mainly independent oviposition pattern).
 - 5 For the system under investigation, our findings suggest that the avoidance of infested fruit does not contribute, or at least not strongly, to the coexistence of the two species.

Keywords Biological invasion, coexistence, EcoSim, female host choice, foraging behaviour, host-marking pheromone, oviposition deterrent pheromone.

Introduction

The introduction and successful adaptation of a species out of its natural range of distribution produce drastic changes in the abundance and distribution of species (Williamson, 1996; Juliano & Lounibos, 2005). Invasive species can modify native

biodiversity, shaping new interspecific interactions either directly or indirectly. Interspecific competition is among the most common interactions induced after a biological invasion, at least for insect species (Kenis *et al.*, 2009). Changes induced by the invasive species in resource distribution within the community can lead to the exclusion of native species (Debach, 1966; Reitz & Trumble, 2002). Alternatively, a stable coexistence of native and invasive species can be attained. Several mechanisms have been put forward to explain such a stable coexistence

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(Ayala *et al.*, 1973; Lawlor & Maynard Smith, 1976; Huston, 1979). Most of these mechanisms rely on processes that ultimately reduce the intensity of competition below a tolerable threshold (such as niche partitioning or the use of refuges by the native species).

Two economically important fruit fly species are present in Argentina: the South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) and the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Anastrepha fraterculus* is native to South America, whereas *C. capitata* is native to Africa and was first detected in Argentina at the beginning of the 20th Century (Vergani, 1952). According to Malvasi *et al.* (1983), *A. fraterculus* females prefer unripe fruit of the Myrtaceae family for oviposition, although they have also adapted to many introduced fruit species (Norrbom, 2004). Similarly, *C. capitata* shows high adaptability to different host species (over 350; Liquido *et al.*, 1991) but prefers ripe over unripe fruit (Joachim-Bravo *et al.*, 2001). Nonetheless, *C. capitata* females do oviposit in unripe fruit using pre-existing punctures or cuts, probably as a means of saving time and avoiding oviscapt wear (Papaj & Messing, 1996). These differences in oviposition preferences that may favour *A. fraterculus* as a result of it infesting the fruit earlier could be compensated to some extent by differences in the developmental times. For *C. capitata*, the duration of the larval stage ranges from 8 to 18 days, depending on the temperature, diet (artificial rearing medium or fruit) and larval density in the diet or the fruit (Shoukry & Hafez, 1979; Carey, 1984; Vargas *et al.*, 1996; Papadopoulos *et al.*, 2002; Liendo, 2013). For *A. fraterculus*, the duration of the larval stage ranges between 11 and 22 days, again depending on the diet and the density (Salles, 1999; Jaldo *et al.*, 2001; Zart *et al.*, 2010; Liendo, 2013). In guava, larval developmental time is 13.0 days for *C. capitata* (Duyck *et al.*, 2006a), whereas, for *A. fraterculus*, Sugayama *et al.* (1998) recorded a mean of 14.7 days. Even when the conditions vary across different studies, it is reasonable to suggest that *A. fraterculus* needs more time to complete the larval stage. The only comparison between the developmental times of these two species that was carried out under identical conditions was reported by Liendo (2013) on an artificial diet where a mean difference of 3 days, favouring *C. capitata*, was found. Such scenario suggests a potential strong interaction between the two species.

When an invasive species is phylogenetically closely related to a native species (implying that they have similar ecological characteristics), a strong competitive interaction is usually expected (Reitz & Trumble, 2002; Thomas & Holway, 2005). After approximately 100 years of having invaded areas of Argentina that were once only inhabited by *A. fraterculus*, *C. capitata* has become established and both species coexist in sympatry, extensively overlapping their host ranges (Ovruski *et al.*, 2003; Segura *et al.*, 2006). The mechanisms by which these species reached a stable coexistence are not known. Field data suggest that *A. fraterculus* and *C. capitata* have not undergone a niche partitioning process, at least at spatial and host species levels (Putruele, 1996; Ovruski *et al.*, 2003; Segura *et al.*, 2006; Oroño *et al.*, 2008). Nonetheless, spatial separation at the microhabitat level has not been evaluated, mainly because previous studies normally grouped infested fruit, and most of the time, the trees and the

orchards where fruit are collected are not considered in the data analysis.

There are several well described cases in the literature in which a Tephritidae fruit fly species has invaded a new area. Displacement of *C. capitata* by *Bactrocera dorsalis* (Hendel) (Fitt, 1989) in Hawaii and that of by *Bactrocera tryoni* (Froggatt) in Australia (Allman, 1939; Andrewartha & Birch, 1954; Bateman, 1971) are among the most cited examples. *Ceratitis capitata* has also been described as an invasive species in the West Indies [where *Anastrepha suspensa* (Loew) was the predominant species] and in Central America [where *Anastrepha ludens* (Loew) predominated] (White & Elson-Harris, 1992). In La Réunion Island (France), the ecology of invasion by several fruit fly species, including *C. capitata*, has been studied thoroughly (Duyck *et al.*, 2006a,b, 2008). This has included an investigation of the coexistence at the regional scale of four fruit fly species: one endemic species [*Ceratitis catiirri* (Guérin-Mèneville)] and three exotic species that have invaded the island at different times [*C. capitata*, *Ceratitis rosa* (Karsch), *Bactrocera zonata* (Saunders)]. In this system, the coexistence of some of the species appears to be related to the ability of species with lower competitive skills (Duyck *et al.*, 2006a) to exploit the most favourable climates (Duyck *et al.*, 2006b) with no niche partitioning associated with host preferences (Duyck *et al.*, 2008). Avoidance of fruit previously infested by *C. capitata* and *C. rosa* by *B. zonata* females could have also contributed to the coexistence (Duyck *et al.*, 2006a). This strategy can be viewed as a partitioning of the resources at the smallest scale (i.e. the fruit).

Both *C. capitata* and *A. fraterculus* deposit host-marking pheromones (HMPs) on the fruit surface after oviposition (Prokopy *et al.*, 1978, 1982). These pheromones have been extensively described in parasitic wasps and phytophagous insects and are known to affect female oviposition behaviour by inducing the rejection of a host that has been previously parasitized or infested by a conspecific female (Nufio & Papaj, 2001). Evidence from at least three genera within the Tephritidae family suggests that HMPs can also act as synomones because they are recognized by members of different species (Prokopy & Papaj, 2000; Aluja & Díaz-Fleischer, 2006; Kachigamba *et al.*, 2012). Liendo (2013) has shown that, under laboratory conditions, *A. fraterculus* and *C. capitata* avoid laying eggs in fruit recently infested by heterospecific females. According to this study, females would assess the infestation status of the fruit on a chemical basis. If the behavioural response towards infested fruit recorded in the laboratory takes place in nature, a pattern of spatial separation of the ovipositions should arise and, consequently, fruit simultaneously infested by both species (co-infested) would be less frequent than expected by chance. Because HMPs would reduce the level of interspecific competition, cross-recognition of these pheromones could act (or at least contribute) to stabilize the coexistence of species with overlapping hosts.

In the present study, we evaluated the pattern of co-occurrence of *A. fraterculus* and *C. capitata* in an area of coexistence. Under the hypothesis that females from both species are able to recognize and avoid fruit infested by heterospecific females in nature, we predicted that the frequency of co-infested fruit would be lower than the frequency expected by chance. To test this hypothesis, we analyzed host fruit utilization patterns across

different scales and at different times during the fruiting season. We further assessed whether variations in the co-occurrence patterns were associated with the level of resource consumption. Our results contribute to an understanding of the mechanisms that might have allowed the two species to reach a stable coexistence.

Materials and methods

Study area

The study was carried out at the Uruguay River basin, in the Department of Concordia in Entre Ríos province, Argentina (31°22'S, 58°09'W), where the two species have been reported (Putruele, 1996; Segura *et al.*, 2006). This area is characterized by a warm and humid climate, and abundant rains (600–1000 mm/year) with no noticeable dry season. The environment is altered by anthropogenic action in two ways: settlements (from small localities of 31 inhabitants to large cities of over 100 000 people) and land use for agriculture, where the most important crops are *Citrus* species.

Sampling

The sampling was focused on fly populations associated with *Psidium guajava* L. (Myrtales: Myrtaceae). Guava was chosen as an appropriate host to evaluate competition avoidance for several reasons. First, it is one of the main (primary) hosts for both *A. fraterculus* and *C. capitata* in the area under study (Putruele, 1996; Segura *et al.*, 2006). Second, guava has been proposed as the ancestral host for the genus *Anastrepha* (Aluja *et al.*, 2000). Third, guava fruit ripening occurs at the end of summer when fly population densities are high and either few or no other hosts are available; competition is thus expected to be high. Fourth, in Concordia, guava is grown without commercial purposes and thus orchards are not treated with pesticides. Finally, guava is a medium-sized fruit (<5 cm in diameter) where HMPs should be detected easily (Díaz-Fleischer *et al.*, 2000).

Within the Department of Concordia, fruit was sampled from four different sites: Concordia City, Villa Adela, Villa Zorraquín and Puerto Yeruá. In each site, four guava trees (separated from each other by at least 200 m) were sampled fortnightly from March to May 2007 (five sampling dates), covering the entire fructification period. Fruit were collected only from the ground because fruit still hanging from the tree could eventually be attacked by flies and infestation would have been underestimated. Because *A. fraterculus* and *C. capitata* females do not lay eggs on fallen fruit, this procedure ensured that sampled fruit were not going to be attacked by females any further. To exclude fruit from which larvae could have exited, damaged fruit (showing holes or cuts in the peel) were not sampled.

The sample size varied between 40 and 50 fruit per tree, depending on fruit availability. Each fruit was placed individually in a capped plastic container. The remaining fallen fruit were first counted (to estimate the availability of fruit in the previous 2 weeks) and then removed from the ground that was beneath the tree canopy.

Processing of samples

Fruit were transported to the Instituto de Genética 'E. A. Favret' (Buenos Aires, Argentina). Each fruit was weighed and placed individually in a small plastic container (500 mL) with sand as pupation substrate. Each container had a lid to avoid other insects from contaminating the sample. Finally, fruit were stored at room temperature.

Twice a week, the sand was sieved and recovered pupae were transferred to small plastic cups (15 mL) that contained vermiculite (to ensure appropriate moisture conditions) and kept under controlled conditions ($25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity) for approximately 3 weeks. Fruit were discarded after two consecutive revisions in which no larvae or pupae were obtained (provided they had been checked at least three times). Emerged adults and puparia (in cases where pupae had failed to emerge) were identified to the species level under a stereoscopic microscope (Olympus, Japan).

Statistical analysis

General description of fruit infestation. To quantify resource availability and use, the temporal and spatial fluctuation of fruit abundance and infestation degree was described at three levels: the trees, the sites (grouping data from different trees) and the whole area (grouping data from different sites). The variables evaluated were: (i) available fruit (quantity and weight); (ii) percentage of infested fruit (without distinction between fruit fly species); (iii) percentage of fruit infested by *A. fraterculus*; (iv) percentage of fruit infested by *C. capitata*; (v) percentage of co-infested fruit (fruit simultaneously infested by both species); (vi) number of pupae (of both species) per kg of fruit; (vii) number of pupae of *A. fraterculus* per kg of fruit; (viii) number of pupae of *C. capitata* per kg of fruit; and (ix) relative abundance index (ratio between the number of pupae of *C. capitata* and the number of pupae of both species) (Segura *et al.*, 2006).

Frequency analyses of co-infestation. To evaluate whether female oviposition choice was conditioned by the infestation status of the fruit, fruit were categorized as co-infested, mono-infested by *A. fraterculus*, mono-infested by *C. capitata* and non-infested. Then, the observed frequency distribution was compared with the expected distributions by means of a chi-squared test of goodness of fit. Expected frequencies were estimated assuming two possible oviposition behaviours: Model (A) independent oviposition by both species (i.e. without avoidance of infested fruit) and Model (B) avoidance of infested fruit (Fig. 1 and Table 1). In Model A, the expected frequency of co-infested fruit was calculated by multiplying the proportion of fruit infested by each species because the model assumes independent oviposition by the two species (Table 1). The expected frequencies of mono-infested fruit were obtained by subtracting the number of co-infested fruit from the number of fruit expected to be infested by each species. In Model B, based on Malavasi *et al.* (1983), we assumed that *A. fraterculus* would be the first species to oviposit in the fruit and therefore only *C. capitata* would have the choice to avoid infested fruit. Under this assumption, Model B predicts that a percentage of the fruit (which should be co-infested if females of both species were

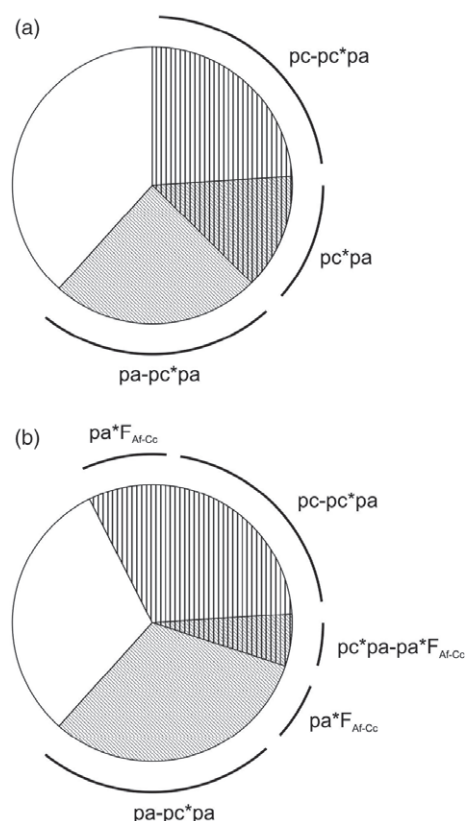


Figure 1 Proportion of non-, mono- and co-infested fruit expected under two models of host acceptance behaviour. The models assume: (A) independent oviposition or (B) avoidance of infested fruit. Grey areas represent the proportion of fruit infested by *Anastrepha fraterculus* (pa), striped areas represent the proportion of fruit infested by *Ceratitis capitata* (pc) and white areas represent the proportion of non-infested fruit. Under the independent oviposition model, the expected proportion of co-infested fruit is derived directly from multiplying the proportion of fruit infested by each species ($pc \times pa$), whereas, in the avoidance model, this proportion is reduced as a result of rejection of *A. fraterculus* infested fruit by *C. capitata* ($pa \times F_{Af-Cc}$). This behaviour would produce an increase of mono-infested fruit (both by *A. fraterculus* and *C. capitata*) and, consequently, a decrease of non-infested fruit.

ovipositing independently) will actually be mono-infested by *A. fraterculus* because *C. capitata* females would have avoided laying eggs in fruit already infested. Therefore, in Model B, the frequency of co-infested fruit is lower than in Model A and this lower value is obtained by multiplying the expected frequency by the rejection rate (Table 1). Model B also assumes that these *C. capitata* females will continue searching and, eventually, will lay eggs in non-infested fruit (Fig. 1). The frequency of rejection of infested fruit (number of females that visited the fruit and laid no eggs/total number of visiting females) obtained by Liendo (2013) for *C. capitata* was used in Model B. Chi-squared tests were performed at each level (tree, site and area for each sampling date). Statistical analyses were performed with STATISTICA, version 6.0 (Statsoft, Inc., 2001).

To simplify the presentation of the data, results from the comparisons between observed and expected infestation frequencies under the two models were summarized:

Table 1 Equations used to obtain expected frequencies of non-, mono- and co-infested fruit, under the independent oviposition and the avoidance of infested fruit models

Type of fruit	Independent oviposition model	Avoidance model
Mono-infested by Cc	$N \times pc - N \times pa \times pc$	$N \times pc - N \times pa \times pc + N \times pa \times pc \times F_{Af-Cc}$
Mono-infested by Af	$N \times pa - N \times pa \times pc$	$N \times pa - N \times pa \times pc + N \times pa \times pc \times F_{Af-Cc}$
Co-infested	$N \times pa \times pc$	$N \times pa \times pc - N \times pa \times pc \times F_{Af-Cc}$
Non-infested	$N - I$	$N - I$

Cc, *Ceratitis capitata*; Af, *Anastrepha fraterculus*; N, sample size; pc, observed proportion of fruit infested by *C. capitata* (considering both co- and mono-infested fruit); pa, observed proportion of fruit infested by *A. fraterculus* (considering co- and mono-infested fruit); I, fruit infested by *C. capitata* + fruit infested by *A. fraterculus* + co-infested fruit; F_{Af-Cc} , *C. capitata* female rate of rejection of fruit previously infested by *A. fraterculus* ($F_{Af-Cc} = 0.571$) (Liendo, 2013).

- Type I: the observed infestation frequencies fitted only the independent oviposition model. The observed frequencies did not fit the avoidance model because of an excess of co-infested fruit.
- Type II: the observed infestation frequencies fitted the independent oviposition model and the avoidance model.
- Type III: the observed infestation frequencies did not fit any model. The frequency of co-infested fruit was higher than the frequency expected under the independent oviposition model or the avoidance model.
- Type IV: the infestation frequencies could not be analyzed because of very low infestation by *C. capitata*. Only fruit infested by *A. fraterculus* were abundant.
- Type V: the infestation frequencies could not be analyzed because of a low percentage of infested fruit (<25%).

Co-occurrence patterns analyzed by means of null models. Null models were used as a parallel approach to study avoidance of competition between *A. fraterculus* and *C. capitata*. First, presence/absence matrices for each tree, site and sampling date were assembled. Species were considered in the rows and fruit in the columns. ECOSIM, version 7.0 (Gotelli & Entsminger, 2001) was used to generate co-occurrence indices (C-Score; Stone & Roberts, 1990) under specific restrictions: the sum of each row was fixed and the sum of each column was allowed to vary randomly. C-Score measures the segregation of the species and does not require a perfect checkerboard distribution (Gotelli, 2000). The observed and expected indices that do not consider biological mechanisms, such as competition, are compared statistically and a P-value calculated. If the C-Score is significantly higher than the score expected by chance, the species tend to infest different hosts more than expected by chance, suggesting a possible underlying mechanism to avoid competition. The analysis was first performed for each sampling date (grouping data for the entire sampling area), then for each site and, finally, for each tree.

Table 2 Infestation levels and summary of chi-squared goodness of fit tests (χ^2 values are shown) considering independent oviposition (Model A) or co-infestation avoidance by *Ceratitidis capitata* (Model B), and the difference between simulated and observed C-Scores of the null model analysis performed using ECOSIM, version 7.0, at the sampling area scale

Sampling date	% infested fruit	% co-infested fruit	Pupae/kg	Model A	Model B	Type	Null model
First	59.78	10.55	77.87 ± 19.89	14.71**	69.55**	III	4586.23*
Second	68.08	15.61	82.46 ± 14.92	37.55**	142.98**	III	10 626.29*
Third	55.36	10.47	62.52 ± 27.38	11.66**	51.75**	III	2621.36*
Fourth	64.61	6.24	41.05 ± 7.29	0.53	1.75	II	-186.79
Fifth	65.86	13.42	77.87 ± 19.89	2.98	12.30**	I	130.11

For the types of model-fitting referring to characteristic infestation patterns, see Materials and methods. * $P < 0.05$; ** $P < 0.01$.

Association between co-infestation patterns and fruit infestation.

To further investigate possible causes of the observed distribution patterns, parameters related to the level of resource utilization (such as fruit availability, percentage of infested fruit, pupae/kg) were compared between trees and sites that showed contrasting infestation patterns (Types I and II) by means of Student's *t*-tests. When statistical assumptions were not met, a Mann–Whitney test was performed. The weight of co-infested fruit and mono-infested fruit was compared, in specific cases, by means of Student's *t*-test. Statistical analyses were performed with STATISTICA, version 6.0 (Statsoft, Inc., 2001).

Results

General infestation

Over the fructification period of *P. guajava*, a total of 1985 fruit were sampled and studied individually. The results demonstrated that 45.74% were infested only by *A. fraterculus*, 3.63% were infested only by *C. capitata*, 14.51% were infested by both species and 36.12% were not infested. On average, we found 60.97 ± 2.07 (mean ± SE) *A. fraterculus* pupae per kg of fruit (4.56 ± 0.14 pupae per fruit) and 11.80 ± 0.91 *C. capitata* pupae per kg of fruit (0.90 ± 0.11 pupae per fruit). The mean relative abundance index was 0.17, indicating an overall predominance of *A. fraterculus*. The presence of females of both species in every tree and sampling date was confirmed on the basis of the fruit infestation patterns (see Supporting information, Table S1).

Sampling area scale

Frequency analyses of co-infestation. When data from the whole sampling area (Department of Concordia) were grouped across different sites, the observed infestation pattern was statistically different from the expected pattern under both Model A (independent oviposition) and Model B (avoidance behaviour) for the first, second and third sampling dates (Type III) (Table 2). On these sampling dates, there was an excess of co-infested fruit compared with that expected under the two models (see Supporting information, Table S1). On the fourth sampling date, the observed frequencies fitted both models (Type II) (Table 2), whereas, on the fifth date, the observed pattern for the entire sampling area fitted only the independent oviposition model (Type I) (Table 2).

Co-occurrence patterns analyzed by means of null models. On the first three sampling dates, the co-occurrence analysis of *A. fraterculus* and *C. capitata* resulted in a pattern that differed from that expected under random occurrence. In all cases, the observed C-Score was significantly lower than the expected index predicted by ECOSIM, version 7.0, showing that there were more cases of co-infested fruit (co-occurrence) than expected by chance (see Supporting information, Table S1). By contrast, the analysis showed no significant differences between the observed and expected indices on the last two sampling dates (Table 2).

Sampling site scale

Frequency analyses of co-infestation. Variability between sites was high, whereas within-site variation across sampling dates was qualitatively lower [i.e. there were more different types (I–V) of results between sites than within sites for a given sampling date] (Table 3). When trees were grouped, Concordia City and Puerto Yeruá showed alternation between Type I and Type II patterns (fitting only to Model A or both models) (Table 3). Villa Zorraquín was the site with the highest frequency of Type III pattern (frequencies did not fit any of the two models). Finally, Villa Adela was the most variable site, showing three different patterns on four sampling dates (Table 3).

Co-occurrence patterns analyzed by means of null models. Deviation from a random co-occurrence pattern was frequently found when data from different trees were grouped within each site (Table 3). Villa Zorraquín showed significant departure from random occurrence on almost every sampling date, whereas the remaining three sites showed significant differences between the expected and the observed co-occurrence patterns on one sampling date (Table 3). Across sampling dates, the deviations from the expected co-occurrence patterns took place preferentially on the second sampling (Table 3). In all cases, the differences between observed and expected C-Scores revealed more cases of co-infestation than expected by chance (see Supporting information, Table S1).

Guava tree scale

Frequency analyses of co-infestation. When data was analyzed at the tree level, four categories were found (Table 4). Although, for some trees, the restriction of the chi-squared test of goodness of fit about a minimum number in the expected frequencies was not met, all the χ^2 values are presented in Table 4, showing

Table 3 Infestation levels and summary of chi-squared goodness of fit tests (χ^2 values are shown) considering independent oviposition (Model A) or co-infestation avoidance by *Ceratitis capitata* (Model B) and the difference between simulated and observed C-Scores of the null model analysis performed using ECOSIM, version 7.0, at the sampling site scale

Site	Sampling	% infested fruit	% co-infested fruit	Pupae/kg	Model A	Model B	Type	Null model
Concordia	1	74.68	13.04	123.36 ± 49.20	2.38	18.97**	I	441.09
	2	67.97	13.03	102.21 ± 44.14	5.57	26.19**	I	668.48*
	3	65.72	2.35	32.81 ± 19.37	2.56	0.39	II	-156.95
Villa	1	27.39	2.13	30.82 ± 22.01	1.19	4.34	II	43.6
Adela	2	51.73	18.60	66.56 ± 44.84	24.47**	66.70**	III	1170.47*
	3 ^a	13.11	0.00	4.05 ± 3.13	0.24	0.12	V	-1.32
	4 ^a	55.56	11.11	54.14	0.9	2.16	II	2.18
Villa	1	64.49	18.69	93.91 ± 24.19	9.10*	39.20**	III	834.07*
Zorraquín	2	77.85	29.73	129.65 ± 45.59	15.96**	59.58**	III	1193.43*
	3	73.98	26.18	105.47 ± 51.53	9.50*	33.21**	III	421.58*
	4 ^a	80.78	2.18	43.65 ± 17.01	0.49	1.99	IV	28.56
Puerto	1 ^a	72.92	8.33	63.41	0.23	2.67	II	15.18
Yerúa	2 ^a	74.79	1.09	48.74 ± 4.20	3.26	0.97	II	-60.73
	3	68.05	13.36	86.54 ± 50.25	3.76	16.74**	I	255.53*
	4	57.50	5.43	25.35 ± 1.65	0.46	4.34	II	61.67
	5	65.86	13.42	34.31 ± 6.12	2.99	12.26**	I	129.16

^aCases that did not fulfil the chi-squared test requirements.

For the types of model-fitting referring to characteristic infestation patterns, see Materials and methods. Asterisks indicate the level of significance (* $P < 0.05$; ** $P < 0.01$).

the degree in which observed frequencies departed from those expected under the two models. Of the total number of cases analyzed, 97.6% fitted Model A (independent oviposition), whereas 67.7% fitted Model B (avoidance behaviour) as well (Type II). The within-date variation among trees from the same site was higher than that among sampling dates for a given tree [only in two cases (out of 13) did all trees from the same site show the same type of response (trees sampled only once were not included), whereas four trees (out of 14) showed the same outcome on different sampling dates] (Table 4).

Co-occurrence patterns analyzed by means of null models. Observed and simulated C-Scores were not statistically different in any case, reflecting a random occurrence pattern for both species (Table 4).

Association between co-infestation patterns and fruit infestation

When the variables that describe the infestation level were compared between trees showing Type I or II patterns, a general trend towards a higher infestation level in trees with a Type I pattern was found (Table 5). These differences were statistically significant ($P < 0.05$) for the percentage of total infestation, the percentage of fruit infested by *A. fraterculus*, the percentage of fruit infested by *C. capitata* and the total number of pupae per kg of fruit (Table 5).

On all five sampling dates, the weight of co-infested fruit was higher than that of mono-infested fruit. This trend was statistically significant ($P < 0.05$) for the second, third and fifth sampling date (Table 6), and marginally nonsignificant for the fourth sampling date.

When the weight of co-infested fruit was compared with that of mono-infested fruit for each combination of sites and sampling

dates, no differences were found in most cases (Table 7). However, in three (out of 14) cases, significant differences were found, with co-infested fruit being heavier than mono-infested fruit.

Discussion

In the present study, we evaluated the co-infestation patterns produced by *A. fraterculus* and *C. capitata*, two sympatric fruit fly species with overlapping host ranges. Laboratory studies (Liendo, 2013) have shown that both species avoid laying eggs in fruit infested by heterospecific females, as reported for other species of Tephritidae (Prokopy & Papaj, 2000; Aluja & Díaz-Fleischer, 2006; Kachigamba *et al.*, 2012). If this behaviour also occurs in nature, a low frequency of co-infested fruit is expected (Duyck *et al.*, 2004; Birke & Aluja, 2011). However, employing two analytical approaches, we found no strong evidence suggesting that avoidance of fruit infested by heterospecifics has an important role in structuring co-occurrence patterns, at least for the system under study. This lack of correspondence appears to be related to the level of niche occupancy (i.e. fruit infestation degree). Also, the spatial scale at which infestation patterns were evaluated proved to be crucial to detect potential cases of interspecific competition avoidance. To our knowledge, this is the first study in which the relevance of this behaviour in terms of competition avoidance in nature is addressed for tephritid flies.

There are many examples in which spatial segregation has been found acting to stabilize populations of competing phytophagous species (Denno *et al.*, 1995; Duyck *et al.*, 2004; Kaplan & Denno, 2007). This mechanism can be overlooked if the scale at which it occurs is different from the scale of analysis (Wiens, 1989; Azovsky, 1996; Gotelli, 1997). For Tephritidae fruit flies, it is generally agreed that the fruit is where interspecific competition

Table 4 Infestation levels and summary of chi-squared goodness of fit tests (χ^2 values are shown) considering independent oviposition (Model A) or co-infestation avoidance by *Ceratitis capitata* (Model B) and the difference between simulated and observed C-Scores of the null model analysis performed using ECOSIM, version 7.0, at the guava tree scale

Tree	Sampling	% infested fruit	% co-infested fruit	Pupae/kg	Model A	Model B	Type	Null model
C1	1	93.62	19.15	174.20 ± 19.92	0.68	5.62	II	20.9
	2 ^a	93.88	6.12	127.02 ± 13.76	1.65	0.52	IV	-28.18
	3 ^a	97.62	7.32	88.70 ± 10.25	0.00	1.62	IV	2.68
C2	1	32.00	2.04	10.45 ± 2.92	0.06	0.13	II	-3.84
	2 ^a	22.00	0.00	8.28 ± 3.02	0.71	0.41	V	-5.35
	3	37.5	2.08	16.92 ± 8.67	0.59	0.07	II	-12.25
C3	1	81.25	16.67	77.60 ± 11.92	2.22	8.40*	I	49.12
	2	66.00	22.88	60.75 ± 10.17	6.44	16.80**	I	91.97
C4	1	91.84	14.29	25.63 ± 7.92	0.73	5.16	II	22.73
	2	90.00	26.00	231.18 ± 27.85	1.95	10.65*	I	44.1
VA1	1 ^a	37.52	0.00	212.79 ± 19.26	1.04	0.62	II	-10.94
	2	97.92	56.25	0.00 ± 0.00	0.06	15.41**	I	3.94
VA2	1	44.68	6.38	19.03 ± 4.39	3.97	8.77*	I	14.81
	2	68.00	12.00	197.55 ± 14.43	0.54	4.41	II	26.33
VA3	2 ^a	25.00	4.17	73.44 ± 3.00	1.65	4.61	V	12.67
	3 ^a	22.22	0.00	51.52 ± 7.30	0.24	0.14	V	-0.93
	4 ^a	55.56	11.11	11.26 ± 3.79	0.92	2.16	IV	2.21
VA4	2 ^a	16.33	2.00	7.18 ± 4.42	5.36	10.03*	V	6.74
VZ1	1	84.00	46.00	54.14 ± 38.26	1.59	16.19**	I	45.36
	2	93.88	53.06	0.00 ± 0.00	1.62	18.03**	I	26.52
VZ2	1	59.57	8.52	5.90 ± 2.42	2.97	7.57	II	42.51
	2	87.5	27.08	0.93 ± 0.66	0.88	9.03*	I	32.17
	3	91.18	47.26	166.36 ± 22.37	2.92	13.91**	I	23.94
VZ3	1 ^a	41.67	2.28	234.64 ± 21.24	0.09	0.78	II	4.14
	2 ^a	30.22	0.00	72.98 ± 2.80	0.40	0.23	II	-4.02
	3 ^a	39.58	2.38	127.91 ± 17.04	0.01	0.27	II	-0.46
	4 ^a	72.92	3.02	187.27 ± 31.14	0.38	1.26	II	9.58
VZ4	1	72.73	18.18	69.79 ± 2.77	0.83	6.25	II	30.82
	2	100	38.78	12.49 ± 3.31	1.48	9.28*	I	-17.37
	3	91.18	29.42	10.27 ± 2.08	0.11	5.54	II	7.02
	4 ^a	88.64	2.27	26.64 ± 3.44	0.13	0.79	IV	4.14
PY1	2	80.00	0.00	66.51 ± 12.69	40.21**	38.91**	III	-4.88
	3	47.73	9.09	143.58 ± 11.47	4.82	10.8*	I	40.92
	4 ^a	66.67	8.89	118.86 ± 17.96	0.06	2.06	II	8.03
	5 ^a	75.62	21.95	60.66 ± 9.81	3.72	11.52**	I	54.55
PY2	3 ^a	66.67	2.78	52.94 ± 19.24	0.18	0.11	IV	-6.02
	4 ^a	53.19	2.23	18.04 ± 5.06	0.92	2.20	II	12.05
	5	56.12	4.88	22.30 ± 4.42	0.00	0.66	II	-0.04
PY3	1	72.92	8.33	40.73 ± 5.60	0.23	2.67	II	14.92
	2	69.57	2.17	57.11 ± 8.90	1.44	0.28	II	-27.93
	3	89.74	28.21	27.95 ± 5.21	0.00	5.05	II	1.22
PY4	4 ^a	52.63	5.26	28.50 ± 5.59	0.01	0.45	II	0.86

^aCases that did not fulfil the chi-squared test requirements.

For the types of model-fitting referring to characteristic infestation patterns, see Materials and methods. Asterisks indicate the level of significance (* $P < 0.05$; ** $P < 0.01$). Codes for trees indicate the site where they were located (C, Concordia; VA, Villa Adela; VZ, Villa Zorraquín; PY, Puerto Yera).

mainly takes place (Fitt, 1989). However, most studies, with the exception of the study by Birke and Aluja (2011), have grouped the fruit and used the level of infestation by different species as indirect evidence of competition (Putruele, 1996; Ovruski *et al.*, 2003; Segura *et al.*, 2006). In the present study, infestation was recorded from single fruit, and co-occurrence patterns were addressed at three spatial scales. Within the tree, the estimated oviposition pattern fitted either the independent oviposition or both the independent and the avoidance (by *C. capitata* females) models (Type I and Type II patterns, respectively). When the scale was expanded (sampling site), a higher proportion of

Type III pattern (higher co-infestation than expected by chance) was found. There are three reasons explaining why aggregation is an artefact that results from grouping fruit from different trees and sites. First, if aggregation had any advantage, such advantage would be restricted by the costs associated with larval competition. In every case in which co-infestation was higher than expected by chance, infestation (measured as number of pupae/kg of fruit) was high (Tables 2–4), indicating a significant fitness cost as a result of larval competition (Duyck *et al.*, 2006a; Liendo, 2013). Second, a preference for larger fruit (Díaz-Fleischer *et al.*, 2000), although undetected during the

Table 5 Mean \pm SE values for variables that describe the resource availability and the infestation level (degree of niche occupancy) found in Type I and Type II cases

Variable	Type I (n = 11)	Type II (n = 12)	t-value	d.f.	P-value
Fruit availability (weight in kg)	17.27 \pm 1.82	13.52 \pm 3.07	1.07	21	0.296
Fruit availability (total quantity of fruit) ^a	388.50 (327.50; 472.50)	250.00 (70.00; 696.00)	55.00	21	0.518
Percentage of total infestation	83.80 \pm 4.51	66.62 \pm 6.26	2.25	21	0.035
Percentage of fruit infested by Af	81.95 \pm 4.50	62.29 \pm 7.12	2.38	21	0.027
Percentage of fruit infested by Cc	33.38 \pm 5.25	16.54 \pm 2.79	2.64	21	0.015
Total pupae/kg of fruit	141.50 \pm 18.72	79.90 \pm 21.43	2.17	21	0.042
Af pupae/kg of fruit	109.16 \pm 17.02	66.03 \pm 190.26	1.68	21	0.107
Cc pupae/kg of fruit	26.06 \pm 7.50	10.14 \pm 3.42	1.87	21	0.075
Relative abundance index	0.15 \pm 0.04	0.15 \pm 0.04	0.00	21	1.000

^aThe U-value (Mann–Whitney test) is presented and the medians (with the first and third quartiles) are informed.

Af, *Anastrepha fraterculus*, Cc, *Ceratitis capitata*.

Table 6 Comparisons between the mean \pm SE weight (n) of fruit infested by one (mono-infested) or both (co-infested) species

Sampling date	Weight (g) of co-infested fruit	Weight (g) of mono-infested fruit	t-value	d.f.	P-value
First	52.31 \pm 2.84 (54)	47.77 \pm 1.39 (186)	1.513	238	0.131
Second	50.81 \pm 1.68 (91)	44.69 \pm 1.29 (206)	2.729	295	0.006
Third	63.54 \pm 4.65 (62)	44.36 \pm 2.49 (126)	3.985	186	<0.001
Fourth	70.03 \pm 2.66 (8)	56.48 \pm 2.18 (93)	1.729	99	0.086
Fifth	66.98 \pm 5.39 (11)	47.46 \pm 4.16 (36)	2.401	45	0.021

Data are grouped by sampling date.

fruit sampling, could explain a tendency to co-infestation, not strictly related to a preference for fruit already infested. This corresponds well with the fact that co-infested fruit are normally heavier than mono-infested fruit. Finally, the dispersal capacity recorded for females of the two species [approximately 2 km for *A. fraterculus* (Utgés, 2012) and 1–3 km for *C. capitata* (Gutiérrez Samperio, 1976; Meats & Smallridge, 2007; Díaz *et al.*, 2008; Navarro-Llopis *et al.*, 2014)], makes it unlikely that females integrate the information about infestation levels at the largest scale. Thus, we conclude that the distribution of mono- and co-infested fruit provides information about the avoidance of interspecific competition only at the smallest scale (i.e. the tree).

Insects integrate a number of external cues and signals with their own internal state to decide whether to lay eggs in a given host or skip it and keep searching (Carriere, 1998; Díaz-Fleischer & Aluja, 2003; Xu *et al.*, 2012). Oviposition in tephritids is a dynamic and plastic process (Mangel & Roitberg, 1989; Aluja *et al.*, 2001) and the response to HMPs is a good example of this plasticity. Nufio and Papaj (2001) reported examples showing that the responsiveness to HMPs is variable and changes according to egg load, fruit size, the experience of the female in terms of proportion of infested/non-infested hosts encountered during foraging and the time since the last host was encountered (Roitberg & Prokopy, 1984; Papaj & Messing, 1996; Aluja *et al.*, 2001). Altogether, these studies suggest that, when the time needed to find an optimal host is long, the female response threshold to HPMs becomes high, accepting already occupied hosts (Papaj *et al.*, 1989). In this scenario, it could be proposed that *C. capitata* and *A. fraterculus* females accept infested fruit as the probability of finding non-infested fruit decreases, even when HMPs are being detected. Such behaviour would produce an independent oviposition pattern. In the present study, the

co-infestation patterns found in some trees fitted the independent oviposition model (Type I), whereas, in others, the distribution fitted the two models (Type II). The Type II response could reflect a transitional situation between avoidance of infested fruit and independent oviposition behaviour and the differences between these two results could be related to an increase in niche occupancy (Roitberg & Prokopy, 1984). Type II trees showed a lower infestation level than Type I trees, supporting the idea that, at lower infestation, rejecting infested fruit probably leads to ovipositing in non-infested fruit and therefore the avoidance of infested fruit pays off in terms of the expected level of larval competition. Yet, the relatively high frequency of non-infested fruit remains to be explained.

The fact that *C. capitata* females use existing conspecific egg laying cavities in unripe coffee berries (Papaj & Messing, 1996) may interact with the avoidance behaviour. We do not know whether existing oviposition punctures made by *A. fraterculus* female elicit the same response in *C. capitata* as those of conspecific females nor if the response would be the same in guava fruit. This needs to be addressed to better understand the patterns of co-occurrence and the reasons underlying the degree of overlapping. According to Prokopy and Roitberg (2001), the advantages obtained by utilizing oviposition cavities of *A. fraterculus* by *C. capitata* may counterbalance the detrimental effects of increasing levels of larval competition. Our data set is not suitable for addressing the real impact of this behaviour. Nonetheless, because unripe fruit is expected to be more abundant at the begging of the fruiting season, if *C. capitata* were profiting from existing oviposition punctures made by *A. fraterculus*, we should find that the frequency of co-occurrence is higher than the frequency expected by chance. However, we found the opposite in the first sampling dates (a weak tendency to avoidance). Thus, even when this behaviour could occur, it does not appear to

Table 7 Comparison between the mean \pm SE weight (n) of fruit infested by one (mono-infested) or both (co-infested) species

Site-sampling date	Weight of co-infested fruit (g)	Weight of mono-infested fruit (g)	t -value	d.f.	P -value
C – first	49.55 \pm 5.44 (24)	50.40 \pm 2.49 (81)	–0.156	103	0.877
C – second	59.00 \pm 3.53 (23)	38.62 \pm 2.28 (73)	4.506	94	0.000
C – third	23.77 \pm 7.01 (4)	32.48 \pm 4.06 (35)	–0.706	37	0.484
VA – first	45.16 (1)	46.75 \pm 3.91 (23)	–	–	–
VA – second	42.28 \pm 2.70 (23)	44.56 \pm 3.35 (41)	–0.462	62	0.646
VZ – first	55.48 \pm 3.12 (25)	46.28 \pm 2.04 (61)	2.443	84	0.017
VZ – second	50.83 \pm 2.29 (44)	49.11 \pm 1.73 (68)	0.609	110	0.544
VZ – third	41.00 \pm 3.30 (21)	41.93 \pm 2.40 (35)	–0.233	54	0.816
VZ – fourth	75.69 \pm 8.90 (2)	58.71 \pm 1.98 (53)	1.635	53	0.108
PY – first	50.77 \pm 6.14 (4)	43.06 \pm 2.22 (21)	1.345	23	0.192
PY – second	57.20 (1)	51.06 \pm 3.40 (23)	–	–	–
PY – third	42.93 \pm 3.56 (14)	40.76 \pm 2.99 (40)	0.395	52	0.695
PY – fourth	53.16 \pm 9.84 (4)	50.29 \pm 4.46 (35)	0.210	37	0.835
PY – fifth ^a	74.39 (60.69; 76.29) (11)	33.14 (25.19; 69.44) (36)	110.50	45	0.028

^aThe U -value (Mann–Whitney test) is presented and the medians (with the first and third quartiles) are informed. C, Concordia City; VA, Villa Adela; VZ, Villa Zorraquín; PY, Puerto Yerúa.

Data are grouped by site and sampling date.

contribute significantly to the co-occurrence patterns. Yet, this is purely speculative and further experiments need to be conducted to clarify the contribution of this behaviour of *C. capitata* females to the interaction with *A. fraterculus*.

Evidence of spatial separation between species with similar niche requirements is based mainly on the study of spatial co-occurrence patterns (Gotelli, 2001). Ecologists have used different analytical approaches to study such patterns (Diamond, 1975; Connor & Simberloff, 1979; Roberts & Stone, 1990; Sanderson *et al.*, 1998). In the present study, we used null models to disentangle patterns that might indicate a preference for un-occupied fruit. Null models support the results of frequency analyses at the tree scale. However, in some cases, the frequency analyses allowed detecting potential cases of avoidance of infested fruit, essentially because Model B allows including a rejection rate (of infested fruit) based on detailed behavioural studies (Liendo, 2013). Therefore, the joint use of these two approaches provided a more accurate tool to evaluate subtle deviations from independent oviposition behaviour.

The vision of the competition as a dominant force that controls the relative abundance and distribution of species with the same requirements (MacArthur, 1972) can be challenged over the central ideas of Andrewartha and Birch (1954), who emphasized the importance of spatial and temporal heterogeneity and stochastic events in natural environments. This vision does not deny that competition acts when resources become scarce, although it questions its role as a force structuring communities in unpredictable and changing environments (Lawton & Strong, 1981; Wiens, 1984). Similar arguments have been proposed to question the importance of competition between insects that feed in discrete and ephemeral habitats (Beaver, 1977; Atkinson & Shorrocks, 1981; Lawton & Hassell, 1984), as many tephritids do. Nevertheless, it can be postulated that competition is important in relatively recent interactions. Our results suggest that, in the system under study, the avoidance of infested fruit is not a phenomenon that promotes spatial segregation, and also females could be avoiding infested fruit only at intermediate densities, whereas, at high densities, they would ignore the infestation status of fruit. Therefore, even when this mechanism could be

contributing to the coexistence of the species, we found no evidence indicating that this contribution is high.

In summary, the present study contributes to understand the mechanisms that enable *A. fraterculus* and *C. capitata* to coexist and presents a novel approach to generate ecological knowledge that aids in defining suitable pest control strategies. This information is of paramount importance for the management of these fruit pests, especially if control programmes are only focused on *C. capitata* (because is normally the most abundant fruit fly pest when commercial hosts are taken into account) (Segura *et al.*, 2006). Such control actions might eventually lead to an outbreak of *A. fraterculus*, whose niche would have been suddenly released of competitors. Unravelling the interaction between these two species should pave the way to assessing to what extent they are conditioning the population density of each other and, ultimately, will provide ecological data that will constitute a valuable tool when deciding a course of action in fruit fly pest control.

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Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12111

Table S1. Percentage of fruit in each of the four categories that were considered regarding their infestation status: (1) Mono-inf. Af: fruit infested exclusively by *Anastrepha fraterculus*; (2) Mono-inf. Cc: fruit infested exclusively by *Ceratitis*

capitata; (3) Co-infested: fruit infested simultaneously by the two species and (4) Non-infested: fruit from which no larvae or pupae were recovered.

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