

Biological Control—Parasitoids and Predators

Temporal Diversity and Abundance Patterns of Parasitoids of Fruit-Infesting Tephritidae (Diptera) in the Argentinean Yungas: Implications for Biological Control

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Received 21 September 2015; Accepted 13 June 2016

Abstract

A 4-yr study was done to analyze seasonal patterns underlying host plant–fruit fly–parasitoid interactions in a secondary forest in the Argentinean Yunga and its importance for the implementation of conservation and augmentative biological control. Larval–pupal hymenopteran parasitoids associated with all host plants and fruit fly species were identified and the seasonal occurrence of fruit, infestation levels, parasitism percentage, and relative parasitoid abundance were determined. Three fruit fly species in two genera were found in association with surveyed plants, two of which (*Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann)) are of major economic importance. Infestation levels were strongly influenced by environmental factors and peak fruit availability. Five fruit fly parasitoid species were recovered from fly pupae, four braconid species, and one figitid. Time windows for fruit fly population growth were pinpointed. Based on results, the present analysis proposes an effective fruit fly biological control strategy tailored for the northwestern Argentinean citrus-producing area.

Key words: area-wide fruit fly control, Braconidae, Figitidae, augmentative and conservation biological control

Insect populations commonly experience strong seasonal variation due to biotic and abiotic factors (Begon et al. 1996). Many ecosystems are highly seasonal, generally because of environmental conditions that cause temporal and spatial variation in availability of ephemeral resources such as seeds, flowers, and fruits, which are relatively rare when compared to resources such as foliage (Wallace et al. 2000). These conditions exert selection pressure on insect life cycles, and the strength of selection depends on plasticity and degree of specialization (Kurota and Shimida 2002).

Andine forests are highly seasonal ecosystems with variation driven at low elevation by an uneven distribution of rainfall and at high elevation by strong fluctuations in mean temperature (Brown et al. 2001). One of these South American mountain cloud forests is locally known as Yungas, which is a humid subtropical mountain forest divided into sections along an altitudinal gradient which extends discontinuously from Venezuela to north-western Argentina. Each altitudinal section has different structural and floristic characteristics (Brown et al. 2001). Although the Yungas represent one of the most valuable biodiversity reservoirs in Argentina, this subtropical rainforest has been partially transformed into crop and pasture

areas as a result of agricultural development and human settlement (Brown et al. 2001). Over the past decades, some of these crop fields have been abandoned and so sites with abundant exotic plants have naturally regenerated; such is the case of *Psidium guajava* L. (guava), *Eriobotrya japonica* (Thunb.) Lindley (loquat), *Prunus persica* (L.) Batsch (peach), and *Citrus aurantium* L. (sour orange) (Grau and Aragón 2000).

Conservation of vegetation surrounding the crop is very important because it offers several ecosystem services, such as biological control of pests by natural enemies (NE), (Bengtsson 2015). Wild plant species play an important role as NE reservoirs during periods of commercial host scarcity, which can contribute substantially to crop production worldwide (Landis et al. 2000). The host plant distribution and abundance, vegetation surrounding crops, and distribution of essential resources (food, shelter, oviposition substrates) strongly influences behavior, distribution, and abundance of the insects and plays an important role in biological control programs (Aluja et al. 2012). The Yungas are therefore an interesting scenario for developing ecological studies that may facilitate the design of effective biorational control strategies. In this region there are two

species of fruit fly of economic importance: *Anastrepha fraterculus* (Wiedemann) (South American fruit fly) and *Ceratitidis capitata* (Wiedemann) (Mediterranean fruit fly). Both species are serious pests of a wide range of commercial fruit crops, and infestations severely limit exportation due to quarantine restrictions in fruit fly-free countries (Guillén and Sánchez 2007).

Patches of secondary forest provide suitable habitats for population growth of pestiferous fruit flies. Many host plants in these areas are of importance for biological conservation (Aluja et al. 2014). They can be classified into three categories: 1) parasitoid multiplier plants, 2) parasitoid reservoir plants, and 3) pest-based parasitoid reservoir plants (Aluja et al. 2014). Movement between wild and cultivated hosts is typical of several important pest fruit fly species and is important to their population survival because no single host species fruits throughout the year; immature stages do not diapause and adults survive for only limited periods; thus they have no mechanisms to bridge fruit-free periods (Aluja et al. 2014). Many parasitoids diapause, and this strategy allows them to endure adverse periods (Aluja et al. 1998, Ovruski et al. 2015). Schliserman et al. (2014b) found that environmental degradation, and introduction and spread of exotic host plants strongly affected distribution patterns, abundance, and phenology of native and exotic tephritids. Monophagous tephritid species and several specialized parasitoids were more sensitive to habitat loss than polyphagous species and parasitoids exhibiting a wide host range.

A series of surveys have been carried out over the last 8 yr to determine frugivorous tephritid species composition, host plant relationships, and the structure of parasitoid guilds in native and exotic host fruits (Ovruski et al. 2003, 2004, 2005, 2006; Schliserman et al. 2004, 2014b; Oroño et al. 2005; Oroño and Ovruski 2007). Even though the latter studies yielded a considerable amount of ecological information at the time, sampling of fruit was not systematic throughout the year and *Anastrepha* and *C. capitata* pupae were not distinguished. To gain further insight into complex trophic interactions, those hosts that yielded the largest numbers of fruit flies were selected and studied systematically over time. The same hosts yielding the most flies also generated 95% of parasitoids. Consequently,

such hosts were sampled over a 4-yr period in a disturbed wild vegetation area of the Yungas.

Here we propose that the parasitoid community of fruit flies present in a mountain cloud forest, exhibit niche segregation based on their foraging preferences for host fruit with different phenological patterns (Fig. 1). So the aim of this work was to analyze the phenology of host plant fruit–fruit fly–parasitoid interactions in a secondary forest and with the long-term goal of implementing conservation and augmentative biological control programs. To achieve this goal, it will be necessary to identify key hosts responsible for population increase and maintenance of pestiferous fly fruit species, and to document the incidence of native parasitoids over time. Both of these are critical elements when designing area-wide fruit fly management strategies.

Materials and Methods

Study Area

The area, located in the southernmost end of the Yungas, can be classified as Low Montane Forest (Brown et al. 2001). It lies between 26° 45' and 26° 49' S latitude and 65° 20' and 65° 18' W longitude at elevations ranging from 600 to 800 m, over a total surface of 12 km² (6 km long by 2 km wide). It is embedded on the eastern slope of Mt. San Javier, a mountain in the “Sierra de San Javier” protected natural area in Horco Molle, Tucumán, Northwestern Argentina. This area is characterized by disturbed secondary vegetation (exotic and native plant species combined) surrounded by large citrus plantations and small fruit orchards. According to Köppen (1918) the climate is classified as “humid warm-temperate” with a rainy-warm season from October through April, and a dry-cold season from May through September. Mean annual rainfall ranges between 1,300 and 1,600 mm, with a mean annual temperature of 18°C.

Fruit Sampling

A total of 130, 158, 70, 200, and 146 *J. australis*, *P. guajava*, *E. japonica*, *C. aurantium*, and *P. persica* trees, respectively, were surveyed, with 25% of the trees of each species chosen randomly on each weekly sampling date from September 1999 to August 2003.

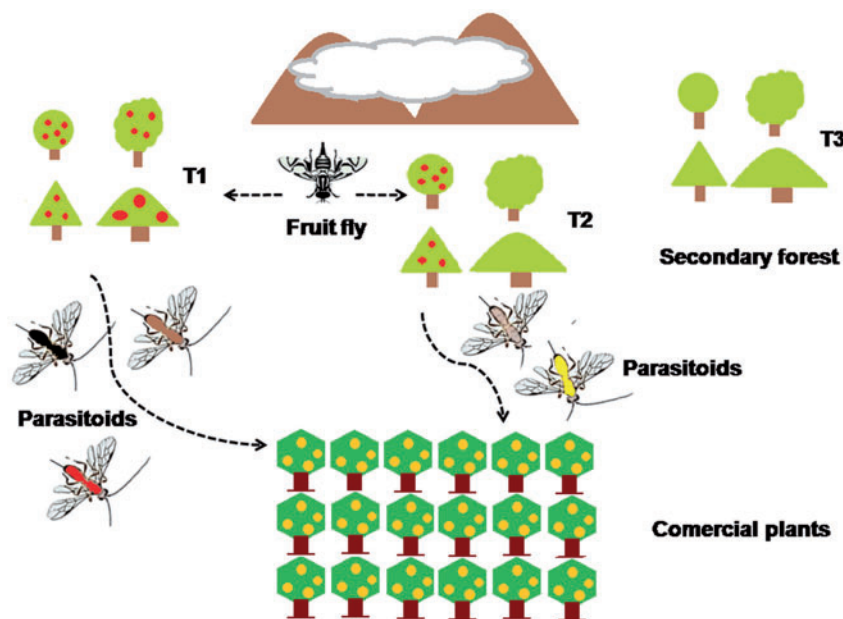


Fig. 1. Schematic host fruit phenology in a secondary forest at three different moments (T1, T2, and T3) and its influence on temporal distribution of fruit flies and parasitoids, which can be used in biological control.

Sample size varied according to relative availability of fruit per host species throughout collecting years and was established over the amount of obtainable ripe fruit (Fig. 2). Half of the ripe fruit in each sample were randomly collected from the tree canopy and the remaining 50% from the ground and handled separately to determine if there were differences in parasitoid species composition at each level. To collect fruit from canopies, a plastic basket attached to an extendable metal pole (3.5 m long) was placed beneath fruit and the branch was shaken. Each fruit sample was placed individually into a cloth bag (20 cm diameter and 30 cm deep) and transported in a plastic crate to the laboratory.

Fruit Processing

Sampled fruits were counted and rinsed with a 30% sodium benzoate solution, and a subsample for each species was weighed (N=10). Mean individual weight was 19.2 ± 3.8 (SD) g, 59.1 ± 20.9 g, 12.5 ± 3.5 g, 34.5 ± 10.0 g, and 144.3 ± 72.4 g for walnut, guava, loquat, sour orange, and peach, respectively. Fruit were placed in a plastic crate (48 by 28 by 15 cm) with a slotted bottom, and piled up over another plastic crate (48 by 28 by 15 cm) with a nonperforated bottom and with a 5 cm sand layer as a pupation medium. Both crates were tightly covered with an organdy lid. The double crate method was used to prevent mixing sand with fruit, fungal growth, and bacterial contamination. Each double crate contained one sample, and all samples of the same collecting date were grouped on shelves and kept in a dark room in natural environmental conditions for 1 mo. Sand was sifted weekly to collect fly pupae. After that, fruit was dissected in search of larvae or pupae remaining in pulp.

Fly Pupae Processing and Identification

Anastrepha Schiner and *C. capitata* pupae were identified by using external characters (White and Elson-Harris 1992), processed separately, and transferred into glass cups (21 cm diameter, 9 cm deep) filled with sterilized moist vermiculite as pupation medium. Each

cup was tightly covered with a piece of organdy cloth and held until eclosion of adults. The number of emerged parasitoids and flies was recorded on a weekly basis.

Adult Parasitoid and Fly Identification

Parasitoids and fruit flies were identified by S.O. and P.S and fruit flies confirmed by Allen Norrbom (Systematic Entomology Laboratory, PSI, USDA-ARS, Washington, DC). Voucher specimens were placed in the entomological collection of the Fundación Miguel Lillo (FML) in San Miguel de Tucumán, Argentina.

Diapausing Parasitoids

The methodology and the criteria for diapause were similar to those reported by Ovruski et al. (2015). *Anastrepha* puparia that contained diapausing and nondiapausing parasitoid larvae were dissected to measure and compare body size. Uneclosed pupae were kept inside cups for 18 mo; after this period of time we dissected them to determine if they contained diapausing parasitoids. As per the procedures of Ovruski et al. (2015), cups were placed inside a wooden frame cage covered with a cloth mesh and held until fly or parasitoid emergence. This emergence cage was kept in the same location as the one where fruits had been collected. The cage was protected from rainfall with corrugated fiberglass. The soil inside the containers was moistened each time it rained. A hygrothermograph was placed near the emergence cage to record daily ambient temperature and relative humidity. Fly and parasitoid emergence was checked three times per week, and all adults (alive and dead) were removed, counted, and identified.

Environmental Conditions

Daily rainfall, as well as maximum and minimum temperatures recorded between September 1999 and August 2003, were obtained from a weather station located at 700 m in the “Parque Sierra de San Javier.”

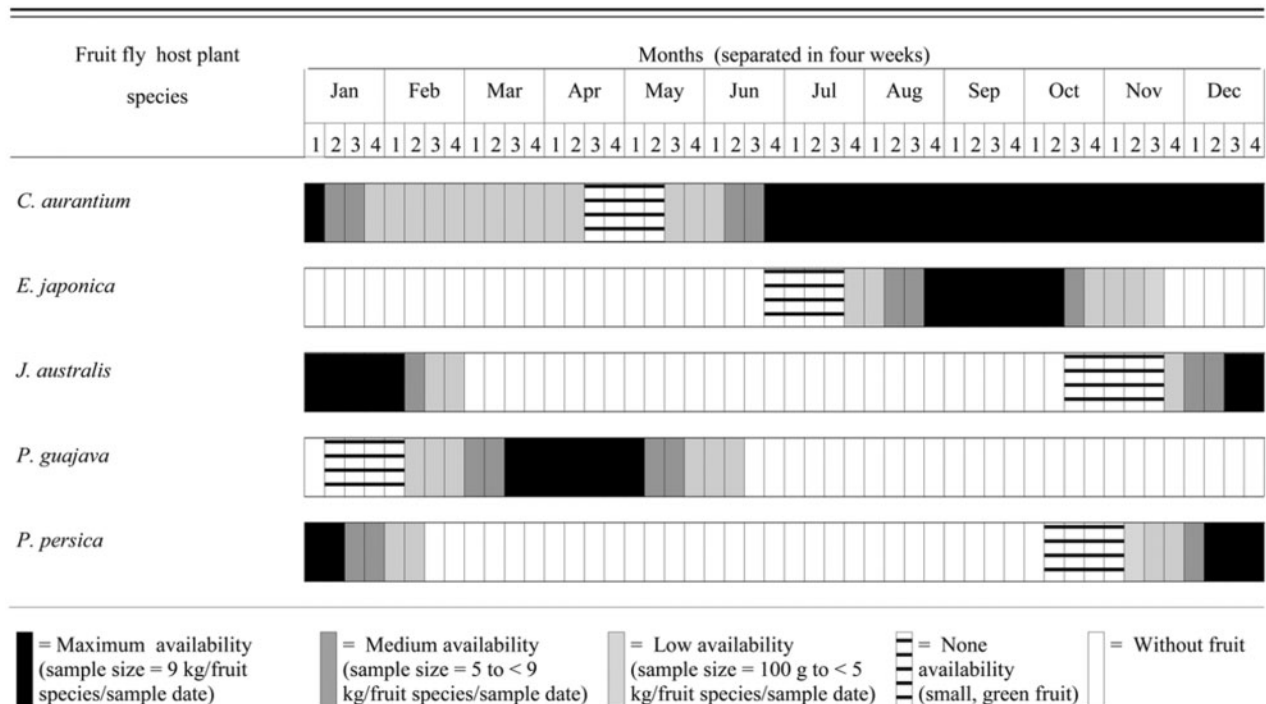


Fig. 2. Ripe fruit availability for five *C. capitata* and *Anastrepha* spp. (*A. fraterculus* and *A. schultzi*) host plant species in Horco Molle, Tucumán, Argentina.

Data Analysis

Parasitism percentage was estimated as the total number of parasitoids over the total number of fly pupae obtained from fruit samples and fruit infestation levels as the number of *Anastrepha* or *C. capitata* pupae obtained per kilogram of fruit. Total emerged parasitoids (abundance), parasitism percentage, and infestation levels recorded from each host plant were compared across sampling months and study period by using a Kruskal–Wallis on ranked data followed by Dunn’s pair wise comparisons. Means and SDs were calculated as summary statistics for percent parasitism, infestation level, and parasitoid abundance. Spearman Rank correlations were used to determine association between parasitism and infestation level for each host plant species. Multiple regression by using linear models to determine the degree of association between fruit infestation levels, parasitism, numbers of emerged parasitoids, and weather conditions (maximum and minimum temperatures, as well as rainfall) was also performed. Prior to regression analysis, abundance of parasitoids and fruit infestation data were transformed to $\ln(x + 0.1)$, whereas parasitism percentage data were subjected to an arcsine square-root transformation to reach normality and homoscedasticity.

Results

Fruit Fly Abundance and Relationship With Host Plants

Of the 93,826 adult fruit flies collected over 4 yr, three tephritid species—*A. fraterculus* (78.6%), *A. schultzi* (4.2%), and *C. capitata*

(17.2%)—were recovered from 40,581 fruits (Table 1). Both *C. capitata* and *A. fraterculus* were recovered from all fruit species sampled each year. *Anastrepha fraterculus* was ~47, 6, 4, and 2 times more abundant than *C. capitata* in guava, walnut, loquat, and peach, respectively, but represented <1% of the total adults obtained from sour orange. *Anastrepha schultzi* was recovered from walnut and guava only, and represent ~30 and <0.5%, respectively, of the total adult *Anastrepha* from the two fruit species. These adult fly abundance patterns remained relatively stable across sampling periods (Table 1).

Fruit Infestation Levels

As shown in Figs. 3A–A.2–B–B.2, fruit fly infestation rates varied sharply across sampling months and host plant. Significantly greater infestation rates by *Anastrepha* spp. were recorded in walnut during both January and February ($H = 28.6$, $df = 3$, $N = 16$, $P < 0.0001$), and in guava during March ($H = 46.2$, $df = 4$, $N = 16$, $P < 0.0001$; Fig. 3A–A.2). A much higher infestation rate by *C. capitata* was also recorded in walnut during January and February ($H = 25.5$, $df = 3$, $N = 16$, $P < 0.0001$), and in guava from February to April ($H = 33.7$, $df = 4$, $N = 16$, $P < 0.0001$; Fig. 3B–B.2). Substantially higher infestation rates for both *A. fraterculus* ($H = 36.1$, $df = 3$, $N = 16$, $P < 0.0001$) and *C. capitata* ($H = 31.4$, $df = 3$, $N = 16$, $P < 0.0001$) were recorded in peach during December and January (Fig. 3A–A.2–B–B.2) with infestation rates by *A. fraterculus* ~20 times higher than those by *C. capitata*. Loquat was infested by

Table 1. Total numbers of *Anastrepha* spp., *Ceratitis capitata*, and their parasitoids recovered from infested fruit of five host plant species collected in Horco Molle, Tucumán, northwestern Argentina, between September 1999 and August 2003

Sampling period	Host fruit	Total no.		<i>Anastrepha</i> parasitoid species											
		Fruit	Weight (kg)	Cc pupae	Cc adults	Cc parasitoid species Ap	An pupae	Af adults	As adults	Da	Db	Ua	Ob	Ap	All species
S/99-A/00	Ca	1,099	252	3,275	1,653	64	65	26	0	0	0	0	0	0	0
	Ej	957	23	558	134	0	295	103	0	0	11	0	0	0	11
	Ja	2,852	76	1,996	459	22	11,656	3,967	456	103	92	3	99	195	492
	Pg	2,401	104	422	145	2	29,314	17,524	0	353	98	50	10	292	803
	Pp	1,738	81	2,555	929	169	5,823	2,266	0	144	123	10	12	397	686
Total		9,047	536	8,806	3,320	257	47,153	23,886	456	600	324	63	121	884	1,992
S/00-A/01	Ca	1,167	353	2,327	1,375	49	86	52	0	0	0	0	0	0	0
	Ej	323	3	0	0	0	4	1	0	0	0	0	0	0	0
	Ja	2,238	61	1,555	926	7	10,160	3,332	1,509	105	187	6	57	249	604
	Pg	2,116	90	736	418	6	39,096	19,846	152	825	277	262	25	797	2,186
	Pp	2,324	107	5,392	3,268	67	7,524	4,753	0	179	181	31	5	335	731
Total		8,168	614	10,010	5,987	129	56,870	27,984	1,661	1,109	645	299	87	1,381	3,521
S/01-A/02	Ca	1,806	535	2,203	935	13	10	5	0	0	0	0	0	0	0
	Ej	7,487	59	498	300	0	2,177	1,174	0	3	116	0	0	6	125
	Ja	1,881	58	535	234	8	8,685	2,576	1,258	35	275	0	30	77	424
	Pg	1,919	103	646	301	16	12,102	6,942	9	57	25	20	4	67	166
	Pp	2,020	119	2,708	1,391	71	7,671	3,228	0	269	216	21	3	244	753
Total		15,203	874	6,590	3,161	108	30,645	13,925	1,267	364	632	41	37	394	1,468
S/02-A/03	Ca	1,228	387	2,474	1,771	4	21	9	0	0	0	0	0	0	0
	Ej	1,899	27	5	1	0	1,015	223	0	0	15	0	0	1	16
	Ja	1,540	38	116	84	0	2,908	1,030	543	2	35	2	4	1	45
	Pg	1,699	82	123	23	0	10,687	7,924	8	25	4	2	0	22	52
	Pp	1,797	60	2,472	1,794	26	3,359	1,849	0	29	18	3	1	50	101
Total		8,163	594	5,190	3,673	30	17,990	11,035	551	56	72	7	5	74	214

S/99-A/00, September 1999–August 2000; S/00-A/01, September 2000–August 2001; S/01-A/02, September 2001–August 2002; S/02-A/03, September 2002–August 2003; Ca, *Citrus aurantium*; Ej, *Eriobotrya japonica*; Ja, *Juglans australis*; Pg, *Psidium guajava*; Pp, *Prunus persica*; Cc, *Ceratitis capitata*; An, *Anastrepha*; Af, *Anastrepha fraterculus*; As, *Anastrepha schultzi*; Ap, *Aganaspis pelleranoi*; Da, *Doryctobracon areolatus*; Db, *Doryctobracon brasiliensis*; Ua, *Utetes anastrephae*; Ob, *Opius bellus*.

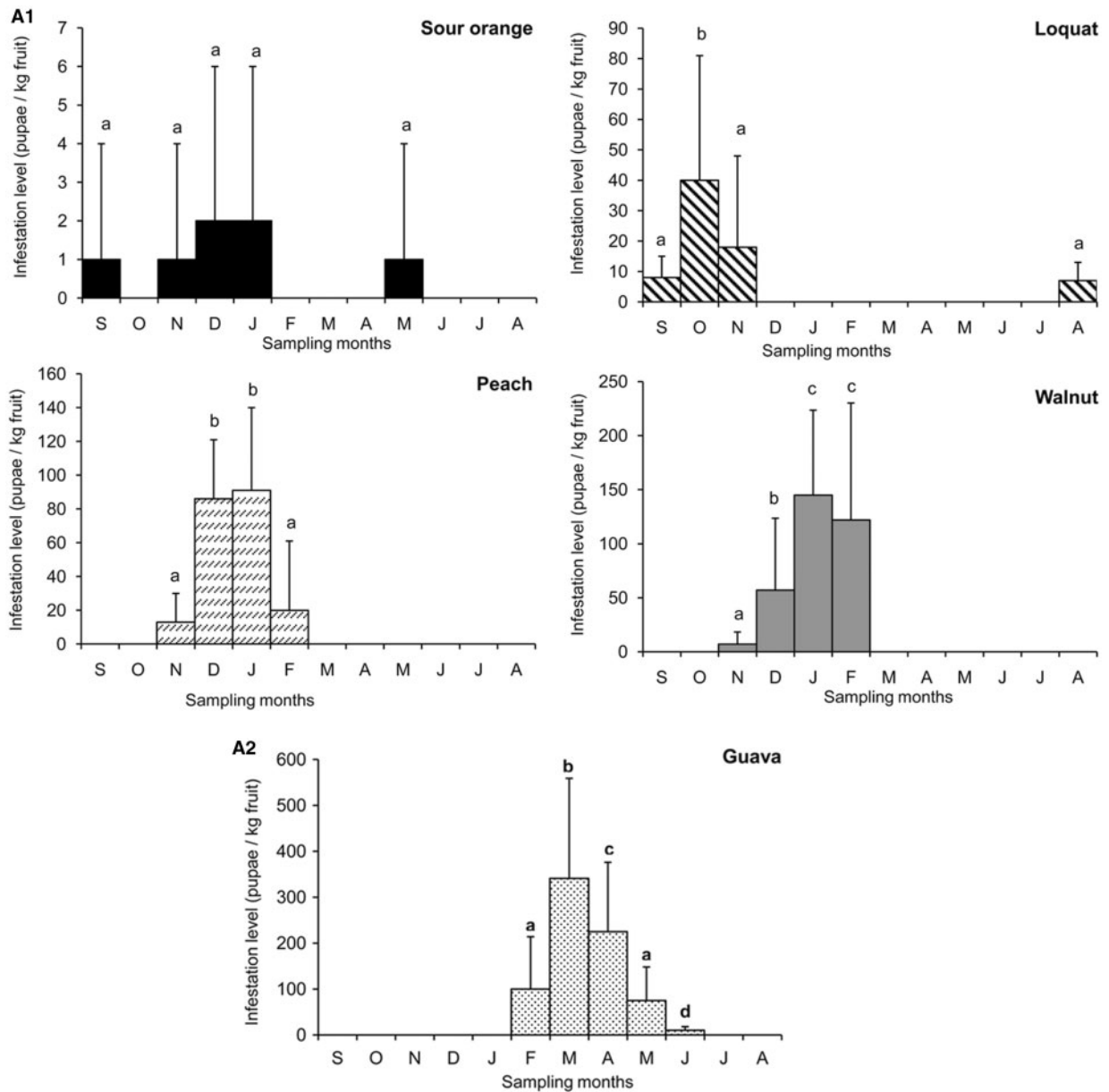


Fig. 3. Mean (\pm SD) monthly infestation levels (larvae/kg fruit) by *Anastrepha* spp. (A) and *C. capitata* (B) per host plant species (walnut, peach, loquat, and sour orange). *Anastrepha* spp. in guava (A.2) and *C. capitata* in guava (B.2) during a 4-yr study period in Horco Molle, Tucumán, Argentina, between September 1999 and August 2003. Bars with the same letter indicate no significant differences (Dunn's test, $P = 0.05$).

A. fraterculus and *C. capitata* between August and November. Remarkably greater infestation levels by *A. fraterculus* ($H = 19.9$, $df = 3$, $N = 16$, $P = 0.0002$) and *C. capitata* ($H = 9.1$, $df = 3$, $N = 16$, $P = 0.0268$) were recorded in October, with infestation rates by *A. fraterculus* 5 times higher than those of *C. capitata* (Fig. 3A-A.2–B-B.2). Sour orange was mostly infested by *C. capitata* throughout the year, with peak infestation levels from November to January ($H = 107.1$, $df = 10$, $N = 16$, $P < 0.0001$; Fig. 3B-B.2). There were no significant differences in monthly infestation rates by *A. fraterculus* in sour orange throughout the year ($H = 12.1$, $df = 4$, $N = 16$, $P = 0.1167$; Fig. 3A-A.2).

Fruit infestation levels by *Anastrepha* spp. and *C. capitata* are shown on a yearly basis in Tables 2 and 3. There were ~4- and ~3-fold differences in the infestation levels of guava and walnut, when comparing the first two study years with the fourth sampling period.

For loquat, infestation level during the second study year was 15–27 times lower than those recorded for the remaining sampling periods. In contrast, infestation rates were relatively stable for sour orange and peach. The lowest infestation rates were recorded for sour orange. As shown in Table 3, infestation rates by *C. capitata* in guava, walnut, peach, and loquat varied significantly across sampling years. Sharp differences in infestation values were recorded for loquat. The highest infestation levels by *C. capitata* were recorded in loquat during first study year and in peach in the remaining three sampling periods. Sour orange was always the second most infested plant by *C. capitata*.

Parasitoid Relative Abundance

Five species of parasitoids, *Aganaspis pelleranoi* (Brèthes) (38%), *Doryctobracon areolatus* (Szèpligeti) (29.6%), *Doryctobracon brasiliensis* (Szèpligeti) (23.3%), *Utetes anastrephae* (Viereck) (5.5%),

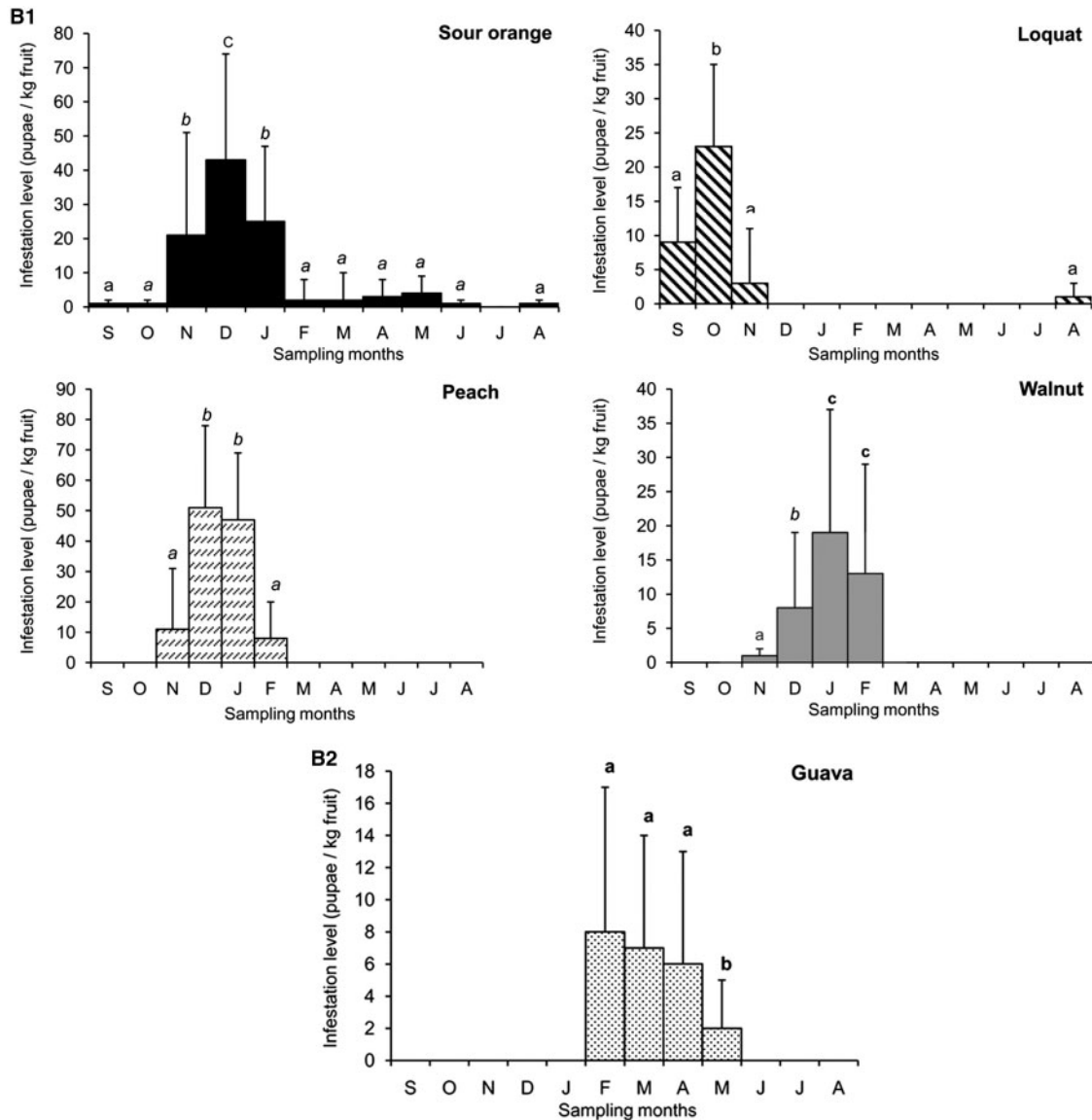


Fig. 3. Continued.

and *Opius bellus* (Gahan) (3.6%), were obtained out of 152,658 *Anastrepha* pupae recovered throughout the 4-yr study (Table 1). However, relative abundance patterns changed over sampling periods. *Doryctobracon brasiliensis* was the most abundant parasitoid between September 2001 and August 2002 followed by *A. pelleranoi* and *D. areolatus*, while *D. brasiliensis* was the second most common species during the fourth sampling period (Table 1). Parasitoids associated with *Anastrepha* pupae decreased significantly in abundance during the fourth year of study, with the most dramatic decreases (22-fold) exhibited by *A. pelleranoi* (Fig. 4). Similarly, the abundance of *A. pelleranoi* from *C. capitata* dropped significantly during the fourth year (Fig. 4). This figitid was the only parasitoid obtained from 30,596 *C. capitata* pupae and sour orange (Table 1). Of the 7,195 parasitoids recovered from all fruit fly pupae over 4 yr, *A. pelleranoi* represented 42% and was recovered from all sampled fruit species, while the braconids *D. areolatus* and *D. brasiliensis* were recovered from four plant species, and *U. anastrephae* and *O. bellus* were only recovered from three fruit species (Table 1). Given the difficulty of distinguishing *A. schultzi* from *A. fraterculus*

pupae, parasitoids from walnut and guava could not be assigned to a specific fruit fly species.

Relative abundances of parasitoids associated with *Anastrepha* differed among plant species (Fig. 5A–D) and sampling method (ground or canopy; Fig. 6A–B). When the 4-yr data were pooled, *D. brasiliensis* was the most abundant species in walnut and loquat, whereas *D. areolatus* and *A. pelleranoi* were the most abundant in guava and peach, respectively. *Aganaspis pelleranoi* was the most abundant parasitoid in peach (72%), guava (56%), and walnut (54%) collected from the ground over 4-yr (Fig. 6A). In contrast, it accounted for 7, 16, and 17% of the total adults recovered from these fruit collected in the tree canopy (Fig. 6B). No *A. pelleranoi* were found in canopy loquat.

Parasitoid relative abundance also varied over time. Both *A. pelleranoi* and *D. areolatus* were the most abundant between January and May, while *D. brasiliensis* from October to December, with *A. pelleranoi* being as abundant as *D. brasiliensis* during December (Fig. 7). *Opius bellus* and *U. anastrephae* were the fourth most abundant species between both January and February, and March

Table 2. Mean (\pm SD) infestation levels by *Anastrepha* spp. (*A. fraterculus* + *A. schultzei*) and mean (\pm SD) parasitism percentages on *Anastrepha* spp. recorded in five host plant species collected in Horco Molle, Tucumán, northwestern Argentina, during 4-yr study period

Sampling period	Host plants									
	Guava ^a		Walnut ^a		Peach ^b		Loquat ^b		Sour orange ^b	
	IL	P	IL	P	IL	P	IL	P	IL	P
S/99-A/00	243.8 \pm 176.7a	3.6 \pm 1.3a	135.2 \pm 89.4a	4.7 \pm 1.5a	57.6 \pm 46.3a	7.9 \pm 5.8a	30.8 \pm 36.1a	0.3 \pm 0.4a	0.6 \pm 1.2a	
S/00-A/01	278.5 \pm 184.2a	5.3 \pm 1.5a	128.9 \pm 82.9a	6.6 \pm 1.9a	70.4 \pm 47.1a	8.4 \pm 4.1a	0.3 \pm 0.1b	0.0a	0.9 \pm 1.5a	
S/01-A/02	141.5 \pm 75.6b	1.7 \pm 0.6b	115.3 \pm 73.3a	4.6 \pm 1.9a	76.3 \pm 51.5a	8.1 \pm 2.8a	33.9 \pm 32.3a	1.0 \pm 0.6a	0.1 \pm 0.2a	
S/02-A/03	63.5 \pm 39.3c	0.5 \pm 0.5c	51.5 \pm 20.7b	0.9 \pm 1.2b	43.8 \pm 30.8a	1.8 \pm 1.8b	45.6 \pm 47.8a	0.4 \pm 0.2a	0.1 \pm 0.2a	
Anova by ranks										
H value	32.2	42.9	8.72	24.1	7.19	20.4	25.1	18.9	7.79	
df	3	3	3	3	3	3	3	3	3	
N	17	13	17	10	12	11	11	8	29	
P value	<0.0001	<0.0001	0.0333	<0.0001	0.0662	0.0001	<0.0001	0.0003	0.0605	

IL, infestation level (pupae/kg fruit); P, parasitism (%).

^aIL by, and P on, *Anastrepha* spp. (*A. fraterculus* + *A. schultzei*).

^bIL by, and P on, *A. fraterculus*.

Within a column values followed by the same letter are not significantly different ($P = 0.05$, Dunn's test).

Table 3. Mean (\pm SD) infestation levels by *C. capitata* and mean (\pm SD) parasitism percentages on *C. capitata* recorded in five host plant species collected in Horco Molle, Tucumán, northwestern Argentina, during 4-yr study period

Sampling period	Host plants									
	Guava		Walnut		Peach		Sour orange		Loquat	
	IL	P	IL	P	IL	P	IL	P	IL	P
S/99-A/00	7.5 \pm 2.6a	0.1 \pm 0.1a	22.0 \pm 14.7a	1.1 \pm 1.8a	21.0 \pm 23.5a	4.1 \pm 3.4a	25.0 \pm 33.1a	2.3 \pm 1.7ab	39.9 \pm 27.1a	
S/00-A/01	8.2 \pm 4.2a	0.6 \pm 1.1a	19.9 \pm 5.3a	0.3 \pm 0.6a	51.8 \pm 20.8a	1.1 \pm 0.8ab	20.5 \pm 17.2a	5.3 \pm 5.2b	0.0b	
S/01-A/02	8.3 \pm 3.7a	1.6 \pm 2.7a	11.1 \pm 7.3ab	1.5 \pm 3.2a	17.4 \pm 11.0b	2.4 \pm 2.1ab	12.8 \pm 11.1a	1.2 \pm 1.9ab	8.9 \pm 11.1a	
S/02-A/03	3.3 \pm 3.4b	0.0a	4.0 \pm 1.1b	0.0a	23.7 \pm 10.0ab	0.3 \pm 0.8b	24.2 \pm 26.4a	0.2 \pm 0.4a	0.4 \pm 0.5b	
Anova by ranks										
H value	12.6	5.78	23.6	6.32	8.53	8.38	4.25	16.3	23.5	
df	3	3	3	3	3	3	3	3	3	
N	17	13	12	10	12	11	29	9	11	
P value	0.0055	0.1230	<0.0001	0.0971	0.0362	0.0387	0.2359	0.0010	<0.0001	

IL, infestation level (pupae/kg fruit); P, parasitism (%).

Within a column values followed by the same letter are not significantly different ($P = 0.05$, Dunn's test).

and April (Fig. 7). No parasitoids were recovered between June and September.

Parasitoid Seasonal Abundance

Seasonal abundance of parasitoid populations is shown in Fig. 8. *Doryctobracon areolatus* and *D. brasiliensis* were found between October and May, with a peak of *D. areolatus* in January and March ($H = 59.1$, $df = 7$, $N = 16$, $P < 0.0001$; Fig. 8A), while *D. brasiliensis* peaked in December and January ($H = 46.8$, $df = 7$, $N = 16$, $P < 0.0001$; Fig. 8B). *Utetes anastrephae* was collected between December and May, and recovered in greater numbers in March and April ($H = 20.3$, $df = 5$, $N = 16$, $P = 0.0011$; Fig. 8C). *O. bellus* was found between December and April, with peaks in January and February ($H = 34.7$, $df = 4$, $N = 16$, $P < 0.0001$; Fig. 8D). Like *Doryctobracon*, *A. pelleranoi* was recovered from *Anastrepha* pupae between October and May, with a peak from December to April ($H = 51.7$, $df = 7$, $N = 16$, $P < 0.0001$; Fig. 8E). This figitid was also recovered from *C. capitata* between November and April and in greater numbers in December and January ($H = 46.0$, $df = 5$, $N = 16$, $P < 0.0001$; Fig. 8F).

Except for *O. bellus* and *U. anastrephae*, all parasitoid species recorded during the study exhibited diapause between June and September as evidenced by their absence from the field (Fig. 8A–F). About 90% of the diapausing individuals were recovered from pupae obtained during the guava fruiting season. The duration of dormancy (length of time from pupation to adult eclosion) was six months approximately.

Parasitization Rates

Monthly parasitism of *Anastrepha* spp. and *C. capitata* is shown in Fig. 9 and average parasitism for each sampling period is shown in Tables 2 and 3. Significantly higher percentages of parasitism on *Anastrepha* spp. were recorded in December and January during both peach and walnut fruiting seasons ($H = 49.1$, $df = 7$, $N = 16$, $P < 0.0001$; Fig. 9). Parasitism values in peach were ~ 2 times greater than those in walnut throughout December and January. A second peak of parasitism was recorded in March and April during the middle of the guava fruiting season. The lowest parasitism rates were recorded in October, November, February, and May (Fig. 9). During November, parasitism percentage in peach and loquat was

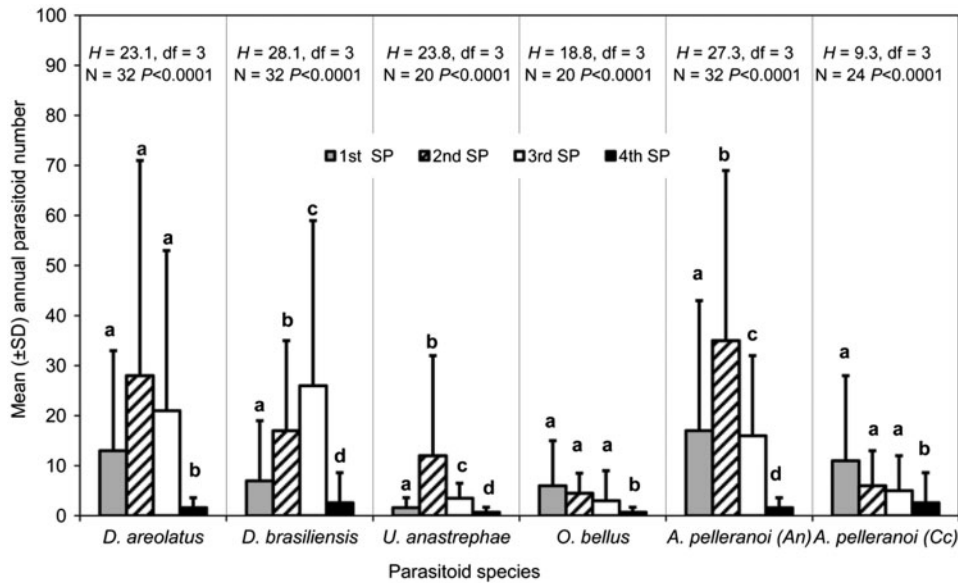


Fig. 4. Mean (\pm SD) number of parasitoid species recovered per yearly sampling period from fruit collections at Horco Molle, Tucumán, Argentina, between September 1999 and August 2003. Results of ANOVA on ranks are shown on top. Bars with the same letter indicate no significant differences (Dunn's test, $P = 0.05$).

similar, while in walnut no parasitism was recorded. Around 70, 20, and 10% of overall parasitism in February was recorded from guava, peach, and walnut, respectively. Regarding parasitism on *C. capitata*, notably higher values were recorded from November to January during peach, sour orange, and walnut fruiting seasons, and in April in guava ($H = 25.9$, $df = 5$, $N = 16$, $P < 0.0001$; Fig. 9). Approximately 50, 30, and 20% of overall parasitism recorded during November, December, and January occurred in peach, sour orange, and walnut, respectively. Noticeable yearly variations in parasitism on *Anastrepha* spp. were documented for all host plant species (Table 2). Low parasitism was recorded in both loquat and sour orange during the 4-yr study period (Table 2). Parasitism rates on *C. capitata* in guava, peach, walnut and sour orange over 4-yr were relatively stable, though the lowest parasitism values always occurred during the fourth sampling period (Table 3).

A significantly strong relationship between parasitization rates on, and infestation levels by, *Anastrepha* spp. was documented for guava, peach, loquat, and walnut throughout the study by means of correlation analyses (guava, $r = 0.41$, $n = 64$, $P < 0.0001$; peach, $r = 0.40$, $n = 64$, $P = 0.0106$; loquat, $r = 0.78$, $n = 64$, $P < 0.0001$; walnut, $r = 0.75$, $n = 64$, $P < 0.0001$). In contrast, there was no relationship between infestation level and parasitism when *C. capitata* was considered per each host fruit species in the correlation analyses (guava, $r = 0.11$, $n = 31$, $P = 0.5540$; peach, $r = 0.19$, $n = 31$, $P = 0.3140$; sour orange, $r = 0.16$, $n = 31$, $P = 0.3800$; walnut, $r = 0.26$, $n = 31$, $P = 0.1590$).

Environmental Conditions, Parasitoid Abundance, Parasitism, and Infestation Level Relationships

Significant and positive relationships were documented between rainfall and minimum temperature with the number of emerged parasitoid adults, parasitization rates, and fruit infestation levels for both *Anastrepha* and *C. capitata* (Table 4).

Discussion

Fruit Fly Abundance and Infestation Levels

Fruit infestation level fluctuations over 4-yr reflected a gradual population increase for both pest species between the cold-dry and the

warm-humid season and a marked preference for particular host plants among tephritid species. *Anastrepha fraterculus* and *C. capitata* population growth was facilitated by the combination of four factors 1) temporal overlapping availability of *E. japonica*, *P. persica*, *J. australis*, and *P. guajava* fruit, 2) almost permanent availability of *C. aurantium*, 3) increases in mean temperature and precipitation, and 4) a high degree of polyphagy of both pest species. In the particular case of *A. fraterculus*, availability of *E. japonica* at the end of the cold-dry season and beginning of the warm-humid season, followed by an overlap of available *P. persica* and *J. australis* from early to mid-season and *P. guajava* at its end produced important population peaks between January and April. Nevertheless, *P. guajava* was the preferred host plant for *A. fraterculus* and the one that allowed the greatest population growth. Although *A. fraterculus* has one of the broadest host plant ranges of all known *Anastrepha* species (Norrbom 2004), *P. guajava* is the most commonly recorded host plant throughout the whole range of the *A. fraterculus* cryptic species complex (Aluja et al. 2003a). Despite the low infestation levels recorded for *A. fraterculus* in *C. aurantium*, this host, along with *E. japonica* serves to bridge fruiting periods between late guavas and the earliest fruit of *P. persica* and *J. australis*. This finding is consistent with those of Ovruski et al. (2003) for *C. paradisi* Mac fadyen (Grapefruit), which is common as a backyard tree and in semicommercial orchards surrounding most native vegetation areas in northwestern Argentina. In the case of *C. capitata*, infestation peaks were practically continuous between October and February due to overlap of *E. japonica*, *P. persica*, *J. australis*, and *C. aurantium*. Nevertheless, the greatest peaks occurred between December and January coinciding with *P. persica* and *C. aurantium* greatest availability. According to infestation levels recorded across 4 yr, *P. persica* would be the primary host for *C. capitata*, with *C. aurantium* as the second most infested. These results confirm records by Ovruski et al. (2003, 2004) and Segura et al. (2006), highlighting *P. persica* and a *C. aurantium* as important multiplying hosts in the Argentinean Northeast. In addition, along with *E. japonica*, *C. aurantium* plays an important bridging role during the entire cold-dry season, a time of the year when no *P. persica* is available. Similar to records in disturbed areas of the current study other

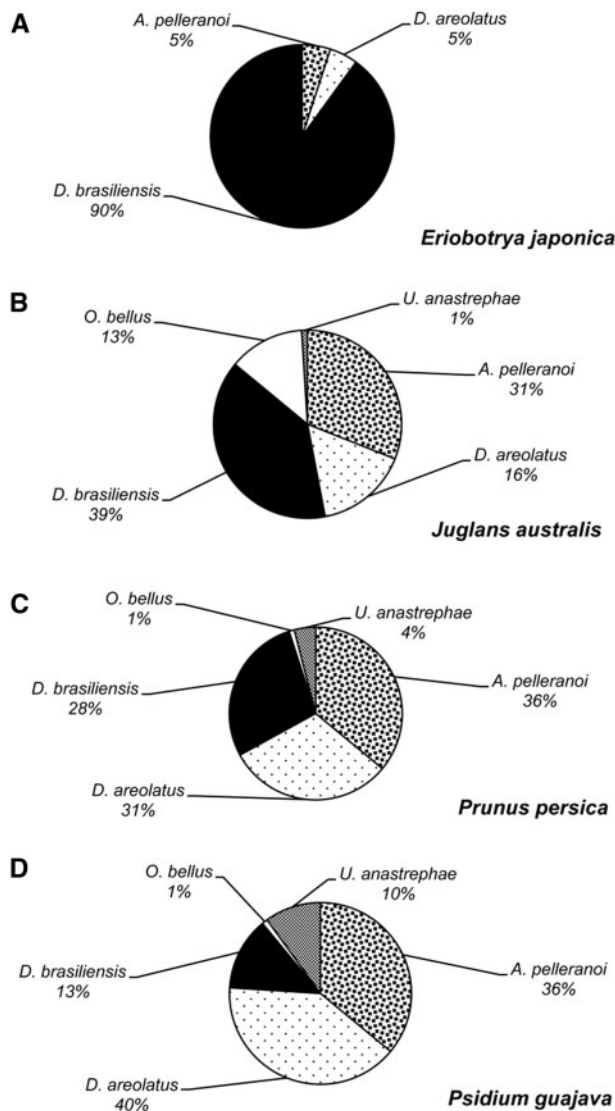


Fig. 5. Relative abundance of *Anastrepha* spp. parasitoids recovered from four host plant species collected in Horco Molle, Tucumán, Argentina, between September 1999 and August 2003. (A) *Eriobotrya japonica*; (B) *Juglans australis*; (C) *Prunus persica*; (D) *Psidium guajava*.

citrus species, such as *C. paradisi*, *C. reticulata* Blanco (Tangerine), and *C. sinensis* (Sweet orange), have been cited as bridging cultivated hosts (Ovruski et al. 2003).

In the case of *A. schultzi*, a species of no economic importance, abundance was limited to *J. australis* fruiting, its primary host (Schliserman et al. 2004), and to a lesser degree *P. guajava*, a species acting as an alternate host allowing to extend presence at least until the beginning of the cold-dry season.

Parasitoid Relative and Seasonal Abundance

Relative seasonal larval-pupal parasitoid abundance depended upon the following factors: 1) Host plants where the larval host developed, 2) tephritid species within host fruit, 3) seasonality in fruit availability, 4) larval infestation level, 5) effect of climate on fruit and tephritid abundance, 6) potential for interspecific competition, and 7) type of environment where host plants were located.

The first claim can be confirmed when examining parasitoid abundance and diversity for *C. aurantium*, which provided

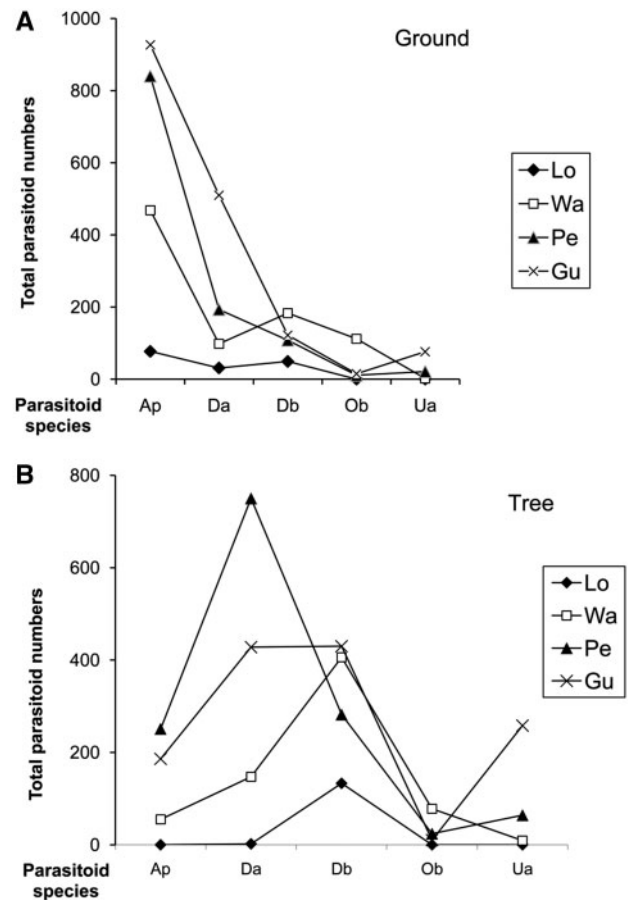


Fig. 6. Total number of *Anastrepha* spp. parasitoids recovered from fruit samples collected from both the ground (A) and the tree canopy (B) in Horco Molle, Tucumán, Argentina, between September 1999 and August 2003.

abundant available fruit all year, and yet 5,300 collected fruit only yielded less than 2% of the total parasitoids with only the figitid *A. pelleranoi* being represented. Such a result can be attributed to the fact that *C. aurantium* is an exotic plant with physical properties such as large fruit size and thick epicarp and mesocarp, which render access to larvae difficult for braconid parasitoids (Ovruski et al. 2004). In general, tephritid larvae infesting large fruit can avoid parasitism by feeding at a greater depth within pulp (Sivinski et al. 1997, Wang et al. 2009). Notwithstanding the above, *A. pelleranoi*, as opposed to the four braconid species recovered during this study, preferentially forages on the ground and penetrates fruit through open peel cracks to find and parasitize its larval host regardless of fruit size and peel thickness (Aluja et al. 2009a).

The second claim is based on data on trophic associations between tephritid and parasitoid species. Only *A. pelleranoi* was recovered from *C. capitata* puparia reared from *C. aurantium*, *P. persica*, *J. australis*, and *P. guajava*, despite the fact that the last three hosts provided suitable conditions for parasitization such as a thin epicarp, soft pulp, and medium size. This information confirms previous observations by Canal and Zucchi (2000) for Brazil, and Ovruski et al. (2004) for Argentina, who reported that with the exception of *A. pelleranoi*, neotropical parasitoids appear to adapt poorly to the exotic *C. capitata*. The evident lack of development of native parasitoid larvae in *C. capitata* is probably brought about by antibiosis or encapsulation as suggested by Ovruski et al. (2004).

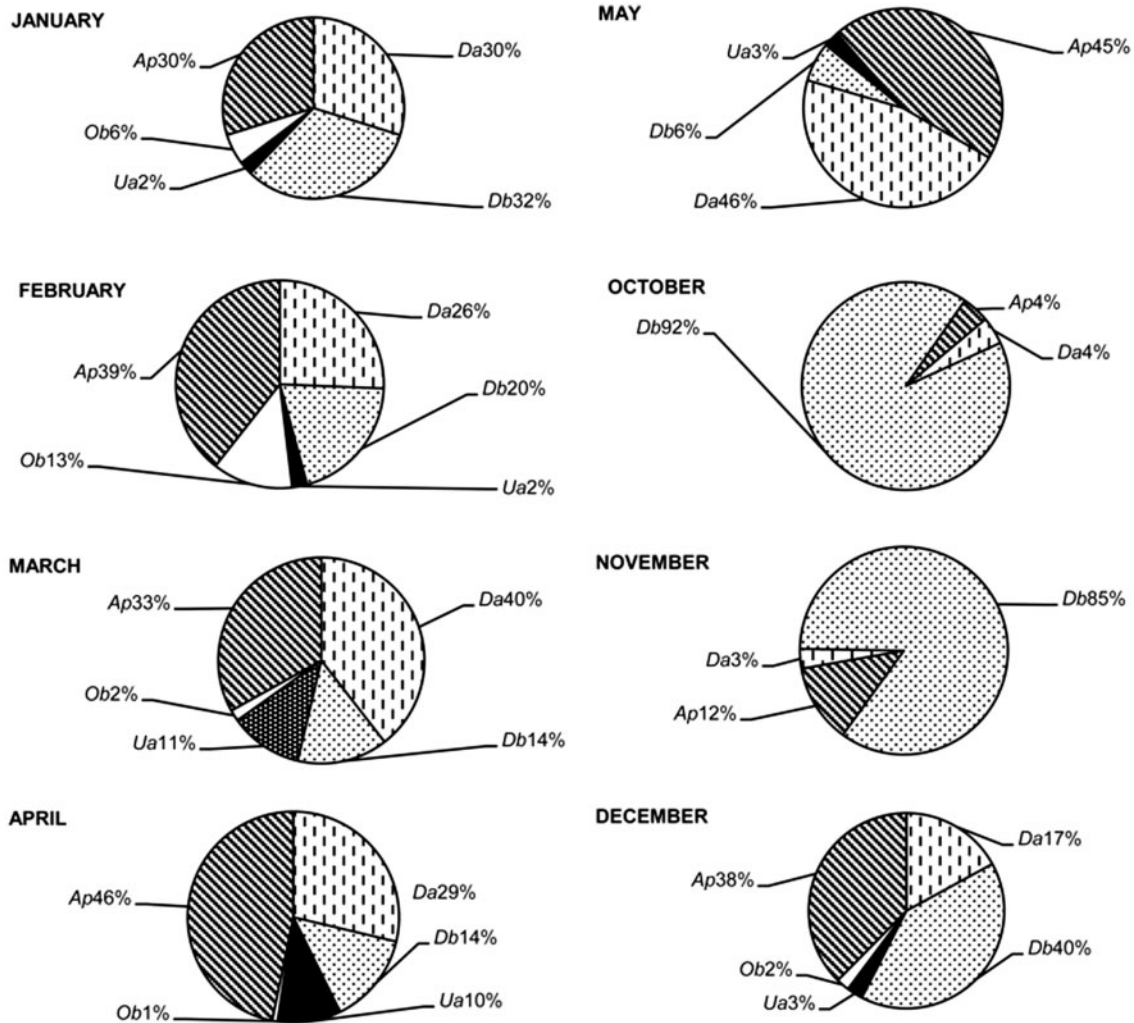


Fig. 7. Relative abundance of *Anastrepha* spp. parasitoids per collecting month in Horco Molle, Tucumán, Argentina, between September 1999 and August 2003.

The three following claims are based on the absence of emerged adult parasitoids during the cold-dry season across the four sampling periods of the study and on confirmation of diapause for all parasitoid species recovered. This finding is linked to the lack of available fruit of the main host species (*P. guajava*, *P. persica*, and *J. australis*) and a sharp reduction of infestation levels for *E. japonica* and *C. aurantium* (Fig. 3), but also and essentially due to scarce rainfall (an average accumulation of 16 mm), daily temperatures below 15°C, and frosting events. These results are similar to those reported by Ovruski et al. (2015).

It is worth noting that during June–September there were fruit available in the study area for *A. fraterculus* and *C. capitata* attack. Late guavas could still be found in June, large quantities of ripe sour orange could be found between June and September, and between August and September large quantities of ripe loquat, a fruit with favorable conditions for parasitization (Sivinski 1991). Nevertheless, recorded infestation levels during such months were notably low and contrastingly high during the warm-humid season. As pointed out by Aluja et al. (1998) diapause in parasitoids associated with *Anastrepha* in the Neotropics allows transition between periods of favorable environmental conditions through periods of poor weather and lack of larval hosts. Weather variation across collection

years also affected abundance. For instance, during the fourth sampling period, rainfall was three times lower than during the first two periods and recovery of adult parasitoids suffered a sharp drop. Similar to the present study, Aguiar-Menezes and Menezes (2001) found a significant negative relationship between low levels of temperature and rainfall, *Anastrepha* spp., and parasitoid population size in Southeast Brazil, an area also characterized by humid warm-temperate (Cwa) type climate. However, as opposed to results from this study Aguiar-Menezes and Menezes (2001) recorded parasitoids from *E. japonica* and *C. aurantium* throughout the cold-dry season (June to August) over two years (1998–1999), a finding perhaps explained by the fact that the region of our study is 1.6 times colder between June and August than Southern Brazil where mean temperature and precipitation during those months surpasses 20°C and 25 mm.

Monitoring the fruiting period of the main host plants of *A. fraterculus* for 4-yr revealed different relative and seasonal abundance patterns among parasitoid species that could be explained by niche partitioning related to interspecific competition. A significant relationship between fruit availability and parasitoid abundance could be established for braconid species—*D. areolatus* clearly peaked in January and March when *P. persica* and *P. guajava* displayed their

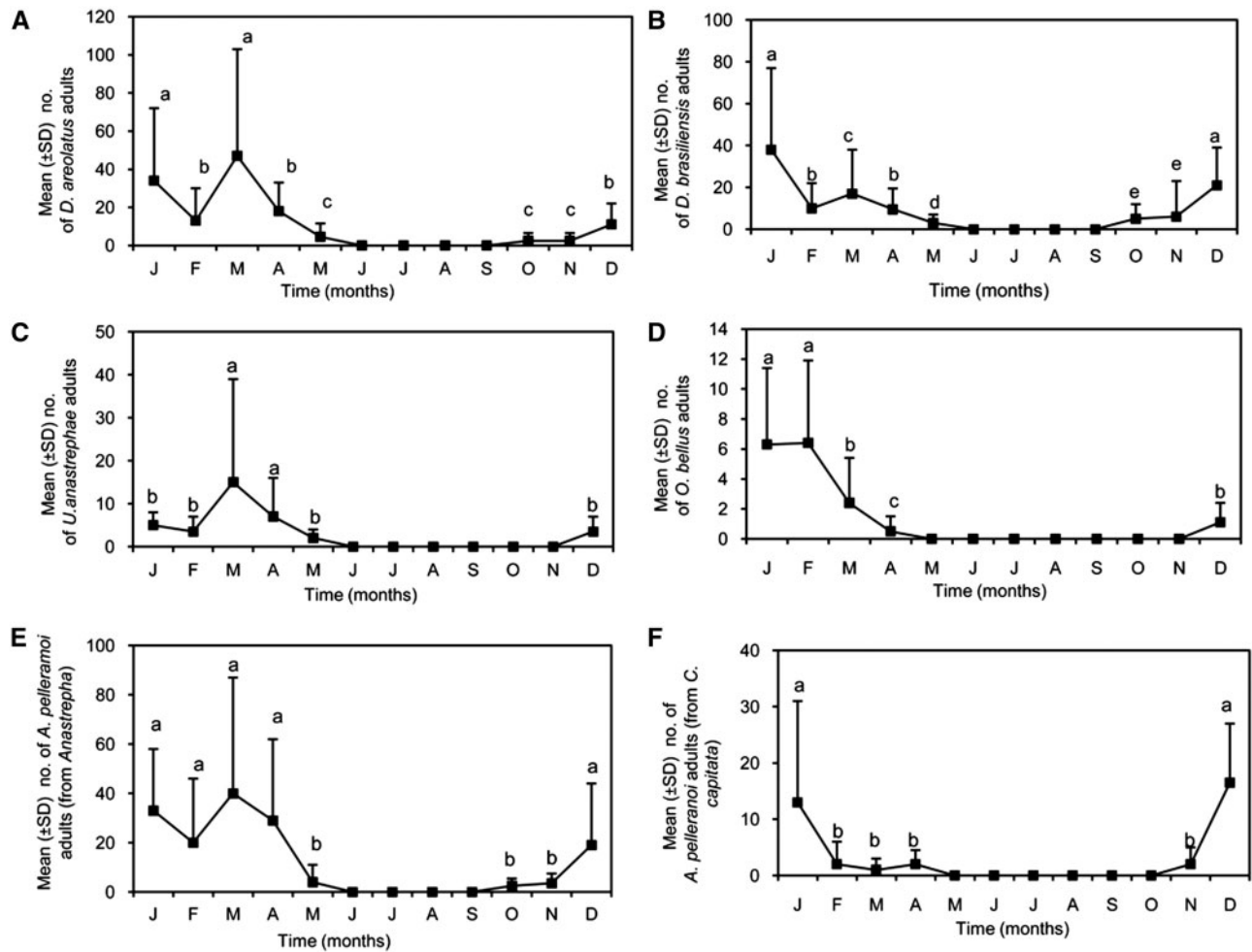


Fig. 8. Mean (\pm SD) monthly numbers of parasitoid adults emerged from fruit fly pupae recovered from five host plant species in Horco Molle, Tucumán, Argentina, between September 1999 and August 2003. (A–E) Parasitoids emerged from *Anastrepha* spp. pupae (*A. fraterculus* and *A. schultzi*). (F) Parasitoid species emerged from *C. capitata* pupae. Bars with the same letter indicate no significant differences (Dunn's test, $P = 0.05$).

respective tephritid infestation peaks, while *D. brasiliensis* peaked in December and January matching *J. australis* and *P. persica* infestation peaks.

Maximal abundance of *D. brasiliensis* and *D. areolatus* only overlapped during maximal *P. persica* infestation. This fact is interesting given that *D. brasiliensis* and *D. areolatus* share morphological and biological features such as a long ovipositor (3.1–3.4 and 2.2–2.6 times longer than the metasoma for *D. brasiliensis* and *D. areolatus*, respectively), a feature allowing females to reach host larvae in both large and small fruit (Sivinski et al. 2001). In addition, according to our results, both species forage for fruit on the tree canopy, as observed in behavioral studies by García-Medel et al. (2007) for *D. areolatus* in México. Additional seasonal segregation was found between *O. bellus* and *U. anastrephae*, while *O. bellus* peaked during *J. australis* highest infestation levels, *U. anastrephae* peaked significantly in March and April during *P. guajava* highest infestation levels. Both parasitoid species have short ovipositors (0.7–0.9 and 0.6–0.8 times longer than the metasoma, respectively), a feature that limits foraging to small fruit hosts (Sivinski et al. 1997, Ovruski et al. 2004). Additionally, on the basis of results for ground and canopy fruit collection, both species forage for canopy fruit, while apparently *O. bellus* also forages for fallen fruit. To sum up, these two species appear to exploit overlapping ecological niches

and avoid potential competition through temporal partitioning and differential host plant specialization.

In general terms, *D. brasiliensis* and *O. bellus* prevail during the beginning of the rainy season, when loquat, peach, and walnut are most abundant. In contrast, *U. anastrephae* and *D. areolatus* thrive during the final months of the warm-humid season, when guava is the dominant fruit. Variation in preponderance of one braconid species over another was also noticed by Aguiar-Menezes and Menezes (1997) for the Itaguaí region of southeast Brazil. These authors recorded *D. brasiliensis* as the most abundant species between May and July (cold-dry season) in coincidence with *P. persica* fruiting, whereas *D. areolatus* predominated the rest of the year in all collected plant species except peach. However, Aguiar-Menezes and Menezes (1997) did not find a succession in predominance between *U. anastrephae* and *O. bellus*.

The seasonal variation in abundance patterns for parasitoid species with similar morphological and biological features documented here reflects some degree of niche differentiation that may allow more efficient use of available resources (*A. fraterculus* larvae). Other strategies aimed at minimizing interspecific competition among braconid parasitoids exploiting *Anastrepha* are through: 1) differential distribution within tree canopies, as has been documented in Mexico between *D. areolatus* and *U. anastrephae*

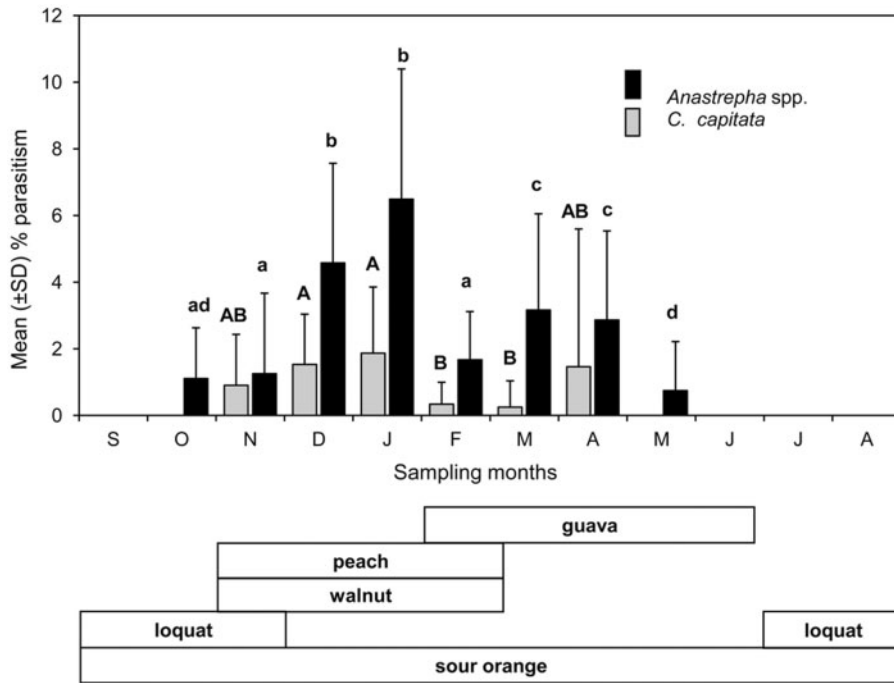


Fig. 9. Mean (\pm SD) monthly parasitism percentages on *Anastrepha* spp. and *C. capitata* during a 4-yr study period in Horco Molle, Tucumán, Argentina, between September 1999 and August 2003. Bars with the same letter indicate no significant differences (Dunn's test, $P = 0.05$).

Table 4. Multiple regression analysis of monthly weather conditions (maximum and minimum temperatures and rainfall) with fruit infestation levels, parasitism, and numbers of parasitoids emerged from pupae collected from five fruit host species in the study area (Horco Molle, Tucumán, Argentina) between September 1999 and August 2003

Response variable	Formula	R ² value	F value	SE	df	P value
Log emerged parasitoid adults	$y = -3.87 + 0.58 \log \text{rainfall} + 0.37 \log \text{min. temp}$	0.68	53.06	0.63	2.46	<0.0001
Log infestation level by <i>Anastrepha</i> spp.	$y = -3.21 + 0.46 \log \text{rainfall} + 0.44 \log \text{min. temp}$	0.61	38.14	0.58	2.46	<0.0001
Log infestation level by <i>C. capitata</i>	$y = -4.23 + 0.47 \log \text{rainfall} + 0.45 \log \text{min. temp}$	0.62	40.69	0.36	2.46	<0.0001
Log parasitism	$y = -1.47 + 0.54 \log \text{rainfall} + 0.34 \log \text{min. temp}$	0.57	33.47	0.31	2.46	<0.0001

(Sivinski et al. 1997); 2) spatial partitioning along an altitudinal gradient as for *D. areolatus*, which prefers warmer environments at low elevations when compared with *D. crawfordi*, which thrives in colder, more humid high elevation environments (Sivinski et al. 2000); 3) distributional differences in latitude (Eitam et al., 2004); 4) different preferences for foraging within tree canopies or fallen fruit (García-Medel et al. 2007); 5) preference for particular fruit species, as is the case for *U. anastrephae*, which forages on a relatively few species generally bearing small fruit (Sivinski et al. 2001).

In contrast to braconid species, the figitid *A. pelleranoi* displayed no preference for any of the five fruit species examined. *A. pelleranoi* adults reared from *Anastrepha* spp. pupae were equally abundant between December and April during periods of highest infestation for walnut, peach, and guava. In addition, *A. pelleranoi* recovered from *C. capitata* pupae, peaked in December and January in synchrony with highest infestation levels for *C. aurantium* and *P. persica*, which yielded the most *C. capitata* pupae. These findings are in accordance with reports by Wharton et al. (1998) on the lack of preference for any fruit species by *A. pelleranoi* rooted on the peculiar foraging behavior of this figitid.

The intrinsic properties of the environment where host plants are found influence both tephritid and parasitoid species. For example,

during studies in Tucuman over 5 yr (Ovruski et al. 2003, 2004) *E. japonica* was collected in untreated semicommercial orchards, public parks, and backyard trees at three urban localities resulting in 70% of *C. capitata* over *A. fraterculus* and had no parasitoid recovery. By contrast, during the present study, *E. japonica* collections from natural areas corresponding to a secondary Yungas forest sector yielded opposite results, with *A. fraterculus* nearing 75% of infestation and the presence of three parasitoid species (Table 1). Previously, Aluja et al. (2003b) for Mexico, and Ovruski et al. (2004) and Schliserman et al. (2014b) for the Argentinean Northwest, found greater diversity and abundance of parasitoids in natural areas with different degrees of disturbance than in highly disturbed agricultural and urban settings.

Parasitization Rates

Parasitization rates on *Anastrepha* spp. (*A. fraterculus* + *A. schultzi*) varied according to changes in larval density for each collected host fruit. Within collections of a single host plant, highest rates were recorded at the time of greatest larval host infestation. However, over the entire season, general patterns of parasitism could be reversed along successive fruiting periods of the five sampled host plants. For

example, there was a fourfold increase in *A. fraterculus* infestation from fruiting of peach to fruiting of guava, but a twofold decrease in parasitization rates across hosts. Another factor that negatively affected parasitism was variation in weather conditions, specifically rainfall. The onset of the dry season has also been reported to affect parasitization in Central Veracruz, México (Sivinski et al. 1997), Central Nuevo León, México (González-Hernández and Tejada 1979), and southeastern Brazil (Aguiar-Menezes and Menezes 1997, 2001).

Practical Implications

Eight findings from this study stand out as having important practical implications for designing fruit fly biological control strategies: 1) *C. aurantium* is one of the main multiplying host plants for *C. capitata*; due to its long fruiting period and seasonality it allows year round maintenance of pest populations. Furthermore, it also functions as an important host bridge for native *A. fraterculus* at times of preferred host scarcity and adverse climatic conditions; 2) *E. japonica* plays a fundamental role for gradual increase of *C. capitata* and *A. fraterculus* populations as climatic conditions improve at the end of the dry season and the beginning of the warm-humid season. Such growth facilitates infestation of early peach fruit and walnut in November for both tephritid pest species whose populations can explode in December; 3) *P. persica* and *C. aurantium* allowed maximal multiplication of *C. capitata*, between December and February; 4) *J. australis* and *P. guajava* allowed maximal multiplication of *A. fraterculus* between January and April; 5) During *A. fraterculus* multiplication in its favorite exotic host guava, natural parasitism rates decreased; 6) Low diversity and abundance of parasitoids attacking *C. capitata* on different host plants, in particular *C. aurantium*, with the largest fruit size in this study; 7) A low percentage of natural parasitism rates was recorded for both *A. fraterculus* and *C. capitata*, across the 4-yr study in an environment characterized by native vegetation patches and nearby Citrus plantations; 8) Niche segregation among different parasitoid species based on foraging preferences for host fruit with different phenological patterns.

Although the annual averages of the abundance of parasitoids and percent parasitoidism were low (Tables 1–2), the conservation and augmentative releases of parasitoids are viable options.

Authors such as Turica and Mallo (1961) concluded that the Yungas are a natural source of parasitoidism, a statement that is supported by our results. Here we show the host fruits that are able to maintain minimum parasitoid populations over the years. So management strategies could be used to conserve natural populations of parasitoids. Instead of removing native host plants, which represent natural parasitoid reservoirs, they should be preserved.

Another point is the accompaniment among populations of parasitoids and flies as well as the phenology of these and their host fruits. All this is important when thinking about augmentative releases.

Another important ecological attribute is diapause. Ovruski et al. (2015) observed for the Yungas that the five parasitoid species recorded in this study exhibit diapause. Less than 10% of the total recovered of individuals entered diapause, with the figitid *A. pelleranoi* showing the highest proportion of diapausing individuals (9.2% of the total), whereas *D. areolatus* had the lowest proportion (2.3%).

Diapause appears to occur for only a small fraction of parasitoid populations and would not represent an obstacle for biological control practices because a portion of the released population would be able to remain in the release area during adverse weather streaks.

Additionally, some of these native parasitoid species have been successfully colonized and reared at the PROIMI insectary.

The findings detailed above allow delineating four scenarios that can help to establish fruit fly biological control strategies based on area-wide management in citrus-producing areas of NW Argentina. First, the use of timed augmentative releases of indigenous parasitoids between September and November in wild vegetation areas adjacent to commercial fruit groves would be useful to suppress population growth of *C. capitata* in orange, loquat, peach, and walnut, and also to diminish population increase of *A. fraterculus* in the last three fruit species. This action would be focused on curtailing maximum infestation peaks during the full warm-humid season before tephritid fly populations spread into commercial crops. Such a goal could be achieved by combining two native parasitoid species that may attack and successfully develop in both tephritid species, such as the figitid *A. pelleranoi* and the diapiid *Coptera haywardi* Loíacono. The former species mainly attacks *A. fraterculus* and *C. capitata* larvae in fallen fruits by entering through holes on the skin (Aluja et al. 2009a), whereas *C. haywardi* attacks *C. capitata* (Baeza-Larios et al. 2002) and *A. fraterculus* pupae (Núñez-Campero et al. 2012). In the case of these two species, fruit size and type are not limiting factors to find and use hosts; therefore, infested fruit with features such as those of *C. aurantium* would not limit the action of these natural enemies. It is also important to emphasize that both species are potential fruit fly biocontrol agents for augmentative releases (Aluja et al. 2009b, Cancino et al. 2012). Currently, *A. pelleranoi* and *C. haywardi* are being successfully reared under laboratory conditions (Núñez-Campero 2011), enabling the implementation of native parasitoid augmentative release programs. Nevertheless, as suggested by Núñez-Campero (2011) more detailed studies on the bioclimatic requirements of both *A. pelleranoi* and *C. haywardi*, as well as preliminary assessment of laboratory-reared parasitoids effectiveness, are still needed before optimal application.

Secondly, the implementation of measures to foster conservation biological control (Aluja and Rull 2009) in wild vegetation areas with mixed stands of native and exotic fruit species, or in areas where native vegetation is still dominant, such as Yungas forest sectors above 1,000 m, should be feasible. It is in these areas where *A. fraterculus* populations increase and from which individuals move to attack commercially grown fruit (Ovruski et al. 2003). In addition to *J. australis*, other native fruit species, such as *Eugenia uniflora* L. and *Myrcianthes pungens* (Berg.) Legrand act as *Anastrepha* parasitoid reservoirs (Ovruski et al. 2004). As previously suggested by Aluja et al. (2003b), in order to promote conservation of nonpest species of tephritids, their host plants, and their associated parasitoids in tropical forests, native host plants can be managed to maintain and multiply fruit fly parasitoids. Thus, instead of removing these native hosts, they can be multiplied through reforestation in partially disturbed or undisturbed environments.

Thirdly, another possibility would be the introduction of exotic specific parasitoids to control *C. capitata* populations on exotic fruits such as Citrus spp., *E. japonica*, and *P. persica*. For example, the two egg-pupal parasitoids *Fopius ceratitivorius* Wharton, an Afrotropical species (López et al. 2003, Bokonon-Ganta et al. 2005), and *Fopius arisanus* (Sonan), an Indo-Pacific species (Wang et al. 2003, Vargas et al. 2007) are good candidates for introduction into Argentina. From two exotic parasitoid species under consideration, *F. arisanus* has been successfully mass-reared in Hawaii (Vargas et al. 2002), Mexico (Zenil et al. 2004), and Guatemala (Rendón et al. 2006). In addition, *F. arisanus* has proven potential to suppress medfly populations in Hawaii (Vargas et al. 2001).

Fourth, as previously demonstrated by Ovruski et al. (2012) the exotic parasitoid species *D. longicaudata* can significantly contribute to *C. capitata* mortality on infested orange and grapefruit. Therefore, *D. longicaudata* is another potential candidate for use in augmentative releases against *C. capitata* in abandoned citrus orchards and in wild vegetation patches containing “feral” plants. Currently, *D. longicaudata* is mass reared at the BioPlanta San Juan facility (Ovruski and Schliserman 2012), a fact which facilitates the implementation of augmentative biological control. Furthermore, *D. longicaudata* could also be useful in suppressing populations of *A. fraterculus* that actively multiply in guava in disturbed environments.

All these biological control measures, in combination with other fruit fly biorational management strategies, as described by Aluja and Rull (2009), could greatly contribute to the establishment of a low *A. fraterculus* and *C. capitata* prevalence area in NW Argentina.

Acknowledgments

We are grateful to Luis Oroño, Nury Ovruski, Segundo Nuñez Campero, Patricia Alborno Medina, and Omar Ulises Chaya (PROIMI) for valuable technical and field assistance. We are grateful to the authorities of the “Reserva Experimental Horco Molle” and “Parque Sierra de San Javier” (Universidad Nacional de Tucumán) for allowing us to collect fruits and providing local weather data. We also thank all the families who allowed us to sample fruit in their homes. We also express our gratitude to the Semicommercial orchard owners. This study was supported by Agencia Nacional de Promoción Científica y Tecnológica de Argentina through Fondo Nacional de Ciencia y Tecnología (FONCyT; grants PICT/2007 No. 01970 and PICT/2010 No. 0393), and by Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET; Grant PIP/2009-2011, No. 1353).

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