



Occurrence of diapause in neotropical parasitoids attacking *Anastrepha fraterculus* (Diptera: Tephritidae) in a subtropical rainforest from Argentina

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Abstract

Incidence of diapause in neotropical parasitoid species associated with the tephritid fruit fly *Anastrepha fraterculus* (Wiedemann) infesting three major host plants collected from the southernmost end of Argentinean Yungas rainforest was studied. Three other objectives were the frequency of diapause according to the fruiting season of *Prunus persica* (L.) Batsch (peach), *Psidium guajava* L. (guava) and *Juglans australis* Grisebach (walnut); the length of diapause period for each recovered parasitoid species; and the proportion of diapausing parasitoid individuals that displayed prolonged diapause. Between 2001 and 2003, infested ripe fruits were weekly collected in wild vegetation areas. A portion of the fruit samples was processed at the same collection site. Recovered *A. fraterculus* puparia were kept under natural environmental conditions inside a wooden frame cage covered with a cloth mesh. The remaining halves of fruit samples were taken to the laboratory and puparia were kept under environmental controlled condition. Unenclosed puparia were kept for 2 years. Diapause period fluctuated between 131 and 426 days and was observed in all recovered parasitoid species (*Doryctobracon areolatus* (Szépligeti), *D. brasiliensis* (Szépligeti), *Opius bellus* Gahan, *Utetes anastrephae* (Viereck) and *Aganaspis pelleranoi* (Brèthes)). All diapausing individuals dissected from host puparia were instar III and were also remarkably different from non-diapausing larvae because of their smaller body size. The 65% of all diapausing individuals was recorded in early autumn and was recovered from *A. fraterculus* larvae that had developed in guava. Prolonged diapause was recorded in a small fraction of the diapausing populations of *D. brasiliensis*, *D. areolatus* and *A. pelleranoi*. Results suggest that diapause is an adaptive mechanism that allows parasitoids to overcome periods of marked host scarcity given that guava is the last widely available host plant before the winter season begins.

Key words biological control, Braconidae, Figitidae, fruit fly, host–parasitoid interaction.

INTRODUCTION

Several hymenopteran parasitoid species have been associated with fruit-infesting tephritids in the neotropical region. Most of these parasitoids have been recorded parasitising fruit fly species in the genus *Anastrepha* Schiner (Wharton *et al.* 1998; Canal & Zucchi 2000; Ovruski *et al.* 2000; Aluja *et al.* 2003), which is endemic to the New World (Norrbom 2004). Approximately 60% of the total parasitoid species recorded from *Anastrepha* belong to Braconidae, and within this family 82% of the species belong to the Opiinae subfamily (Wharton 1997; Ovruski *et al.* 2000; Schliserman *et al.* 2010). Some of these Opiinae species, such as *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti), *Opius bellus* Gahan and *Utetes anastrephae* (Viereck), as well as the figitid eucoiline *Aganaspis pelleranoi* (Brèthes), are

commonly found throughout north-western Argentina and are known to attack the native South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Ovruski *et al.* 2004, 2005). This tephritid species and the exotic Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), are among the most important pests affecting commercial fruit production in Argentina (Guillén & Sánchez 2007).

The original native vegetation in north-western Argentina is subtropical montane rainforest, locally known as ‘Yungas’ (Brown *et al.* 2001), which is characterised by a high diversity of *A. fraterculus* host plants growing in the remaining stands of pristine forest and in disturbed native vegetation areas surrounding citrus crops and orchards (Ovruski *et al.* 2003). This phytogeographical region represents an important source of neotropical parasitoids of the genus *Anastrepha*. It is in these areas that *A. fraterculus* populations grow and from which individuals move to lay eggs on the introduced fruit crops (Schliserman 2005). Recent laboratory studies have revealed that two local species of native parasitoids, *A. pelleranoi* and

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O. bellus, could be used for starting a mass-rearing system focused on developing a biological control program either augmentative or conservative or both (Núñez-Campero *et al.* 2014).

Conservation measures can contribute to safer and more effective biological control practices if there is prior knowledge of the relationships between the pest and its natural enemies (Landis *et al.* 2000). Therefore, it is essential to deepen the studies on the biology and ecology of parasitoids associated with *A. fraterculus* in their native habitat. The lack of ecological information on these natural enemies can lead to failures in establishing biorational methods for controlling pestiferous tephritids (Aluja & Rull 2009). Diapause is an important ecological aspect to study due to the close relationship between tephritid fly and their parasitoids.

Many insects that are required to overcome stressful periods have diapause. This phenomenon involves the disruption of morphogenesis not directly controlled by local environmental factors (Tauber *et al.* 1983, 1986). Diapause induction occurs in a genetically predetermined stage of the insect life cycle and occurs in anticipation of environmental stress (Kostal 2006). Diapause may implicate growth retardation during a season with unfavourable environmental conditions (such as winter), or during 1 year or more. The former type is described as simple diapause, while the latter is named as prolonged diapause (Tauber *et al.* 1986; Turgeon *et al.* 1994; Corley *et al.* 2004). Diapause regulation may be mediated by environmental, hormonal and molecular regulators (Danks 1987; Denlinger 2002).

Diapause in fruit fly parasitoids has been mainly reported in temperate regions (Prokopy 1968; Maier 1981; AliNiazee 1985; Hoffmeister 1992; Gut & Brunner 1994), but this phenomenon has also been recorded in tropical environments (Pemberton & Willard 1918; Darby & Knapp 1934; Aluja *et al.* 1998; Carvahlo 2005). Aluja *et al.* (1998) and Carvahlo (2005) working in tropical forests from Mexico and Brazil, respectively, found ecological evidence for diapause in *D. areolatus*, *U. anastrephae* and *A. pelleranoi*, three of the five most abundant parasitoid species spread throughout Yungas forest in north-western Argentina. In addition, Ovruski *et al.* (2004) also found that Argentinean populations of both *D. areolatus* and *A. pelleranoi* exhibited diapause, but this was not exhibited by *D. brasiliensis*, *O. bellus* or *U. anastrephae*.

Therefore, the prediction that all parasitoid species associated with *A. fraterculus* in the southernmost sector of the Yungas forest would display diapause was tested. This prediction was based on two scenarios: (1) the bioclimatic conditions of the austral sector of the Yungas, in which a dry-cold season alternating with a rainy-warm season, but with significant variations of temperature within each season (Brown *et al.* 2001; Ovruski *et al.* 2004), and (2) the fruiting phenology of *A. fraterculus* host plants, which involves an extended period of suitable host unavailability (Ovruski *et al.* 2003). Particularly, the study aimed at determining (1) the incidence of diapause (percentage of individuals entering diapause) in each parasitoid species collected from the three major *A. fraterculus* host plants, (2) the incidence of diapause according to the

fruiting period of each fruit species sampled, (3) the length of diapause period for each parasitoid species, and (4) the proportion of diapausing parasitoid individuals that displayed prolonged diapause.

MATERIALS AND METHODS

Fruit collection sites

The sites of study are located between 26°45' and 26°49' S in latitude and 65°20' and 65°18' W in longitude, with an elevation ranging from 600 to 800 m, a total surface of 26 km² on the eastern slope of the San Javier Mountain within both 'Sierra de San Javier Park' and 'Horco Molle Natural Reserve', protected natural areas in the locality of Horco Molle, Tucumán Province, north-western Argentina. These collecting areas are located at the southernmost end of the Yungas rainforest and are characterised by disturbed secondary vegetation, with exotic and native plant species combined. The region has a temperate-warm humid climate, with a rainy-warm season from October to April and a dry-cold season from May through September. Mean annual rainfall is between 1300 and 1600 mm. Mean annual rainfall ranges between 1300 and 1600 mm, while mean temperature in the warmest month is 24°C, and mean annual temperature is 18°C (Sesma *et al.* 1998). Mean weekly temperature, day length (Fig. 1a), relative humidity and accumulative rainfall (Fig. 1b) were

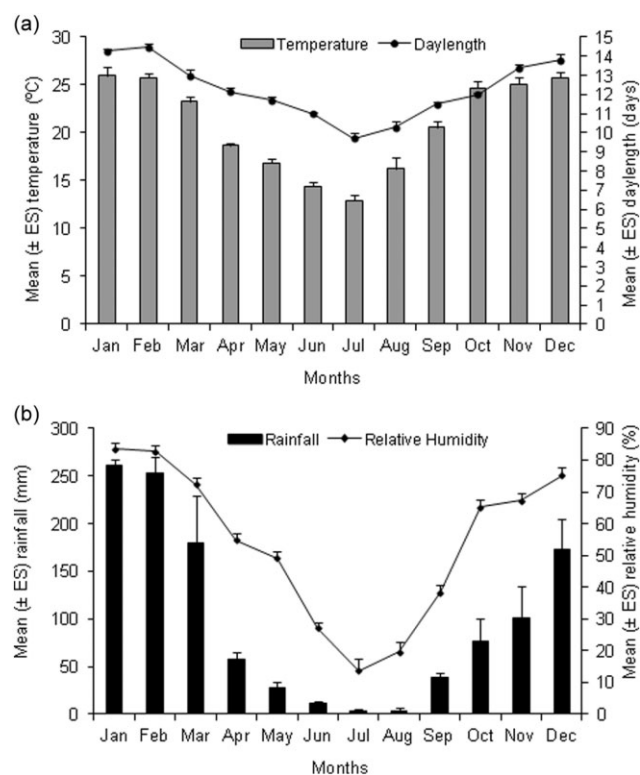


Fig. 1. Mean (\pm ES) weekly temperature ($^{\circ}$ C), day length (days) (a), accumulative rainfall (mm) and relative humidity (%) (b) in the study area throughout 2001, 2002 and 2003.

recorded throughout 2001, 2002 and 2003 in study areas. Data were provided by the local weather station in the Sierra de San Javier Park belongs to the National University of Tucumán.

Fruit sampling and processing

From December 2001 to December 2003, ripe fruits of *Prunus persica* (L.) Batsch (peach) (Rosaceae), *Psidium guajava* L. (guava) (Myrtaceae) and *Juglans australis* Grisebach (wild walnut) (Juglandaceae) were weekly and randomly collected from the canopy of trees and the ground below when available. These three fruit species are among the most common and widespread *A. fraterculus* host plants growing in wild vegetation areas in Argentina's north-western region, and harbour a large parasitoid assemblage (Ovruski *et al.* 2004). The fruit collecting period comprised the peach, wild walnut and guava fruiting months in the study region, which ranged from November to January, from December to February, and from February to May, respectively. Fruit was collected according to availability during fruiting period, and the number varied between 50 and 300 per week and per fruit species. Fruit harvested from the tree canopy and fruit that had fallen from the tree to the ground were included in the same sample. Fruit samples were placed in cloth bags. One half of each fruit sample was directly processed in the 'Horco Molle Natural Reserve' and maintained under natural ambient conditions in a warehouse. Fruit of each sample were weighed, placed in a plastic crate (48 × 28 × 15 cm) with a slotted bottom, and placed on top of another plastic crate (48 × 28 × 15 cm) with a non-perforated bottom lined with 2 cm of moistened sand as a pupation medium for the fly larvae. Both of the crates were fully covered with an organdy cloth lid. During 1 month, fruit samples were kept in the warehouse and the sand was sifted every 2 days to collect *A. fraterculus* puparia. The remaining halves of fruit samples were transported to the laboratory at the Research Centre for Harmful Organism Population Regulation (CIRPON) belongs to the Miguel Lillo Foundation. This institution is located in San Miguel de Tucumán between 26°48' S latitude and 65°14' W longitude, with an elevation of 482 m. Fruit processing method was identical to that described above, but the fruit samples were always kept inside a room at 25 ± 1°C, 75 ± 2% relative humidity and a photoperiod of 14 : 10 (L : D) h.

Experimental procedures

The *A. fraterculus* puparia recovered from the crates kept in the 'Horco Molle Natural Reserve' were transferred to plastic containers (500 mL) with 200 mL of soil in the bottom (ca. 500 pupae per container). The soil used was from the site where the fruits had been collected. The containers were covered with organdy cloth and it was tied with a rubber band. The containers were then placed inside a wooden frame cage (65 × 67 × 70 cm) covered with a cloth mesh (1 mm), until fly or parasitoid emergence. This emergence cage was placed 80 cm above the ground below a guava tree in the same place where the fruits had been collected. The cage was protected

from rainfall with corrugated fibreglass. 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 the adults (alive and dead) were removed, counted and identified by the authors Pablo Schliserman and Sergio Ovruski. Voucher specimens were placed in the entomological collection of the Miguel Lillo Foundation in Tucumán, Argentina. Unenclosed puparia were kept in the containers for 2 years at the collection site to determine if they contained diapausing parasitoids. Puparia recovered at the CIRPON's laboratory were processed as described above but were maintained under controlled environment conditions (25 ± 1°C, 75 ± 2% RH, 14 : 10 (L : D) h) throughout 2 years. Separate subsamples of 25 non-diapausing and 25 diapausing parasitoids were measured for size comparison. Non-diapausing parasitoids were taken from unenclosed *Anastrepha* puparia kept under controlled laboratory conditions, whereas diapausing parasitoids were taken from unenclosed puparia kept for 1 year under natural environmental conditions.

Data analysis

For data analysis, the percentage of diapausing parasitoids and pre-imaginal development time (egg to adult) of both non-diapausing and diapausing parasitoids were estimated. The percentage of diapausing parasitoids was calculated as the number of parasitoid individuals entering diapause from the total number of parasitoids (non-diapausing and diapausing) recovered under both controlled (laboratory) and natural (field) conditions. The proportion of individuals entering either simple diapause or prolonged diapause over the total diapausing parasitoid population was also calculated. The sex ratios were recorded in all parasitoid species. Pearson's chi-square tests ($P = 0.05$) were performed to statistically compare the sex ratio in adult parasitoids recovered from diapause periods, as well as between the number of individuals that entered simple diapause and prolonged diapause. The non-diapausing and diapausing parasitoid size was compared using a *t*-test at $P = 0.05$. Statistical analysis was performed using the data analysis software system STATISTICA, version 8.0 (StatSoft, Inc. 2007).

RESULTS

A total of 24 246 fruits (848.3 kg) corresponding to the three major host plants species of *A. fraterculus* was processed during the 25-month study period. The total number of *Anastrepha* puparia recovered from the infested fruit was 121 799. Of this total, 62 727 (~51.5%) were *A. fraterculus*, 826 (~0.7%) *A. schultzi*, 4789 (~3.9%) hymenopterous parasitoids, and from 53 457 puparia (~43.9%) no insects emerged (Table 1). Considering all adult parasitoids recovered, 37.3% were *A. pelleranoi* of which 58.2% were females,

Table 1 Total number of *Anastrepha* pupae, fly adults and parasitoid individuals recovered from three *A. fraterculus* host plants collected (*Juglans australis*, *Prunus persica* and *Psidium guajava*) between 2001 and 2003 in the austral sector of Argentinean Yungas forest and kept under both controlled (laboratory) (CLC) and natural environmental conditions (NEC)

Host plant species	No. of fruit sampled	Weight (kg) of fruit	No. of <i>Anastrepha</i> pupae recovered under:		No. of <i>Anastrepha</i> [†] adults recovered under:		Parasitoid species	No. of individuals of each parasitoid species [‡] recovered under:		Per cent and (no.) of parasitoid entering diapause recovered under NEC
			CLC	NEC	CLC	NEC		NEC	CLC	
<i>J. australis</i>	6842	194.5	9031	11 593	3808	3606	<i>Ap</i>	77	102	2.6 (2)
							<i>Da</i>	37	45	0.0
							<i>Db</i>	310	269	9.4 (29)
							<i>Ob</i>	42	31	7.1 (3)
<i>P. persica</i>	9518	394.2	11 523	13 816	5316	6701	2	9	0.0	
						<i>Ap</i>	328	362	2.4 (8)	
						<i>Da</i>	354	232	0.8 (3)	
						<i>Db</i>	342	128	1.8 (6)	
<i>P. guajava</i>	7886	259.6	35 153	40 683	20 105	24 017	8	2	0.0	
						<i>Ua</i>	28	12	0.0	
						<i>Ap</i>	505	412	14.7 (74)	
						<i>Da</i>	435	303	3.7 (16)	
							<i>Db</i>	109	127	8.3 (9)
							<i>Ob</i>	26	34	11.5 (3)
							<i>Ua</i>	67	51	10.4 (7)

[†]Two *Anastrepha* species were recovered from *J. australis* (98.7% *A. fraterculus*, 1.3% *A. schultzei*); from both *P. guajava* and *P. persica* was only *A. fraterculus* recovered. [‡]Non-diapausing and diapausing parasitoids. *Ap*, *Agamaspis pelleranoi*; *Da*, *Doryctobracon areolatus*; *Db*, *Doryctobracon brasiliensis*; *Ob*, *Opius bellus*; *Ua*, *U. anastrephae*.

Table 2 Comparison of pre-imaginal development times (from egg to adult) of non-diapausing parasitoid individuals collected in the field and kept under laboratory conditions or under field conditions, and diapausing parasitoid individuals kept under field conditions

Parasitoid species	Mean (\pm SE) pre-imaginal development time under varying rearing conditions					
	Non-diapausing parasitoid				Diapausing parasitoid	
	CLC†		NEC‡		NEC§	
	Tmax = 26°C Tmin = 24°C Tmean (\pm ES) = 25.1 \pm 0.1°C RH (\pm ES) = 75.1 \pm 0.4%		Tmax = 40.8°C Tmin = 6.2°C Tmean (\pm ES) = 23.9 \pm 0.3°C RH (\pm ES) = 65.5 \pm 1.4%		Tmax = 40.8°C Tmin = 1.8°C Tmean (\pm ES) = 21.0 \pm 0.5°C RH (\pm ES) = 58.9 \pm 1.1%	
	PDT (days)	No.¶	PDT (days)	No.	PDT (days)	No.
<i>A. pelleranoi</i>	29.5 \pm 0.7	60	31.8 \pm 0.6	60	305.1 \pm 5.7	57
<i>D. areolatus</i>	25.4 \pm 0.3	60	27.6 \pm 0.3	60	289.1 \pm 13.4	19
<i>D. brasiliensis</i>	26.8 \pm 0.5	60	29.9 \pm 0.7	60	277.3 \pm 12.3	44
<i>O. bellus</i>	22.5 \pm 0.4	40	27.2 \pm 0.3	35	311.2 \pm 16.3	6
<i>U. anastrephae</i>	24.4 \pm 0.5	40	26.8 \pm 0.3	35	239.3 \pm 5.5	7

†Controlled laboratory conditions. ‡Natural environmental conditions (from November to April; adult emergence period). §Natural environmental conditions (from January to December; adult emergence period). ¶n, total number of parasitoid individuals that emerged from *A. fraterculus* puparia that the sampled fruit had yielded. PDT, pre-imaginal development time; RH, relative humidity; Tmax, maximum temperature; Tmean, mean temperature; Tmin, minimum temperature.

29.4% *D. areolatus* of which 48.7% were females, 26.8% *D. brasiliensis* of which 56.3% were females, 3.5% *U. anastrephae* of which 60.8% were females, and 3.0% *O. bellus* of which 45.2% were females (Table 1). All these species, which are koinobiont larval–prepupal endoparasitoids, exhibited diapause. There was no significant evidence of deviation from a sex ratio of 1 : 1 in adult parasitoids recovered from diapause periods ($\chi^2 = 0.40$, Pearson's coefficient = 0.05, $df = 1$, $P = 0.527$).

Overall (taking into account all diapausing parasitoid species recovered from all host fruit species), *A. pelleranoi* was the species with the highest proportion of individuals entering diapause (52.5%), followed by *D. brasiliensis* (27.5%), *D. areolatus* (11.9%), *U. anastrephae* (4.3%) and *O. bellus* (3.8%). Nonetheless, this trend varied according to the *Anastrepha* host fruit species. For example, *D. brasiliensis* had the highest proportion of diapausing individuals stemming from *Anastrepha* larvae that had developed in *J. australis* (Table 1).

A striking contrast between pre-imaginal development time in diapausing and non-diapausing individuals was determined (Table 2). Of the five parasitoid species recovered under controlled laboratory conditions, non-diapause pre-imaginal development times fluctuated between 22.5 and 29.5 days. Such development periods were similar to those exhibited by parasitoid individuals recovered under natural environmental conditions but which did not enter diapause (Table 2). Overall, the pre-imaginal development times of diapausing individuals lasted an average of 216.8–282.7 more days than those recorded from non-diapausing individuals (Table 2).

When *Anastrepha* puparia that contained diapausing parasitoid were dissected, only third instars were found. Diapausing parasitoid larvae were also significantly distinguished from non-diapausing larvae due to their smaller body size (3.6 \pm 0.04 mm for non-diapausing larvae, 2.7 \pm 0.06 mm for diapausing larvae, $t = 16.0$, $df = 48$, $P < 0.0001$, $n = 25$).

Individuals of *D. brasiliensis*, *A. pelleranoi* and *D. areolatus* exhibited prolonged diapause, and it had prevalence from 27.6%, 11.3% and 10.5% of the diapausing population of each respective parasitoid species. However, statistical analyses showed that significantly more parasitoids entered in simple diapause (Table 3). Prolonged diapause varied between 369 and 426 days in *D. brasiliensis*, 369 and 373 days in *D. areolatus*, and 365 and 387 in *A. pelleranoi*.

Although diapausing individuals were found between December and April, most of them were commonly recorded during March and April (Table 4). However, 65% of diapausing parasitoids were only recorded in April. These individuals were recovered from *A. fraterculus* larvae that had developed in *P. guajava*. Emergence schedules of diapausing individuals showed different trends among parasitoid species. *Aganaspis pelleranoi* exhibited a series of peak emergences from October to April, but the highest proportion of adults (67%) emerged between January and March (Fig. 2a). *Doryctobracon areolatus* exhibited an emergence period relatively shorter than that of *A. pelleranoi* because adults emerged between November and March. However, the highest proportion of adults (74%) also emerged between January and March (Fig. 2b). The longest emergence period of diapausing individuals was recorded in *D. brasiliensis*. Adults of this braconid parasitoid species emerged between September and April, but the highest proportion (84%) emerged between September and January (Fig. 2c). The shortest emergence period of diapausing individuals was exhibited by *O. bellus*. Adults emerged from December (37%) to January (67%) (Fig. 2d). Similarly to both *A. pelleranoi* and *D. areolatus*, diapausing individuals of *U. anastrephae* emerged between November and March. However, proportion of emerged adults was similar among the different months (Fig. 2e). None of the two *Anastrepha* species (*A. fraterculus* and *A. schultzi*) serving as host of the parasitoids entered diapause. All adult flies emerged 26–46 days after pupation under field environmental conditions.

Table 3 Summary of Pearson's chi-square tests on the number of individuals entering simple and prolonged diapause in each parasitoid species

Parasitoid species	Type of diapause and no. of diapausing individuals emerged from host puparia		χ^2	Pearson's coefficient	df	P
	Simple	Prolonged				
<i>A. pelleranoi</i>	74	10	48.76	0.61	1	<0.0001†
<i>D. areolatus</i>	17	2	11.84	0.62	1	<0.0001†
<i>D. brasiliensis</i>	32	12	9.09	0.41	1	<0.0001†
<i>O. bellus</i>	6	0	–	–	–	–
<i>U. anastrephae</i>	7	0	–	–	–	–

†Significant variables.

Table 4 Incidence of diapause in each parasitoid species according to fruiting season of each *A. fraterculus* host plant sampled during the study

Time of year (month) and fruit species available†	Per cent individuals entering diapause for each parasitoid species‡				
	<i>A. pelleranoi</i>	<i>D. areolatus</i>	<i>D. brasiliensis</i>	<i>O. bellus</i>	<i>U. anastrephae</i>
December (<i>Pp</i> and <i>Ja</i>)	2.6 (302)	0.8 (119)	3.1 (160)	0 (12)	0 (13)
January (<i>Pp</i> and <i>Ja</i>)	1.9 (107)	0.7 (271)	7.3 (254)	6.4 (31)	0 (7)
February (<i>Ja</i> and <i>Pg</i>)	0 (13)	0 (4)	0 (67)	14.3 (7)	0 (0)
March (<i>Pg</i>)	10.2 (447)	3.6 (393)	8.1 (99)	8.7 (23)	10.2 (59)
April (<i>Pg</i>)	46.2 (39)	5.6 (36)	40.0 (10)	33.4 (3)	12.5 (8)
May (<i>Pg</i>)	0 (2)	0 (3)	0 (0)	0 (0)	0 (0)

†Months with fruit available for oviposition of *A. fraterculus* females. ‡Numbers in parenthesis represent total number of parasitoids (diapausing and non-diapausing individuals) recovered from each collecting month under natural environmental conditions. *Pp*, *Prunus persica*; *Ja*, *Juglans australis*; *Pg*, *Psidium guajava*.

DISCUSSION

This study reports for the first time diapausing individuals in *O. bellus*, *D. brasiliensis* and *U. anastrephae*, parasitoid species associated with *A. fraterculus* in the southernmost section of Yungas. In addition, the present study confirmed that the most abundant native parasitoid species in this Argentinean region, *A. pelleranoi* and *D. areolatus*, exhibited diapause, as reported previously by Ovruski *et al.* (2004). These findings contrast with information on parasitoid species recovered from *A. fraterculus* puparia in the northernmost Yungas of Argentina, a forest region close to the border with Bolivia where environmental conditions are warmer and wetter (Ovruski *et al.* 2005). None of the five parasitoid species here studied exhibited diapause in that sector of Yungas. As suggested by Aluja *et al.* (1998) working in tropical sub-deciduous and deciduous forests in Veracruz, Mexico, this difference between regions may be the result of different patterns of host fruit abundance throughout the year as well as by disparities in environmental conditions. Similar to those reported by Aluja *et al.* (1998) for Mexico and Carvahlo (2005) for Brazil, diapause was exclusively found in parasitoids.

The emergence of diapausing individuals was distributed between spring (September–December), summer (January–March) and early autumn (April). This emergence pattern coincides with the fruiting periods of most host plants of *A. fraterculus* widely dispersed in the southern sector of Yungas (Ovruski *et al.* 2003). For example, between September and October, it is possible to find an abundance of ripe fruit

of the exotic species *Eriobotrya japonica* [Thunb.] Lindley, and also of the native *Eugenia uniflora* L.; November and December mark the fructification of exotic plants *Prunus domestica* L. and *P. persica* and the indigenous species *Myrcianthes pungens* (Berg.); between December and February, ripe fruits of *J. australis* are abundant, whereas from February fructification of both *Annona cherimola* and *P. guajava* occurs. Both plant species may keep ripe fruit until early May (Ovruski *et al.* 2003). In contrast, between May and August (late autumn and winter), there is a predominance of solely ripe citrus fruits, such as *Citrus aurantium* L. (Rootstock), *C. paradisi* Macfadyn, *C. reticulata* Blanco, *C. sinensis* (L.) Osbeck and *C. limon* (L.) Burman, which comprise suitable hosts for the development of *A. fraterculus* larvae (Ovruski *et al.* 2003, 2004; Segura *et al.* 2006). Furthermore, as pointed out by Schliserman (2005), the *A. fraterculus* adult population levels are extremely low from May to August but particularly high throughout the summer in the austral sector of the Yungas forest. In addition, Schliserman (2005) highlighted that larval infestation levels significantly increase from spring to summer in this section of Yungas. Given the discontinuity of the availability of *A. fraterculus* larvae brought about by the winter's scarcity of suitable host fruits, appears to be need for the five parasitoid species recovered in the Yungas enter diapause in the study region. Coinciding with the statements above, when examining the emergence schedules of diapausing individuals of the five parasitoid species, it is noted that diapause ends coinciding with the time of the year in which fruit infestations by fly larvae are increased.

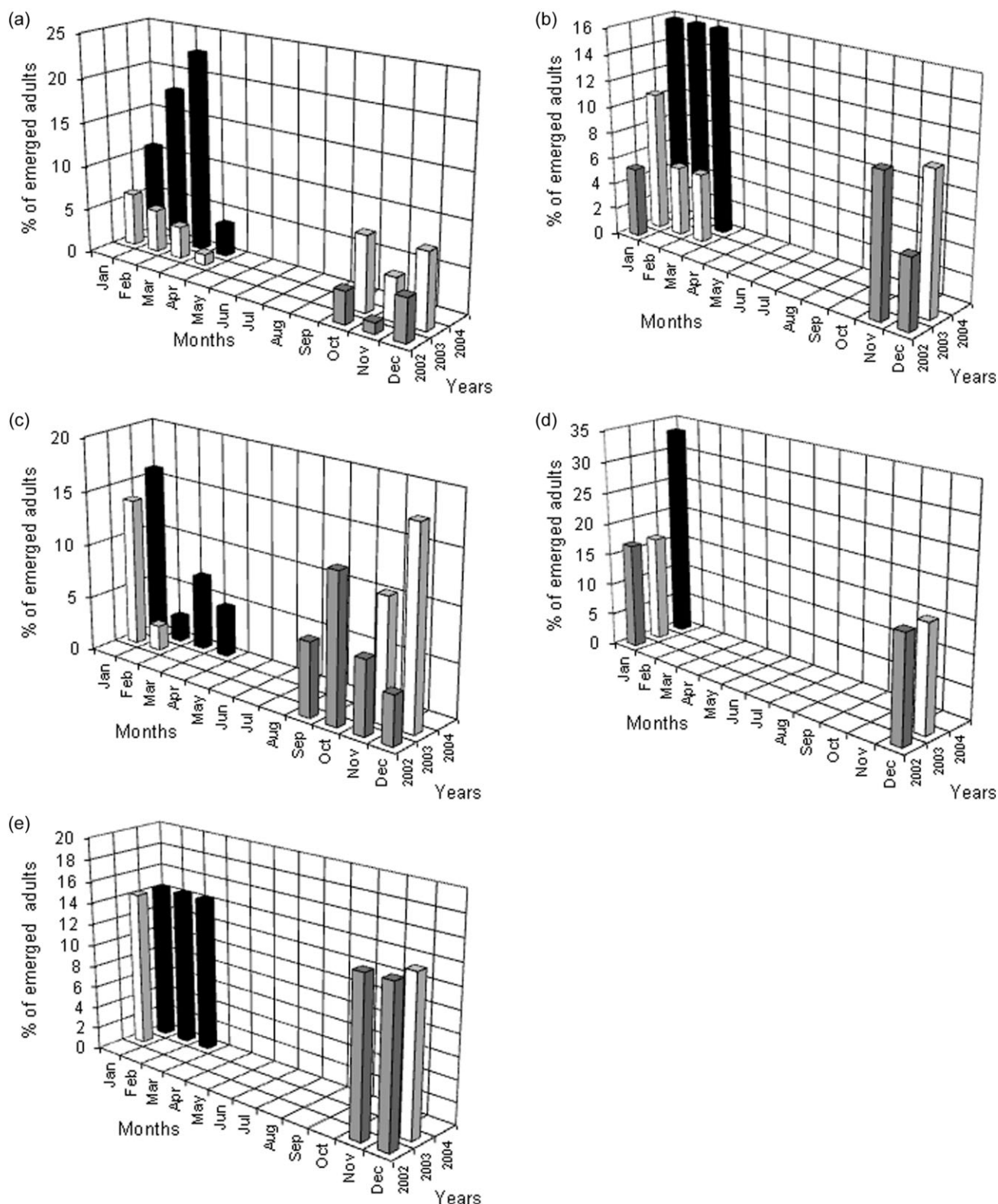


Fig. 2. Emergence schedules of *A. pelleranoi* (a), *D. areolatus* (b), *D. brasiliensis* (c), *O. bellus* (d) and *U. anastrephae* (e) diapausing individuals stemming from three *A. fraterculus* host plants (*Juglans australis*, *Prunus persica* and *Psidium guajava*).

It should be pointed out that the lack of host fly larvae during the lean period of fruit species suitable for the development of the pest was closely associated with marked temporal changes in certain environmental abiotic factors, such as

the decrease of both temperature and rainfall, low relative humidity, and day length drop. Both scenarios would facilitate diapause induction in all of the *A. fraterculus* parasitoid species discussed here. Although this study only provides

comparative evidence by biological material kept under field and laboratory conditions, it is possible to consider the influence of the abovementioned abiotic stimuli in the induction of diapause. Those parasitoid individuals stemming from host larvae that had developed in late fruiting species such as *P. guajava* in early autumn (April) were more likely to enter diapause. For example, the largest proportion of diapausing individuals of each parasitoid species was recorded in April. During this time of year, the mean temperature was below 19°C, day length was shorter than summer months (Fig. 1a), relative humidity was about 55%, and the accumulated rainfall was lower than 60 mm (Fig. 1b). All these abiotic factors decrease during the period of April–August. Despite the fact that diapause needs to be more thoroughly studied in fruit fly parasitoid species from the Yungas forest, it is highly likely that this phenomenon is an adaptive mechanism that allows them to overcome periods of pronounced host scarcity given that *P. guajava* is the last widely available *A. fraterculus* host plant before the winter season begins in June.

Parasitoid individuals of the five parasitoid species recovered from laboratory conditions never entered diapause. Under artificial conditions, temperature, relative humidity and photoperiod were kept relatively constant when compared with field environmental conditions. However, Aluja *et al.* (1998) studying a guild of *Anastrepha* parasitoids in a tropical rainforest from Mexico recorded diapausing individuals of both *U. anastrephae* and *D. areolatus* under laboratory conditions. Probably, certain cues important for the induction of diapause were present under artificial conditions and affected the development time of both *D. areolatus* and *U. anastrephae*. For example, Aluja *et al.* (1998) considered it likely that diapause in *D. areolatus* individuals was due to a noticeable decrease in the day length, which might have been perceived through the laboratory windows. In the present study, the *A. fraterculus* puparia were kept in a room completely isolated from external light and with a photoperiod of 14 : 10 (L : D) h exclusively provided by artificial light six fluorescent tubes of 60 W each one. Several authors (Pemberton & Willard 1918; Darby & Knapp 1934; Ashley *et al.* 1976) related the incidence of diapause in fruit fly parasitoid flies with soil moisture and low temperature. Similarly, Aluja *et al.* (1998) found that a higher proportion of *Anastrepha* parasitoid individuals entered diapause in areas with mean temperature lower than 20°C but with high rainfall. This only partially coincides with findings recorded in the present study. The increase in diapausing individuals appeared to be related to the decrease in temperature, as well as the reduction in relative humidity in the study region.

Interestingly, diapause duration varied not only among different parasitoid species but also within the same species. The majority of diapausing individuals (86%, involving the five parasitoid species found in this study) extended diapause for a given predictable unsuitable season, like winter, whereas a minor proportion (including only *D. brasiliensis*, *D. areolatus* and *A. pelleranoi*) extended diapause between 12 and 14 months. A very long diapause period in *D. areolatus* (414 days) was previously reported by Carvahlo (2005) working in

a tropical forest from north-eastern Brazil, whereas Aluja *et al.* (1998) in Central Mexico reported that diapause in both *D. areolatus* and *A. pelleranoi* is common and that it can last up to 11 months. Prolonged diapause enables the emergence of a given genotype of the insect population to provide a better emergency distribution in time, thereby allowing certain progenies to reproduce in more suitable conditions (Hanski 1988; Hopper 1999; Menu *et al.* 2000), whether it is at the competitive level regarding its peers, increased availability of hosts or by abrupt environmental changes. Simultaneously, prolonged diapause may have consequences on the dynamics of populations interacting in a host–parasitoid system (Ringel *et al.* 1998). These authors suggested that prolonged diapause markedly increases the stability of host–parasitoid interaction. Nonetheless, Corley *et al.* (2004) suggested that prolonged diapause does not necessarily compensate for the destabilising effects of time lags on the influence of parasitoids on the dynamics of interacting populations.

Recent studies on local parasitoid species associated with *A. fraterculus* (Núñez-Campero *et al.* 2012, 2014; Schliserman *et al.* 2014) open up the possibility of implementing a native parasitoids mass-rearing and release program in Argentina. Therefore, diapause schedules of parasitoids should be specifically considered in future native parasitoid release programs. Diapause is a significant ecological attribute because a portion of the released population would be able to remain in the release area during unfavourable weather.

The five parasitoid species recorded in this study exhibited diapause. However, less than 10% of total recovered individuals entered diapause. The figitid *A. pelleranoi* showed the highest proportion of diapausing individuals (9.2% of the total individuals), whereas *D. areolatus* had the lowest proportion (2.3%). Only parasitoid individuals kept under natural environmental conditions exhibited diapause. In general, diapause patterns were polymodal exhibiting different peak emergences, although most of them occurred in early autumn. Guava was the *Anastrepha* host fruit species that yielded the highest proportion of diapausing parasitoid individuals. Preimaginal development times in diapausing parasitoids varied between 239 and 311 days, depending on the parasitoid species. Prolonged diapause was a common condition but in a small fraction of the diapausing populations of *D. brasiliensis*, *D. areolatus* and *A. pelleranoi*, three *A. fraterculus* parasitoid species widely spread over Yungas. In general, this prolongation of diapause is for a single year. The decrease of both temperature and relative humidity, as well as the reduction in day length in the study area, would facilitate diapause induction in all of the *A. fraterculus* parasitoid species studied here.

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