

Full Length Research Paper

Biological control of *Anastrepha* Schiner (Díptera: Tephritidae) through augmentative releases of *Diachasmimorpha longicaudata* Ashmead (Hymenoptera; Braconidae) in fruit-producing marginal areas of Northern Nayarit, México

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In Mexico, fruit flies (*Anastrepha* Schiner) are one of the main pests that affect production and limit the commercialization of fresh fruits. In regions producing municipalities such as Ruiz (Nayarit), the insect is actively multiplying in marginal areas, in fruits such as *Mangifera indica* L. (mango), *Psidium guajava* L. (guava), *Spondias* sp. (jobo) and *Psidium sartorianum* (Berg), from where they move to commercial orchards. An alternative solution is the use of biological control agents, which is why, in 2012, the population fluctuation of *Anastrepha* species and the effectiveness of augmentative releases of the parasite, *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae) on the pest were determined. Of 2,853 fruits and 1,690 larvae, 740 adults of *Anastrepha* emerged (395 females, 345 males: sexual ratio of 1: 0.87): *Anastrepha obliqua* (422), *Anastrepha striata* (208) and *Anastrepha ludens* (110), with 545 parasitoids recovered (265 females, 280 males: sexual ratio 1: 1.06), and annual mean parasitism of 42.41%, where the highest value corresponded to December (69.57%) and the greatest impacts of parasitism was on *P. sartorianum* (62.50%).

Key words: Fruit flies, Diptera-Tephritidae, *Anastrepha* species, Hymenoptera-Braconidae, parasitoid releases, parasitism.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are major pests affecting fruits (Aluja, 1993), and are among the 10 most important agricultural pests affecting the world economy (Purcell,

2005). Therefore, its presence requires implementing effective methods of control, with minimal side effects on the ecosystem. In spite of the traditional use of toxic bait

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sprays, which, although effective, haul problems encountered in society for apparent pollution to the environment and toxicity to the associated entomofauna, there is search for alternatives that can minimize these effects (Montoya and Cancino, 2004).

In Mexico, species of economic importance included in the genus *Anastrepha* Schiner, constitute a limitation to the production and commercialization of fresh fruits (Aluja, 1994; SAGARPA, 1999). The presence of fruit flies causes damage by feeding on fruit, and consequently, economic losses if control is not efficient (SENASICA, 2012a). Based on the current problem, since 1992, the Federal Government through the National Service of Health, Safety and Agro-Food Quality launched the National Campaign against Fruit Flies (NCFE), with the aim of conserving and/or improving phytosanitary status and developing the competitiveness of national agriculture, with actions aimed at controlling, suppressing and eradicating species of fruit flies. The strategy is based on a concept of integrated pest management, including trapping (adult) and fruit sampling (for larvae) and mechanical, chemical, autocidal and biological control methods (SAGARPA, 1999; SENASICA, 2014). Of the 200 species of *Anastrepha* identified in Mexico, the main species recognized as pests are *Anastrepha striata* Schiner (guava), *Anastrepha obliqua* (Macquart) (mango and jobo), *Anastrepha ludens* (Loew) (bitter orange, lime and sweet orange), *Anastrepha serpentina* (Wiedemann) (caimito, medlar and sapote) and *Anastrepha fraterculus* (Wiedemann) (peach, mango, and jobo) (Aluja, 1994).

As an essential element within the pest management component, parasitoids are considered as efficient candidates for biological control of insects (Liu et al., 2015). In this sense, biological control represents one of the strategies with greater ecological orientation that can be integrated with control programs or eradication (Montoya and Cancino, 2004). In the case of fruit flies, induced biological control is an alternative to provide control solutions based on reports indicating low effectiveness of classical biological control in cases such as Florida, USA (Montoya and Toledo, 2010). Classical biological control does not represent a viable option against the tephritid by low levels of parasitism (Wharton, 1989), which is why the development strategy of the mass rearing of different species of parasitoids is initiated for its periodic release in the field; thus, as part of the strategy of actions of the NCFE in Mexico is conduction of massive releases with the solitary endoparasitoid *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae), native to Southeast Asia, introduced to the country in 1992 and raised massively since 1993 on the ground of fruit flies (Moscafrut), located in Metapa de Domínguez, Chiapas, where implementation requires the handling of large amounts of parasites that are released in field, as an efficient way to ensure the maintenance of its main biological attributes (SENASICA, 2012b).

In Nayarit, land releases were carried out since 1996 within urban and marginal areas bordering on commercial mango, belonging to municipalities such as Tepic, Compostela, Banderas Bay, San Blas, Tecuala and Acaponeta. In January, 2005, it was published in official journal of the federation, an agreement of the declaration of area of low prevalence in fruit flies in seven municipalities in the north of the state (SAGARPA, 2005). From the declaration of the agreement, the strategy was to intensify the release of parasitoids, but to date, no evaluation has been carried out to determine the impact of the parasitoid on the insect pest.

The objective of this study was to assess the effectiveness of augmentative releases of *D. longicaudata* made during 2012 on populations of native species of *Anastrepha* in marginal areas of the municipality of Ruiz, Nayarit, México.

MATERIALS AND METHODS

Study site

The parasitoid releases were carried out in 2012, in 16 localities: El Pozolillo, El Zopilote, El Cordón del Jilguero, El Quemado, El Volantín, El Caracol, La Copa, Puerta de Platanares, Patolpa, Laguna del Mar, San Pedro Ixcatán, El Taixte, Presidio de los Reyes, La Bolita, El Refugio and El Carrizal. The study region is located in the north-central part of the state, between the extreme geographical coordinates: 22° 10' to 21° 52' of latitude north and 104° 47' to 105° 14' of longitude west, with an average height of 20 masl (<http://www.ocdemexico.org.mx/Nayarit/Ruiz/>).

Fruit species considered in release

The augmentative releases of the parasitoid were carried out on various fruit species, such as creole mango (*Mangifera indica* L.), Anacardiaceae), guava (*Psidium guajava* L.: Myrtaceae), jobo (*Spondias* sp.: Anacardiaceae), myrtle (*Psidium sartorianum* (Berg) Niad: Myrtaceae), sour orange (*Citrus aurantium* L.: Rutaceae) and grapefruit (*Citrus paradisi* Macfad: Rutaceae). These fruit species are found in marginal areas in the mountainous area of the municipality of Ruiz, Nayarit, México.

Reception, packaging and release of parasitoids

The parasitoids were received weekly from the mass production plant located in Metapa de Domínguez, Chiapas. Previously, the quality of each shipment was verified, as much the physical state of the boxes and seal of irradiation for zones with similar temperature. The biological material was transported terrestrially in vehicles equipped with thermo-king (22 ± 2°C) to the facilities of the Adult Cold Packing Center (PCAC) in Tuxpan, Nayarit, in charge of the State Committee on plant health in the State of Nayarit. In this center, temperature was recorded for 11% of the containers with pupae for quality control; the contents of material was subsequently placed in a single box and weighed to verify the data of the plant. Material was deposited in kraft paper bags No. 20 with reinforced base KP 80-68 21 × 12 × 39 cm, and placed on average of 3,500 parasites per bag. As recommended by Montoya and Toledo (2010), once emerged for their release, the parasitoids were transported in vehicles equally equipped to maintain fresh

Table 1. Monthly emergency level of *Anastrepha* spp. in the municipality of Ruíz, Nayarit, 2012.

Month	Fruits		Number of larvae	<i>Anastrepha</i> adults emerged								Sexual proportion
	Number	Kg		<i>A. ludens</i>		<i>A. obliqua</i>		<i>A. striata</i>		Total		
				♀	♂	♀	♂	♀	♂	♀	♂	
J	167	15.9	85	26	20	0	0	0	0	26	20	1: 0.77
F	550	10.4	69	10	8	0	0	5	12	15	20	1: 1.33
M	233	13.7	106	4	9	21	15	1	1	26	25	1: 0.96
A	264	22.5	285	13	10	55	51	0	0	68	61	1: 0.90
M	413	49.0	357	5	2	101	65	0	0	106	67	1: 0.63
J	218	32.2	185	0	0	37	30	0	0	37	30	1: 0.81
J	152	27.8	139	0	0	20	20	3	5	23	25	1: 1.09
A	174	11.9	121	1	1	3	4	18	30	22	35	1: 1.59
S	72	3.5	35	0	0	0	0	5	6	5	6	1: 1.2
O	99	4.1	125	0	0	0	0	34	29	34	29	1: 0.85
N	270	4.6	145	1	0	0	0	28	24	29	24	1: 0.83
D	241	2.6	38	0	0	0	0	4	3	4	3	1: 0.75
Total	2,853	198.2	1,690	60	50	237	185	98	110	395	345	1: 0.87

♀ = Females; ♂ = males.

temperature ($22 \pm 2^\circ\text{C}$).

The releases were carried out during the first hours of the morning, in order to allow the parasitoids to better adapt to the environment when they were removed from the bags. The containers of the parasitoids were placed on various fruit hosts in the fruiting stage, within a range of 100 m, with periodic releases and a frequency of every three weeks in each locality.

Sampled fruits and parasitism rates

To determine the impact of the parasitoids on the pest, after the releases, an average of 10 fruit samples were collected per locality; each sample was composed of variable quantities of fruits, which were transferred to the Laboratory of Agricultural Parasitology of Agricultural Academic Unit of the Autonomous University of Nayarit for the dissection and separation of tephritid larvae. The larvae obtained were deposited in thermal vessels (No. 10) with pupation substrate (vermiculite), covered with tulle mesh, and placed on shelves in emergency chambers with

appropriate conditions of temperature ($24 \pm 2^\circ\text{C}$) and relative humidity (60 to 70%), with photoperiods of 12 h light and 12 h of darkness for the emergence of adult insects. The percentage of emerged parasitoids was calculated as the total number of emerged progeny divided by the number of recovered pupae (Van Nieuwenhove et al., 2012), or as the total number of parasitoids emerged divided by the total number of fruit flies and parasitoids emerged (Cancino and Yoc, 1993). The sexual ratio of both fruit flies and parasitoids was estimated (Van Nieuwenhove et al., 2012). For identification at species level by comparison with specimens preserved, a stereoscopic microscope was used and the dichotomous keys and criteria of authors like Steyskal (1977), Hernández-Ortiz (1992) and SENASICA-DMF (2010), were used.

RESULTS

During the year, 205.759 million parasitoids were released (an average of 2,617 parasitoids per

hectare) in an accumulated area of 78,625 ha. The monthly fruit sampling (Table 1) showed that of 2,853 fruits sampled (198.2 kg), 1,690 *Anastrepha* spp. larvae were separated, of which 740 adults emerged. Relative dominances correspond to: *A. obliqua* (422: 57.03%), *A. striata* (208: 28.11%) and *A. ludens* (110: 14.86%). The overall sex ratio (PS) of females (♀) against males (♂) was 1: 0.87 (395 ♀ and 345 ♂); by species, the PS was as follows: *A. ludens* 1: 0.83 (60 ♀ and 50 ♂), *A. obliqua* 1: 0.72 (237 ♀ and 185 ♂), and *A. striata* 1: 1.12 (98 ♀ and 110 ♂). The monthly incidence of insect pest in the larval stage showed the highest densities during the months of May (357), April (285) and June (185), while the lowest ones corresponded to the months of September (35), December (38) and February (69).

Results from monthly parasitism (Table 2) although 726 fruits (25.45%) showed presence of

Table 2. Monthly level of parasitism by *D. longicaudata* on *Anastrepha* spp. in the municipality of Ruíz, Nayarit, 2012.

Month	Fruits				Larvae			Parasitoids			
	Number	Kg	Infested	Percentage of infestation	Number	Larvae per fruit	Larvae per kg	♀	♂	Sexual proportion	Percentage of parasitism
J	167	15.9	35	20.96	85	0.51	5.35	10	11	1: 1.10	34.43
F	550	10.4	38	6.91	69	0.13	6.63	8	12	1: 1.50	36.36
M	233	13.7	50	21.46	106	0.45	7.74	13	18	1: 1.38	37.80
A	264	22.5	110	41.67	285	1.08	12.67	32	43	1: 1.34	36.76
M	413	49.0	131	31.72	357	0.86	7.29	59	58	1: 0.98	40.34
J	218	32.2	58	26.61	185	0.85	5.74	38	29	1: 0.76	50.00
J	152	27.8	52	91.44	139	0.91	5.00	27	21	1: 0.78	50.00
A	174	11.9	87	50.00	121	0.70	10.17	20	17	1: 0.85	39.36
S	72	3.5	21	29.17	35	0.49	10.00	4	4	1: 1	42.11
O	99	4.1	45	45.45	125	1.26	3.05	25	30	1: 1.20	46.61
N	270	4.6	70	53.70	145	0.54	31.52	22	28	1: 1.27	48.54
D	241	2.6	29	15.77	38	0.16	14.62	7	9	1: 1.29	69.57
Total	2,853	198.2	726	25.45	1,690	0.59	8.53	265	280	1: 1.06	42.41

♀ = Females; ♂ = males.

larvae, the highest percentages of infestation corresponded to the months of July (91.44%), November (53.70%) and August (50%); with an annual average of 42.41% parasitism, the highest percentages of monthly parasitism corresponded to the months of December (69.57%), June (50%) and July (50%), coinciding with the phenological stage of fruiting of smaller size, like guava, myrtle and creole mango. Of the 1,690 larvae harvested, with an annual average of 0.59 larvae per fruit and 8.53 larvae per kg, the highest monthly averages of larvae per fruit corresponded to October (1.26), April (1.08) and July (0.91). Based on larvae per kg, the highest infestations were recorded in November (31.52), December (14.62) and April (12.67). From the total of emerged adult parasitoids (265 ♀ and 280 ♂; PS 1: 1.06), dominance in emergencies corresponded to May (117), April (75) and June (67), without altogether

coinciding with the monthly PS, since the highest PS proportions were recorded in June (1: 0.76), July (1: 0.78) and August (1: 0.85).

Based on emergencies of adult flies by fruit species (Table 3), the highest percentages of infestation were recorded in guava (42.46%), sour orange (34.72%) and creole mango (31.49%). Of the 740 emerged fruit flies, 57.43% were obtained from creole mango. The global average of larvae/fruit was 0.59 and the highest rates of infestation corresponded to sour orange, creole mango and guava, with 0.89, 0.88 and 0.78 larvae/fruit, respectively. Regarding the percentage of infestation by fruit species, with a global average of 25.42%, the relative abundance corresponded to guava, sour orange and creole mango, with 42.46, 34.72 and 31.49%, respectively. In relation to the differentiation of adults obtained in each of the fruit species, the

relative dominance of emergencies corresponded to creole mango (425), guava (196), sour orange (103), myrtle (12) and grapefruit (4).

As regards the emergence of parasitoids by fruit species (Table 4), a total of 545 parasitoids emerged, with monthly abundances corresponding to May (117), April (75) and June (67). The emergences of parasitoids by fruit species in descending order corresponded to creole mango (314), guava (161), sour orange (47), myrtle (20) and grapefruit (3). In the case of creole mango, a species with relative dominance in emergencies, the presence of the braconid was recorded in six months (March to August), which coincides with the fruiting stage. With respect to guava, emergencies were obtained in January and February and from July to December, which also coincides with its fruitless period and almost uninterrupted during most months of the year, a

Table 3. General adult emergency level of *Anastrepha* spp. by fruit species in Ruiz, Nayarit, 2012.

Fruit specie	Fruits		Emerged adults of <i>Anastrepha</i>													
	Number	kg	Infested	Number of larvae	Percentage of infestation	<i>A. ludens</i>		<i>A. obliqua</i>		<i>A. striata</i>		<i>A. serpentina</i>		Total		Sexual proportion
						♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
Guava	617	25.50	262	480	42.46	-	-	-	-	94	102	-	-	94	102	1:1.09
Myrtle	915	3.40	40	47	4.37	-	-	-	-	4	8	-	-	4	8	1:2
Creole mango	1,121	137.10	353	983	31.49	2	1	237	185	-	-	-	-	239	186	1:0.78
Sour orange	216	30.40	75	193	34.72	55	48	-	-	-	-	-	-	55	48	1:0.87
Grapefruit	22	3.90	5	8	22.73	3	1	-	-	-	-	-	-	3	1	1:0.33
Total	2,891	200.30	735	1,711	25.42	60	50	237	185	98	110	0	0	395	345	1:0.87

♀ = Females; ♂ = males.

Table 4. Monthly level of parasitism of *D. longicaudata* by fruit species on *Anastrepha* spp. in Ruiz, Nayarit, 2012.

Fruit species	J		F		M		A		M		J		J		A		S		O		N		D		Total		
	np	%	np	%	np	%	np	%	np	%	np	%	np	%	np	%	np	%	np	%	np	%	np	%	np	%	
Guava	-	-	6	33	4	80	-	-	-	-	-	-	3	27.27	29	37.66	8	42.11	55	46.61	43	46.74	13	76.47	161	35.70	
Myrtle	1	100	7	58.33	3	75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	66.67	3	50	20	62.50	
Creole mango	-	-	-	17	32.08	67	38.73	113	40.07	67	50	45	52.94	5	46.67	-	-	-	-	-	-	-	-	-	-	314	42.49
Sour orange	17	28.81	7	28	7	35	8	25.81	4	50	-	-	-	-	3	60	-	-	-	-	1	50	-	-	47	31.33	
Grapefruit	3	42.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	42.86	
Total	21	34.43	20	36.36	31	37.80	75	36.76	117	40.34	67	50	48	50	37	39.36	8	42.11	55	46.61	50	48.54	16	69.57	545	42.41	

Np = Number of parasitoids; % = percentage of parasitism.

situation similar to that recorded for sour orange, where emergencies of parasitoid were recorded from January to May and August to November. Finally, in terms of the percentage of parasitism, the highest parasitoid efficiencies corresponded to myrtle, grapefruit and creole mango (62.50, 42.86 and 42.49%, respectively), while the lowest percentages of parasitism were recorded in sour orange (31.33%) and guava (35.70%). In this study, the outstanding results of parasitism recorded on some fruit species, such as myrtle,

showed high levels of parasitism during all the months sampled, with cases of 100% (January), 75% (March) and 66.67% (November). Similar situation for guava in March registered a parasitism of 80%; creole mango in July, 52.94%; sour orange in August, 60%.

DISCUSSION

Marginal or backyard fruit refers to plant species

established outside commercial areas, pens and recreational areas of rural society. For these areas, chemical applications pose a health risk, as well as promote the elimination of native natural enemies of agricultural pests that interact in a natural way and cause biological imbalance (Vargas et al., 2001). Based on the results, the percentage of infestation by fruit flies was 25.45%, the predominant species was *A. obliqua*, followed by *A. striata*, and *A. ludens*. In this regard, Tucuch et al. (2008) found that abiotic factors are

correlated with the presence of fruit flies, specifically precipitation, temperature and relative humidity. The results differ because the *A. ludens* species was the most abundant species, while in the present study it was *A. obliqua*, whereas *A. ludens* was the one with the lowest populations; although, the present peaks are not coincident, the behaviors of insect in both studies are similar.

Several authors agree that abiotic factors such as temperature, relative humidity and rainfall have an effect on the population fluctuation of fruit flies (Tucuch et al., 2008; Vanoye-Eligio et al., 2015), while other factors such as food availability and controllers are important for the presence or absence of the pest (Ovruski et al., 2003; Ledesma et al., 2013). When registering the months of May to August as the fruiting periods of guava, mango, Mexican plum and jobo, among other fruits in Nayarit as there is an increase in food availability, the populations of the insect pests increase and the probability of capture of the parasitoid decreases. This is because, between the months of January and December, the average percentage of parasitism is the highest (65.03%), due both to the competition of the braconid for parasitizing the few larvae present and to its efficiency by ovipositing on larvae of the tefritido present in fruits of small size, such as guava and myrtle. Months of food availability (May to September) recorded an average fruit fly infestation of 45.78%, and the average parasitism in the same period was 44.36%, a situation that shows that parasitism decreased with respect to the months that there is no fructification, or that the availability of food for fruit flies is lower. These results confirm the observations of Montoya et al. (2000), noting that at low densities of larvae of *A. ludens*, females of *D. longicaudata* increase their oviposition activity; however, it is a well-known fact that *D. longicaudata* is a parasitoid with a strong tendency towards super parasitism, both in laboratory and field conditions (González et al., 2007, 2010). The release densities fluctuate between 1500 and 2500 parasitoids per hectare, depending on the ecological complexity of the area. Apparently, the effect of releases is similar in control areas, showing high rates of parasitism in *Anastrepha* species (e.g. 33.5 to 64.7% in the state of Nayarit) and notable reductions in flies by trap per day (Montoya et al., 2007).

Based on the analysis of infestation per host, *A. striata* is confirmed as an oligophaga species, as it affects Myrtaceae fruits such as guava and myrtle; in this sense, for the case of guava, Nunez et al. (2004) reported an infestation of 91.72% of *A. striata* and 8.26% of *A. fraterculus* on fruits belonging to Myrtaceae, confirming their preference for these fruit species, whereas in the case of *A. obliqua*, despite being recognized as a polyphagous species (Programa de Sanidad Vegetal-SAGARPA-Guanajuato, 2004), its presence in this study was related only to a fruit species, pertaining to Anacardiaceae, while *A. ludens*, as a polyphagous

species, was registered to infest fruits belonging to the families Anacardiaceae and Rutaceae.

The augmentative releases of parasitoids carried out by the NCFE in Mexico are focused on specific areas where ecological and social conditions are considered propitious, which are done in a directed way to host trees located in marginal areas previously identified as reservoirs of *Anastrepha* spp. (Montoya and Toledo, 2010). The parasitoids recovered from the generality of host fruits indicated the wide range of fruit accepted by the parasitoid, whose preference in fruit species was according to the size of the fruits, with preferences of the females of small to medium sizes.

As shown in Table 2, between the months of May and September, during which the fruiting period occurs in the State, 277 adult parasitoids were recovered, which represent an average of 55.4 adults parasitoids emerged per month, with a mean parasitism of 44.3% in this period; during the rest of the year (October to December and January to April), 268 parasitoids were collected, corresponding to 38.3 specimens per month, and a parasitism rate of 44.3%, similar to the period of presence of fruits. In Mazapa de Madero, Chiapas, Mexico, Enkerlin et al. (1990) carried out two-year releases of *D. longicaudata* and *D. tryoni* on populations of *Anastrepha* spp., with parasitism records above 90% and significant reductions in fruit infestation rates. Montoya et al. (2000) reported a 2.7-fold suppression in populations of *Anastrepha* spp. In backyard mango orchards in fruit areas of Chiapas, Mexico on an area of 1,600 ha, the parasitoid *D. longicaudata* was released at densities of 1,000 specimens per hectare, where *A. obliqua* populations were mostly suppressed than *A. ludens*, which correlated mainly with the type of fruit each species of fly prefers to oviposit.

Different conditions of the fruit and the environment affect the level of parasitism and are related to the fruit size, shell thickness, fruit color, maturity, among other factors.

Cancino et al. (2014) reported that the sequential exposure of *A. ludens* and *A. serpentina* larvae to the parasitoids *D. longicaudata* and *C. haywardi* contributed to a decrease in the emergence of tephritid due to higher levels of parasitism, which varied according to the type of fruit, with higher percentages of *D. longicaudata* on creole mango, whereas the parasitism of *C. haywardi* was higher on pupae on mango cv. Ataulfo and sapote, where the size and volume of the pulp acted as refuge, which allowed the larvae of fly to escape and leave a larger number of non-parasitized pupae available for *C. haywardi*. Similar results were obtained in field cage conditions, but the level of parasitism by *C. haywardi* was lower, suggesting that its effectiveness has some limitations under natural conditions. The results suggest that both species may exert complementary parasitism, which represents a valuable alternative to investigation under open field conditions.

Conclusion

Based on the rates of parasitism obtained with the decrease of the native populations of *Anastrepha* spp. in each of the fruit species studied, the results demonstrate that augmentative releases of *D. longicaudata* can contribute efficiently to the control programs of fruit flies if they are carried out under the appropriate conditions and densities within sites with the presence of tephritid.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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