

ORIGINAL CONTRIBUTION

Pre-release diet effect on field survival and dispersal of *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: Tephritidae)

M. E. Utgés¹, J. C. Vilardi¹, A. Oropeza², J. Toledo² & P. Liedo²

¹ Laboratorio de Genética de Poblaciones Aplicada, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

² Departamento de Entomología Tropical, El Colegio de la Frontera Sur, Tapachula, Chiapas, México

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Correspondence

María Eugenia Utgés (corresponding author), Laboratorio de Genética de Poblaciones Aplicada, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. 2, 4to. piso, C1428EHA, Buenos Aires, Argentina.
E-mail: meutges@ege.fcen.uba.ar

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Abstract

The effect of pre-release diets on starvation resistance, field survival and dispersal of sterile *Anastrepha ludens* and *Anastrepha obliqua* fruit flies was investigated. Protein-enriched diets resulted in reduced longevity under laboratory and field conditions. Flies exposed to a combination of sugar and fresh mango fruit pulp showed greater longevity and field survival. Release–recapture experiments showed that this mango plus sugar diet resulted in the greatest trap capture and the longest life expectancy when compared with the other treatments. Per cent recapture ranged from 0.24% to 17.50%. More females than males were recaptured. Spatial distribution was not affected by diet treatment, sex or replicate, but was affected by environmental conditions, such as vegetation cover or shade in the case of *A. ludens* or prevalent winds in the case of *A. obliqua*. Our results confirm the trade-offs between better mating performance and reduced survival produced by protein-rich diets and suggest fresh mango fruits, their products or derivatives as an alternative to be developed to overcome this problem for sterile insect technique programmes.

Introduction

The Mexican fruit fly *Anastrepha ludens* (Loew) and the West Indies fruit fly *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), are the two major pests of fruits in Mexico (Aluja and Liedo 1986; Aluja et al. 1987). The National Fruit Fly Campaign has developed and is using the Sterile Insect Technique (SIT) to control these two pests in Mexico (Rull et al. 1996; Reyes et al. 2000; Orozco et al. 2004). A requirement for the successful application of the SIT is that the released sterile males should be able to survive, disperse and compete with wild males for mating with wild females. Because of the possible harmful effects of mass rearing and irradiation for sterilization, there is great interest in methods that can improve the performance of sterile males in the field.

Pre-release diets have been shown to improve male mating performance in a number of tephritid fruit flies, and this could contribute to enhanced SIT (Blay and Yuval 1997; Papadopoulos et al. 1998; Yuval et al. 1998, 2002, 2007; Field and Yuval 1999; Taylor and Yuval 1999; Kaspi and Yuval 2000; Kaspi et al. 2000; Shelly and Kennelly 2002; Shelly et al. 2002, 2005, 2006; Shelly and McInnis 2003; Cresoni-Pereira and Zucoloto 2006; Manrakhan and Lux 2006; Pérez-Staples et al. 2007, 2009; Gavriel et al. 2009). However, it has also been found that longevity of males fed on yeast-enriched diets could be adversely affected compared with males fed on sugar only, and mortality of males fed on enriched diets is increased if they are food deprived (Carey et al. 1999, 2008; Jácome et al. 1999; Kaspi and Yuval 2000; Prabhu et al. 2008).

Released tephritid fruit flies must forage for natural sources of nutrition, and their success at finding

food may influence their ability to participate in reproductive activities (Yuval et al. 1998). Dispersal to a given distance depends upon ability to survive long enough to get there; thus even in the absence of environmental causes of death, there will be an intrinsic limit imposed by a maximum potential life-span (Meats and Smallridge 2007). Improved pre-release feeding could increase longevity and dispersal of sterile Mediterranean fruit fly *Ceratitis capitata* (Wied.) (Meats and Smallridge 2007). Thus, the trade-off between mating performance and longevity associated with protein-rich diets could be optimized by improving pre-release diets.

In this study, our goal was to evaluate the survival and dispersal ability of sterile *A. ludens* and *A. obliqua* fruit flies subjected to different pre-release diets. The central point release and capture methodology was used, assuming that the time between release and capture was an indication of fly survival, and the distance of the traps to the central point of release was an estimation of the distance flies moved from the central point. Therefore, we used the number of flies captured and their location as estimators of field survival and dispersal. This allowed us to identify an improved pre-release diet for SIT programmes.

Material and Methods

Insects

Flies of both species were obtained as pupae from the Moscafrut mass-rearing facility at Metapa de Dominguez, Chiapas, Mexico. Forty-eight hours before adult emergence, pupae were gamma-irradiated with 80-Gy dose using a JS 7400 irradiator with ^{60}Co (Nordion International Inc., Peterborough, Ontario, Canada), at 25°C under hypoxia conditions, as is carried out regularly in the control programme.

Starvation resistance under laboratory conditions

Dietary treatments supplied to flies during the first 6 days of adult life for the starvation resistance experiment consisted of

- (a) 6 days of sugar diet (sugar diet, S);
- (b) 6 days of proteinaceous full diet (3 : 1, sugar/hydrolysed yeast as used at the Moscafrut facility) (full diet, F);
- (c) 3 days of sugar diet and following 3 days of proteinaceous full diet (sugar–full, SF);
- (d) 3 days of proteinaceous full diet and following 3 days of sugar diet (full–sugar, FS);

- (e) 6 days of mango diet (*Mangifera indica* cv. Ataulfo) (M);
- (f) 3 days of sugar diet and following 3 days of mango diet (sugar–mango, SM); and
- (g) 3 days of mango diet and following 3 days of sugar diet (mango–sugar, MS).

In treatments e–g, mature mango fruit was offered in slices that were replaced every second day.

To determine the effects of treatments on starvation resistance, groups of 10 males fed with each one of these diets were confined in 3-l plastic containers. For each dietary treatment, 10 replicates with *ad libitum* water till the end of the experiment were established. After food removal, flies were not moved to a new container. Containers were distributed randomly in a room with controlled conditions ($26 \pm 1^\circ\text{C}$, $70 \pm 5\%$ relative humidity). The number of dead flies was recorded daily starting at 09:00 hours.

Male survival was analysed by two variables: (i) comparison among treatments of average survival (days) by one-way ANOVA and Tukey contrasts (R Development Core Team 2009); and (ii) comparison of the proportion surviving by the Kaplan–Meier survivor function and the log-rank test between groups (Bonferroni correction, $\alpha_{\text{lab}} = 0.0031$; $\alpha_{\text{field cages}} = 0.017$). The first analysis compares average survival (days) among groups, whereas the Kaplan–Meier method evaluates the differences in the shape of the survival plots over time. These analyses were performed with the package *survival* of the R software ver. 2.9 (R Development Core Team 2009; Therneau and Lumley 2009).

Emergence and flight ability

To estimate the emergence and sex ratio of *A. ludens*, five Petri dishes with 100 pupae each were left till all flies had emerged and died. The percentage of non-emerged pupae and adults per sex were recorded. Percentage of fliers of *A. ludens* was obtained from routine tests conducted at Metapa facility according to the quality control manual (FAO-IAEA-USDA 2003). For *A. obliqua*, a black tube (10 cm height by 8.5 cm diameter) with unscented talcum powder was placed inside a mesh cage (30 × 30 × 30 cm) and 100 pupae were placed inside the tube (FAO-IAEA-USDA 2003). Two days later, malformed, non-emerged, fliers and non-flier flies per sex were recorded. All these measurements were taken for each release.

Mean parameters were calculated for each replicate and sex where necessary. An effective sex ratio was calculated taking into account only those flies capable

of flight. Estimated numbers of released flies per treatment and sex were calculated by multiplying the estimated number of pupae assigned to each treatment by the sex ratio and the proportion of emergence and fliers. The estimated percentages of captured flies for each treatment and replicate were calculated using the estimated number of released flies.

Field survival and dispersal

Adult diet treatments

Adult flies were held in the laboratory in $100 \times 100 \times 50$ cm mesh cages from emergence till 6 days old, when they were released. Water and food – according to each treatment – were provided during this period. Three different diets were tested:

- (1) only sugar (S);
- (2) full diet (F); and
- (3) exposure to mango fruit for 3 days, followed by sugar only for other 3 days (MS).

Samples of 100 pupae were weighted to the nearest 0.0001 g. Based on this information, the total number of pupae per treatment per replicate was estimated at approximately 20 000. Three fluorescent dyes were used to mark flies on the *ptilinum* at the moment of their emergence (4 g/kg of pupae, Neon Red: Day-Glo Color Corp., Cleveland, OH, USA; Green and Orange: Carnuba Wax Fluorescent Dye, University of Southampton, UK). Cages were covered at the top with paper to avoid direct sunlight, and extra places for resting were offered by hanging stripes of paper inside the cage.

Starvation resistance under small field cage conditions

On the release day of each replicate, 100 individuals of each sex of *A. ludens* were placed in $30 \times 30 \times 30$ cm wood frame mesh-covered cages and water was provided. In the case of *A. obliqua*, 250 individuals of each sex were placed in each cage. Two cages of each treatment were hung from the mango tree at the release point (for *A. ludens* first release, only one cage per treatment was set up). Dead flies were collected and recorded daily, until the last fly was dead.

The same statistical approaches described for laboratory starvation resistance tests were applied to compare the effects of different diet treatments. ANOVA analyses were not performed because of the low number of replicates (2).

Field releases

A central point release and recapture design was used in mango (*M. indica* L. cv. Ataulfo) orchards

located in the outskirts of Tapachula, Mexico. This area has a tropical climate with average annual rainfall of 2450 mm and a rainy season from late April to November. The average annual temperature is 26.2 °C, with April and May being the hottest months (Garcia 2004). For *A. ludens*, a 7-ha mango orchard was used, with the centre located at 14°46'50"N, 92°22'35"W, situated 21 m above sea level. For *A. obliqua*, a 8.7-ha mango orchard was used, with its central point located at 14°41'7"N, 92°16'54"W, also at 21 m above sea level. Trees were approximately 8 m tall in both orchards.

Seven-day-old flies were released in the morning at the central point of the orchard. The experiment was repeated three times for *A. ludens* (June–July 2007) and six times for *A. obliqua* (August–November 2008).

Two days after release, 52 Multilure© traps were hung in the orchard following a design of 13 circles at radial distances of approximately 15 m between consecutive circles. Distance to the point of release varied from 20 to 129 m for *A. ludens*, and from 21 to 187 m for *A. obliqua*. Traps were placed at a height of 4 m protected from direct sun exposure. We used liquid hydrolysed protein Captor 300 (Promotora Agropecuaria Universal S. A. de C. V., Guadalajara, Mexico) prepared according to the recommendations of the Mexican National Fruit Fly Campaign (235 ml of water, 10 ml of hydrolysed protein and 5 g of borax) as attractant for each trap (Gutiérrez et al. 1992). Traps were checked daily for a 15-day period for *A. ludens*. This experiment showed that after the 10th day, fly capture drops to near zero. Taking into account this experience, in the case of *A. obliqua*, traps were checked for only 10 days. Caught individuals were conserved dried and frozen for identification. Captured flies were observed under stereoscopic microscope with epifluorescence adaptation (60×, Nikon SMZ1500) to determine their diet treatment by colour identification. When fluorescence was not visible, the head was squashed on filter paper so as to see color remains of the head.

Information on climatic conditions (temperature, relative humidity, precipitation, direction and intensity of winds) during the trial periods was provided by the Tapachula International Airport that was located at <2 km from the orchards.

Statistical analysis of life expectancy:

Life expectancy at day of release (e_0) of each treatment was calculated by Life Table's formulas (Carey

1989; Hernández et al. 2007). This method is the best approach to estimate life expectancies under field conditions, although it has some limitations. In particular, the data actually obtained are the expected mean age of captured flies, and the method is based on the following assumptions: all ages are equally trappable, and flies do not leave the trapping grid. The number of flies captured on a given day (Y_x) was used to calculate the accumulated number of flies captured from a given day by the expressions

$$n_0 = \Sigma Y_x \text{ and}$$

$$n_{x+1} = n_x - Y_x$$

Survival rate up to a given day was found by the following equation:

$$l_x = n_x/n_0$$

Mean life expectancy at the day of release was estimated as:

$$e_0 = \frac{1}{2} + (l_1 + l_2 + \dots + l_w)/l_0,$$

where w denotes the last day of capture and l_0 was equal to 1.

Life expectancies were estimated for each replicate and for the whole sample. The differences in e_0 among diet treatments and sexes were compared by two-way ANOVA.

To compare the effects of sex or pre-release diet on survival rate trends throughout the observed period, we conducted an analysis of covariance (ANCOVA) with l_x as the response variable, days after release as the continuous explanatory variable, and sex and diet as the fixed covariate factors. Survival rate was log-transformed as $\ln(l_x + 0.00001)$ for linearity. The amount 1×10^{-5} was added to deal with the indetermination of log transformation when $l_x = 0$. Whenever possible, the model was simplified by dropping non-significant interactions. The analysis was conducted with the package *stats* of R software ver. 2.9 (R Development Core Team 2009).

The sex ratio of both emerged and captured flies was compared with the expected 1:1 ratio by means of goodness-of-fit chi-squared test. In the captured sample, we also compared the frequency of flies fed on different diets with the expected ratio (1:1:1) assuming equal survival and attraction of traps for all treatments. In all cases, consistency among replicates was evaluated by heterogeneity chi-squared tests.

Statistical analysis of average dispersal distance:

The effects of diet on average dispersal distance were evaluated by applying a generalized linear model expressed as:

$$d_{ijkn} = \mu + t_i + s_k + r_j + e_n,$$

where d_{ijkn} represents the dispersal of the individual n , sex k , replicate j , and fed on diet i , μ is the overall mean, t_i and s_k are the fixed effects of diet and sex, respectively, r_j is the random effect of replicate, and e_n is the random residual error. The different fixed effects and variance components were estimated by restricted maximum likelihood (REML) by means of the package *nlme* of the R software ver. 2.9 (Pinheiro et al. 2008; R Development Core Team 2009).

Statistical analysis of dispersal patterns:

The patterns of dispersal of flies fed on different diets were analysed by a spatial generalized linear mixed model with spatially correlated random effects. As our data consisted of number of individuals captured, we used the package *geoRglm* of the R software ver. 2.9 (Christensen and Ribeiro 2002; R Development Core Team 2009), assuming Poisson distribution of data and logarithmic link. The algorithm of the model was tuned by scaling the proposal variance, so that acceptance rate was approximately 60%. Plotting was performed with the package *geoR* of the same software (Ribeiro and Diggle 2001). Spatial coordinates were rescaled between 0 and 10 ($(\text{coord. } x_1 - \min x)/(\max x - \min x) \times 10$). In *A. ludens*, each dispersal unit on the x and y axes represents, respectively, 17.58 and 23.76 m in the real scale, and in *A. obliqua*, they correspond to 29.20 and 37.60 m respectively. In this analysis, replicates were averaged for analysis simplicity.

Additionally, we analysed the distribution of captured flies throughout each of the two spatial coordinates (x and y) of the experimental area. We evaluated possible effects of diet on dispersal trends by analysis of covariance, with number of captured flies as the response variable and position on the x (or y) axis as the continuous explanatory variable and sex and diet as the fixed covariate factors. Whenever possible, the model was simplified by dropping non-significant interactions. We applied a generalized model assuming Poisson distribution for the response variable using the R software ver. 2.9 (R Development Core Team 2009).

Table 1 Mean and standard error of survival (days) of *Anastrepha ludens* and *Anastrepha obliqua* males fed on different diets in the laboratory

Diet	<i>A. ludens</i>		<i>A. obliqua</i>	
	Mean	SE	Mean	SE
Sugar	12.30	0.14aA	10.44	0.21*aA
Mango	10.00	0.12cdB	8.41	0.13cB
Full	9.67	0.10dB	8.72	0.15cC
Sugar–Mango	10.61	0.18bC	9.00	0.19*bcD
Mango–Sugar	11.76	0.14aD	10.21	0.21aA
Sugar–Full	10.30	0.11bcBC	9.87	0.33abE
Full–Sugar	10.52	0.20bB	10.00	0.21*aAE

Different lowercase letters indicate significant ($P < 0.05$) differences according to Tukey contrasts. Different uppercase letters indicate significant ($P < 0.0031$) differences in survival curves according to Log Rank tests.

*One replicate was considered outlier.

Results

Starvation resistance in laboratory conditions

The comparison of average survival of *A. ludens* by ANOVA yielded highly significant results ($F = 42.76$; d.f. = 6, 63; $P < 0.0001$). Tukey contrasts indicated that males fed on S or MS lived longer than any other treatment (table 1). We found an effect of the feeding regime (diet plus moment at which it was offered) between treatments MS and SM: flies fed first on mango lived longer than those fed first on sugar. However, this effect was not observed for treatments SF and FS. There was no significant difference in the survival of flies fed mango (M) diet compared with flies fed F. Flies fed FS or SF lived

significantly longer than flies fed only F, resulting in an intermediate value between S and F.

The comparison of the proportion surviving by the Kaplan–Meier survivor function and the log-rank test also indicated highly significant differences among groups in the survival curves ($\chi^2 = 300$; d.f. = 6; $P = 0$). Consistent with the results of ANOVA, feeding regime had a significant effect in the case of MS and SM, but not in the combination of SF and FS.

The analysis of average survival of *A. obliqua* by ANOVA showed significant results ($F = 14.08$; d.f. = 6, 60; $P < 0.0001$). Contrasts indicated that males fed on S or MS differed in mean survival from M, F and SM. In this species, we also found an effect of the feeding regime in treatments SM and MS (table 1). The log-rank analysis indicated highly significant differences among groups in the survival curves ($\chi^2 = 304$; d.f. = 6; $P = 0$). A significant effect of regime was observed only for MS–SM.

Field survival and dispersal

Quality parameters

Considering both emergence and proportion of fliers, the estimated number of released flies per treatment per replicate varied approximately from 6800 to 10 300 flies for *A. ludens* and from 4200 to 7600 flies for *A. obliqua* (table 2). In the first replicate of *A. ludens*, treatment MS suffered an unusual high mortality during the 6 days before release, leaving approximately only 7500 flies alive of each sex.

Starvation resistance under field cage conditions

The analysis of starvation resistance of *A. ludens* in small field cages, set in the orchard during the field-release experiments, showed similar results as the

Table 2 Average data and SE for *Anastrepha ludens* and *Anastrepha obliqua* from quality control tests and estimated number of males (m) and females released per treatment for each replicate

Replicate	Emergence (%)	Fliers males (%)	Fliers females (%)	Sex ratio (m/total)	Estimated released males	Estimated released females
<i>A. ludens</i>						
1	95.20 (0.95)	90.00	90.00	0.60 (0.03)	10 299	6837
2	94.60 (1.35)	90.00	90.00	0.54 (0.02)	9128	7900
3	93.20 (0.86)	90.00	90.00	0.49 (0.01)	8276	8500
<i>A. obliqua</i>						
1	69.80 (0.03)	65.81 (0.05)	74.94 (0.03)	0.48 (0.02)	4448	5396
2	80.20 (0.05)	55.95 (0.08)	65.58 (0.06)	0.47 (0.02)	4213	5580
3	86.60 (0.02)	86.92 (0.03)	83.63 (0.02)	0.50 (0.03)	7574	7197
4	84.60 (0.04)	75.11 (0.05)	78.75 (0.04)	0.49 (0.03)	6245	6777
5	86.40 (0.02)	56.53 (0.05)	67.41 (0.05)	0.49 (0.03)	4828	5892
6	90.02 (0.02)	78.75 (0.05)	79.51 (0.04)	0.54 (0.02)	7653	6588

ones obtained in the laboratory. Without food, 50% of mortality was reached at the ages of 9.33 (SD = 0.56) days for males and 9.37 (SD = 0.60) for females fed on F and more than one day later for those fed on MS (males: mean = 10.62, SD = 0.93; females: mean = 10.94, SD = 0.97) and S (males: mean = 10.59, SD = 0.94; females: mean = 10.75, SD = 1.06) diets. The log-rank analysis showed significant differences between MS and F diets for both sexes (males: $\chi^2 = 342$; females: $\chi^2 = 406$; d.f. = 1; $P = 0$), and S and F (males: $\chi^2 = 296$; females: $\chi^2 = 298$; d.f. = 1; $P = 0$). Differences in survival between females and males were roughly half a day, although they were highly significant ($\chi^2 = 15.2$; d.f. = 1; $P < 10^{-5}$).

Anastrepha obliqua starvation resistance in field cages showed that the MS diet was associated with the highest values. Also, females lived longer than males but the difference was less than a day. A difference of approximately 1 day between F and MS diets in reaching 50% mortality was observed. There was also approximately 1-day difference between F and S when survival was reduced to 0.4 (60% mortality). These trends were evaluated by the log-rank test. The analysis showed significant differences between MS and F (males: $\chi^2 = 819$; females: $\chi^2 = 1241$; d.f. = 1; $P = 0$), MS and S (males: $\chi^2 = 28.6$; females: $\chi^2 = 111$; d.f. = 1; $P = 0$) and F and S diets (males: $\chi^2 = 620$; females: $\chi^2 = 725$; d.f. = 1; $P = 0$). Differences in survival between females and males were highly significant ($\chi^2 = 1028$; d.f. = 1; $P = 0$).

Field survival

Climatic data for both sites in 2007 ranged from 22 to 34°C, 55% to 100% relative humidity and 660 (June) to 740 mm (July) of precipitation and for 2008, from 21 to 32°C, 48% to 100% relative humidity and 298 to 439 mm of precipitation. In 2007, during the months of June and July, approximately 40% of records indicated calm weather and 41% gentle breeze (2–9 knots). The exception was June 2nd when the tropical storm 'Barbara' produced winds of more than 20 knots. The predominant directions were W (41% of records) and WSW (28%). In 2008, during the period August–November, 64% of records indicated calm weather, 13% gentle breeze, and 22% moderate breeze. Predominant wind directions were W (36% of records) and WSW (34%).

The estimates of life expectancy of *A. ludens* at release time yielded values within the range from

Table 3 Weighted average of life expectancies from release day (e_0) (days) of *Anastrepha ludens* and *Anastrepha obliqua* males and females. Standard deviations in brackets

Treatment	Males	Females
<i>A. ludens</i>		
S	4.81 (0.50)	5.58 (0.98)
MS	4.92 (0.57)	5.69 (0.82)
F	4.72 (0.57)	5.32 (0.78)
<i>A. obliqua</i>		
S	4.38 (0.42)	4.33 (0.32)
MS	4.51 (0.82)	4.81 (0.57)
F	4.43 (0.88)	4.49 (0.50)

MS, mango-sugar; S, sugar; F, full diet.

4.7 to 5.7 days after release (table 3) (equivalent to 11.7 and 12.7 days after emergence). The comparison of life expectancies by two-way ANOVA indicated that the interaction between sex and diet was non-significant ($F = 0.002$, $P = 0.99$), females live approximately 1 day more than males ($F = 5.21$; d.f. = 1, 14; $P = 0.04$) and the differences among diets were non-significant ($F = 0.08$; d.f. = 2, 14; $P = 0.92$).

The ANCOVA revealed significant triple interaction among days, sex and diet ($F = 3.85$; d.f. = 2, 78; $P = 0.03$), indicating that the slope of \ln of survival rate on days after release was affected by the interaction between diet and sex. Therefore, we decided to conduct the analysis separately for each sex, to consider in each case only the effect of diet on survival rate. When we analysed the effects of diet on survival rate for males only, we observed that the regression of log-transformed survival rate on days after release was highly significant ($t = -11.67$; d.f. = 39; $P = 3 \times 10^{-14}$). The difference in slope between males fed on MS or S and males fed on F was highly significant ($t = 2.84$; d.f. = 39; $P = 0.007$; and $t = 2.78$; d.f. = 39; $P = 0.008$, respectively), but no differences were observed between MS and S ($t = 0.05$; d.f. = 39; $P = 0.96$) (fig. 1a). These results indicate that survival rate declined faster for F than for the other treatments. In females, the results were similar, maximum (negative) slope corresponded to F ($t = -30.42$; d.f. = 39; $P < 1 \times 10^{-15}$), but in this case, no significant differences were detected between S and F ($t = 0.02$; d.f. = 39; $P = 0.53$) (fig. 1b). The slope for MS differed significantly ($t = 2.65$; d.f. = 39; $P = 0.011$) from F, whereas the difference with respect to S was marginal ($t = 2.01$; d.f. = 39; $P = 0.051$).

Life expectancy estimates of *A. obliqua* fed on different adult diets (table 3) showed in both males

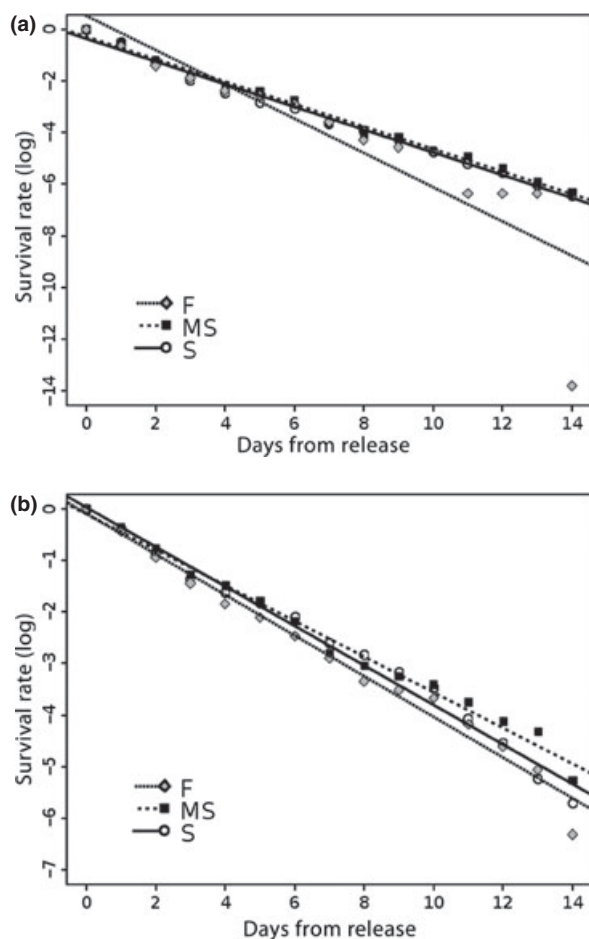


Fig. 1 Field survival (natural log scale) of *Anastrepha ludens* males (a) and females (b) as a function of time for each dietary treatment. (a) $y_{(F)} = 0.53 - 0.67x$; $y_{(MS)} = -0.27 - 0.44x$; $y_{(S)} = -0.37 - 0.44x$; (b) $y_{(F)} = -0.09 - 0.39x$; $y_{(MS)} = -0.10 - 0.35x$; $y_{(S)} = 0.03 - 0.38x$. MS, mango-sugar; S, sugar; F, full diet.

and females that the highest values, similar to *A. ludens*, corresponded to MS, but in this case, the differences among treatments ($F = 0.69$; d.f. = 2, 30; $P = 0.51$) or sexes ($F = 0.81$; d.f. = 1, 30; $P = 0.37$) were non-significant. According to these results, median survival time from release in all cases was close to 4.5 days (i.e. 11.5 days after emergence).

The analysis of covariance indicated that there was no significant interaction between days, sex and diet ($F = 0.91$; d.f. = 2, 48; $P = 0.41$), and the slope of the survival rate was similar between males and females ($t = -0.04$; d.f. = 54; $P = 0.97$) (fig. 2). Highly significant differences were observed between F and MS ($t = -3.02$; d.f. = 54; $P = 0.005$) and between F and S ($t = 3.68$; d.f. = 54; $P = 0.001$), whereas S and MS exhibited similar slopes of survival rate on days after release ($t = 0.66$; d.f. = 54; $P = 0.512$). The main

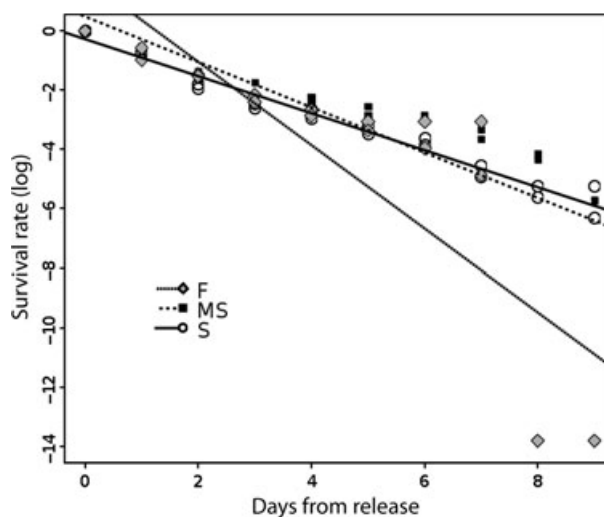


Fig. 2 Field survival (natural log scale) of *Anastrepha obliqua* individuals as a function of time (no differences were observed between sexes) for each dietary treatment $y_{(F)} = 1.77 - 1.41x$; $y_{(MS)} = 0.48 - 0.77x$; $y_{(S)} = -0.28 - 0.63x$. MS, mango-sugar; S, sugar; F, full diet.

cause for these differences is that no captures of F females or males were recorded the last 2 days, whereas for the other treatments, at least one fly was captured until the last experimental day.

Sex ratio and proportions of flies captured

For both species, sex ratio of fliers at emergence adjusted to a 1 : 1 ratio with no heterogeneity between replicates (table 2). By contrast, the sex ratio of the captured sample (table 4) showed a significant departure from the expected in both species. The number of females captured was higher than the one for males with highly significant differences (*A. ludens* global: $\chi^2 = 177.3$, $P = 2 \times 10^{-16}$; *A. obliqua*

Table 4 Mean number and standard error (SE) of flies recaptured and recapture percentage per sex and treatment. Recapture % was estimated according to estimated released numbers per diet for each replicate (see table 2)

Treatment		m	f	% m	% f
<i>A. ludens</i>					
S	Mean	416 (150)	503 (160)	4.36 (1.68)	5.78 (1.72)
MS	Mean	347 (138)	451 (157)	3.76 (1.41)	5.17 (1.71)
F	Mean	194 (84)	369 (111)	2.04 (0.96)	4.22 (1.07)
<i>A. obliqua</i>					
S	Mean	31 (7)	93 (22)	0.53 (0.08)	1.42 (0.28)
MS	Mean	32 (5)	154 (50)	0.55 (0.08)	2.38 (0.73)
F	Mean	7 (2)	44 (17)	0.13 (0.04)	0.72 (0.29)

m, males; f, females; F, full diet; MS, mango-sugar; S, sugar.

global: $\chi^2 = 801.9$, $P = 2 \times 10^{-16}$). For both species, highly significant heterogeneity was observed in the sex ratio among replicates (*A. ludens*: $\chi^2 = 48.3$, $P = 3 \times 10^{-11}$; *A. obliqua*: $\chi^2 = 38.7$, $P = 2.7 \times 10^{-7}$); however, in all cases, the number of females was much higher than that of males.

In *A. ludens*, the proportion of males captured differed between replicates ($\chi^2 = 1075$, $P < 0.0001$) in the same way as for females ($\chi^2 = 567$, $P < 0.0001$) because total capture in replicate 1 was lower because of the effects of the tropical storm Barbara. Estimated number of released flies of each sex and diet, per replicate, is shown in table 2.

The proportions of flies captured for each treatment differed from the expected 1 : 1 : 1 in both sexes (male: $\chi^2 = 243$, $P < 0.0001$; female: $\chi^2 = 61.52$, $P < 0.0001$) showing a global trend of a higher-than-expected number of S and lower-than-expected number of F. However, there was a significant heterogeneity between replicates (male: $\chi^2 = 41.66$, $P < 0.0001$; female: $\chi^2 = 57.68$, $P < 0.0001$), and there was non-significant difference between the observed and the expected for replicate 3 in females ($\chi^2 = 2.56$, $P = 0.28$).

A partial chi-squared test to compare MS and S treatments also yielded a highly significant departure from the expected 1 : 1 ratio both in males (global: $\chi^2 = 18.55$, $P = 2 \times 10^{-5}$) and in females (global: $\chi^2 = 8.29$, $P = 0.004$). In these comparisons, the results were homogeneous among replicates (heterogeneity: $\chi^2 = 4.33$, $P = 0.12$ and $\chi^2 = 2.58$, $P = 0.28$ for males and females, respectively). A similar analysis to compare MS and F treatments indicated highly significant excess of MS-fed individuals (males: $\chi^2 = 130.5$, $P = 2 \times 10^{-16}$; females: $\chi^2 = 24.58$, $P = 7 \times 10^{-7}$). Heterogeneity among replicates was highly significant in both males ($\chi^2 = 44.9$, $P = 2 \times 10^{-10}$) and females ($\chi^2 = 52.2$, $P = 5 \times 10^{-12}$), due to the fact that in the first replicate, the differences between treatments were non-significant in both sexes and the same occurred in replicate 3 for females.

In the case of *A. obliqua* (table 4), there was a clear asymmetry in the number of flies captured from the different diets within each sex. In females, the pooled data from the six replicates showed a higher-than-expected percentage of flies fed on MS (53%) and lower-than-expected percentage of F-fed females (15%), while the proportion of S-fed flies (32%) was close to the expected. The global chi-squared test indicated that the proportion of captured females for each treatment departed in a highly significant fashion from the expected (1 : 1 : 1) ($\chi^2 = 378.3$, $P = 2 \times 10^{-16}$). There was

highly significant heterogeneity among replicates ($P = 4.2 \times 10^{-50}$); however, the frequency of F flies was the lowest in five of the six replicates. A partial chi-squared test to compare MS and S treatments also yielded a highly significant departure from the expected 1 : 1 ratio (global: $\chi^2 = 91.6$, $P = 2 \times 10^{-16}$). As in two replicates, the number of S flies was higher than MS and heterogeneity was also highly significant (heterogeneity: $\chi^2 = 77.6$, $P < 0.00001$).

In the case of *A. obliqua* males, there was also deficiency in F-captured flies (10%), and although contrasting with females, the proportion of MS (45%) and S males (44%) was similar. The chi-squared analysis revealed highly significant differences with respect to the expected when all groups were considered (global: $\chi^2 = 102.2$, $P = 2 \times 10^{-16}$). In this case, although the heterogeneity among replicates was highly significant (heterogeneity: $\chi^2 = 37.4$, $P = 5 \times 10^{-5}$), in all cases, the F group exhibited the lowest frequency. By contrast, the proportion MS/S was not significantly different from 1 : 1 (global: $\chi^2 = 0.04$, $P = 0.84$). The highly significant heterogeneity ($\chi^2 = 22.1$, $P = 0.0005$) reflected that the frequency of MS (or S) was the highest in 50% of the cases.

Average dispersal distance

The statistical analysis of the dispersal distance indicated significant effects of sex and diet, but non-significant interaction. As the comparison between the complete model (including interaction) and the simplified one did not yield significant differences (*A. ludens*: $\chi^2 = 3.64$, d.f. = 2, $P = 0.16$; *A. obliqua*: $\chi^2 = 4.48$, d.f. = 2, $P = 0.11$), we simplified the model by dropping the interaction term (following Crawley 2007). The average dispersal distance from the release point classified by sex and diet treatment (table 5) suggests that both *A. ludens* ($F_{1, 6720} = 24.47$, $P < 0.0001$) and *A. obliqua* ($F_{1, 6720} = 6.09$, $P = 0.0135$) females tend to disperse further than males. Also, the mean dispersal distance of MS-fed flies of both species (averaging males and females) was higher than that of the remaining treatments ($F_{2, 6720} = 7.23$, $P = 0.0007$ and $F_{2, 6720} = 7.64$, $P = 0.0005$ for *A. ludens* and *A. obliqua*, respectively). Pairwise contrasts among diets for *A. ludens* showed non-significant differences between MS and S ($t = -0.88$; d.f. = 6720, $P = 0.38$), significant differences between S and F ($t = 3.78$; d.f. = 6720, $P = 0.0002$) and highly significant differences between MS and F ($t = -4.11$; d.f. = 6720, $P < 0.0001$), indicating that the relative dispersal ability associated with diet treatments was $MS = S > F$.

Table 5 Basic statistics for dispersal distance (m) from the release point for *Anastrepha ludens* and *Anastrepha obliqua* males and females for each treatment

Treatment	Males		Females	
	Mean	SE	Mean	SE
<i>A. ludens</i>				
S	66.10	0.78	70.71	0.76
MS	68.40	0.83	70.04	0.88
F	63.21	1.15	67.28	0.88
<i>A. obliqua</i>				
S	68.75	3.16	81.83	1.94
MS	85.06	3.42	88.55	1.54
F	75.91	7.77	81.66	2.86

F, full diet; MS, mango-sugar; S, sugar.

However, the results for *A. obliqua* showed no evidence of difference between MS and F ($t = -0.99$; d.f. = 2154; $P = 0.32$) and significant differences between MS and F with respect to S ($t = -3.74$; d.f. = 2154; $P = 0.0002$ and $t = -2.34$; d.f. = 2154; $P = 0.019$, respectively), indicating that the relative dispersal ability associated with diet treatments was $MS = F > S$.

Analysis of dispersal patterns

Spatial statistics allowed a more detailed analysis of dispersal patterns at a two-dimensional scale. The estimated dispersal pattern of *A. ludens* did not show differences between treatments. Spatial analysis showed that males and females in all treatments moved towards the South East — South South East edge (fig. 3). Winds were mostly from the West, SW and WSW.

The graphical analysis of *A. obliqua* dispersal showed a different pattern (fig. 4). Captures were close to the release point. The differences between treatments are reflected in the number of captured flies, which can be observed in table 4 and in the scale below each plot. There seems to be a trend to disperse from the release point to the East and NE, with limited movement on the y (North–South) axis. During the period when the *A. obliqua*'s releases took place, predominant winds were from the West and WSW.

The analysis of covariance to evaluate the association of coordinate x, diet and sex on the number of captured flies indicated a highly significant dispersal trend for both species (*A. ludens*: $t = -5.57$, d.f. = 924, $P < 0.0001$; *A. obliqua*: $t = 3.20$, d.f. = 1855, $P = 0.0014$). For *A. ludens*, slopes for males and females were different ($t = 2.19$, d.f. = 924, $P =$

0.029) in contrast to *A. obliqua* where the difference was marginal ($t = -1.95$, d.f. = 1855, $P = 0.0509$). The slopes for diets F and MS differed in both species (*A. ludens*: $t = -3.59$, d.f. = 924, $P = 0.0004$; *A. obliqua*: $t = 4.07$, d.f. = 1855, $P < 0.0001$), but those for S and MS only differed for *A. obliqua* ($t = -2.15$, d.f. = 1855, $P = 0.032$). Differences between slopes for F and S diet were significant for *A. ludens* ($t = -2.98$, d.f. = 924, $P = 0.003$) but marginal for *A. obliqua* ($t = 1.92$, d.f. = 1855, $P = 0.0547$). The analysis for the coordinate y showed a significant trend in *A. ludens* ($t = 2.03$, d.f. = 929, $P = 0.043$) but not at all in *A. obliqua*. However, there were no significant differences between slopes in either species.

Discussion

The effects of different post-teneral diets were evaluated under different experimental conditions, including laboratory tests, outdoor small field cages and open-field assays. Under controlled laboratory conditions, the effect of protein-rich vs. sugar-only diets on survival or longevity has been studied by several authors on tephritid species including *C. capitata*, *Anastrepha suspensa* and *Bactrocera tryoni* (Froggatt) (Carey et al. 2002a,b; Teal et al. 2004; Yuval et al. 2007; Pérez-Staples et al. 2008). In most cases, protein-rich diets have been associated with better male mating performance but in some others, they have also been associated with reduced survival (Kaspi and Yuval 2000). Our results with *A. ludens* under laboratory conditions were consistent with those observed in other fruit fly species. The protein-rich (F) diet was associated with increased susceptibility to starvation. Flies fed on S or a combination of S and mango (MS) had the highest survival.

Resistance to starvation recorded under small field cages hung near the release point produced results highly consistent with those obtained under laboratory conditions, in showing the lowest survival for F diet, and small differences between S and MS diets that vary between species. In all cases, females tend to survive longer than males.

Due to a tropical storm with strong winds of more than 20 knots (most frequent winds are between 2 and 9 knots for that time of the year), traps for the *A. ludens* experiment had to be hung 2 days after release. This deviation from the original protocol was maintained through the other replicates and for the *A. obliqua* releases to maintain consistency. The impact of this decision may have been advantageous. Enkerlin (1987) recommended hanging the traps

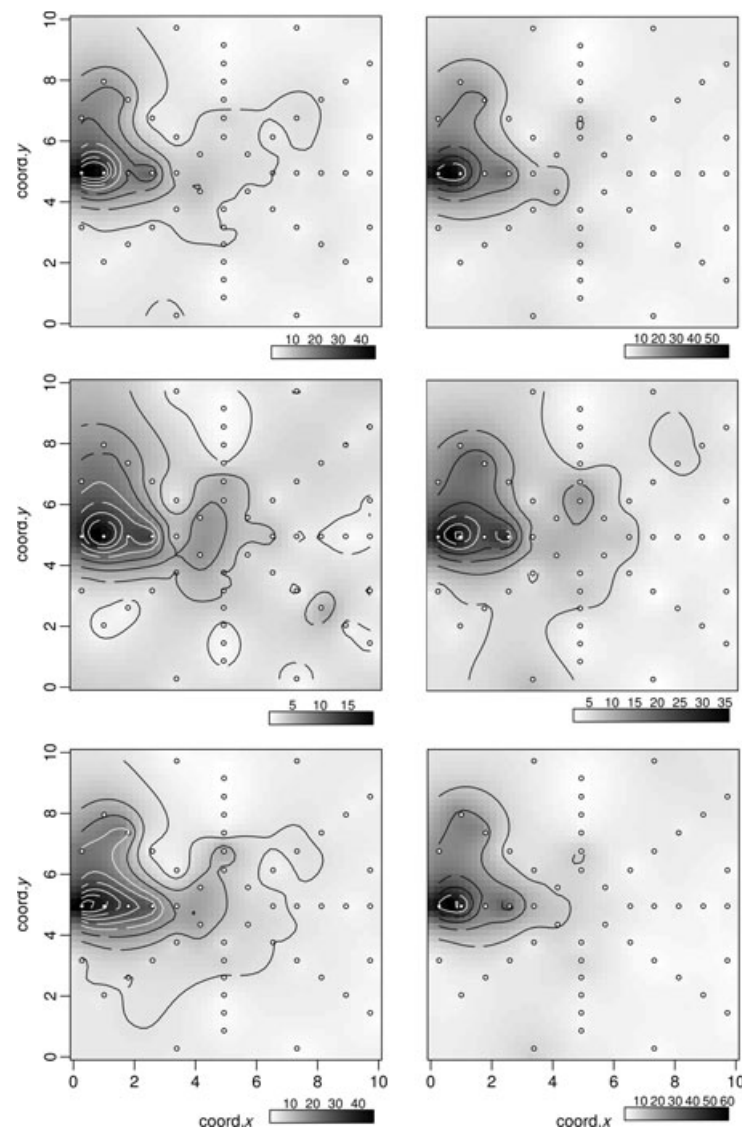


Fig. 3 Mean estimated dispersal pattern for *Anastrepha ludens* males (left) and females (right). Original coordinates were rescaled to the 0–10 range. Top: MS, centre: F, bottom: S. MS, mango-sugar; F, full diet; S, sugar.

near the release point 3 days after the release of flies so that flies can disperse, avoiding overtrapping on the first day.

With a similar central point release and recapture experimental design, Baker and Chan (1991) found that disappearance of *C. capitata* and *A. ludens* flies was surprisingly fast and they suspected that mortality was high because of extreme temperatures and predation. Baker et al. (1986) caught twice as many *A. ludens* females as males using protein-baited McP-hail traps, with an overall recapture rate of 2%. In our study, overall recapture rates for *A. obliqua* were close to 1% and close to 4% in *A. ludens*. Rates for

both species varied among replicates, sex and diet treatments, from 0.08% (*A. obliqua* F-fed males, replicate 3) to 17.50% (*A. obliqua* MS-fed females, replicate 6), and in some cases, the number of recaptured females was more than twice the number of recaptured males, which was expected because females respond stronger than males to protein baits. S and MS groups presented a significantly greater number of captures than treatment F for both species. Assuming that all treatment groups are equally attracted to the bait, the result suggests that flies fed on protein-rich diet have shorter longevity in the field than those fed on sugar or natural fruits plus

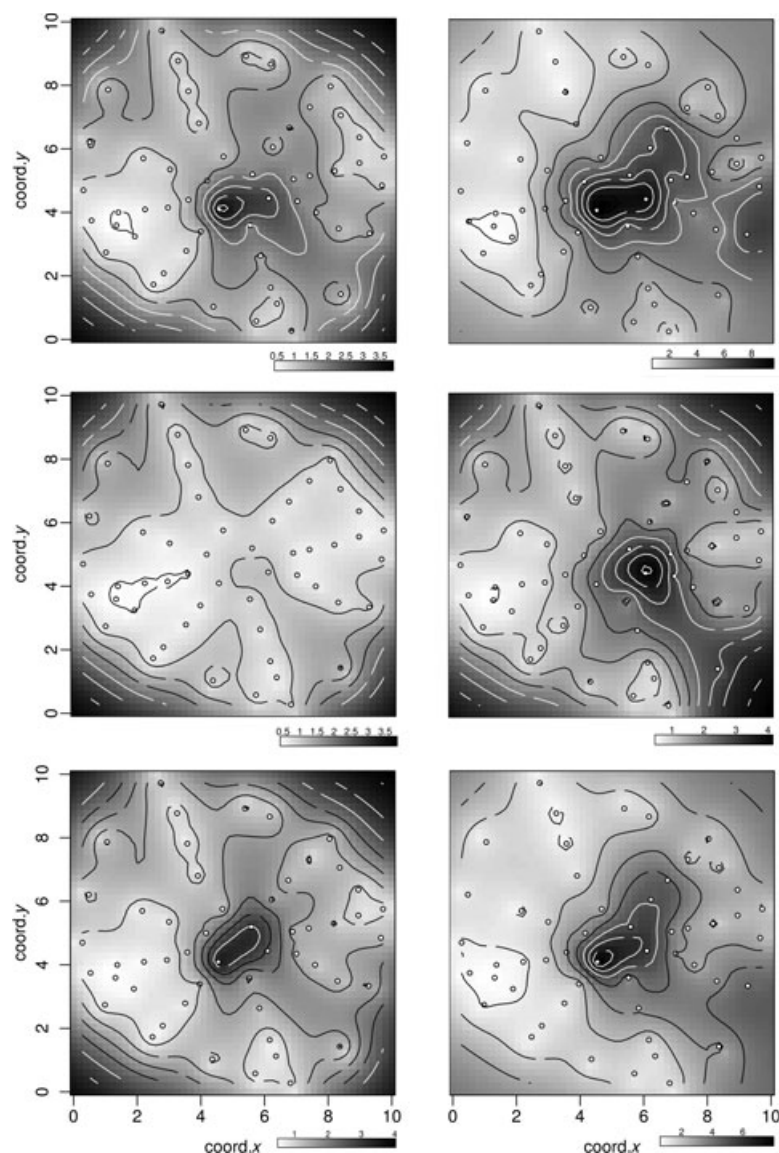


Fig. 4 Mean estimated dispersal pattern of *Anastrepha obliqua* males (left) and females (right). Original coordinates were rescaled to the 0–10 range. Top: MS, centre: F, bottom: S. MS, mango–sugar; F, full diet; S, sugar.

sugar diets and, within each group, that females survive longer than males. An alternative explanation for the low capture of F flies could be that these are less attracted to the protein-based lure in the traps. Our laboratory and field cage data showed reduced survival of F-fed flies, so the lower capture could also be a combination of lower survival and lower attraction. To gain deeper insight into this issue, non-protein-baited traps should be used, but these types of baits for *Anastrepha* species are not available or are still under development (Toledo et al. 2009). In a recent field experiment on sterile *C. capitata*

using non-protein baits, Gavriel et al. (2010) found no effect of protein diet on recapture percentages both in enclosures and in open field.

Field longevity estimates were within the range of similar studies. In a previous study conducted with 5-day-old *A. ludens* flies from the same strain fed with sugar and released near the location as in the present study, Hernández et al. (2007) obtained an estimated life expectancy from release day of 4.0 days (SE = 0.3 day) for males and 4.4 days (SE = 0.2 day) for females. Our results were close to those values, e_0 estimates were from 4.8 days for

males to 5.8 days for females, depending on diet. In our case, the difference between males and females was also a little higher. Thomas and Loera-Gallardo (1998) found that the average life expectancy of irradiated 4-day-old *A. ludens* fed on a diet similar to F, released at Santa Rosa Canyon (Nuevo León, México), was 9.85 days (13.85 days from emergence), whereas flies released at an orange grove at Río Bravo (Tamaulipas, México) had a life expectancy of 4.8 days (8.8 days from emergence). However, the results of this work are not completely comparable to ours as the authors serviced the traps on a weekly instead of a daily basis.

The ranking of e_0 estimates is partially consistent with the conclusions based on the proportion of recaptured flies of each diet treatment. The differences between these two approaches are due to the fact that e_0 estimates are based only on the number of captured individuals within each treatment, without taking into account the number of individuals captured in the other treatments.

Spatial analysis did not show differences in directionality between diet treatments. As in Baker and Chan (1991), no difference in distribution between sexes was found. After releases of *C. capitata* in Chiapas, Baker et al. (1986) found a significant movement from the release point towards a particular direction, usually in the direction of prevailing day-time winds. The dispersal pattern of *A. obliqua* obtained in this study could be explained partially by the direction of prevalent winds (mostly from the W and WSW). However, in the case of *A. ludens*, the areas with the highest numbers of captures were those with more shade (field observation), next to an untended mango orchard, and the pattern cannot be explained on the basis of the direction of prevailing winds. This observation suggests active displacement of flies to particular areas of the orchard as the distribution in the field was not random. The causes of such distribution could be behavioural, because of intraspecific interactions (Meats et al. 2006). For released *C. capitata*, a known cause of contagious distribution is the tendency of most flies to remain within a few hundred metres from the release point (Meats et al. 2006). This contagious pattern was observed here for *A. obliqua*.

Although the general dispersal pattern was similar for all treatments, average dispersal distances differed significantly among them and between sexes. These results indicate that the ability to disperse from the release point is dependent on diet and sex. In particular, in both species, the maximum average dispersal distance corresponded to flies fed on MS, and

females dispersed significantly further than males. The average dispersal distances estimated in the present study are not to be extrapolated to conditions different from those applied in the present work because they are dependent on the experimental grid. However, the results indicate that dispersal ability is dependent on the diet. The greater dispersal of MS and S than F in *A. ludens* could be attributed to their greater survival or their greater need for protein or a combination of both factors. However, in *A. obliqua*, flies fed on S did not show higher dispersal than F, suggesting that the effect of diet on dispersal is species specific and cannot be explained solely on the basis of survival or need of protein. Sex differentials were similar in both species and could be attributed to females' greater need of protein food, greater survival or the combination/interaction of both factors. Differences in survival between *A. ludens* males and females have been also observed under laboratory conditions with sugar-only diet, where life expectancy of females was greater than that of males (Carey et al. 2008). However, *C. capitata* females have been found to be more sensitive than males to the effects of protein deprivation on longevity (Müller et al. 1997; Carey et al. 2002b).

The results obtained suggest different ability to survive and disperse under field conditions of flies that underwent different pre-release dietary conditions. However, some methodological concerns should be considered inherent to trap capture. Particularly, the active space of the trap is continually changing, so that it is impossible to ascertain whether variation in catch is real or apparent (Baker and Chan 1991). Food traps might catch hungry flies rather than dispersing flies. These points cannot be ignored in an attempt to account for density distributions. A possible explanation for the relative low capture of flies fed on F is that they may not be attracted by traps baited with protein as they have received protein before release. The increased proportion of females captured in protein-baited traps might also be explained by their higher need of protein supply in comparison with males.

From laboratory and field cage experiments, we can conclude that sterile *Anastrepha* flies fed with protein-rich diets alone or in combination with other diet elements showed reduced starvation resistance compared with those fed with sugar, or mango and sugar. Mango diet alone did not improve starvation resistance but in combination with sugar, did so. This tendency was also seen in the survival of both males and females in the cages that were placed in

the orchard. This greater survival of flies fed on S or MS, compared with F, was also evident in the release–recapture data in both species analysed. These results confirm the trade-off between better mating performance of males fed on protein-rich diets but reduced survival ability found by Yuval et al. (2007), in *C. capitata*.

Fruit-based diets, such as the MS diet in this study, represent an alternative to deal with this trade-off in SIT programmes, because flies exposed to fruits have shown good mating performance (Liedo et al. 2013) and good survival ability. The mode of action of fresh fruit diets needs to be investigated to develop standard methods for pre-release feeding of sterile flies. Fresh fruits could provide nitrogen/protein, vitamins, pheromone precursors, might be the media for symbiotic microorganisms or could produce volatiles that affect male's mating competitiveness (Aluja et al. 2001; Shelly 2001; Ben-Yosef et al. 2008). Given the low protein proportion in mango (22 : 1 according to an analysis conducted at Laboratory of Bromatology, El Colegio de la Frontera Sur), an alternative could be to optimize the sugar/yeast ratio in the diet. Recent research results have found that 9 : 1 or 24 : 1 sugar/yeast diets resulted in improved male mating performance and no detrimental effect on their survival ability or longevity under laboratory conditions (Prabhu et al. 2008; Liedo et al. 2013).

The positive effect of mango on the mating performance of these two species (Liedo et al. 2013) and the favourable effect of MS on field survival and dispersal suggest that it is worth to further investigate its mode of action and to develop methods for standard pre-release feeding in SIT operational programmes. Fresh fruits, their products or sub-products could represent a good alternative for SIT improvement in other fruit fly species.

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