Costly Nutritious Diets do not Necessarily Translate into Better Performance of Artificially Reared Fruit Flies (Diptera: Tephritidae)

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ABSTRACT Protein, lipid, carbohydrate, and energy contents of three artificial diets (Xal2, Met1, and Met2) used for laboratory-rearing and mass-rearing the Mexican fruit fly, *Anastrepha ludens* (Loew), for a sterile insect technique program were measured. The larval survival, pupation, pupal weight, adult emergence, sex ratio, and flight capacity of the flies reared on each of these diets were also quantified. The diet with the highest nutrient and energy content was Xal2 followed by Met2 and Met1, but larval recovery and percent pupation was significantly higher in flies reared on either the Met1 or Met2 diets. *A. ludens* reared on Xal2 exhibited the highest proportion of adults capable of flight. No other response variable differed significantly among the three diets tested. This suggests that a high content of nutrients and multiple sources of protein (dried yeast and wheat germ in the case of the Xal2 diet) do not necessarily improve overall performance or fly quality. We conclude that nutritious diets for *A. ludens* can be modified to reduce their cost without compromising the performance of artificially reared flies.

KEY WORDS artificial diet, nutrient content, diet cost, mass rearing, *Anastrepha ludens*

Introduction

Fruit flies (Diptera: Tephritidae) are major pests of fruit crops, and some species in the economically important genera *Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis* have been colonized for laboratory or mass rearing on artificial diets (Christenson et al. 1956, Tzanakakis et al. 1968, Hernández et al. 2009, Köppler et al. 2009, Domínguez et al. 2010, Gutiérrez et al. 2013, Sacchetti et al. 2013). Mass rearing of tephritid flies on an artificial diet is critical for the application of area-wide management programs such as the sterile insect technique (SIT) (Gutiérrez et al. 2013), and also provides the necessary substrate on which to mass rear parasitoids used for inundative biological control (Montoya et al. 2000, Zamek et al. 2012, Gutiérrez et al. 2013). In addition, fly colonies support studies on the defensive role of host plant secondary metabolites (Pascacio-Villafán et al. 2014), life-history strategies (Salum et al. 2014), nutrition physiology (Nestel et al. 2004), and facilitate the development of new parasitoid rearing and colonization techniques (Aluja et al. 2009).

Tephritid artificial diets, as with many other insect diets, are usually mixtures of various ingredients that provide nutrients such as proteins, lipids, carbohydrates, vitamins, and minerals, together with preservatives, pH modifiers, bulking agents, gelling agents, and water (Cohen 2004, Hernández et al. 2010). While fly species differ in their nutritional requirements and need larval growing media varying in consistency and texture (Vera et al. 2007, Hernández et al. 2010), diets are often generated from existing formulations developed for other species (Hernández et al. 2010). This is the case of artificial diets for rearing the Mexican fruit fly, *Anastrepha ludens* (Loew), which were originally adapted from a diet used to rear the oriental fruit fly, *Dacus* (*Bactrocera*) *dorsalis* (Hendel) (Spishakoff and Hernández-Dávila 1968).

A. ludens is a tropical, polyphagous, and multivoltine fruit fly (Aluja et al. 2001) distributed from southern Texas to Central America (Birke et al. 2013), where it attacks fruit such as citrus (*Citrus* spp.), mango (*Mangifera indica* L.), and peach (*Prunus persica* [L.] Batsch; Aluja et al. 2000). *A. ludens* is considered a pest of economic importance across most of its range (Aluja 1993, Aluja and Mangan 2008), and it is likely that it will expand its range because of global climate change (Birke et al. 2013, Aluja et al. 2014). Under such circumstances, *A. ludens* could exploit new hosts including some apple (*Malus* × *domestica* Borkh) cultivars (Aluja et al. 2014). As part of governmental management programs aimed at suppressing *A. ludens* populations, millions of artificially reared flies are produced and sterilized on a daily basis at the Programa Moscafrut facilities located at Metapa de Domínguez, Chiapas, Mexico. These are employed in SIT releases, and used to mass rear the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) for augmentative biological

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control (Gutiérrez et al. 2013). Artificial *A. ludens* diets have also been used in the fruit fly laboratories of the Red de Manejo Biorracional de Plagas y Vectores (RMBPV) of the Instituto de Ecología, A.C., in Xalapa, Veracruz, Mexico, to provide experimental subjects, and as hosts for 14 species of hymenopteran parasitoids colonized at the RMBPV (Aluja et al. 2009).

Artificial diets from Moscafrut and RMBPV are oligidic (i.e., their ingredients are not fully chemically defined) and differ in the content and proportion of some ingredients (Table 1). The nutrient content in terms of protein, lipid, and carbohydrate percent of the RMBPV diets is often greater than that of *A. ludens* host fruit (Cicero 2011). In the case of the Moscafrut diets, we are not aware of any published information on its exact nutrient content. We suspected that the high nutrient content in artificial diets may not be fully utilized by artificially reared flies, resulting in an unnecessarily expensive diet. Given this, we recognized the need to determine whether the nutritional content of *A. ludens* artificial diets exceeds the needs of the flies that they are used to rear. A first step in this direction was to define the nutrient content of the Moscafrut and RMBPV diets and evaluate a number of quality control parameters of the flies reared on each diet. We predicted a priori that diet Xal2, which contained dried yeast and wheat germ (Table 1), would have the higher nutritional content, and that the overall performance of the flies reared on this diet would be superior to that of Met1 and Met2.

Materials and Methods

Experimental Flies. *A. ludens* used in this study were obtained from a strain maintained on an artificial diet at the RMBPV, originally provided by the Comité Estatal de Sanidad Vegetal (DGSV-SÁGARPA) in Xalapa, Veracruz, Mexico (Aluja et al. 2009). Parental flies aged 13–16 d were held in Plexiglas cages (30 by 30 by 60 cm) with ad libitum access to water and food (a mixture of hydrolyzed protein and refined sugar). Flies oviposited on transparent silicon substrates and eggs were washed and incubated for 4 d until hatch (for rearing details see Aluja et al. 2009). Larvae were subsequently reared on the experimental diets described in Artificial Diets section.

Artificial Diets. Two artificial diet formulations based on the recipes used at Moscafrut (hereafter Met1 and Met2; Domínguez et al. 2010) and one from the RMBPV (hereafter Xal2; Aluja et al. 2009) were tested (Table 1).

To prepare the Xal2 diet, all ingredients except water and hydrochloric acid were weighed individually on a

Table 1. Composition and cost of 1 kg of each of the artificial diets tested

Diet components	Diet type					
	Xal2		Met1		Met2	
	% by weight	Cost[a] (USD)	% by weight	Cost[a] (USD)	% by weight	Cost[a] (USD)
Nutrients						
Dried yeast[b]	9.70	$0.44	6.00	$0.27	7.00	$0.32
Sugar[c]	9.70	$0.05	8.20	$0.04	9.20	$0.05
Wheat germ[d]	9.70	$0.34	–	–	–	–
Vitamins						
Viterra plus capsules[e]	0.14	$0.23	–	–	–	–
Bulking and texturizing agents						
Corn cob fractions[f]	14.55	$0.17	19.00	$0.22	19.00	$0.22
Corn flour[g,h]	–	–	5.30	$0.04	5.30	$0.04
Preservatives						
Sodium benzoate[i]	0.78	$1.32	0.40	$0.68	0.40	$0.68
Methylparaben[j]	–	–	0.10	$0.008	0.20	$0.02
Acidifying agents						
Citric acid[k]	–	–	0.44	$0.007	0.44	$0.007
Hydrochloric acid[l]	0.58	$0.06	–	–	–	–
Gelling agents						
Guar gum[m]	–	–	0.10	$0.014	0.10	$0.014
Purified water[n]	54.83	$0.03	60.46	$0.032	58.36	$0.032
Total cost		$2.64		$1.31		$1.37

[a] Costs were estimated from the unit price of single ingredients in Mexican pesos as indicated below, and then transformed to USD (exchange rate at the time of writing: 1 MXN = 0.077 USD).

[b] Lake States Div. Rhinelander, WI, USA (22.7 kg cost $1,331.00 MXN).

[c] Refined sugar (50 kg cost $345.00 MXN).

[d] Fit grain Nutrisa, Mexico City (600 g cost $27.00 MXN).

[e] Pfizer SA de CV, Toluca, Mexico (30 capsules cost $46.14 MXN, 1 capsule = ca. 710.8 mg).

[f] Mt. Corn cob fractions 100 Pulaski, Products Inc., Chicago, IL (23.33 kg cost $356.82 MXN).

[g] Minsa, Grupo Minsa, Mexico (1 kg cost $8.86 MXN).

[h] Although corn flour has been employed as a texturizing agent (Rivera et al. 2012), it also provides nutrients to the diet—mainly protein and carbohydrate.

[i] Baker, J.T. Baker S.A. de C.V., Xalostoc, Mexico (500 g cost $1,099.35 MXN).

[j] Alfa Delta, Mexico (1 kg cost $112.8 MXN).

[k] Cava Nutrialimentos, S.A. de C.V., Mexico (1 kg cost $21.74 MXN).

[l] Baker, J.T. Baker S.A. de C.V., Xalostoc, Mexico (2.5 liter cost $350.05 MXN, 1 liter = 1.448 kg).

[m] Cava Nutrialimentos, S.A. de C.V, Mexico (1 kg cost $179.59 MXN).

[n] Econopura, Mexico (20 liter cost $14.00 MXN).

digital scale (Ohaus TP4KD) and then mixed by hand for 5 min in a plastic tray (30 cm in length by 25 cm in width by 13 cm in height). Next, water and hydrochloric acid diluted in 25% of the total volume of water were added to the tray and hand mixed with the other ingredients until a homogeneous mixture free of lumps was obtained. Met1 and Met2 diets were prepared by mixing the nutrients, bulking and texturizing agents, and guar gum (Table 1) for 5 min in a plastic tray of the same size as above. Preservatives and citric acid were dissolved in 25% of the total water (Rivera et al. 2012) prior to incorporation, together with the rest of the water, to the plastic tray containing the rest of the diet ingredients. Met1 and Met2 diets were also hand mixed until no lumps were present.

Nutrient Determination. Samples of 500 g of each artificial diet were analyzed following the Association of Official Analytical Chemists (AOAC) (1975) and the Norma Oficial Mexicana NOM-051-SCFI-1994 (1994) standard analytical procedures, at the Laboratorios de Alta Tecnología de Xalapa, S.C. (LATEX), in Xalapa, Veracruz, Mexico. Protein content was determined by the Kjeldahl method; lipids were determined directly with a Soxhlet extractor; the nitrogen-free extract was considered as total carbohydrates and was calculated using the following equation: percentage carbohydrate = 100 – (% protein + % lipid + % moisture + % ashes). The energy calculation was performed using the following conversion factors: carbohydrates 4 kcal/g, proteins 4 kcal/g, and lipids 9 kcal/g. Each diet was analyzed four times to assess potential experimental error due to diet preparation or processing.

Experimental Procedure. The experiments were performed at the laboratories of the RMBPV on four separate days. Each artificial diet was replicated 14–18 times.

Portions of 200 g of each artificial diet were placed in plastic containers (11 cm in diameter by 7 cm in height) together with 250 neonate larvae (<10 h old) of *A. ludens.* Containers were covered with pieces of pantyhose (Cannon Mills) and incubated in a dark room at $30 \pm 1°C$ and $70 \pm 5\%$ relative humidity (RH). Following standard rearing procedures (Rivera et al. 2012), 9 d after the beginning of the experiment, larvae were recovered from the diet by gently washing it with tap water through a plastic strainer (18 cm in diameter) with nylon mesh (1 mm). Recovered larvae were counted and placed in a plastic container (7 cm in diameter by 6 cm in height) with vermiculite (1:1, larvae:vermiculite). The container was closed with a lid that had a 5-cm-diameter hole covered with organdy cloth, and placed in a laboratory at $22 \pm 1°C$, $70 \pm 5\%$ RH, and a photoperiod of 12:12 (L: D) h to promote pupation. After 24 h, pupae were counted and moved to a laboratory at $26 \pm 1°C$, $60 \pm 5\%$ RH, and a photoperiod of 12:12 (L: D) h. To standardize the age of the recovered pupae, larvae that did not pupate after the 24-h period were discarded. Three days following pupation, pupae were weighted on an analytical scale (Sartorius CP64). Ten days later (13 d after pupation), samples of 100 pupae from each diet were placed

inside individual cells (1.6 by 1.6 cm) of a grid until adult emergence. In addition, samples of pupae (range of 14–100 pupae) from each diet were placed inside PVC cylinders to evaluate flight capacity (black PVC cylinders [9 cm in diameter by 10 cm in height] coated with unscented talcum powder on the interior, on the top of Petri dishes). Cylinders with pupae were placed in a 90-cm-long- by 100-cm-wide- by 90-cm-tall mesh cage, and adults were allowed to emerge and fly out the tubes. To minimize the incidence of flies that escaped from the PVC cylinders (i.e., fliers) falling back inside a tube, they were removed twice a day. In addition, two traps consisting of transparent plastic bottles (600 ml capacity) with three round perforations (fly entrance) on the top, and 300 ml of grape soft drink (Sangria Casera) as bait, plus four sticky traps were hung on the ceiling of the cage. Flightless individuals died inside the tubes. When emergence had ceased, the remaining contents of the cylinders were counted.

Response Variables. The response variables were based on the following six quality control parameters previously established for mass-reared tephritid flies (Food and Agriculture Organisation–International Atomic Energy Agency–U.S. Department of Agriculture [FAO-IAEA-USDA] 2003): 1) larval recovery, estimated as the proportion of larvae recovered from diet after 9 d in relationship to the total number of larvae seeded in each diet; 2) pupation at 24 h, expressed as the percentage of larvae that pupated 24 h after being separated from the diet; 3) mean pupal weight (mg), estimated by weighting all pupae from each diet and dividing the total weight by the total number of pupae weighed; 4) adult emergence, calculated as the percentage of adults that emerged from the pupae placed individually inside cells of grids; 5) sex ratio, expressed as the proportion of males relative to the total adults emerged from the pupae placed individually inside cells of grids; and 6) flight capacity (fliers), expressed as the proportion of adults that emerged and flew outside the black PVC cylinders in relation to the total pupae placed inside them.

Statistical Analyses. The software R (R Core Team 2013) was used in all analyses. Mixed model ANOVAs were used to identify significant effects of diet type on the response variables. The fixed component of the model was diet type with three levels (Xal2, Met1, and Met2), whereas the random component was block with four levels (days 1–4). The mixed model tested for significance of the fixed components (Xal2, Met1, and Met2) and allowed us to account for error variability due to daily (block) variation in fly characteristics. To meet the model assumptions, variance was modeled using a power function and a compound symmetry correlation structure (Pinheiro et al. 2009). When significant effects were detected, *t*-test contrasts were used to explore differences among diet levels (Warnes 2009). Replicate plastic containers, 100 cell grids and PVC black cylinders were the observational units, so all analyses were performed on replicate means.

One-way ANOVA was used to identify significant differences among protein, lipid, carbohydrate, and energy content in the diets. After ANOVA, the

assumptions of the model were verified using diagnostic plots (Crawley 2007), confirming homoscedasticity of the residuals and normality of errors in all cases. When significant effects were detected, t-test contrasts were performed.

Results

Protein ($F = 84.63$; df = 2, 9; $P < 0.0001$), lipid ($F = 5.07$; df = 2, 9; $P = 0.0335$), and energy ($F = 18.67$; df = 2, 9; $P < 0.001$) content differed significantly among diets, but the carbohydrate content did not ($F = 0.701$; df = 2, 9; $P = 0.521$; Fig. 1A–D). Protein content in Xal2 was about twice that of either Met1 ($t = 11.92$; df = 9; $P < 0.0001$) or Met2 ($t = 10.48$; df = 9; $P < 0.0001$). Xal2 also had significantly more lipid than did Met1 ($t = 3.14$; df = 9; $P = 0.012$), but was similar to that of Met2 ($t = 2.04$; df = 9; $P = 0.071$). The energy content of Xal2 was significantly higher than either Met1 ($t = 5.82$; df = 9; $P < 0.001$) or Met2 ($t = 4.52$; df = 9; $P < 0.001$). No significant differences were observed between Met1 and Met2 for protein ($t = 1.44$; df = 9; $P = 0.1846$), lipid ($t = 1.09$; df = 9; $P = 0.3037$), or energy ($t = 1.3$; df = 9; $P = 0.2268$) content.

Larval recovery differed significantly among diet types ($F = 19.94$; df = 2, 46; $P < 0.0001$; Fig. 2A), with more larvae recovered from Met1 ($t = 5.8$; df = 46; $P < 0.0001$) and Met2 ($t = 6.3$; df = 46; $P < 0.0001$) than from Xal2. Percent pupation ($F = 17.73$; df = 2, 46; $P < 0.0001$; Fig. 2B) on Met1 ($t = 5.9$; df = 46; $P < 0.0001$) and Met2 ($t = 5.8$; df = 46; $P < 0.0001$) was significantly superior to Xal2. No significant differences were observed for pupal weight ($F = 0.675$; df = 2, 44; $P = 0.52$), adult emergence ($F = 0.80$; df = 2, 44; $P = 0.46$), or sex ratio ($F = 0.17$; df = 2, 44; $P = 0.84$; Figs. 2C-E). Flight capacity ($F = 4.7$; df = 2, 44; $P = 0.01$; Fig. 2F) differed significantly among diets; higher proportions of flies reared on Xal2 flew out of PVC cylinders compared to flies reared on Met1 ($t = 2.8$; df = 44; $P = 0.007$) or Met2 ($t = 2.4$; df = 44; $P = 0.0229$).

Discussion

Significant differences were detected in the nutrient content of the three diets tested and in the performance of flies reared on them. As predicted, the Xal2 diet containing both dried yeast and wheat germ had the highest nutrient content. Contrary to our prediction, the Met1 and Met2 diets were superior in terms of larval recovery and pupation, whereas the Xal2 diet yielded the highest proportion of flying adults. Despite the differences observed in percent pupation and flight capacity among flies reared on the different diets, these proportions were all near or above 80%, which meet the recommended quality control values in *A. ludens* mass rearing protocols (Dirección General de Sanidad Vegetal-Dirección de Moscas de la Fruta [DGSV-DMF] 2009, Santiago et al. 2012). We recognize that discarding larvae that did not pupate after 9 d, as performed in our study, could have possibly biased our
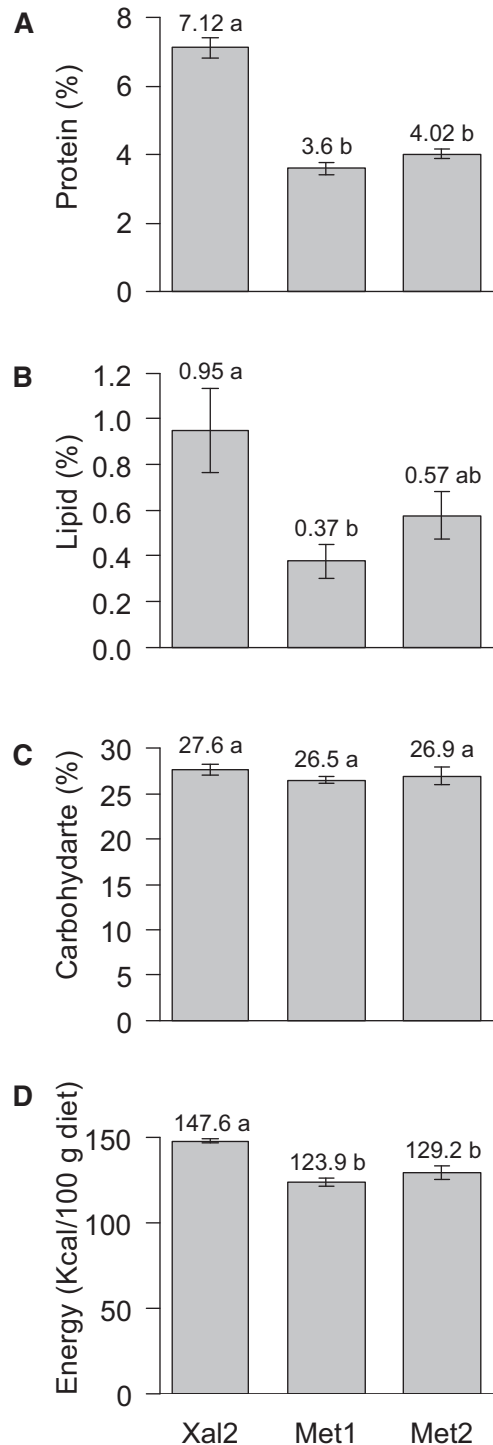

Fig. 1. Mean (±SE) content of (A) protein (%), (B) lipid (%), (C) carbohydrate (%), and (D) energy (kcal/100 g diet) in three artificial diets (Xal2, Met1, and Met2) used for rearing *A. ludens*. Values above bars indicate mean values. Means labeled with identical letters indicate no significant differences for mixed-effect models (t-test contrasts, $P > 0.05$).
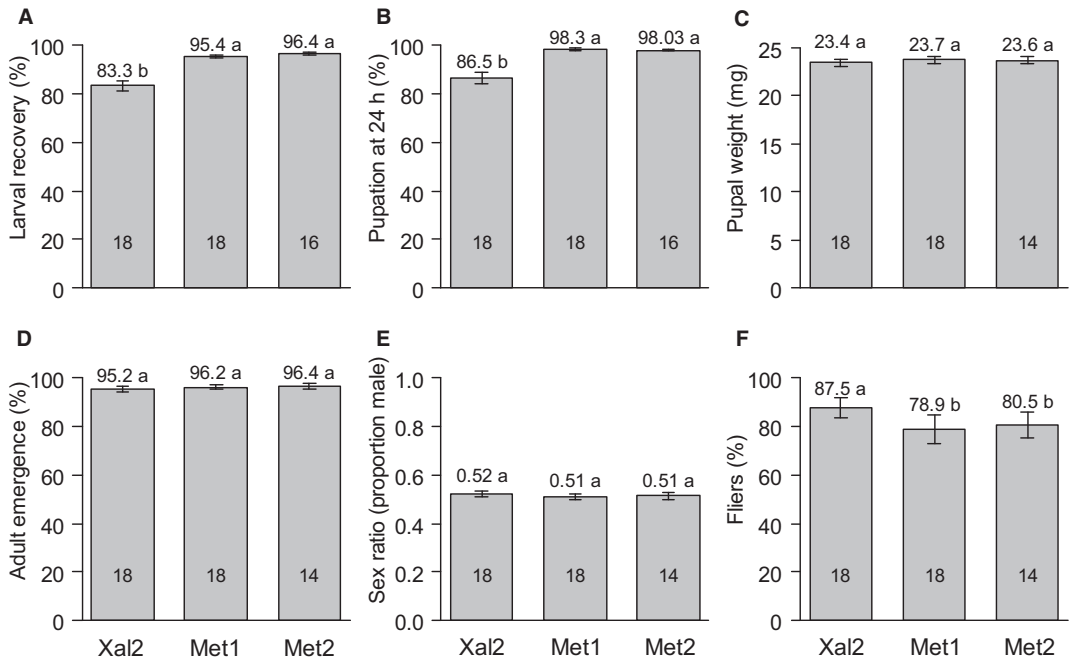
Fig. 2. Mean (±SE) of (A) larval recovery (%), (B) pupation at 24 h (%), (C) pupal weight (mg), (D) adult emergence (%), (E) sex ratio (proportion male), and (F) fliers (%) of *A. ludens* reared on three artificial diets (Xal2, Met1, and Met2) differing in their nutrient and energy content. Values above and within bars indicate mean values and number of replicates, respectively. Means labeled with identical letters indicate no significant differences for mixed-effect models (*t*-test contrasts, $P > 0.05$).

comparison on percent pupation, as it is likely that development in Met1 and Met2 was more rapid than in Xal2.

Flight ability is of paramount importance in flies used for SIT (Collins and Taylor 2010), and this represents one of the most sensitive and informative quality control parameters (Rull et al. 2012). Wheat germ is known to have high protein content, and it contains carbohydrates and lipids that are rich in fatty acids and phytosterols (Cohen 2004). Consequently, wheat germ may have been the ingredient responsible for the increased flight of flies reared on Xal2. Fatty acids in wheat germ could contribute to energy levels required for flight muscle functioning (Arrese and Soulages 2010), and its absence in Met1 and Met2 may have induced an overexpression of the flightless-I protein (fli-I; Cho et al. 2013), and so reduced the prevalence of flight-capable adults. Indeed, the flight ability of *B. dorsalis* increased with the amount of wheat germ oil in the larval diet, and Chang and Vargas (2007) suggested that fatty acids and vitamin E were involved in this effect. Similarly, *Ceratitis capitata* (Wiedemann) reared without fatty acids had flight capacity of only ∼20%, in contrast to ∼90% when reared on a diet containing fatty acids (Cho et al. 2013).

In addition to fatty acids, vitamins also influenced *C. capitata* flight ability (Chang et al. 2001). If true for *A. ludens*, we suggest that Met1 (with the lowest cost and nutrient content, and overall high fly performance) could serve as a base diet to evaluate the effect of

wheat germ and vitamins on flight capacity. This should be done estimating the cost:benefit ratio of incorporating both ingredients into the diet, and analyzing any tradeoffs between flight capacity and other traits such as male sexual competitiveness (Marden 2000).

The protein content of 3.6% present in the Met1 diet was sufficient to rear high-quality *A. ludens*. However, the minimum protein content required might be still lower, and future studies should test concentrations below 3.6%. While protein is essential to dipteran development (Nash and Chapman 2014), high concentrations can have a detrimental effect (Sentinella et al. 2013). Perhaps the low larval recovery observed in Xal2 (∼80% in contrast to ∼100% in Met1 and Met2) was the result of its relatively high protein content (7.12%). However, our results are insufficient to establish such a relationship, and other components may also have influenced this parameter. For example, guar gum in Met1 and Met2 might have a positive influence on larval survival, as it improves the consistency of diets by modifying high water content into a gel so that insects do not die if the food substrate collapses on them when tunneling (Cohen 2004). Also, the inclusion of certain preservatives (sodium benzoate) in artificial diets have been observed to be lethal to *A. fraterculus* (Wiedemann) unhatched eggs (J. Rull, personal communication), and perhaps the quantity of sodium benzoate in Xal2 (Table 1) affected neonate larvae as well.

As observed in Table 1, sodium benzoate was the ingredient that contributed the most to the high cost of

Xal2, followed by dried yeast, wheat germ, and vitamins. If, as suggested above, the high amount of sodium benzoate in Xal2 affected larval survival, and as our data showed, high protein content do not improve artificial *A. ludens* rearing and might even be detrimental, the amounts of such components in Xal2 needs to be reduced. We also question if the dried yeast levels in Met1 and Met2 diets could be reduced maintaining its high quality and production of flies. Additional research is necessary to address these questions, as artificial rearing of *A. ludens* would benefit from any diet modification that result in high-quality flies from efficient and more economical diets.

In summary, the large amounts of nutrients in some *A. ludens* artificial diets do not generate higher numbers or a better quality of adult flies, and are, therefore, not necessary for artificial rearing of this fly pest. Diets now in use for mass rearing, Met1 and Met2, yielded higher larval recoveries and percent pupations than the research diet Xal2; however, they were inferior in terms of flight capacity. Because flight capacity is critical for mass-reared flies used in SIT programs, the identification of the compound(s) responsible for differences among diets could result in further improvements. Further experiments to elucidate minimum levels of nutrients are warranted considering the high costs associated with ingredients such as dried yeast and wheat germ, and the preservative sodium benzoate (Table 1). We conclude that systematic studies on *A. ludens* nutritional requirements are required, as the results of our study suggest that nutrient-rich diets may be unnecessary for rearing high-quality flies suitable for use in SIT-based programs of area-wide pest control.

Acknowledgments

References Cited

Aluja, M. 1993. Manejo integrado de la mosca de la fruta. Editorial Trillas, Mexico City, Mexico.

Aluja, M., and R. L. Mangan. 2008. Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. Annu. Rev. Entomol. 53: 473–502.

Aluja, M., J. Piñero, M. López, C. Ruíz, A. Zúñiga, E. Piedra, F. Díaz-Fleischer, and J. Sivinski. 2000. New host plant and distribution records in Mexico for *Anastrepha* spp., *Toxotrypana curvicauda* Gerstacker, *Rhagoletis zoqui* Bush, *Rhagoletis* sp., and *Hexachaeta* sp. (Diptera: Tephritidae). Proc. Entomol. Soc. Wash. 102: 802–815.

Aluja, M., J. Piñero, I. Jácome, F. Díaz-Fleischer, and J. Sivinski. 2001. Behavior of the flies in the genus *Anastrepha* (Trypetinae: Toxotrypanini). pp. 375–406. *In* M. Aluja and A. L. Norrbom (eds.), Fruit flies (Tephritidae): phylogeny and evolution of behavior. CRC Press, Boca Raton, FL.

Aluja, M., J. Sivinski, S. Ovruski, L. Guillén, M. López, J. Cancino, V. Torres-Anaya, G. Gallegos-Chan, and L. Ruiz. 2009. Colonization and domestication of seven species of native New World Hymenopterous larval-prepupal and pupal fruit fly (Diptera: Tephritidae) parasitoids. Biocontrol Sci. Technol. 19: 49–79.

Aluja, M., A. Birke, M. Ceyman, L. Guillén, E. Arrigoni, D. Baumgartner, C. Pascacio-Villafán, and J. Samietz. 2014. Agroecosystem resilience to an invasive insect species that could expand its geographical range in response to global climate change. Agric. Ecosyst. Environ. 186: 54–63.

Arrese, E. L., and J. L. Soulages 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55: 207–225.

Association of Official Analytical Chemists (AOAC). 1975. Official methods of analysis, 12th ed., Association of Official Analytical Chemists, Washington, DC.

Birke, A., L. Guillén, D. Midgarden, and M. Aluja. 2013. Fruit flies, *Anastrepha ludens* (Loew), *A. obliqua* (Macquart) and *A. grandis* (Macquart) (Diptera: Tephritidae): three pestiferous tropical fruit flies that could potentially expand their range to temperate areas, pp. 192–213. *In* J. Peña (ed.), Potential invasive pests of agricultural crops. CABI International, Boca Raton, FL.

Chang, C. L., and R. I. Vargas. 2007. Wheat germ oil and its effects on a liquid larval rearing diet for Oriental fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 100: 322–326.

Chang, C. L., R. Kurashima, and C. P. Albrecht. 2001. Larval development of *Ceratitis capitata* (Diptera: Tephritidae) on a meridic diet. Ann. Entomol. Soc. Am. 94: 433–437.

Christenson, L. D., S. Maeda, and J. R. Holloway. 1956. Substitution of dehydrated for fresh carrots in medium for rearing fruit flies. J. Econ. Entomol. 49: 135–136.

Cho, I. K., C. L. Chang, and Q. X. Li. 2013. Diet-induced over-expression of flightless-I protein and its relation to flightlessness in Mediterranean fruit fly, *Ceratitis capitata*. PLoS ONE 8: e81099.

Cohen, A. C. 2004. Insect diets: science and technology. CRC Press, Boca Raton, FL.

Cicero, L. 2011. Efecto de la calidad del hospedero en la dinámica ovárica y contenido nutricional de cuatro parasitoides (Hymenoptera: Braconidae) de *Anastrepha ludens* (Diptera: Tephritidae). Unpublished Ph.D. dissertation, Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico.

Collins, S. R., and P. W. Taylor. 2010. Flight ability procedures for mass-reared Queensland fruit flies, *Bactrocera tryoni*: an assessment of some variations. Entomol. Exp. Appl. 136: 308–311.

Crawley, M. J. 2007. The R book. Wiley, Chichester, United Kingdom.

Dirección General de Sanidad Vegetal-Dirección de Moscas de la Fruta (DGSV-DMF). 2009. Cría masiva de *Anastrepha ludens*. SENASICA-Dirección de Moscas de la Fruta. Mexico City, Mexico.

Domínguez, J., T. Artiaga-López, E. Solís, and E. Hernández. 2010. Métodos de colonización y cría masiva, pp. 259–276. *In* P. Montoya, J. Toledo and E. Hernández (eds.), Moscas de la fruta: fundamentos y procedimientos para su manejo. S y G editores, Mexico City, Mexico.

(FAO-IAEA-USDA) Food and Agriculture Organisation–International Atomic Energy Agency–U.S. Department of Agriculture. 2003. Manual for product quality control

and shipping procedures for sterile mass reared tephritid fruit flies. Version 5.0, IAEA, Vienna, Austria.

Gutiérrez, J. M., G. Santiago, A. Villaseñor, W. R. Enkerlin, and F. Hernández. 2013. Los programas de moscas de la fruta en México: su historia reciente. Instituto Interamericano de Cooperación para la Agricultura (IICA). Mexico City, Mexico.

Hernández, E., J. Toledo, T. Artiaga-López, and S. Flores. 2009. Demographic changes in *Anastrepha obliqua* (Diptera: Tephritidae) throughout the laboratory colonization process. J. Econ. Entomol. 102: 542–551.

Hernández, E., J. P. Rivera, D. Orozco-Dávila, M. Salvador, and J. Toledo. 2010. An artificial diet for rearing of *Anastrepha striata* (Diptera: Tephritidae). Fla. Entomol. 93: 167–174.

Köppler, K., T. Kaffer, and H. Vogt. 2009. Substantial progress made in the rearing of the European cherry fruit fly, *Rhagoletis cerasi*. Entomol. Exp. Appl. 132: 283–289.

Marden, J. H. 2000. Variability in the size, composition, and function of insect flight muscles. Annu. Rev. Physiol. 62: 157–178.

Montoya, P., P. Liedo, B. Benrey, J. Cancino, J. F. Barrera, J. Sivinski, and M. Aluja. 2000. Biological control of *Anastrepha* spp. (Diptera: Tephritidae) in mango orchards trough augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 18: 216–224.

Nash, W. J., and T. Chapman. 2014. Effect of dietary components on larval life history characteristics in the Medfly (*Ceratitis capitata*: Diptera, Tephritidae). PLoS ONE 9: e86029.

Nestel, D., E. Nemny-Lavy, and C. L. Chang. 2004. Lipid and protein loads in pupating larvae and emerging adults as affected by the composition of Mediterranean fruit fly (*Ceratitis capitata*) meridic larval diets. Arch. Insect Biochem. 56: 97–109.

Norma Oficial Mexicana NOM-051-SCFI-1994. 1994. Especificaciones generales de etiquetado para alimentos y bebidas no alcohólicas preenvasados. Diario Oficial de la Federación, 29 March 1994, Mexico City, Mexico.

Pascacio-Villafán, C., S. Lapointe, T. Williams, J. Sivinski, R. Niedz, and M. Aluja. 2014. Mixture-amount design and response surface modeling to assess the effects of flavonoids and phenolic acids on developmental performance of *Anastrepha ludens*. J. Chem. Ecol. 40: 297–306.

Pinheiro, J., B. Douglas, D. Saikat, S. Deepayan, and the R. Core team. 2009. nlme: Linear and nonlinear mixed effects models. R package ver. 3.1-92. (http://www.R-project.org)

R Core Team. 2013. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. (http://www.R-project.org/)

Rivera, J. P., E. Hernández, J. Toledo, B. Bravo, M. Salvador, and Y. Gómez. 2012. Optimización del proceso de cría de *Anastrepha ludens* Loew (Diptera: Tephritidae) utilizando una dieta larvaria a base de almidón pre-gelatinizado. Acta Zool. Mex. 28: 102–117.

Rull, J., A. Birke, R. Ortega, P. Montoya, and L. López. 2012. Quantity and safety vs. quality and performance: conflicting interests during mass rearing and transport affect the efficiency of sterile insect technique programs. Entomol. Exp. Appl. 142: 78–86.

Sacchetti, P., B. Ghiardi, A. Granchietti, F. M. Stefanini, and A. Belcari. 2013. Development of probiotic diets for the olive fly: evaluation of their effects on fly longevity and fecundity. Ann. Appl. Biol. 164: 138–150.

Salum, J. K., M. W. Mwatawala, P. M. Kusolwa, and M. D. Meyer. 2014. Demographic parameters of the two main fruit fly (Diptera: Tephritidae) species attacking mango in Central Tanzania. J. Appl. Entomol. 138: 441–448.

Santiago, M. G., D. D. Orozco, I. M del R. Hernández, A.J.H. Luis, and L.J.J. Bravo. 2012. Manual de control de calidad de moscas de la fruta estériles (procedimientos para evaluar el producto en los centros de empaque). SENASICA-Dirección de Moscas de la Fruta. Mexico City, Mexico.

Sentinella, A. T., A. J. Crean, and R. Bonduriansky. 2013. Dietary protein mediates a trade-off between larval survival and the development of male secondary sexual traits. Funct. Ecol. 27: 1134–1144.

Spishakoff, L. M., and J. G. Hernández-Dávila. 1968. Dried torula yeast as a substitute for brewer's yeast in the larval rearing medium for the Mexican fruit fly. J. Econ. Entomol. 61: 859–860.

Tzanakakis, M. E., A. P. Economopoulos, and J. A. Tsitsipis. 1968. Artificial rearing of the olive fly, progress report, pp. 123–150. *In* Proceedings of a panel on Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests, 17-21 October 1966, Tel Aviv. International Atomic Agency, Vienna.

Vera, T., S. Abraham, A. Ovideo, and E. Willink. 2007. Demographic and quality control parameters of *Anastrepha fraterculus* (Diptera: Tephritidae) maintained under artificial rearing. Fla. Entomol. 90: 53–57.

Warnes, G. R. 2009. Gmodels: various R programming tools for model fitting. R package version 2.15.0. (http://www.R-project.org/)

Zamek, A. L., J. E. Spinner, J. L. Micallef, G. M. Gurr, and O. L. Reynolds. 2012. Parasitoids of Queensland fruit fly *Bactrocera tryoni* in Australia and prospects for improved biological control. Insects 3: 1056–1083.