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IMPROVING MASS REARING TECHNOLOGY FOR SOUTH AMERICAN FRUIT FLY (DIPTERA:TEPHRITIDAE)

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ABSTRACT Studies on availability of suitable and economic diets for adults and larvae of the South American fruit fly *Anastrepha fraterculus* (Wiedemann, 1830) were carried out at the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria with the aim to find the best diets to fit in a large scale mass rearing production. The best diet for adult was the combination of Hydrolysate Corn Protein + Yeast Hydrolysate Enzymatic + Sugar (3:1:3). This diet resulted in the highest numbers of egg/female/day, spermatozoid in the spermathecae, percentages of egg hatch, the lowest mortality rate of adults and the highest average mating duration compared with the standard adult diet based on Yeast Hydrolysate Enzymatic + Sugar (1:3). Among eleven larval diets tested, diets based on sugarcane and sugarbeet bagases plus 7% brewer yeast, 8% sugar, 0.2% sodium benzoate, 0.8% of hydrochloric acid and 60% water (adjusted), yielded the highest percentages of egg hatching, pupal recovery, pupal weight and adult emergence. There was no statistical difference with the standard larval diet based on wheat germ 3%, corncob 15%, corn flower 8%, brewer yeast 6%, sugar 8%, sodium benzoate 0.23%, hydrochloric acid 0.63%, nipagin 0.14% and water 59% (adjusted). The significant performance of these adult and larval diets open discussion for future researches on improvement of rearing techniques required for the establishment of sterile insect technique (SIT) program focused on the South American fruit fly.
Key words: *Anastrepha fraterculus*, diet, pupal recovery, egg hatching, pupal weight.

AVANÇOS TECNOLÓGICOS NA CRIAÇÃO MASSAL DA MOSCA DAS FRUTAS SUL-AMERICANA (DIPTERA:TEPHRITIDAE)

RESUMO Estudos com o objetivo de testar dietas adequadas e econômicas para criação massal de adultos e larvas da mosca das frutas sul-americana, *Anastrepha fraterculus* (Wiedemann, 1830) foram desenvolvidos nos Laboratórios de Entomologia, Agricultura e Biotecnologia da Agência Internacional de Energia Atômica e da Organização das Nações Unidas para a Agricultura e Alimentação FAO/IAEA em Seibersdorf, Áustria. A melhor dieta para adultos foi a combinação de proteína hidrolisada de milho, hidrolisado enzimático de fermento e açúcar cristalizado na proporção de (3:1:3). Esta dieta proporcionou maiores quantidades de ovos diários por fêmea, maior quantidade de espermatozóide na espermateca, altas percentagens de eclosão de larvas, menores taxas de mortalidade de adultos, e maior duração de cópula quando comparada com a dieta padrão para adultos baseada em proteína hidrolisada mais açúcar cristalizada na proporção (3:1).Entre onze dietas testadas para larvas, a melhor foi aquela baseada em bagaço de cana-de-açúcar mais 7% de levedura de cerveja, 8% de açúcar cristalizado, 0,2% de benzoato de sódio, 0,8% de ácido hidroclorídrico e 60% de água (ajustado), produziu as maiores percentagens de eclosão de larvas, recuperação de pupas, peso de pupas e emergência de adultos. Os resultados desta dieta não foram estatisticamente diferentes da dieta padrão usada para *A. fraterculus*, baseada em 3% gérmen de trigo, 15% farelo de sabugo de milho, 8% de farinho de milho, 6% de levedura de cerveja, 8% de açúcar cristalizado, 0,23% de benzoato de sódio, 0,63% de ácido hidroclorídrico, 0,14% de nipagin e 59% de água (ajustado). Os excelentes resultados obtidos com as dietas para adultos e larvas abrem perspectivas para futuras pesquisas sobre avanços nas

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técnicas de criação massal necessárias para o estabelecimento da técnica do inseto estéril (TIE) com foco em programa direcionado para a espécie da mosca sul-americana das frutas.
Palavras chave: *Anastrepha fraterculus*, dieta, recuperação de pupa, eclosão de larvas, peso de pupas.

INTRODUCTION

The genus *Anastrepha* (Diptera: Tephritidae) is the largest and most economically important genus of true fruit flies in the American tropics and subtropics, and for some countries some species of *Anastrepha* are of quarantine importance (NORRBOM & KIM 1988, ZUCCHI 2000). This genus consists of 183 known species being only six of economic importance. It is indigenous to the Americas, presently with no distribution outside the Western Hemisphere. It has been a major pest of citrus, mangos, guavas and other subtropical fruits. Among other species, the South American fruit fly, *Anastrepha fraterculus* (WIEDEMANN, 1830) is highly economic important in South America (IAEA 1999, QUILAN *et al*. 2002).

Nutritional conditions in insects may play a significant role in the ability of males to attract females and result in mating. The relation between nutrition and male reproductive behavior is shown by production of signs for courtship and males may use nutrients to synthesize materials for production of pheromones or substances transferred during copulation such as sperm (LANDOLT & SIVINSKY 1992, EPSKY & HEATH 1993, PITNICK & MARKOW 1994, NISHIDA *et al*. 1997, Shelly *et al*. 2002) or accessory glands secretion (JANG, 2002). Adult diets for *Ceratitis capitata* (WIEDEMANN, 1824) (Diptera: Tephritidae) with high content of sugar and protein resulted in better male reproductive behavior, higher number of lekking males than males fed on diet with smaller percent of sugar and protein. In addition protein-fed males sustained long "bouts" of pheromone-calling and courted more often than protein-deprived males (PROKOPY & HENDRICKS, 1979, WARBURG & YUVAL 1996, 1997, YUVAL *et al*. 1998, FIELD & YUVAL 1999). There is a general agreement that protein-fed males are more likely to copulate. Vienna-7 sterile Medfly males copulated much more frequently in cages with protein than did the regular sugar fed colony. On the other hand wild males that emerged in the laboratory and were tested for copulation in a field cage also succeeded significantly better than sugar fed males (KASPI & YUVAL 2000, YUVAL *et al*. 2002). Sperm transfer cannot mean sperm storage by females. Sperm in spermathecae was found in 94% of females that had copulated. Females were significantly less likely to store sperm of protein deprived-males (SEO *et al*. 1990, TAYLOR & YUVAL 1999, YUVAl *et al*. 2002). These results strongly speculate that adult diet has a strong effect on the ability of males of fruit fly species to meet the requirement of energy from diets associated with the production of pheromone, courtship behavior and mating.

By examining the effect of age of the Mediterranean fruit fly on its propensity to mate in field cages, Liedo *et al*. (2002), showed that the optimal age for mating ranged between 7 to 13 days for wild flies and 3 to 5 days for mass reared flies. They also showed that when sexes are held separately, flies are more prone to mate. Theses results support finds by Hendricks *et al*. (1995) and Rendon *et al*. (2000). For South American fruit fly, Salles (1999) showed that sexual maturation of males began at the age of 5 days, and full sexual maturation was reached at 11 days old.

Bulking and nutritive components in an insect diet can be very costly and in some countries very difficult to import. The replacement of imported components by local products has been the concern of many researchers in countries that have fruit fly mass rearing factory. Several attempts were made by Tanaka *et al*. (1969) in order to find suitable and inexpensive substitutes for dehydrated carrot powder and brewer yeast in rearing diet of the Mexican fruit fly, *Anastrepha ludens* (Loew). Insect diet has a profound effect on the performance of immature and adult phases. The omission of essential amino acids from *C. capitata* diets inhibited and delayed development and growth. Pupal recovery, adult emergence and flight ability were affected by removing vitamins or cholesterol. Increasing the sugar content in a diet did not affect egg production and hatch, but influenced fly survival (CHANG *et al*. 2001).

Attempts to improve diet quality for mass rearing *C. capitata* have been ongoing for more than a half century. The diets currently used throughout the world were based on combined ingredients taken in account their availability, physical properties and economics with little emphasis on nutrient value. The essential information on nutritional requirements is still lacking because of the inability to rear larvae in a

complete purified diet (CHANG et al., 2000).

A mass rearing system and Sterile Male Technique SIT for *A. fraterculus* are underway and will remain a challenge. A technique to mass rear *A. fraterculus* is the start point for the development of SIT (JALDO *et al*., 2001, QUINLAN *et al*. 2002). In very few places in the world efforts have been focused on its mass rearing technology. The objective of the present work was to identify appropriate ingredients for suitable larval and adult diets to be used as a platform to develop a protocol for mass rearing the South American fruit fly.

MATERIALS AND METHODS

Studies were conducted at FAO/IAEA Entomology Unit Laboratories in Seibersdorf, Austria. Eggs were obtained from an *A. fraterculus* colony introduction from Tucuman, Argentina (JALDO *et al*. 2001). This colony was in the fifth generation at FAO/IAEA Entomology Laboratories. Adults were being fed on sugar plus Yeast Hydrolysate Enzymatic^{R} (ICN Biomedicals, Inc. Aurora, Oh 44202) YHE (3:1). The larvae were being fed on Mexican fruit fly (*Anastrepha ludens* Loew) larval diet (Tanaka *et al*. 1969) based on wheat germ, corn flower and brewer yeast. Data from this research will be summarized as mean numbers and percentages of egg hatch, pupal recovery, adult emergence, egg/ female/day, weight of pupa, and estimation of spermatozoids in the spermathecae. Standard analysis of variance ANOVA and Tukeys's HSD tests at 5% level was used. Data will be reported as means ±SE. Nonparametric data was analyzed by Kruskal-Wallis test (MINITAB STATISTICAL SOFTWARE, 2000).

Adult diet

For this study research actions were undertaken in order to find a source of protein that could promote a better egg production and hatchability. Three adult diets were used as follow:

Diet A – Yeast Hydrolysate Enzymatic ^{R} (YHE) + Sugar (1:3) standard

Diet B – Hydrolysate Corn Protein ^{R} (ARCOR – Buenos Aires, Argentina) + YHE ^{R} + Sugar (3:1:3)

Diet G– Soy Hydrolysate Enzymatic^{R} (ICN Biomedicals, Inc.Aurora, Oh 44202) + YHE ^{R} + Sugar (3:1:3)

Three days before adult emergence, pupae were placed into emergence plastic cages. In the first day of adult emergence, 100 virgin females and 100 virgin males were separated in different boxes, comprising 6 cages for the three diets. Each cage was supplied with water and the correspondent above diet. After ten days of emergence which has reached sexual maturity, Salles (1999), fifty lekking males and fifty females of the same treatment were placed into a standard USDA cage (Plexigas cage 30x30x40cm) for mating. Mating pairs of each treatment were separated in an individual box for behavior studies. The courtship behavior was recorded from 10:00 am to 12:00 pm (laboratory condition). All pairs that mated for more than eight minutes were selected for biology studies. Further, ten mated females from each treatment were placed into a small plastic cage (16x10x10cm) with an oviposition silicone panel on front. Each cage was supplied with water and the corresponding adult diet.

The study had three treatments (diets), five replicates (cages with ten females), comprising fifteen cages and a total of one hundred fifty females. After five days, eggs were daily collected from each silicone panel counted and spread with a small camel hair brush on moist blotter paper in Petri dishes for egg hatching studies. After ten days of egg laying, females from each treatment were dissected, taken the spermathecae and placed onto a slide, then softly squashed with a cover slip for observation of presence of sperm. Each of the three spermathecae of each female was observed under a light (40x) microscopy and evaluated the number of spermatozoids in each spermathec. The criteria for number of spermatozoid estimation was used a scale of one to four. Number one stands for zero spermatozoid; number two less than one hundred spermatozoids; number three more than one hundred and less than thousand, and number four over a thousand spermatozoids.

Larval diet

A screening of eleven different larval diets (Table 1) was performed in order to find suitable and economic diets for further comparison tests. Thirty grams of each diet were poured in a plastic Petri dish with 8.5cm of diameter and 2.0cm high. The composition and formulation of each diet are listed in Table 1. The standard Mexican fruit fly larval diet (Tanaka et al. 1969) (diet 1) based on wheat germ, corncob fraction, cornflower, brewer yeast, sugar, sodium benzoate, hydrochloric acid, nipagin and water, was used as the control treatment throughout this study. Diets were mixed in a domestic blender.

Table 1. Different diets and their ingredients for larvae of *Anastrepha fraterculus* Diets.

Ingredients (%)	**1**[1]	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Wheat germ	3.0	8.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Corn cob	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wheat bran	0.0	14.0	18	0.0	4.0	0.0	2.0	2.0	0.0	0.0	0.0
Corn flower	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Beet bagasse	0.0	0.0	0.0	24.0	14.0	18.0	18.0	0.0	0.0	0.0	0.0
Cane bagasse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.0	18.0	18.0	18.0
Yeast	6.0	5.0	5.0	7.0	13.0	13.0	5.0	5.0	5.0	7.0	9.0
Sugar	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Na.Benzoate	0.23	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Hydchl. acid	0.63	0.6	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Nipagin	0.14	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water	59.0	64	65.8	59.8	59.8	60.6	65.8	65.8	67.8	65.8	64.0

[1]Seibersdorf standard diet.

All diets had the pH adjusted to a value between 3.8 to 4.0.

The rearing room with photoperiod of 10:14 h (L:D) was maintained com fluorescent lights. The general procedure for diet preparation was based on Tanaka *et al*. (1969) and quality control test were run followed the protocols specified on the International Fruit Fly Quality Control Manual (FAO/IAEA/USDA 1998). Five replicates of 100 eggs each (bubbled for 48 hours) were seeded onto a fine strip blotting paper placed on top of diet. After the eggs were applied to the diets they were held at 29°C and 90% relative humidity. After 6 days, strip blotting papers were removed from Petri dishes and checked for egg hatching. After 8 days, Petri dishes were removed to other room with temperature of 21°C and 75% of relative humidity until larval development was complete. After 10 days individual Petri dish was put in plastic box with saw dust for pupation. Pupae were recorded and transferred to individual box for adult emergence.

The efficacy of the diets of Table 1 was determined by evaluating pupal recovery, pupal weight and adult emergence. From the eleven diets on Table 1, six diets (diets 1, 4, 7, 9, 10 and 11) that had over 50% of pupal recovery were selected for a simulate rearing scale comparison test in plastic trays (30x19x2cm) with 250g of the correspondent diet. Trays with a strip blotting paper on the diet were seeded with 200 eggs bubbled for 48 hours. This experiment was set up with 6 treatments (diets) and 5 replications. Data from this trial were, egg hatching, pupal recovering, pupa weight and adult emergence.

RESULTS AND DISCUSSION

The final goal of a fruit fly mass rearing production is a consistent result of healthy and competitive adults. This success is very dependable of high control quality of all laboratory procedures and specially a suitable and economic diet.

Adult males fed on diet B had faster response in searching for females compared those fed on diets A and C. They also presented longer mating time. The maximum mating duration was 35 minutes for pairs fed on diet B. The average mating time for adults fed on diet B was 19 minutes. The least mating duration time was 10 minutes for mostly pairs fed on diet C. The overall average mating duration was 14 minutes. Number of eggs per female per day was higher in diet B and was significantly different from diets A and C (Table 2). The highest percentages of egg hatching were obtained from diet B that showed significantly different when compared with other diets. From 50 mated females fed on each diet, ten days after feeding and egging, the highest numbers of dead females were found in cages with diet C, followed by diet A. Diet B presented only 12 (24%) dead females while diet C presented 27 (54%) dead and diet A, with 21 (42%) dead females. Only 11% of spermathecae of females fed on diet A presented more than 100 spermatozoids stored. Diet B presented the highest percentages (53%) of spermathecae with more than 100 spermatozoids. In this study, Hydrolysed Corn Protein^{R} deprived males fed on diets A and C, presented very low searching ability and mating time. Possibly explanation for significant results of numbers of egg/female/day, percentage of egg hatching and number of sperm

Table 2. Adult diets for *Anastrepha fraterculus*, number of eggs per female per day, percentage of egg hatching and spermatozoid density in the spermathecae.

DIET	Egg/female/day (±SE)*	Egg Hatching (%) (±SE)*	Sperm in Spermathecaec**
Diet A Yeast Hydrolysate Enzymatic(YHE) + Sugar (1:3)	4.13±1.11 **b**	14.28±1.87 **b**	<100
Diet B Hydrolysate Corn Protein + YHE + Sugar (3:1:3)	16.34±0.39**a**	59.98±1.97**a**	>100
Diet C Oliver Hydrolysate Protein ^{R} + YHE + Sugar (3:13)	9.77±0.88 **b**	13.39±2.35 **b**	<100

*Data followed by the same letter in the same column do not differ significantly according to Tukey`s HSD test (P>0.05).
**Used nonparametric' Kruskal-Wallis test

transferred is that Hydrolysed Corn Protein^{R} in diet B fulfils some base requirements for both male and female to expand more energy and court more vigorously than flies fed on other sources of protein. In addition females fed on diet B had less mortality (24%) than on diet A (42%) and diet C (54%).

Data in Table 3 shows the results of pupal recovery, pupal weight and adult emergence of five of those diets from Table 1 (4, 7, 9,10 and 11). These diets were compared with standard diet 1 also from (Table 1). All diets presented acceptable values for adult emergence. Diet 4, 10 and 11 were not significantly different from the standard diet regarding percentage of pupal recovery. However, the standard diet was statistically different from diet 7 and 9. Pupal weights from diets 10 and 11 were not significantly different. Diet 11 presented pupal weights significantly higher than those from diets 1, 4, 7 and 9. Diet 10 with 7% of protein was not significantly different from standard diet 1 also with 7% of protein.

The use of bulking agent like sugarbeet bagasse, sugar cane bagasse and corncob in diets for Tephritidae was studied by Vargas et al. (1983,1994). They emphasized that these bulking agents defray some of the cost of the large amounts of diet needed to mass-rear billions of flies for SIT programs. These products absorb liquid and provide suitable physical consistency and texture for larval feeding and growth. These significant results of of pupal recovery, pupal weight and adult emergence from diets 4, 9 and 10 are supported by the finds of Vargas et al. (1983,1994).

The diets described in Table 3 yielded high quality adults and they can be suitable for a mass release program based on a future SIT. These results were based on a small scale test and they were also validated in large USDA-APHIS MISSION TEXAS cages (215cmx185cmx31cm) with 900mL of pupae which gave similar and acceptable results of egg production, pupal recovery and adult quality.

Table 3. Pupal recovery, pupal weight and adult emergence from diets for larvae of the *Anastrepha fraterculus*

Diet	Pupal recovery (%) (±SE)	Pupal weight (mg) (±SE)	Adult Emergence (%)
1 -Standard diet (based on wheat germ + corn-cob + corn flower + 6% yeast)	66.0 ± 2.75 **a**	16,45 ± 0.58 **b**	92.6
4 - Based on sugarbeet bagasse + 7% brewer yeast	68.0 ± 1.34 **a**	17.06 ± 0.33 **b**	91.8
7 - Based on sugarbeet bagasse + 5% brewer yeast	58.0 ± 1.09 **b**	13.55 ± 0.24 **c**	91.0
9 - Based on sugarcane bagasse + 5% brewer yeast	61.0 ± 0.71 **b**	14.61 ± 0.28 **c**	91.0
10 - Based on sugarcane bagasse + 7% brewer yeast	67.8 ± 0.49**a**	17.68 ± 0.22**ab**	91.4
11 - Based on sugarcane bagasse + 9% brewer yeast	62.4 ± 1.32**ab**	18.43 ± 0.25**a**	91.6

Data followed by the same letter in the same column do not differ significantly according to Tukey`s HSD test (P<0.05).

CONCLUSIONS

Diets for adults of *Anastrepha fraterculus* based on combination of Hydrolisate Corn Protein, Yeast Hidrolisate Enzimatic plus sugar (3:1:3) present the best results regarding eggs/ female/day and egg viability.

Larval diets based on sugarcane bagasse plus 7,0% of brewer yeast presented significant results and can be used in a mass rearing program for *Anastrepha fraterculus.*

The significant performance of these adult and larval diets open discussion for future researches on improvement of rearing techniques required for the establishment of sterile insect technique (SIT) program focused on the South American fruit fly.

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