Notas Científicas

Diets based on soybean protein for Mediterranean fruit fly

Raimundo Braga Sobrinho[(1)], Carlos Caceres[(2)], Amirul Islam[(2)], Vivat Wornoayporn[(2)] and Walter Enkerlin[(3)]

[(1)]Embrapa Agroindústria Tropical, Rua Dra. Sara Mesquita, nº 2270, CEP 60511-110 Fortaleza, CE, Brazil. E-mail: braga@cnpat.embrapa.br [(2)]Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria. E-mail: C.Caceres@iaea.org [(3)]Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria. E-mail: W.Enkerlin@iaea.org

Abstract – The objective of this work was to develop suitable and economic diets for mass rearing Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Diets containing sugar beet bagase, wheat bran, brewer yeast, and others with wheat bran and palletized soybean protein from Brazil were tested. Diets based on soybean protein have shown promising results regarding pupal recovery, pupal weight and adult emergence. Soybean bagase in the form of pellets with 60% of protein can be a very important substitute for other expensive sources of protein.

Index terms: *Ceratitis capitata*, soybean bagase, sugar beet bagase, brewer yeast, biology.

Dietas baseadas em proteína de soja para moscas do Mediterrâneo

Resumo – O objetivo deste trabalho foi desenvolver dietas adequadas e econômicas para a criação massal de moscas de frutas do Mediterrâneo, *Ceratitis capitata* (Diptera: Tephritidae). Foram testados dietas com bagaço de beterraba açucareira, farelo de trigo, levedura de cerveja e outras dietas de farelo de trigo e proteína de soja prensada brasileira. Dietas compostas por proteína de soja apresentaram resultados positivos de recuperação de pupas, pesos de pupa e emergência de adultos. O bagaço de soja, na forma de pellet com 60% de proteína, pode ser um importante substituto de outras fontes de proteína.

Termos para indexação: *Ceratitis capitata*, bagaço de soja, bagaço de beterraba açucareira, levedura de cerveja, biologia.

The Sterile Insect Technique (SIT) is one of the most promising control approaches for the future of fruit fly integrated management (Enkerlin & Munford, 1997; Hendrichs et al., 2002). In addition to SIT, another important step was the development of fruit fly male only strains through the temperature sensitive lethal (TSL) (Caceres, 2002). All these advances in fruit flies mass rearing and releasing programs are highly dependable on efficient larval and adult diets. The essential information on nutritional requirements is still lacking, because of the inability to rear fruit fly larvae in a complete purified diet (Chang et al., 2000).

The larvae of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824) or Medfly have been reared for SIT program on a variety of artificial diets for decades. Available information on larval nutritional requirements for Tephritid has been focused on improving survival, growth, or development by using dry plant materials and yeasts (Vargas et al., 1994; Manoukas & Zografou, 1997). In general, nutrients from some diets apparently are sufficient to support larval development. However, for optimization of growth and development, a completely chemically defined diet is required for better understanding of nutritional responses for *C. capitata* (Chang et al., 2001).

Bulking and nutritive components in an insect diet can be very costly and in some countries are difficult to import. The replacement of imported components by local products has been the concern of researchers in many countries that have fruit fly (Diptera: Tephritidae) mass rearing factory.

Insect diet has a profound effect on the performance of immature and efficiency of sterile adult released on the field (Yuval et al., 2002). The omission of essential amino acids from Medfly diet inhibited and delayed development and growth. Pupal recovery, adult emergence and flight ability were affected by removing vitamins or cholesterol. Increasing sugar content in a diet did not affect egg production and hatch, but influenced fly survival (Chang et al., 2000, 2001). A diet with an adequate amount of protein is essential for larvae of *C. capitata*. The absence of essential amino acids resulted in no survivorship. Vitamins improved larval development, pupal recovery, pupal weight, adult emergence and flight ability. Absence of sugar not only delayed larval developmental period, but also reduced the pupal weight (Chang et al., 2001).

The SIT has become a major area-wide control strategy against some key insect pests. The first requirement for such activity is the ability to mass-producing millions of insects at an economically practical cost.

The objective of the present work was to develop suitable and economic diets, using an alternative source of protein (Brazilian soybean protein) and bulking agents (wheat bran, sugar beet and sugarcane bagase), for mass rearing Mediterranean fruit fly under requirement of a SIT mass release program.

The studies were conducted at the FAO/IAEA, Entomology Laboratory in Seibersdorf, Austria, following standard procedures (A Manual..., 1998). Eggs were obtained from a fruit fly colony of Genetic Sexing Strain (GSS), based on TSL mutation Medfly colony Vienna-8, mass reared for 12 generations. Adults were fed on sugar plus yeast hydrolysate enzymatic YHE (3:1) (ICN Biomedical, Inc. Aurora, Oh 44202), and larvae fed on a standard Seibersdorf's diet based on wheat bran.

The first test involved the comparison of two different diet formulations, based on blended and nonblended sugar beet bagase and wheat bran, with the standard Seibersdorf's diet (diet 1).

Second test involved the comparison of four diets, based on wheat bran and Brazilian soybean protein with the standard Seibersdorf's diet. The composition and formulation of each diet are listed in Table 1. For each test, 200 g of each diet were poured in a plastic tray (30x19x2 cm).

All diets had their pH adjusted to a value between 3.8 and 4.0. Six replicates of 200 eggs, previously incubated for 48 hours, were seeded onto a strip blotting paper placed on top of diet. Afterwards, they were held at room, with a temperature of 29°C and 90% relative humidity.

After four days, strip blotting papers were removed from trays and checked for egg hatching. Eight days later, the trays were removed to other room, with temperature of 21°C and 75% of relative humidity, until larval development was complete.

After ten days, individual trays were placed in a plastic box with saw-dust as pupation substrate. Pupae were recorded and transferred to individual box for adult emergence. The best diet was selected for a simulation test of mass production, based on these results, wich were always compared with those obtained using the standard Seibersdorf's diet. For this test, plastic trays (30x19x3 cm) were used with 350 g of the correspondent diet. A strip of blotting paper was placed on the diet in each tray, and was seeded with 0.3 mL of eggs that had been previously incubated for 48 hours.

Data were summarized as mean numbers and percentages of egg hatch, white and brown pupae recovery, weight of 100 white and brown pupae, total weight of 100 pupae and adult female and male recovery. Standard analysis of variance ANOVA and Turkey's HSD tests at 5% level were used. Data were reported as means±SE (Minitab Statistical Software, 2000).

Pupal recovery from diet based on nonblended sugar beet bagase (diet 2) did not present significant difference from the standard diet (diet 1). However, blended sugar beet bagase (diet 3) was inferior to standard and nonblended diet (Table 2). Diets 1 and 2 were statistically different from diet 3, based on blended sugar beet bagase. Although these three diets used the same source and amount of protein, the statistical difference was detected only in diet 3 with sugar beet bagase blended. This inferiority is probably due to the change in the diet texture,

Table 1. Different diets and their percentages of ingredients tested for larvae of *Ceratitis capitata*.

Ingredients (%)	Diets						
	1(1)	2(2)	3(3)	4(4)	5(4)	6(4)	7(4)
Wheat bran	28.0	4.0	4.0	28.0	28.0	28.0	28.0
Soybean protein	-	-	-	7.0	9.0	14.0	17.0
Sugar beet bagase	0.0	21.0	21.0	0.0	0.0	0.0	0.0
Brewer yeast	7.0	7.0	7.0	0.0	0.0	0.0	0.0
Sugar	13.0	13.0	13.0	13.0	13.0	13.0	13.0
NaBenz	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Hyd. acid	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Water	50.2	53.2	53.2	50.2	48.2	43.2	40.2
Total (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0

(1)Standard Seibersdorf's diet. (2)Sugar beet bagase not blended. (3)Sugar beet bagase blended. (4)Soybean protein diet.

increasing the metabolic heat and making difficult the movement of larvae in the diet (Tanaka et al., 1972). Percentages and weights of white and brown pupae did not present statistical differences when compared with standard diet.

The results of other four diets selected for tests are presented in Table 3. These diets present the same amount of wheat bran, and different percentage of soybean protein. Except for weight of 100 white and brown pupae, the other parameters were not significantly different from the standard diet. Weight of white pupae in diet 1 was not significantly different from diets 4, 5, 6 and 7. For weight of 100 white pupae, diet 5 was statistically different from diet 4. Even though statistical differences were found in weight of pupae, no differences were detected in pupal and adult recoveries. It seems that there is an optimum or a balance value for amount of protein regarding larval development. Although these diets are not considered yet a chemically-based diet for *C. capitata*, the choice of any amount of soybean protein can be used with similar results to the standard diet.

Diet 4 based on soybean protein presents potential to be used in a mass rearing production system for *C. capitata* (Table 4). The results were similar to the previous small-scale experiment regarding larval development (Table 3). There were no statistical differences for all larval development parameters between the two diets (1 and 4).

It seems that Brazilian soybean protein has chemical components that fulfill basic requirements for Medfly development, comparing with brewer yeast of the standard diet. The final goal of mass production is to continuously maintain production of healthy and sterile adults for release in large-scale SIT programs. These results highlight the need for a cost-effective mass rearing process, a fundamental aspect for the sustainability of a SIT program. The diets tested, based on bulking and nonbulking agents, yielded high quality pupae and adults and can be suitable for mass rearing Medfly under a SIT release program.

Table 2. Percentages of egg hatch, pupal recovery, white and brown pupae, weight of white and brown pupae, and total weight of pupae of *Ceratitis capitata* obtained from different diets(1).

Diet	Egg hatch (%)	Pupal recovery (%)	White pupae (%)	Brown pupae (%)	Weight 100 white pupae (g)	Weight 100 brown pupae (g)	Total weight 100 pupae (g)
1(2)	73.7±2.4a	60.5±2.2a	51.2±3.8a	48.8±3.8a	0.9899±0.01a	1.0236±0.02a	0.9471±0.01a
2(3)	77.3±2.1a	57.6±2.9a	45.6±2.1a	54.3±2.1a	0.9657±0.01a	0.9624±0.01a	0.9716±0.04a
3(4)	74.8±3.0a	48.0±2.3b	48.2±1.9a	51.9±1.9a	0.9665±0.02a	1.0274±0.01a	0.8985±0.02a

(1)Data followed by the same letter, in the same column, do not differ significantly according to Turkey's HSD test (P>0.05). (2)Standard Seibersdorf's diet. (3)Sugar beet bagase not blended. (4)Sugar beet bagase blended.

Table 3. Percentage of egg hatch, percentage of white and brown pupae recovery, weight of 100 white and brown pupae, and adult female and male recoveries of *Ceratitis capitata*, obtained from different diets(1).

Diet	Egg hatch (%)	White pupae recovery (%)	Brown pupae recovery (%)	Weight 100 white pupa (g)	Weight 100 brown pupa (g)	Adult female recovery (%)	Adult male recovery (%)
1(2)	71.3±0.5a	26.8±2.1a	22.0±1.0a	0.8995±0.03ab	0.8345± 0.2b	74.06±33a	76.90±1.1a
4(3)	66.4±1.5a	27.0±2.9a	22.4±4.5a	0.8330±0.02b	0.8278± 0.2b	74.01±2.9a	74.48±4.5a
5(3)	66.4±1.9a	25.8±3.8a	22.0±1.1a	0.9189±0.02a	0.8794± 0.1ab	86.00±3.8a	86.20±1.2a
6(3)	67.8±0.9a	27.2±2.4a	20.0±2.5a	0.8626±0.02ab	0.8555±0.2b	76.91±2.4a	84.71±2.5a
7(3)	70.4±1.2a	24.2±3.4a	21.6±3.4a	0.8795±0.01ab	0.8267±0.2b	71.96±3.4a	80.64±3.4a

(1)Data followed by the same letter, in the same column, do not differ significantly, according to Tukey's HSD test (P>0.05). (2)Standard Seibersdorf's diet. (3)Soybean protein diet.

Table 4. Comparative test of Standard Seibersdorf's diet (diet 1), and best Soybean protein diet (diet 4) for *C. capitata*, averages and standard error of pupal production, number of white and brown pupae in 5 mL, total of pupae in 5 mL, weight of 100 white and brown pupae(1).

Diet	Pupal production (mL)	White pupae in 5 mL	Brown pupae in 5 mL	Pupae total in 5 mL	Weight 100 white pupae (g)	Weight 100 brown pupae (g)
1(2)	35.8±0.87a	126.8±4.66a	144.4±6.38a	271.2±5.00a	0.8563±0.009a	0.8138±0.003a
4(3)	35.0±0.59a	119.2±3.61a	155.0±9.51a	274.8±12.7a	0.8217±0.008a	0.8027±0.005a

(1)Data followed by the same letter, in the same column, do not differ significantly, according to Tukey's HSD test (P>0.05). (2)Standard Seibersdorf's diet. (3)Soybean protein diet.

Acknowledgements

To Salvador Meza Hernandez and Maria Teresa Vera, for providing useful suggestions and for their laboratory assistance; to CNPq, Embrapa and the International Atomic Energy Agency (IAEA), Joint FAO/IAEA Division of Nuclear Technique in Food and Agriculture, for supporting this research.

References

A MANUAL of quality control for fruit flies. Version 4.0. Vienna: FAO: IAEA: USDA, 1998.

CACERES, C. Mass rearing of temperature sensitive genetic sexing strains in the Mediterranean fruit fly (*Ceratitis capitata*). **Genetica**, v.116, p.107-116, 2002.

CHANG, C.L.; KURASHIMA, R.; ALBRECHT, C.P. Effect of limiting concentrations of growth factors in mass rearing diets for *Ceratitis capitata* larvae (Diptera: Tephritidae). **Annals of the Entomological Society of America**, v.94, p.898-903, 2000.

CHANG, C.L.; MANGINE, T.E.; BROWN, C.R.; HARRIS, E.J.; BAUTISTA, R.C.; VARGAS, R.I. Larval development of *Ceratitis capitata* (Diptera: Tephritidae) on a meridic diet. **Annals of the Entomological Society of America**, v.94, p.433-437, 2001.

ENKERLIN, W.; MUNFORD, J. Economic evaluation of three alternative methods for control of the Mediterranean fruit fly (Diptera: Tephritidae) in Israel, Palestinian territories, and Jordan. **Journal of Economic Entomology**, v.90, p.1066-1072, 1997.

HENDRICHS, J.; ROBINSON, A.S.; CAYOL, J.P.; ENKERLIN, W. Medfly area-wide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behaviour studies. **Florida Entomologist**, v.85, p.1-13, 2002.

MANOUKAS, A.G.; ZOGRAFOU, E.N. Utilization of low cost media for rearing the Mediterranean fruit fly. **Bulletin OILB SROP**, v.20, p.122-127, 1997.

MINITAB statistical software: user's guide [State College, PA], 2000.

TANAKA, N.; HART, R.A.; OKAMOTO, R.Y.; STEINER, L.F. Control of excessive metabolic heat produced by a high density of larvae of the Mediterranean fruit fly. **Journal of Economic Entomology**, v.65, p.866-867, 1972.

VARGAS, R.I.; MITCHELL, S.; HSU, C.L.; WALSH, W.A. Laboratory evaluation of diets of processed corncob, torula yeast, and wheat germ on four developmental stages of Mediterranean fruit fly (Diptera: Tephritidae). **Journal of Economic Entomology**, v.87, p.91-95, 1994.

YUVAL, B.; KASPI, R.; FIELD, S.A.; BLAY, S.; TAYLOR, P. Effects of post-teneral nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). **Florida Entomologist**, v.85, p.165-170, 2002.

Received on February 4, 2005 and accepted on August 8, 2005