

## SCIENTIFIC NOTE

Use of Polyester Pad as a New Physical Substrate for Rearing *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae) LarvaeCARLOS E. SILVA<sup>1</sup>, GONZALO E. MOYA-BORJA<sup>2</sup> AND PATRICIA AZAMBUJA<sup>3</sup><sup>1</sup>Depto . Microbiologia, Instituto de Ciências Básicas da Saúde, Univ . Federal do Rio Grande do Sul Sarmiento Leite, 500, 90070-150, Porto Alegre, RS; ceusilva@ufrgs .br<sup>2</sup>Faculdade de Veterinária, Curso de Pós-Graduação em Ciências Veterinárias, Univ . Federal Rural do Rio de Janeiro Instituto de Veterinária – UFRRJ, BR 465, km 7, 23 .890-000, Seropédica, RJ; gmoya@ufrj .br<sup>3</sup>Depto . Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil, 4365, 40-360 Manginhos, RJ; azambuja@ioc.fiocruz.br

---

*Neotropical Entomology* 37(3):349-351 (2008)Uso de Manta de Poliéster como Substrato Novo para Criação de Larvas de *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae)

RESUMO - A utilização de manta de poliéster como substrato para a criação de larvas de *Cochliomyia hominivorax* Coquerel em dieta líquida foi comparada com dieta à base de carne moída. Não foram verificadas diferenças na média dos pesos de larvas de terceiro estágio e pupas, número de pupas formadas, emergência e eficiência de criação. Os resultados foram obtidos por quatro gerações consecutivas, não demonstrando interferência em nenhum parâmetro biológico avaliado. Propõe-se que manta de poliéster pode ser utilizada como suporte para a criação laboratorial de *C. hominivorax* em dieta líquida, reduzindo os custos de criação, uma vez que pode ser reutilizada.

PALAVRAS-CHAVE: Mosca da bicheira, criação larval, matriz sintética, dieta líquida

ABSTRACT - Polyester pad was utilized as solid support for rearing *Cochliomyia hominivorax* Coquerel larvae in liquid diet and compared with the ground meat diet. There were no significant differences in the mean third instar larvae and pupal weights, number of pupae, emergence and rearing efficiency. The tests were conducted through four consecutive generations, presenting no detectable effect in any of the life history parameters. We propose that the polyester pad can be used as solid support for rearing *C. hominivorax* larvae in liquid diets and can be reutilized reducing the costs of mass rearing.

KEY WORDS: Screwworm, larval rearing, synthetic matrix, liquid diet

*Cochliomyia hominivorax* Coquerel also known as screwworm fly is an agent of primary myiasis originated in America. Screwworms are obligatory parasites of warm-blooded animals which cause great economical losses to livestock production (Baumhover 1966, Snow *et al.* 1985, Grisi *et al.* 2002) and several cases of human infestation are related annually (Hall & Wall 1995, Nascimento *et al.* 2005).

To reduce the losses caused to livestock production, Knippling (1955) proposed an eradication program of this fly releasing in the field sexually sterile males, which will compete with wild males for mates. This program has been developed successfully for 40 years and resulted in eradication of *C. hominivorax* from some Caribbean countries as well as North and Central America up to Panama (Galvin & Wyss 1996).

The possibility of expanding this eradication program to South-America is under study but problems such as the

environmental impact (Moya Borja 2003) and questions related to the mass rearing of sterile insects have to be taken into consideration. To give an example, the weekly release of millions of sterile males would require complex facilities for keeping the screwworm fly colonies and consume great quantities of raw material for rearing the larvae.

In nature, the larvae of the screwworm fly only develop in living animal tissues. *C. hominivorax* however can be reared in the lab at 37°C on a low-fat meat diet (Graham & Dudley 1959). The diet currently used in the mass rearing of the screwworm fly is a hydroponic (liquid) diet, similar to that proposed by Brown & Snow (1979). It contains starch grafted sodium polyacrylate (Chaudhury & Alvarez 1999), which provides a consistence similar to that of viable tissue, ideal for larval development.

In this experiment, we studied the substitution of the meat-based diet by liquid diet in the *C. hominivorax* colony maintained in our laboratory to facilitate the handling and

rearing of the larval stage. However, the difficulty to obtain starch grafted sodium polyacrylate on the Brazilian market led us to try substituting it by a polyester pad. The objective of this trial was to compare larval development of *C. hominivorax* reared on meat diet with larval development on liquid diet using a new synthetic matrix to give the larvae physical support.

In this study, we compared larval development of *C. hominivorax* reared on two different diets. The meat diet (Graham & Dudley 1959) contained 55 g ground bovine meat, 15 ml citrated bovine blood, 29.9 ml distilled water and 0.1 ml of formalin. The liquid diet was similar to that used by Taylor et al. (1991), and was composed of 20 ml of citrated bovine blood, 5 g dried egg, 5 g non fat spray dried milk, 69.9 ml distilled water, 0.1 ml formalin and a polyester substrate, class ABNT G2 or G3 (comparable to ASHRAE 52-76), white color, 0.5 inch thickness, in a proportion of 1 gram per 20 ml of liquid diet.

On both diets larval development were originated in similar conditions to that proposed by Taylor & Mangan (1987). Immediately after oviposition, the *C. hominivorax* eggs were placed on a petri dish (5 cm diameter) containing meat diet. The eggs were incubated at  $37 \pm 2^\circ\text{C}$  and relative humidity of  $70 \pm 5\%$  for 24h (1<sup>st</sup> day). After this period the first instars (recently hatched larvae) were separated to be used in the trial.

Larvae were reared on both meat and liquid diets. Initially, two hundred recently hatched larvae were transferred to a plastic pan containing meat diet, which was added at 24h intervals as follows: 50 g at day 2 and 100 g at days 3 and 4. The used diet was discarded when larvae changed to fresh diet. The larval rearing was kept in an incubator ( $37 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH).

For studying larval development on hydroponic rearing medium, two hundred recently hatched larvae were transferred to a plastic pan containing polyester pad in liquid diet, which was added as follows: 50 ml at 48h intervals (days 2 and 3); 100 ml at 24h intervals (days 4 and 5). At each change, the old diet was removed before the fresh diet addition. The larval rearing was kept in an incubator ( $37 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH).

After 96h (day 4), the pans containing larvae from both meat and liquid diets were placed into individual vats with

Table 1. Mean ( $\pm$ SD) number of insects formed in different instars of *C. hominivorax* after rearing on meat or liquid diets.

	Days of rearing	Percentage of insects	
		Meat diet <sup>1</sup>	Liquid diet <sup>2</sup>
Mature larva	4	141.3 $\pm$ 15.31	152.7 $\pm$ 11.72
Pupa	10	140.3 $\pm$ 14.57	150.7 $\pm$ 10.69
Adult	18	131.7 $\pm$ 11.15	144.3 $\pm$ 7.57

<sup>1</sup>Meat diet based on Graham & Dudley (1959); <sup>2</sup>Liquid diet modified from Taylor et al. (1991); the experiments were repeated four times using 200 1<sup>st</sup>-instar larvae; both experiments with the rearing media were kept in an incubator ( $37 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH) for 196h.

vermiculite for pupation. After 120h of rearing (day 5), third instar (mature) larvae that crawled out the media from both groups were weighed. After six days of rearing, the insects from both groups were transferred to an incubator at  $25 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  RH. At day 8 (192h) the larvae from each group remaining on the rearing vats were removed, washed, and placed on the vermiculite. At day 10, all pupae were weighed. Finally, on day 18 (three days after emergence), all adult insects were counted.

Up to the end of the experiment, the used polyester substrate was washed many times under running water. Afterwards, the pad was immersed for 24h in 2% sodium hypochlorite solution for bleaching and disinfecting. Finally, the pad was submitted to another washing step under running water to remove the liquid cleanser and was dried in the laboratory to be reutilized.

All experiments were repeated four times and the results were analyzed by Student *t* test with confidence interval of 95% using Stats Direct statistic software (version 3.6.2).

The mean weight and standard deviation (SD) of mature larvae in meat and liquid diets are respectively  $63.33 \pm 2.76$  mg and  $64.50 \pm 2.89$  mg. The mean weight and standard deviation (SD) of pupae formed from meat and liquid diets are respectively  $48.17 \pm 1.76$  mg and  $49.29 \pm 1.99$  mg. Comparison of the results obtained from mature larvae and pupae indicated no significant differences between treatments (*t* test,  $P > 0.05$ ).

The number of mature larvae, pupae and adults are shown in Table 1. The mean number of all instars reared in liquid diet was greater than when reared in meat diet, but the difference was not significant (*t* test;  $P > 0.05$ ).

No significant difference was observed between meat and liquid diets (*t* test;  $P > 0.05$ ) in relation to the percentage of larval survival (number of mature larvae in relation to the 200 L1 in the beginning of experiment), pupation (number of pupae in relation to mature larvae), emergence (number of adults in relation to the number of pupae) and rearing efficiency (number of emerged adults in relation to the 200 L1 of the beginning of experiment) (Table 2).

Our present findings are similar to those obtained by Taylor & Mangan (1987), when these authors compared meat

Table 2. Percentage of survivorship of different developmental *C. hominivorax* stages in meat or liquid diets.

Stage	Survival percentage <sup>1</sup>	
	Meat diet <sup>2</sup>	Liquid diet <sup>3</sup>
Until mature larva	70.7	76.3
Mature larva to pupa	99.3	98.7
Pupa to adult	94.0	95.9
Larva to adult	65.8	72.2

<sup>1</sup>The survival percentage refers to the number of insects that completed the stage, in relation to those that started; <sup>2</sup>Meat diet based on Graham & Dudley (1959); <sup>3</sup>Liquid diet modified from Taylor et al. (1991). The experiments were repeated four times, using 200 1<sup>st</sup>-instar larvae. Both rearing media tested were kept in an incubator ( $37 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH) for 196h.

diet and gelled diet (hydroponic diet with sodium polyacrylate as a substrate). Interestingly, we observed high mortality in most mature larvae that had not left the culture pans in the group of larvae reared on meat diet. As expected, most of mature larvae pupated even if they had not left the liquid diet (data not shown).

As mass production of *C. hominivorax* is improving, production cost and environmental impact become increasingly relevant (Chaudhury & Alvarez 1999). Recently, Chaudhury *et al.* (2002) proposed a less costly and less polluting diet, using cellulose residues instead of sodium polyacrylate as a substrate.

Because in our experiments the used polyester pad could be reutilized, the cost of this substrate per liter of rearing medium was reduced to less than a third. Furthermore, the reutilization of this matrix would result in less environmental pollution in comparison to acrylic polymers.

Substitution of the acrylic matrix by the polyester pad still offers other advantages. The product is easily available in the Brazilian market and there is the possibility of producing meshes of different density allowing better adaptation to the needs of the rearing system. A further possibility could be the use of the polyester pad for hydroponic diets for rearing other myiasis causing insects.

In conclusion, our observations indicate that polyester pad does not interfere with the development of *C. hominivorax* larvae, it is easy to handle, reduces the production cost, and causes less environmental impact due to the possibility of its reutilization. All these factors indicate that the polyester pad can substitute the acrylic matrix for handling and rearing *C. hominivorax*.

## References

- Baumhover, A.H. 1966. Eradication of the screwworm fly. An agent of myiasis. *J. Am. Med. Assoc.* 196: 240-248.
- Brown, H.E. & J.W. Snow. 1979. Screwworms (Diptera: Calliphoridae): A new liquid media for rearing screwworm larvae. *J. Med. Entomol.* 16: 29-32.
- Chaudhury, M.F. & L.A. Alvarez 1999. A new starch-grafted gelling agent for screwworm (Diptera: Calliphoridae) larval diet. *J. Econ. Entomol.* 92: 1138-1141.
- Chaudhury, M.F., L.A. Alvarez & L.L. Velazquez. 2002. Recycled paper products as substitutes for gelling agent in screwworm (Diptera: Calliphoridae) larval diet. *J. Econ. Entomol.* 95: 1337-1341.
- Galvin, T.J. & J.H. Wyss. 1996. Screwworm eradication program in Central America. *Ann. NY Acad. Sci.* 791: 241-247.
- Graham, A.J. & F.H. Dudley. 1959. Culture methods for mass rearing of screw-worm larvae. *J. Econ. Entomol.* 52: 1006-1008.
- Grisi, L., C.L. Massard, G.E. Moya-Borja & J.B. Pereira. 2002. Impacto econômico das principais ectoparasitoses em bovinos no Brasil. *Hora Veterinária* 21: 8-10.
- Hall, M. & R. Wall. 1995. Myiasis of humans and domestic animals. *Adv. Parasit.* 35: 257-334.
- Knipling, E.F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.* 48: 459-462.
- Moya-Borja, G.E. 2003. Erradicação ou manejo integrado das miíases neotrópicas das américas? *Pesq. Vet. Bras.* 23: 131-138.
- Nascimento, E.M.F., J.B. Oliveira, M.J. Paes, A.P. Lobo, A.L.A. Silva, E.R. Santos Jr, J.L.F. Leal & G.E. Moya-Borja. 2005. Miíases humanas por *Cochliomyia hominivorax* (Coquerel, 1858) (Diptera, Calliphoridae) em hospitais públicos na cidade do Recife, Pernambuco, Brasil. *Entomol. Vect.* 12: 37-51.
- Snow, J.W., C.J. Whitten, A. Salinas, J. Ferrer & W.H. Sudlow. 1985. The screwworm, *Cochliomyia hominivorax* (Diptera: Calliphoridae), in Central America and proposed plans for its eradication south to the Darien Gap in Panama. *J. Econ. Entomol.* 22: 353-360.
- Taylor, D.B. & R.L. Mangan. 1987. Comparison of gelled and meat diets for rearing screwworm, *Cochliomyia hominivorax* (Diptera: Calliphoridae) larvae. *J. Econ. Entomol.* 80: 427-432.
- Taylor, D.B., J.C. Bruce & R. Garcia. 1991. Gelled diet for screwworm (Diptera: Calliphoridae) mass production. *J. Econ. Entomol.* 84: 927-935.

Received 09/V/07. Accepted 21/XII/07.