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# Laboratory environment effects on the reproduction and mortality of adult screwworm (Diptera: Calliphoridae)

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### SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

## Laboratory environment effects on the reproduction and mortality of adult screwworm (Diptera: Calliphoridae)

### Efeitos de condições de laboratório na reprodução e mortalidade de *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae)

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#### ABSTRACT

The New World screwworm, *Cochliomyia hominivorax* Coquerel, is mass reared for screwworm eradication initiatives that use the sterile insect technique. New methods for rearing have helped to reduce the cost of the eradication program. We examined the effect and interaction of three temperatures (24.5, 29.5 and 34.5°C), two diets (2% spray-dried blood plus 0.05% vitamins and corn syrup carrageenan) and three population densities (300, 400, and 500 flies/cage) on egg production, egg hatch, number of observable fertilized eggs, mortality (male and female) and ovarian development. The three population densities did not affect any of the parameters monitored. Using the protein diet increased egg production

at all temperatures. Diet did not affect egg hatch or female mortality. Male mortality was significantly greater when fed the protein diet and reared at 24.5°C and 34.5°C. Egg hatch was significantly less when the flies were reared at 34.5°C. When exposed to high temperatures (37°C and 40°C) egg production, egg hatch, fertility and mortality were adversely affected. At the higher temperatures, yolk did not adequately form during oogenesis. When compared to the normal rearing photoperiod (12 L: 12 D), short photoperiod (1 L: 23 D) increased egg production, egg hatch and fertility but lowered mortality.

**Key words:** Temperature, *Cochliomyia hominivorax*, egg production, ovarian development

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## RESUMO

A mosca-da-bicheira, *Cochliomyia hominivorax* Coquerel, é criada em escala massal com o objetivo de fornecer insetos para programas para sua erradicação, através da técnica do inseto estéril. Novos métodos de criação têm auxiliado na redução dos custos desses programas de erradicação. Nós examinamos o efeito e a interação de três temperaturas (24,5, 29,5 e 34,5°C), duas dietas (carboidrato e carboidrato + proteína) e três densidades populacionais (300, 400 e 500 moscas/gaiola), na fecundidade eclosão de larvas, número de ovos fertilizados, mortalidade (macho e fêmea) e desenvolvimento dos ovários. As três densidades populacionais não afetaram nenhum dos parâmetros avaliados. O uso da dieta protéica aumentou a produção de ovos em todas as temperaturas testadas. O tipo de dieta não afetou a eclosão de larvas e mortalidade de fêmeas. A mortalidade dos machos foi significativamente maior quando alimentados com a dieta protéica e criados a 24,5°C e 34,5°C. A eclosão de larvas foi significativamente menor quando as moscas foram criadas a 34,5°C. Nas temperaturas mais elevadas (37°C e 40°C), a produção de ovos, eclosão de larvas, fertilidade e mortalidade foram afetadas desfavoravelmente. Ainda nessas mesmas temperaturas, durante a oogênese, não houve a formação adequada do estoque de alimento para os embriões. O fotoperíodo curto (1L: 23E) propiciou maior produção de ovos, eclosão de larvas e fertilidade, e diminuiu a mortalidade quando comparado ao fotoperíodo padrão em criações (12L: 12E).

**Palavras-chave:** Temperatura, produção de ovos, desenvolvimento do ovário

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The primary screwworm, *Cochliomyia hominivorax* Coquerel, is an obligate parasite that affects all warm-blooded animals including humans. The eggs are laid on the periphery of wounds and the larvae feed on live tissues of living animals. Prior to its eradication from the United States, American livestock producers lost nearly \$500 million annually because of death, low milk and meat production, increased veterinary costs and other factors caused by screwworms (Wyss & Galvin 1994). The sterile insect technique (SIT) (Knipling 1955) has been used to eradicate this pest from the United States, Mexico and Central America. The western portion of the Republic of Panama was technically declared screwworm free in 2001, and a screwworm barrier at the Darien Gap of Panama has been established to avoid future infestations of screwworms migrating from South America. In SIT, mass releasing sterile screwworm adults is used to eradicate the pest and to maintain a barrier. Continuous efforts to improve the techniques for rearing screwworm through applicable research have enhanced the success attained by the eradication program (Taylor 1992).

Oogenesis and survival are affected by temperature (Vogt & Walker 1987), and photoperiod (Cunha-e-Silva *et al.* 1992). The percentage of total time (hours to complete the 1<sup>st</sup> cycle of oogenesis) for each stage of egg development was not affected significantly when screwworm females were held at 24°C and 30°C (Adams & Reinecke 1979). Krafur *et al.* (1978) reported that screwworms held at 30°C have a 6-day preovipositional period but Thomas (1993) found that some females (27.9%) were gravid after four days at this temperature. Screwworm eggs successfully develop at temperatures between 12.3°C and 47.2°C; the optimum temperature range for screwworm oogenesis is between 21.8°C and 37.8°C and egg development is fastest (completed in 81h) at 32.2°C (Adams & Reinecke 1979).

Adult screwworms are inactive in trees and shrubs at night (Hightower 1963). Cunha-e-Silva *et al.* (1992) found that adult screwworms produced more eggs and survived longer when reared under 1:23 (light:

dark) photoperiod regime.

Population density also affects adult screwworm survival (Crystal 1967). Female mortality increased considerably when the mating competition between males was increased (Baumhover 1965). The mean longevity of flies from mixed populations of males and females was about 2.5 weeks (Crystal 1967).

In a previous study a protein diet of spray-dried blood was developed to optimize fecundity, hatching, fertility, ovarian development and male and female mortality (Sagel *et al.* 2002). This diet and a carbohydrate diet (corn syrup carrageenan) were used in this study. The objectives of the present study were to evaluate the effects and interactions of these diets, temperature and population density on these parameters. The effect of two photoperiods was also evaluated.

## Material and Methods

**Insect rearing and preparation of diets.** We used a screwworm strain (Panama 95) originated from females collected in Panama in 1995. The larvae were reared on gel media using standard rearing protocol (Taylor *et al.* 1991). The adult screwworms were maintained in cotton gauze-covered cages (29.9 cm L X 17.8 cm W X 24.1 cm H).

**Effect of temperature, population density and diet on egg production, fertility, and ovarian development.** Adult screwworms were grouped into three different fly population densities (300, 400 and 500 flies) with 1:1 sex ratios, maintained at one of three temperatures (24.5, 29.5 and 34.5°C) and 50% RH, and fed one of two diets (corn syrup carrageenan or 2% spray-dried blood plus 0.05% vitamins) and water (Sagel *et al.* 2002). From days one through five, ten female flies per cage were collected daily, placed in glass vials and kept at -20°C until the ovaries could be examined. Fifty additional female flies of the same age were placed in each of the cages to maintain the assigned fly population density. The flies in each treatment were allowed to oviposit when six days old on 10 g of fresh ground beef laced with ~100 µl of putrefied blood. The total weight of the eggs collected and the hatch of a 100 egg sample were used as a measure of egg production and fertility, respectively. The eggs were scored as hatched, embryonated (identifiable development) and unfertilized after incubating for 18-22h at 37°C. The dead flies in each cage were counted and sexed after the eggs were collected.

Ovarian development was determined by dissecting ovaries from the frozen females collected each day and examining them under a dissecting microscope (12X). We slightly modified the physiological age grading method of Scholl (1980) for scoring ovarian development by assigning a numerical value to the 15 stages in the gonotrophic cycle (0 to 14; 14 corresponded to gravid, stage 5 of Scholl, 1980): additional values from 15 to 23 were used to identify the stages of ovarian development in the second gonotrophic cycle. This scale simplified data analysis.

Multivariate analysis of variance was used for this 2 x 3 x 3 factorial completely randomized design to determine the effects and interactions among the factors in our study. Data were analyzed with PROC GLM (SAS Institute 1999). Mean separations were accomplished by using Fishers Least Significant Differences and Duncans Multiple Range Tests (SAS Institute 1999). This experiment was replicated three times.

**The effect of diet on egg production, fertility, ovarian development and female mortality of adult screwworms reared at 37°C and 40°C.** Two groups of 400 flies (1:1 sex ratio) were held at 37°C ± 0.9 for six days. Another two groups were held at 40°C ± 0.9 for the same period. One of two diets (corn syrup carrageenan or 2% spray-dried blood plus 0.05% vitamins) was fed to each group of flies at each temperature. The treatment cages were sampled four times by randomly collecting ten females from each cage on days one, two, three and five. The samples were placed in glass vials and frozen at -20°C until ovaries were examined. Forty additional females were placed in each of the cages at the time of setup to replace those removed for ovary examination. The ovaries were scored as previously described. Data were analyzed with PROC GLM and mean separations were accomplished using Fishers Least Significant Differences and Duncans Multiple Range Tests (SAS Institute 1999) as previously described. This study was replicated three times.

**The effect of two photoperiod regimes on adult screwworms.** Two cages of three hundred flies

(1:1 sex ratio) were fed the carbohydrate with 2% spray-dried blood plus 0.05% vitamin diet and reared at 24.5°C. One cage was exposed to the normal 12:12 (light: dark) photoperiod and the second cage was placed in an incubator set to a 1:23 (light: dark) photoperiod. All flies were allowed to oviposit when six days old. The quantity of eggs, egg hatchability, fertility and mortality were recorded as before. We deviated from Cunha-e-Silva *et al.* (1992) protocol by allowing oviposition during scotophase. This test was replicated three times and data were analyzed using a *t* Test in PROC GLM (SAS Institute 1999).

## Results

**Effect of temperature, population density and diet on egg production, fertility, and ovarian development.** The quantity of eggs produced by each surviving female was not affected significantly by the three fly population densities ( $F = 0.89$ ;  $df = 2, 36$ ;  $P = 0.42$ ). Temperature affected egg production ( $F = 3.36$ ;  $df = 2, 36$ ;  $P = 0.046$ ) but diet had the most pronounced affect ( $F = 34.73$ ;  $df = 1, 36$ ;  $P < 0.0001$ ). The flies fed the spray-dried blood diet produced a significantly greater amount of eggs at all temperatures compared to flies fed the corn syrup carrageenan diet ([Table 1](#)). Flies reared at 29.5°C and 34.5°C produced significantly more eggs than the flies reared at 24.5°C ([Table 1](#)).

Table 1. Effect of temperature and diet on egg production, egg hatch, fertility, and mortality.

	24.5°C		29.5°C		34.5°C	
	Standard	Protein	Standard	Protein	Standard	Protein
Av. egg wt./female (mg)	2.01 ± 0.16c	2.82 ± 0.24b	2.09 ± 0.25c	3.59 ± 0.26a	2.42 ± 0.30bc	3.64 ± 0.18a
Egg hatch (%)	69.78 ± 3.56a	64.44 ± 5.64a	70.11 ± 3.65a	67.00 ± 3.17a	44.22 ± 4.57b	44.33 ± 5.24b
Fertility (%)	84.44 ± 1.42ab	83.00 ± 2.80ab	88.00 ± 1.94a	83.00 ± 1.77ab	85.44 ± 1.13ab	80.89 ± 1.90b
Female Mortality (%)	3.81 ± 0.59b	4.33 ± 1.14b	4.56 ± 1.19b	5.48 ± 0.61b	9.77 ± 1.46a	12.07 ± 1.04a
Male mortality (%)	7.33 ± 0.77c	10.47 ± 1.31cb	7.10 ± 0.88c	9.05 ± 1.04c	13.14 ± 1.99b	20.09 ± 1.61a

No significant interactions were detected (all  $P > 0.05$ ) in contrasts between the treatment factors of temperature and protein levels of adult diet and the response variables.

Means (± SEM) followed by the same letter within a row do not differ using Fisher's Least Significant Differences ( $P > 0.05$ ).

Temperature had a significant effect on egg hatchability ( $F = 20.37$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) but not fertility (identifiable embryonic development) ( $F = 0.87$ ;  $df = 2, 36$ ;  $P = 0.43$ ). The lowest hatch was observed in eggs from flies reared at 34.5°C ([Table 1](#)). Neither population density ( $F = 1.32$ ;  $df = 2, 36$ ;  $P = 0.28$ ) nor diet ( $F = 0.64$ ;  $df = 1, 36$ ;  $P = 0.43$ ) had a significant effect on egg hatchability within each temperature range tested.

Ovarian development was significantly affected by temperature ( $F = 139.61$ ;  $df = 2, 2890$ ;  $P < 0.0001$ ) and diet ( $F = 7.71$ ;  $df = 1, 2890$ ;  $P = 0.0055$ ) but not population density ( $F = 0.28$ ;  $df = 2, 2890$ ;  $P = 0.7529$ ). The highest mean stage of ovarian development was observed in those flies reared at 34.5°C and fed protein ([Table 2](#)). The ovaries of the flies fed the protein diet and reared at 34.5°C developed more quickly than in the other treatments ([Table 2](#)). The slowest rate of development was observed in flies fed only carbohydrate kept at 24.5°C ([Table 2](#)).

Table 2. Effect of temperature and diet on ovarian development at different ages post emergence.

Age (day)	24.5°C		29.5°C		34.5°C	
	Standard	Protein	Standard	Protein	Standard	Protein
1	3.09 ± 0.07d <sup>1</sup>	3.19 ± 0.08d	4.83 ± 0.13c	5.00 ± 0.13c	5.49 ± 0.14b	5.82 ± 0.12a
2	6.07 ± 0.16e	6.64 ± 0.16d	7.37 ± 0.14c	7.54 ± 0.14c	9.50 ± 0.23b	9.99 ± 0.18a
3	8.06 ± 0.14d	8.57 ± 0.14d	9.22 ± 0.16c	9.45 ± 0.15c	11.70 ± 0.29b	12.76 ± 0.25a
4	9.81 ± 0.18d	10.32 ± 0.15d	10.32 ± 0.30d	11.38 ± 0.26c	14.17 ± 0.25b	15.17 ± 0.27a
5	10.65 ± 0.29d	11.25 ± 0.32cd	12.14 ± 0.36bc	12.48 ± 0.34b	15.55 ± 0.34a	15.93 ± 0.31a

No significant interactions were detected (all  $P > 0.05$ ) in contrasts between the treatment factors of temperature and protein levels of adult diet and the response variables.

Means ( $\pm$ SEM) followed by the same letter within a row do not differ using Fisher's Least Significant Differences ( $P > 0.05$ ).

<sup>1</sup>Rate of ovarian development. An ovary rated 14 is gravid.

Male mortality was significantly affected by temperature ( $F = 25.02$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) and diet ( $F = 13.62$ ;  $df = 1, 36$ ;  $P = 0.0007$ ). The males showed greater mortality when fed the protein diet. Female mortality was significantly affected by temperature ( $F = 30.71$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) but not diet ( $F = 2.60$ ;  $df = 1, 36$ ;  $P = 0.1158$ ). The greatest mortality was observed at the highest temperature (Table 1). Male mortality was greater than female mortality ( $F = 26.61$ ,  $df = 1, 72$ ;  $P < 0.0001$ ) at all temperatures tested (Table 1). Fly population density had no significant effect on male ( $F = 2.80$ ;  $df = 2, 36$ ;  $P = 0.0738$ ) or female mortality ( $F = 1.96$ ;  $df = 2, 36$ ;  $P = 0.1553$ ).

**The effect of diet on egg production, fertility, ovarian development and female mortality of adult screwworms reared at 37°C and 40°C.** Temperature ( $F = 6.31$ ;  $df = 1, 8$ ;  $P = 0.0363$ ) and diet ( $F = 5.99$ ;  $df = 1, 8$ ;  $P = 0.0401$ ) had a significant effect on the amount of eggs produced by each female. Protein fed flies held at 40°C produced significantly more eggs than in the other treatments (Table 3). Protein fed flies produced an average of 1.85 mg and 0.14 mg more eggs per female when reared at 40°C and 37°C, respectively, than those fed the carbohydrate diet (Table 3).

Table 3. Ovarian development, egg production, egg hatch, fertility, and mortality of screwworms fed two diets and reared at 37°C and 40°C.

	37°C		40°C	
	Control diet	Protein diet	Control diet	Protein diet
Ovarian score <sup>1</sup>				
Day 1	5.27 ± 0.66	5.47 ± 0.72	5.37 ± 0.50	5.47 ± 0.60
Day 2	9.19 ± 0.75	9.77 ± 0.47	9.47 ± 1.23	9.90 ± 0.20
Day 3	12.27 ± 0.98	13.97 ± 0.76	13.53 ± 0.80	13.50 ± 0.26
Day 5	13.87 ± 0.56	15.40 ± 1.01	14.03 ± 1.05	16.77 ± 0.37
Av. egg wt./female (mg)	1.23 ± 0.49a	1.37 ± 0.17a	1.40 ± 0.40a	3.25 ± 0.48b
Egg hatch (%)	15.33 ± 4.37a	23.33 ± 6.94a	8.0 ± 3.61a	11.0 ± 3.21a
Fertility (%)	31.0 ± 8.54ab	43.67 ± 6.84a	15.67 ± 5.21b	22.33 ± 7.36ab
Female mortality (%)	25.11 ± 2.12a	19.56 ± 7.95a	34.00 ± 7.06a	35.11 ± 11.57a
Male mortality (%)	24.44 ± 10.47a	17.33 ± 4.54a	35.78 ± 6.76a	32.44 ± 4.64a

No significant interactions were detected (all  $P > 0.05$ ) in contrasts between the treatment factors of temperature and protein levels of adult diet and the response variables.

Means ( $\pm$ SEM) followed by the same letter within a row do not differ using Fisher's Least Significant Differences ( $P > 0.05$ ).

<sup>1</sup>Mean ovarian score for ten females.

The diet did not have a significant effect on the overall rate of ovarian development ( $F = 2.76$ ;  $df = 1, 412$ ;  $P = 0.0971$ ). Gravid females appeared four days after emergence when fed protein and not until five (40°C) or six (37°C) days when fed only sugar. No interaction between temperature and diet was found

( $F = 0.24$ ;  $df = 1, 412$ ;  $P = 0.6280$ ). The extreme temperatures tested affected yolk formation after the oocyte reached the last stages of development. Many of the eggs appeared shortened, translucent and flattened, probably due to yolk degradation by the temperature. At the time of the first oviposition, secondary oocytes were at the earliest stages of development in adults reared at 37°C and 40°C. About 60% of ovaries studied from flies held at 40°C had poor yolk formation while that condition was recorded in about 30% of flies held at 37°C.

Egg hatch was not significantly different between the two temperatures ( $F = 4.27$ ;  $df = 1, 8$ ;  $P = 0.0726$ ) or diets ( $F = 1.34$ ;  $df = 1, 8$ ;  $P = 0.2811$ ) tested. Temperature had a significant effect on fertility ( $F = 6.69$ ;  $df = 1, 8$ ;  $P = 0.0323$ ) but diet did not ( $F = 1.86$ ;  $df = 1, 8$ ;  $P = 0.2098$ ) (Table 3). The number of fertile embryos was significantly greater from flies reared at 37°C than those reared at 40°C.

Neither male ( $F = 1.39$ ;  $df = 3, 8$ ;  $P = 0.3149$ ) nor female mortality ( $F = 0.88$ ;  $df = 3, 8$ ;  $P = 0.4923$ ) were significantly different at these temperatures. The addition of protein to the diet of flies held at either temperature did not have a significant effect on adult mortality (Table 3).

**The effect of two photoperiod regimes on adult screwworms.** Photoperiod had an effect on egg production, egg hatchability and male mortality. Females held at 1:23 (L:D) photoperiod produced significantly more eggs ( $F = 11.85$ ;  $df = 1, 4$ ;  $P = 0.03$ ) that hatched significantly better than those reared under 12:12 (L:D) photoperiod (Table 4). Fertility was not significantly effected by photoperiod ( $F = 6.15$ ;  $df = 1, 4$ ;  $P = 0.0683$ ). Male mortality was greater than female mortality ( $F = 11.70$ ;  $df = 1, 8$ ;  $P = 0.0091$ ) with no dead females found in the 1:23 (L:D) photoperiod (Table 4). No interaction between gender and photoperiod was observed ( $F = 2.93$ ;  $df = 1, 8$ ;  $P = 0.1256$ ). The lowest mortality for both sexes was observed in the group reared under the long scotophase ( $F = 12.36$ ;  $df = 1, 8$ ;  $P = 0.0079$ ). The number of dead males found in the long scotophase treatment was less than that obtained in the other experiments. It is also important to note that the number of dead males was greater than that of females throughout these experiments.

Table 4. The effect of photoperiod on screwworm egg production, egg hatch, fertility and mortality.

	Photoperiod	
	12 L: 12 D	1 L: 23 D
Av. egg wt./ female (mg)	4.36 ± 0.36b <sup>1</sup>	6.05 ± 0.33a
Egg hatch (%)	56.47 ± 1.92b	66.91 ± 1.62a
Fertility (%)	86.16 ± 1.02a	92.40 ± 2.30a
Female mortality (%) <sup>1</sup>	4.22 ± 1.82a	0b
Male mortality (%)	16.22 ± 3.86a	4.00 ± 1.92b

Means (± SEM) followed by the same letter within a row do not differ using Fisher's Least Significant Differences ( $P > 0.05$ ).

<sup>1</sup>Actual means are reported. ANOVA and means separation procedures were conducted on arcsine square root-transformed proportions.

## Discussion

Screwworms have been reared in research colonies for generations using only carbohydrates as the adult diet with no observed reduction in their oogenetic potential and with the production of normal offspring. That fact has supported the idea of autogeny in this species (Crystal 1966, Mackley & Snow 1982). Ground meat has traditionally been used in the adult diet at the screwworm mass rearing facility in Mexico with the assumption that it helped to promote egg production (Marroquin 1985, Thomas 1993).

In the present study, the reproductive features during the first gonotrophic cycle of screwworms were improved when levels of 2% protein were included in the diet. Other researchers have reported dietary protein positively enhancing reproductive output of adult screwworms: Hammack (1999) reported significant differences using raw meat; Chaudhury *et al.* (2000) observed improvements when dried egg was incorporated in the adult diet. Dry milk (35.7% protein content in dry matter) and dried eggs (46% protein content in dry matter) were the protein sources investigated during our preliminary studies, but

we observed that they were not completely accepted by adult screwworms. Although raw meat has been used in mass rearing for the screwworm eradication program, it is more laborious, requires refrigeration and it is more expensive than spray-dried blood. In addition, raw meat needs to be replaced daily. The use of spray-dried blood is advantageous because of its availability at the laboratory facility (it is an ingredient of the larval media). Its low cost and its water solubility allows easy incorporation into the mixture prepared in our rearing diet. Although the screwworm is capable of completing the first gonotrophic cycle without protein, the addition of protein to the diet increases the quantity and quality of the eggs produced. The importance of a protein diet undoubtedly increases with subsequent gonotrophic cycles. To optimize production the screwworm is normally maintained for only the first cycle.

High temperatures increase the rate of ovarian development in screwworms (Adams & Reinecke 1979). In this study, some females were gravid four days after adult emergence when flies were held at 34.5, 37 and 40°C but hatching and fertility were adversely affected by the two highest temperatures as seen in interrupted yolk formation/deposition. Fly mortality increased as the temperature was increased; but the final egg production obtained per female was not affected if compared with mean oviposition per female from flies held at the lower temperatures (24.5°C and 29.5°C). The quality of the eggs at 34.5°C (based on our fertility measurement) did not differ from eggs obtained when rearing flies under current normal conditions (six day pre-oviposition period at 24.5°C) or at the intermediate temperature (29.5°C). But mortality of both sexes was significantly higher at 34.5°C.

The pre-oviposition period was reduced to four days at the highest temperatures (37°C and 40°C), but yolk formation was severely hindered resulting in a reduction of screwworm fertility. Extreme temperatures used to rear screwworms have deleterious effects on oogenesis. At our normal rearing temperature (24.5°C), the secondary oocytes reach either stage 2 or 3 at the time of the first oviposition. However, the oocytes were at earlier stages of development (including early stage 0) in gravid females held at 37°C and 40°C. Thus, it might be expected a second clutch would be delayed (or fail to develop under these stressful conditions) in flies exposed to high temperatures. This might be an adaptive reaction to maintain energy reserves to overcome extreme conditions and yet propagate at least one time.

No significant effect on the reproductive potential was seen by population densities used in this study. The interaction between diet, temperature and fly population density was not significant. The 500 flies/cage density used here is similar (based on volume available/fly) to that used in mass production (Baumhover *et al.* 1966).

Rearing protein fed flies under long scotophase (1 L: 23 D) and at 24.5°C reduced the general activity of adult screwworms but their reproductive capacity (egg production) was increased and their mortality decreased. In this experiment, three consecutive generations of protein fed flies produced more viable eggs than other protein fed flies reared at 24.5°C and under 12h:12h photoperiod. This indicates that the males were less aggressive, but they were still successful in mating. Fly mortality (both sexes) observed under long scotophase was reduced; in fact, no females perished in this treatment. This is similar to that found by Cunha-e-Silva *et al.* (1992). Physical damage and premature death to mated females caused by sexually aggressive males (Spates & Hightower 1967) might have been reduced because of the increased scotophase.

Based on these results, spray-dried blood could be considered a superior source of protein for adult screwworms, temperatures between 24.5°C and 30°C appear optimal for adult screwworms in mass production, and the current density of adults in cages does not adversely affect mass rearing. Although long scotophase improved all of the parameters measured, such an artificial environment could possibly have long-term detrimental effects on the mass reared colonies. It is important when rearing insects for release that all reasonable measures are taken to imitate natural conditions. This precaution reduces negative selection pressure, relative to the mass rearing program, which can lead to laboratory adapted insects that no longer are competitive with their wild counterparts. Temperature, photoperiod and feeding regimes need to be within the limits found in their natural habitat. Although more rapid ovarian development may be economically advantageous for mass rearing in the short run it may lead to an insect that is ineffective in the field (especially in a SIT program). Unintentional selection could result in an unfit population requiring the development of a new strain for mass production. Therefore, before changing the photoperiod currently used in mass rearing, future research should be done with light intensity that mimics dense forest environments and to determine any metabolic changes in adult



screwworms that might lead to strain deterioration if 1:23 (L:D) were to be used.

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