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Rearing systems for screwworm mass production

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Rearing Systems for
Screwworm Mass Production

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The screwworm, Cochliomyia hominivorax (Diptera: Calliphoridae), eradication effort employs the largest insect mass production program in the world. Approximately 400 billion insects have been reared, sterilized, and released since 1954, resulting in the eradication of screwworm from the United States and most of Mexico. The mass production factory, located at Tuxtla Gutierrez, Mexico, is designed to produce 500 million screwworm flies per week. When at peak production, the factory uses more than 450 metric tons of dry products to produce nearly 3.5 million liters of larval diet per month (Marroquin 1985). At that level of production, diet components cost $8.5 to $10 million per year. The Mexican eradication program achieved its goal of eliminating screwworm from Mexico west of the Isthmus of Tehuantepec in 1985 and switched from an eradication to barrier maintenance program. Production quotas for barrier maintenance have been between 200 and 300 million flies per week. Although extension of the eradication program into Central America (Belize and Guatemala) has already begun, increased production should not be needed (Snow et al. 1985, Krafsur et al. 1987).

The larval stage is the most critical part of the screwworm life cycle for mass production. The quality of the mass produced flies is determined primarily by the diet and care given during this stage, and the majority of consumable products, labor, energy, and physical facilities used for mass production is dedicated to the larvae. Therefore, improvements in larval rearing technology offer the greatest opportunity for improving the quality of the mass produced flies and reducing the cost of mass production.

The resources used for insect mass production are diet, labor, and facilities. The specific application of these resources to a mass rearing situation constitutes a rearing system. In most cases, facilities and labor are designed around the
diet and the interaction of the biology of the insect with the diet. Major changes in diet, for example from meat to liquid or from liquid to gel, usually require modification of methods and facilities. When comparing diets for use in a mass rearing program, the interaction of the diet with the other resources must be considered. Optimal labor practices (techniques, schedules, etc.) and physical facilities (environment) should be used for each diet tested. Thus, rearing systems, not diets, must be compared.

The first artificial rearing system for screwworm used a diet consisting of lean beef, citrated beef blood, milk, water, and formalin (Melvin & Bushland 1936). Later, milk was eliminated from the diet (Melvin & Bushland 1940). The resulting rearing system was used for screwworm mass production with little modification until 1970 (Graham & Dudley 1959, Smith 1960, Baumhover et al. 1966). Several sources of meat were tested and used for varying periods of time, including cattle, horses, pigs, whales, and nutria (Myocaster coypus). Of these, horse meat was the most readily available, cheap, and conducive to good screwworm production. However, meat was expensive to store, and the supply was not reliable. Competition with fur ranchers and dog food manufacturers reduced the supply further and inflated the price of high quality meats (Gingrich et al. 1971). This prompted the search for alternative diets composed of stabilized proteins which could be shipped and stored without refrigeration. After testing hundreds of formulations, a liquid (hydroponic) diet, consisting of dried whole chicken egg, dried whole bovine blood, calf suckle (a milk substitute), sucrose, dried cottage cheese, and formalin suspended in water was developed (Gingrich et al. 1971). The hydroponic diet was initially suspended in cotton and later acetate fiber mats to provide support for the larvae. Waste hydroponic diet was vacuumed from the larval rearing vats every 4 h and replaced with fresh diet. The hydroponic rearing system, with minor modifications (Brown & Snow 1979), has been used for mass production up to the present time (Brown 1984, Goodenough et al. 1983, Marroquin 1985).

Though cheaper and easier to handle than meat, the hydroponic rearing system has several disadvantages, most importantly, it is labor dependent and intensive. Waste diet must be removed and replaced with fresh diet every 4 hours. Failure to properly remove waste diet results in accumulation of metabolic wastes, primarily ammonium bicarbonate (Brown & Snow 1978), forcing the larvae to leave the rearing vats before they are mature. Vacuuming and feeding are done by several crews dispersed throughout the larval rearing floor (about 4000 m²), making supervision difficult. In addition, the environment on the larval rearing floor is less than conducive to quality workmanship. It is hot (about 35 °C), humid (about 70% relative humidity) and ammonia concentrations in the air can be high (Merkle 1985).

There is little doubt that labor quality has a significant impact on the quantity and quality of screwworms produced with the hydroponic rearing
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system. During the past 3 years yield in the Tuxtla Gutierrez mass production facility has ranged from more than 9 liters to less than 3 liters of pupae per vat. During the same time period pupal weight has exceeded 50 mg and been below 40 mg. Pupal weight and yield are positively correlated \((r^2 = 0.78)\), indicating that the variability is not due to competition. About 50% of the variability in pupal yield and weight during the last 3 years can be explained by one quantitative measure of labor quality (D. B. T. Taylor, unpublished data). Because of the limitations of the hydroponic system, a program was initiated to look for alternate diets and rearing systems.

Harris et al. (1984, 1985) tested nine gelling or solidifying agents for use in screwworm diets, six of which produced larvae of acceptable size (> 60 mg [Hightower et al. 1972]). A synthetic superabsorbent (poly [2-propenamide-co-2-propenoic acid, sodium salt]) marketed under the brand name Water-Lock G-400 yielded the most promising results. Initial tests indicated that the Water-Lock diet was roughly comparable with the meat diet being used for research rearing and the hydroponic diet used for mass production. Advantages of a gelled diet rearing system over the hydroponic rearing system were elimination of the acetate mats, reduced diet usage, and reduced labor.

The procedure used for rearing screwworm larvae on gelled diet in the research laboratory at Tuxtla Gutierrez, Mexico is as follows:

1. 100 mg of screwworm eggs (about 2,200 eggs) are incubated in a petri dish with a small piece of lean meat (5 g) 2 cm above a heated water bath (39°C) for 18 to 24 h (day 0).
2. First instar larvae with meat are transferred to a 19 by 16 by 9 cm deep plastic pan with 0.5 liters of gelled diet consisting of 70 g dried bovine blood, 30 g dried whole egg, 30 g nonfat dried milk, 1.2 ml formalin, 15 g Water-Lock G-400 gel, and 1000 ml of water. The pan is floated in a heated water bath (39°C) (day 1).
3. The second day after transferring the larvae to the diet, an additional 0.5 liters of diet is poured into the vats on top of the old diet and the larvae (day 3).
4. The next day one liter of gelled diet is added to the vat (day 4).
5. The following day the rearing vat is placed inside a larger plastic vat with 2 cm of sawdust in the bottom. Mature larvae crawl from the rearing vat and fall into the sawdust (day 5).
6. After 3 days of crawl-off, the rearing vat is discarded (day 8).
7. On day 10 the pupae are sifted from the sawdust. The volume of pupae recovered, weight of 10 ml of pupae, and number of pupae in 10 ml are recorded.
8. Pupae are then placed into cages. Adult eclosion usually begins on day 12.
Between 1,000 and 1,500 pupae (50 - 70% survival) weighing 50 - 55 mg are produced in each pan. A total of 2 liters of diet are used per plastic pan.

Factors critical to the successful use of the gelled diet are temperature of the water used to mix the diet, mixing the diet thoroughly, and the ambient environment, especially temperature and humidity. Mixing the diet with warm water (35° - 40° C) is necessary to get a gel of the proper consistency and avoid "chilling" the larvae with cold medium. Agitation of the diet throughout the gelling period (3 - 5 min) prevents the gel particles from sticking together and forming a hard, unsuitable gel. High temperatures (>38° C) and humidities (>90% relative humidity) cause excessive syneresis. The latter condition disrupts larval development and results in high mortality if the larvae are not transferred to fresh diet quickly. The gelled diet rearing system was compared with the meat rearing system used for research rearing by Taylor and Mangan (1987). They found that the gelled diet produced more consistent screwworms between rearing groups and over time than did the meat diet. Mean pupal weights and yields did not significantly differ between the two diets. Hydroponic diet was never adopted for research rearing of screwworms because it required 24 h per day attention and was not well suited to rearing many small discrete groups as was necessary in the laboratory. The Water-Lock diet offered advantages over the meat diet in that it was cheaper, the components were readily available and could be shipped and stored without refrigeration, while still being suited to rearing small discrete groups and fitting into the schedule of an 8 h per day operation. In addition, gelled diet was easier to handle, produced less odor, required less labor, and because there was no need to remove waste diet before refeeding, less opportunity for interstrain contamination. Gelled diet was adopted for all routine research rearing of screwworms at the Tuxtla Gutierrez laboratory in 1985.

Tests were initiated in 1986 to explore the use of gelled diet for screwworm mass production. Initial studies emphasized the use of gelled diet for initiation (first 2 days of development) because no modifications in equipment or procedures were necessary to implement gelled diet in this area of the rearing facility; whereas major modifications would be needed to introduce gelled diet onto the main rearing floor (last 3 days of larval development). Two obstacles were immediately encountered. At the high densities (8 grams of eggs per 5 liters of diet) being used in the starting rooms at that time, competition for nutrients and space had a greater effect on the development of the larvae than did the various diets tested (Taylor 1988). Once the densities in the starting rooms were reduced to 3 grams of eggs per 5 liters of diet, a second problem was encountered with the Water-Lock diet. The waste gelled diet clogged the vacuum heads when the larvae were transferred to the hydroponic vats on the main rearing floor. Inefficient removal of waste hydroponic diet resulted in reduced pupal yields and weights (R. Garcia, personal communication). A second gelling agent, carrageenan, which is soluble in warm hydroponic media...
was therefore adopted for use during initiation. Carrageenan starting diet produced smaller larvae at the end of the starting period, 4.7 mg verses 6.7 mg for Water-Lock, however, due to more efficient vacuuming on the hydroponic floor vats, carrageenan produced higher pupal weights and yields than did Water-Lock. Unfortunately, carrageenan was clearly inferior to Water-Lock for rearing screwworms through to pupation (Taylor 1988).

In mid 1987, tests were initiated to explore the use of Water-Lock gelled diet for rearing larvae in mass production from egg to pupation. Initial tests concentrated on using unmodified production floor vats. These vats are 91 by 152 by 4 cm deep and hold 55 liters of diet. In the first test, production of vats infested with 3 g of eggs and provided 55 liters of Walter-Lock diet were compared with production of vats infested with 6 g of eggs and provided 218 liters of hydroponic diet. The Water-Lock system produced an average of 32,400 pupae per vat (49% survival) with a mean weight of 50.3 mg while the hydroponic system produced an average of 78,900 pupae per vat (60% survival) with a mean weight of 53.6 mg. Though the hydroponic system produced higher pupal weights and yields, 27.8 liters of diet per 10,000 pupae (about 1 liter) produced were required for the hydroponic vats versus 17.3 liters of diet per 10,000 pupae with the Water-Lock system. In addition, the hydroponic system required 22 feedings occupying 21.25 min of labor versus 3 feedings for the Water-Lock system occupying 2.5 min of labor. After adjustment for yield per vat, 3.8 times more labor and 1.6 times more diet were required to produce a unit of pupae with the hydroponic system compared with the Water-Lock system. Experimental hydroponic vats were given better care than routine production vats. During the course of this experiment, production hydroponic rearing was averaging 49,500 pupae per vat (38% survival) with a mean weight of 45.9 mg. Calculations based on raw diet component consumption and direct observation indicate that production hydroponic vats received 107 liters of diet per vat (21.6 liters per 10,000 pupae) and 11.5 min of labor. Though the results of these tests were promising, especially with respect to diet usage and labor, survival and pupal weights were below what was expected based on the results of research rearing. Reducing the infestation rate from 3 to 2 g of eggs per vat increase survival to 59% and pupal weights to 52.6 mg. However, this also increased diet consumption (21 liters of diet per 10,000 pupae), labor, and required more space than was available in the mass production facility to maintain production quotas.

The most probable cause for the less than optimal production from the Water-Lock rearing system appeared to be the dimensions of the vats on the rearing floor. These vats were 4 cm deep whereas the vats used for research rearing were 9 cm deep. Therefore smaller, deeper vats, 66 by 46 by 9 cm deep, were tested. Each of these vats was infested with 1 g of screwworm eggs and provided 20 liters of diet. An average of 14,400 pupae (65% survival) weighing 53.5 mg were produced per vat. 13.9 liters of diet were required per
10,000 pupae. The smaller vats offered several advantages. They were not fixed to permanent supports and as such could be moved for feeding and cleaning, and they weighed less than 22 kg when full of diet so that one person could lift them. The ability to remove the vats from their support structure for feeding and cleaning allowed them to be packed closer together, thus reducing the space needed for larval rearing.

Temperature and humidity of the production larval rearing floor adversely affected the gelled diet rearing system as well. A test was conducted to compare rearing on the production floor (35°C) with rearing in the pupation room where the temperature was lower (30°C). Pupal yields did not significantly differ between the two rooms, however pupal weights increased from a mean of 50 mg on the production floor to 53.8 mg in the pupation room.

Based on these results, an area in the pupation room was modified to accommodate a pilot test for screwworm mass production with the Water-Lock rearing system. The pilot test was designed to produce 15 million pupae per week. This level of production was accomplished by preparing 160, 66 by 46 by 9 cm high plastic rearing pans. Each pan was infested with 1.25 g of screwworm eggs, provided 2 liters of diet, and placed in the first initiation room (39°C, 75%). An additional 2 and 6 liters of diet were added to the pans after 32 and 56 h. The pans were transferred to the main rearing floor at 56 h (35°C, 70%). An additional 10 liters of diet were added at 80 h and the pans were transferred to the pupation room (30°C, 60%). Mature larvae began to leave the rearing pans at approximately 96 h. The larvae that fell into metal pans under the rearing racks were collected every 4 h and placed in sawdust to pupate. For the 51 weeks during which the pilot test operated, an average of 19.2 million pupae averaging 47.1 mg (5.5 days postpupation) were produced each week. Yield averaged 1.38 liters of pupae per gram of eggs (63% survival) and diet usage was 11.7 liters per liter of pupae produced. During the same period, mass production pupae averaged 46.2 mg with a yield of 1.06 liters of pupae per gram of eggs (48% survival). Hydroponic diet usage was 25.3 liters per liter of pupae (Fig. 1). The gelled diet rearing system produced 2% heavier pupae, 32% higher yield, and consumed 54% less diet than mass production.

A cost comparison for hydroponic and gelled diet rearing systems is presented in Table 1. The cost for diet components to produce 300 million screwworm flies per week for one year with the hydroponic rearing system is $5,252,915 whereas the cost for the same number of flies using the Water-Lock rearing system is $3,805,312. Conversion to gelled diet will reduce the cost of diet components by $1,447,605 per year.

In addition to the savings in diet components, significant savings are foreseen in the costs of labor, facilities, and energy. Quantifying these savings is difficult. Conservative estimates indicate a 50% reduction in work force from 231 to 108 workers per day (H. Chris Hofmann, personal communication). Plant
Fig. 1. Comparison of pupal yield (liters of pupae per gram of eggs), pupal weight, and diet consumption (liters of diet per liter of pupae produced) for screwworm mass production with hydroponic diet and a pilot test with gelled diet.
Table 1. Cost of diet components for gelled and hydroponic diet rearing systems

<table>
<thead>
<tr>
<th>Diet Component</th>
<th>Blood Cost</th>
<th>Egg Cost</th>
<th>Milk Cost</th>
<th>Acetate Cost</th>
<th>Water-Lock Cost</th>
<th>Total Cost</th>
</tr>
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<tbody>
<tr>
<td>Kilogram</td>
<td>$0.95</td>
<td>$1.54</td>
<td>$1.03</td>
<td>$4.73</td>
<td>$7.01</td>
<td>--</td>
</tr>
<tr>
<td>Gelled Diet System</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liter diet</td>
<td>0.057</td>
<td>0.046</td>
<td>0.015</td>
<td>0.000</td>
<td>0.091</td>
<td>0.209</td>
</tr>
<tr>
<td>Vat</td>
<td>1.14</td>
<td>0.92</td>
<td>0.31</td>
<td>0.00</td>
<td>1.82</td>
<td>4.20</td>
</tr>
<tr>
<td>Liter pupae</td>
<td>0.66</td>
<td>0.53</td>
<td>0.18</td>
<td>0.00</td>
<td>1.06</td>
<td>2.44</td>
</tr>
<tr>
<td>Hydroponic System</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liter of diet</td>
<td>0.057</td>
<td>0.046</td>
<td>0.015</td>
<td>0.00</td>
<td>0.00</td>
<td>0.118</td>
</tr>
<tr>
<td>Vat</td>
<td>9.01</td>
<td>7.30</td>
<td>2.44</td>
<td>2.47</td>
<td>0.00</td>
<td>21.21</td>
</tr>
<tr>
<td>Liter of pupae</td>
<td>1.43</td>
<td>1.16</td>
<td>0.39</td>
<td>0.39</td>
<td>0.00</td>
<td>3.37</td>
</tr>
</tbody>
</table>

Note: Diet usage and pupal yield data are for the 51 weeks beginning October 2, 1988 and ending September 17, 1989. Costs include purchase price and transportation to the rearing facility at Tuxtla Gutierrez, Mexico.

*20 liters of diet per vat.
*b1.72 liters of pupae per vat.
*c158 liters of diet per vat.
*d6.3 liters of pupae per vat.

operation will be reduced from three full shifts per day to one full shift and two partial shifts. Energy and physical plant savings will be substantial, although insufficient information exists to make projections at this time.

Lower diet usage will reduce the amount of organic waste produced by the mass production facility. Waste gelled diet is semisolid, making handling, treatment, and disposal easier and reducing the organic load on the waste water treatment lagoons. This should improve the quality of the effluent being discharged. In addition, the waste diet still has a high protein content (Brown Snow 1978). Tests will be initiated soon to evaluate the use of waste screwworm diet as a protein supplement for livestock feed. Another potential
use of the waste gelled diet is as a combination fertilizer-soil conditioner. Similar gelling agents are marketed for use as conditioners for sandy soil. Processing waste screwworm diet into a usable by-product will provide economic input to the local community as well as reduce the waste water treatment needs and environmental impact of the rearing facility.

Conversion of the mass production facility at Tuxtla Gutierrez from hydroponic to gelled diet will cost between $500,000 and $750,000. Modifications to the existing rearing floor, vat support structures, larval collection system, diet transport system, and feeding stations will cost approximately $35,000. Equipment for mixing the gelled diet, new pans and racks to hold them, and diet dispensers will cost about $675,000. Savings in expenditures for diet components during the first year should be sufficient to pay for the conversion.

A bilateral agreement was signed between the Mexican and American managers of the eradication program in June 1989 to convert the mass production facility to gelled diet. Modifications were initiated in October 1989 and conversion to the gelled diet rearing system was completed in April 1990.

Gelled diet produces screwworm pupae of equal or better quality than hydroponic diet with less labor and diet. Use of the gelled diet will improve the environment on the larval rearing floor and reduce the amount of time workers must spend on the floor, thus improving the overall working conditions in the mass production facility. Gelled diet will allow centralization of the feeding process and elimination of vacuuming, the two most critical steps in screwworm rearing. This will result in improved supervision and reduce the impact of labor on fly quality. Lower diet consumption will reduce waste production and improve the quality of the effluent discharged into the environment. Processing the waste diet into livestock feed supplements or soil conditioners will provide a badly needed boost to the local economy and improve the agricultural output of the region.

Acknowledgment

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