New Method of Processing Diet for Mass Rearing Pink Bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelichiidae)\(^1\)

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**ABSTRACT** Pink bollworm (PBW) (*Pectinophora gossypiella* Saunders) diet processed with a scraped surface heat exchanger (SSHE) was compared to the standard batch-making system currently used in the Pink Bollworm Rearing Facility (PBWRF) in Phoenix, Arizona. Diet processed by standard methods and by the SSHE method produced similar yields of pupae per rearing container in two of the three tests conducted. However, larger pupae were produced with the SSHE-processed diet than with the standard diet. Pupal eclosion rates were similar, but wing deformities in adults reared on SSHE diet were higher than those reared on the standard diet. Production and the advantages of using the SSHE method of PBW diet processing are discussed.

**KEY WORDS** Lepidoptera, Gelichiidae, *Pectinophora gossypiella*, pink bollworm mass rearing, scraped surface heat exchanger

The San Joaquin Valley in California is the only major cotton-growing area in the southwest that is not generally infested with pink bollworm (PBW) (*Pectinophora gossypiella* Saunders). The release of sterile pink bollworm moths in the valley from 1968 to present is one of several methods that have successfully prevented PBW from establishing damaging endemic populations (Miller et al. 1983, Flint et al. 1974, Stewart 1984). Sterile PBW moths released in the San Joaquin Valley are supplied by the U. S. Department of Agriculture, Animal and Plant Health Inspection Service’s Pink Bollworm Rearing Facility (PBWRF) located in Phoenix, Arizona.

Numerous modifications in rearing technologies in recent years have increased the yields per container and also the quality of laboratory-reared PBW (Stewart 1984). Despite substantial improvements in rearing PBW, the method of rearing has remained essentially a hand labor operation with piecemeal automation. Modified automated processing systems used by the food industry could replace the diet-making

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system used by the PBWRF. The current method entails making 21 individual batches (558 liters each) per week (Stewart 1984). Each batch is processed in approximately 1.5 h. Implementation of a continuous diet-processing system would significantly reduce labor costs, one of the most costly components in rearing PBW. It would also increase speed of the operation, improve product consistency, and better utilize rearing space. We report here on a continuous processing system for preparing PBW diet, a first step in a totally automated system for rearing PBW moths.

Materials and Methods

Test 1. Diets used as controls were prepared by personnel in the PBWRF by using ingredients listed in Table 1. The methods used in preparing the diet, filling the rearing containers, and implanting the eggs are the same as those described by Stewart (1984), unless otherwise noted. Test diets were prepared in 30-liter batches and were formulated as usual, except that formaldehyde was omitted. Agar, methyl paraben, and potassium sorbate were added to 22 liters of water in a 189 liter stainless steel kettle. A 0.33 HP variable-speed Lightning blender (Mixing Equipment Co., Rochester, New York) was operated at 10 rpm to mix the above ingredients as they were added to the water. After adequate mixing, 950 ml of the slurry was removed and poured into a Waring Blender® with 100 ml of corn oil and 4.5 g of Calco red No. 1700 dye (American Cyanamid Corp., Princeton, New Jersey). The dye was used to internally mark sterile moths. After thorough blending with the oil and dye, the slurry was poured back into the kettle. The remaining ingredients were added separately in the order given in Table 1 and blended at 350 rpm for 5 min to ensure thorough mixing.

The slurry was pumped into a closed, steam-heated scraped surface heat exchanger (SSHE) (model 3 X 12 Cherry-Burrell, Louisville, Kentucky), where the mixture was exposed to a temperature of 132°C for ca. 48 sec. The SSHE works on the concept of continuously removing and renewing a thin film of the product against a large surface area that is either heated or cooled. Inside the heat exchanger, a direct drive shaft fitted with scraper blades rotates within a tube. The product passes through an annulus formed by the shaft and the tube; the surface serves as the contact area for heating or cooling the product. The tube is jacketed, and steam or cold water flows through the jacket to heat or cool the product. The unit is insulated to minimize energy loss and a stainless steel cover protects the insulation. The temperature and exposure time selected to cook and sterilize the diet were derived from a table of sterilization values (M. Burns, Engineer, Cherry Burrell, personal communication). The sterilization value of 10 was chosen because this value is used in processing aseptic puddings for human consumption, a product similar in consistency to PBW diet.

The diet was pumped from the first heat exchanger into a second, water-cooled SSHE, which cooled the diet to 79.5°C. The diet was then pumped into stacking fiberglass trays (76 cm X 26 cm) to the depth of 1.2 cm per tray. Trays in regular production are filled to a depth of 4 cm. The trays were then placed under a clean air bench at a temperature of 32°C. The thin layer of diet in each
Table 1. Quantities of ingredients for a 30-liter batch of artificial PBW diet listed in the order they are added to the diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>22.0</td>
<td>liters</td>
</tr>
<tr>
<td>Agar</td>
<td>726.0</td>
<td>g</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>57.0</td>
<td>g</td>
</tr>
<tr>
<td>Potassium sorbate</td>
<td>75.0</td>
<td>g</td>
</tr>
<tr>
<td>Dye</td>
<td>4.5</td>
<td>g</td>
</tr>
<tr>
<td>Corn oil</td>
<td>100.0</td>
<td>g</td>
</tr>
<tr>
<td>Locust bean gum</td>
<td>36.6</td>
<td>g</td>
</tr>
<tr>
<td>Sugar</td>
<td>528.0</td>
<td>g</td>
</tr>
<tr>
<td>Wesson salts mixture</td>
<td>348.0</td>
<td>g</td>
</tr>
<tr>
<td>Alphacel™</td>
<td>90.0</td>
<td>g</td>
</tr>
<tr>
<td>Toasted soy flour</td>
<td>2400.0</td>
<td>g</td>
</tr>
<tr>
<td>Wheat germ flour</td>
<td>1044.0</td>
<td>g</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>174.0</td>
<td>ml</td>
</tr>
<tr>
<td>(22% solution in water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>348.0</td>
<td>ml</td>
</tr>
<tr>
<td>(10% solution in water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>144.0</td>
<td>ml</td>
</tr>
<tr>
<td>(10% solution in water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aureomycin™</td>
<td>1.61</td>
<td>g</td>
</tr>
<tr>
<td>Fumidil B (Fumagillin)™</td>
<td>6.0</td>
<td>g</td>
</tr>
<tr>
<td>Potassium pantothenate</td>
<td>2.7290</td>
<td>g</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>1.3989</td>
<td>g</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.6968</td>
<td>g</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.6968</td>
<td>g</td>
</tr>
<tr>
<td>Thiamin hydrochloride</td>
<td>0.3484</td>
<td>g</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>0.3484</td>
<td>g</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.0279</td>
<td>g</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.0014</td>
<td>g</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>492.0</td>
<td>ml</td>
</tr>
<tr>
<td>(25% solution in water)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not used in test diets.
tray and the high volume of circulating air accelerated the drying of the diet. The test diet was allowed to solidify and dry for 2 h or 16 h. Control diet was held for 5 d in a drying tunnel maintained at 32–43°C with continuous air movement maintained by circulating fans. This is standard protocol in the rearing facility and facilitates the removal of formaldehyde fumes over the first 24 h. The diet is then dried in the same tunnel over the next 4 d to reduce the moisture content of the diet from 82% to ca. 75%.

The SSHE diet preparation method delivers an end product that is similar to our batch-made diet in form and texture. The diet must be shredded before it can be fed to PBW larvae. Attempts to feed PBW larvae solid blocks of diet with the surface area scarred with a knife to induce neonate larval feeding reduced yields by 50% (E. Miller, unpublished data).

Therefore, the test diets were shredded using a speed cutter S-500 (Valmont Corp., Grass Valley, California) light-duty vegetable and meat chopper. The shredded diet was manually placed into rearing containers at 350 g per rearing container. PBW eggs were implanted on the diet surface of each container with a 5-ml glass pipette at a rate of $3,200 \pm 20$ eggs suspended in 4 ml of a 0.1% agar solution (Richmond & Martin 1966).

The rearing containers for each treatment were isolated on a rearing cart and placed in a darkened larval incubation room for 8 d at 28.9°C, 40 ± 5% RH. On day eight, the carts were moved to an incubation room for pupae, maintained at the same environmental conditions except for the addition of constant, subdued overhead lighting. To pupate, mature PBW larvae exit the rearing containers and drop onto two pieces of 58 cm × 38 cm × 6.5 cm honey comb cell (Foster et al. 1978) placed in galvanized trays beneath each set of rearing containers. Twenty grams of pupae was taken from each treatment, and individual pupal weights were determined. Thirty pupae of mixed sex per treatment from these samples were placed individually in culture tubes (10 mm × 75 mm) and held 7 d to determine percent eclosion and presence of deformities. Treatments were replicated six times, with 15 rearing containers in each replicate.

Tests 2 and 3. The 5-d holding period for the batch-made diet (our current standard diet) in the PBWRF requires a 30 square meter drying tunnel and one person about 14 h a week to move daily diet production trays through the diet drying system. In the course of a production year, the drying system produces a considerable amount of variability in the moisture content of the diet due to influences of the ambient humidity. In a given year the moisture content of the diet has ranged from 58% to 80% (E. Miller, unpublished data). Diet with high moisture content (78%–80%) invariably produces lower yields (F. D. Stewart, personal communication). Optimal diet moisture content is approximately 75%.

Therefore, tests 2 and 3 were designed to determine if reducing the amount of water during diet processing with the SSHE system would produce a diet comparable in moisture content to our current standard diet without a significant holding period to dry the diet. Reducing water content in the batch-making system (current diet-making method) is not a viable option because the pumping system cannot move a product with a higher viscosity than diet with a moisture content of 80%.
In test 1, it was noted that diet ingredient mixing efficiency of the SSHE was much improved over the batch making system. Therefore, reduced amounts of agar—the gelling agent in our diet—and the fat soluble dye Calco red were tested. Diet preparations used in these tests were the same as those described in test 1, except in test 2, water was reduced by 25% (16.5 liters vs. 22 liters) and red Calco dye by 24.4% (3.4 g vs. 4.5 g). In test 3, water was reduced by 32% (15 liters vs. 22 liters), Calco red dye by 60% (1.8 g vs. 4.5 g), and agar by 25% (545 g vs. 726 g). Diet shredding, rearing-container filling, and egg implantation followed the methods described in test 1.

Data were analyzed either by t-test or analysis of variance (ANOVA). In ANOVA tests with significant differences, means were separated by a least significant differences (LSD) test at $P = 0.05$. All data were transformed by using a rank transformation as described by Conover & Iman (1981).

Results and Discussion

Test 1. Mean pupal yields per rearing container in the standard diet were significantly higher than those of the 2-h-old and the 16-h-old SSHE-processed diets (Table 2). Furthermore, SSHE-processed diet held 16 h had significantly higher yields than SSHE diet held 2 h. However, the 2-h-old SSHE diet produced pupae with a mean weight of 18.89 mg/pupa, which was significantly larger than pupae produced on the standard diet or 16-h-old SSHE diet (Table 2). In previous tests Miller et al. (1994) found pupal size to be larger in rearing containers when yields were less than 1,000 pupae per rearing container. At higher yields, pupae tend to be smaller, suggesting that size is density dependent. Historically, the PBWRF produces pupae with a mean weight of 15.5 mg. Pupal weights become a concern in the facility when they fall below 14 mg.

Pupal eclosion did not differ significantly among any of the diets tested (Table 2). However, PBW moths reared in the 2-h-old SSHE diet had wing or leg deformities in 4.8% of the individuals. This rate was significantly higher than in moths reared on standard diet or 16-h-old SSHE diet, which did not differ significantly from each other (Table 2). Although wing and leg deformities of moths reared on 16-h-old SSHE diet were not statistically different from those reared on the standard diet, deformities were numerically higher (2.29-fold).

Tests 2 and 3. The standard diet in test 2 produced 1,194 pupae per container with a mean weight of 15.05 mg per pupa. These data were not significantly different from those of the SSHE diet (Table 3). Differences in pupal eclosion rates were also not statistically different among the diets, respectively (Table 3).

In test 3, mean pupal yields per rearing container were 684 for the standard diet and 716 for the test diet. However, pupae weighed significantly more when reared on the test diet with a mean pupal weight of 17.3 mg compared to 15.7 mg for the standard diet (Table 3).

In tests 2 and 3, adult deformities of PBW reared on the test diets were again significantly higher than PBW reared on the standard diet. For example, in test 2 and test 3 of the SSHE-manufactured diet, 3.3% and 2.4% of the
Table 2. Comparison of mean pupal weights (mg), yields per rearing container, and eclosion rates of PBW reared on artificial diet processed by two different methods.

<table>
<thead>
<tr>
<th>Method diet processed</th>
<th>Diet age</th>
<th>Mean no. pupae/container ± SEM</th>
<th>Mean pupal weight (mg) ± SEM</th>
<th>% Eclosion</th>
<th>% Deformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSHE</td>
<td>2 h</td>
<td>1,003.0 ± 52.15c</td>
<td>18.89 ± 0.51a</td>
<td>96.7 ± 0.004a</td>
<td>4.8 ± 1.34a</td>
</tr>
<tr>
<td>SSHE</td>
<td>16 h</td>
<td>1,405.0 ± 150.29b</td>
<td>17.68 ± 0.70b</td>
<td>90.6 ± 2.14a</td>
<td>3.2 ± 0.89b</td>
</tr>
<tr>
<td>Standard</td>
<td>5 d</td>
<td>1,899.2 ± 98.98a</td>
<td>17.46 ± 0.36b</td>
<td>95.3 ± 2.64a</td>
<td>1.4 ± 0.68b</td>
</tr>
</tbody>
</table>

a Diet age defined as period from processing to time diet is implanted with PBW eggs. Means in columns followed by the same letter are not significantly different at the 0.05 level (LSD).

b Percent eclosion from examination of 180 pupae per treatment.

Table 3. Comparison of mean pupal weights (mg), yield per container, and eclosion rate from rearing containers filled with modified diets processed in a SSHE or by standard methods.

<table>
<thead>
<tr>
<th>Processing method</th>
<th>Diet age</th>
<th>Mean no. pupae/container ± SEM</th>
<th>Mean pupal weight ± SEM</th>
<th>% Eclosion</th>
<th>% Deformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSHE</td>
<td>2 h</td>
<td>1,222 ± 79</td>
<td>15.56 ± 0.36</td>
<td>96.7</td>
<td>3.3 ± 1.1c</td>
</tr>
<tr>
<td>Standard</td>
<td>5 d</td>
<td>1,195 ± 150</td>
<td>15.05 ± 0.41</td>
<td>93.3</td>
<td>0.0 ± 0</td>
</tr>
</tbody>
</table>

Test 2

| SSHE              | 2 h      | 716 ± 115                      | 17.3 ± 0.25c           | 96.7       | 2.4 ± 0.73c |
| Standard          | 5 d      | 648 ± 168                      | 15.7 ± 0.52            | 96.7       | 0.0 ± 0    |

Test 3

a Test 2—water reduced by 25%, dye by 24.4%.
b Test 3—water reduced by 32%, agar by 25%, and dye by 60%.
c Means in each test are significantly different from each other at the .05 level (t-test).
adults, respectively, had deformities of the wings or legs while none of the adults reared on the standard diet batches had deformities.

At this time, it is unclear what caused the increased rate of deformities. The high temperature (132°C) used in the SSHE process may have caused a partial inactivation of one or more of the vitamins. In normal batch processing, vitamins are added to PBW diet at a temperature of 79.5°C. Additional testing will be necessary to identify the cause of this problem.

Yield differences that were evident between the test and control diet in test 1 were not present in tests 2 and 3. These differences in results can be attributed to the moisture content of the diet. In test 1, both control and test diets were prepared with the same amount of water (82%). However, test diets were dried for 2 h or 16 h while the control was held 5 d before use. The 2 h old test diet appeared wet (78%–80% moisture content) with a softer texture than the control or the 16 h old test diet, which in two of the six replications was similar in texture and moisture content (73%–75% moisture content). In the other four replications, the control diet was much drier (65%–68% moisture content) than diet normally used in the PBWRF but within the range of moisture content produced by the facility over the course of a season. When diet this dry prevails in the rearing facility, yields per rearing containers can exceed the standard yield of ca. 1,250 pupae/container by as much as 60% (F. D. Stewart, personal communication).

In tests 2 and 3, the amount of water was reduced 25% and 32%, respectively, in the test diets. This resulted in diets that were prepared with moisture contents of 77.6% and 75.9%, respectively. The 2-h solidifying and drying period resulted in another 2%–4% loss in moisture content. Thus, the test diets were similar in moisture content or slightly drier than control diet normally produced in PBWRF.

Yields from test diets in tests 2 and 3 dried just 2-h were not different from those of the standard (Table 3). Therefore, implementation of the SSHE system of diet preparation in the PBWRF would eliminate the need of a drying tunnel that accommodates five production days worth of diet. It would also eliminate the labor required to handle the movement of diet trays over the course of 5 d, which is ca. 14 labor h per week or 320 labor h for a 160 d production season. Furthermore, it appears from observations made from our test diet that the moisture content of the diet is more consistent using the SSHE system than our current system due to the reduction in the amount of moisture that has to be evaporated from the diet and the time frame to accomplish this task.

Thirty adults reared on test diets in tests 2 and 3 were examined for dye retention at death (20–30 d old) by crushing the moths on white filter paper. The results showed that moths reared on diets containing 24.4% and 60% less dye than used in the standard diet were 100% tagged. Dye retention could be distinguished by the naked eye in all cases.

The main objective in reducing the amount of Calco red dye in the diet formula was to reduce the overall cost of the diet while maintaining moths for release that would be readily identifiable in field survey traps. The savings per year in dye costs would be ca. $880.

In test 3 we also reduced the amount of agar by 25% in the SSHE-processed diet. This reduction was directed at reducing the overall costs of the diet. In
the case of agar this represents a significant savings to the program, since agar represents 52% of our total ingredient costs. Reducing the amount of agar by 25% provides the facility a savings of $56,148 per year.

The deletion of formaldehyde as a diet component eliminated the 24-h-holding time necessary to evaporate and/or allow formaldehyde to react with the diet ingredients, rendering the diet nontoxic to PBW larvae (Stewart 1984). Deletion of formaldehyde from the SSHE-processed diet did not increase susceptibility to microbial contamination. Comparative microbiological tests (agar plate and Broth tests), with the control diet as a standard, revealed no microbial contamination of either diet type through the aging, shredding, and rearing container filling processes. Similar results were obtained by David et al. (1972) in comparing a semisynthetic diet with or without formaldehyde. Furthermore, the deletion of formaldehyde reduces diet ingredient costs by $1,715 per year and eliminates the exposure of employees to a known carcinogen (Ulsamer 1984).

The SSHE also significantly reduces the time required to process the 11,718 liters of diet used each week in the PBWRF from 31.5 h to 8 h per week. Over the course of the 160-d production season this equates to a savings of 537 labor h.

The cost of implementing the SSHE system into the PBWRF would be approximately $295,000. If yield per rearing container remain the same as our data indicated in tests 2 and 3, the equipment would pay for itself in 4.53 yr. This is based on a savings in diet ingredients of $58,743 per year and a labor savings of $6,427.50 per year. The labor savings is based on 857 labor h saved per year at an average cost of $7.50 per hour.

The SSHE system is the first of three changes that would be necessary to automate two operations in the PBWRF. The second would be to automate the placement of diet in each rearing container. Once this task was completed the third change would be to develop an automated diet-conveying system that would link the SSHE system to the diet shredder and then to the container filling station. The conveying system between the SSHE and diet shredder would be equipped with hoods to dry and cool the diet before shredding.

One disadvantage of the SSHE system is the higher deformity rates we encountered in all test diets when compared to the control diet. If future work identifies the cause as reduced vitamin potency due to heat damage, the problem could be resolved by moving vitamin injection to a downstream zone in the process where temperature of the diet has cooled to $\leq 79.5^\circ C$. However, the results of our tests indicate that the SSHE system of PBW diet preparation offers a practical alternative to the current batch-making method of diet preparation in the PBWRF.

References Cited


