MASS PRODUCTION OF *TRICHOGRAMMA PRETIOSUM* RILEY[1/2/]

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ABSTRACT

The egg parasite, *Trichogramma pretiosum* Riley was mass-reared on eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), using a production system that yielded parasitized eggs unattached to a substrate. During a specific 7-wk period during three consecutive yrs of production, a mean of 13,465,000 parasites were produced daily for field release. Production efficiency (parasites available for field release from host eggs exposed) was 63.7%. A cost analysis of the system showed that the cost of the parasites was ca. $.0178/1000.

INTRODUCTION

A modified system for efficient mass production of the egg parasite, *Trichogramma pretiosum* Riley, was reported by Morrison et al. (1978). This system produced parasites in a form that was more amenable to aerial release systems because the parasitized eggs were unattached to a substrate, and could be programmed for rapid, uniform emergence of adults with the use of cold temperature (Stinner et al. 1974). This small prototype system was expanded into one with true mass production potential and this paper reports the methods of production and the resulting production of parasites used in an ARS 3-yr pilot program, "Management of *Heliothis* spp. in Cotton by Augmentative Releases of *Trichogramma*."

MATERIALS AND METHODS

Host Egg Production and Preparation for Parasitization. All *T. pretiosum* produced for field release during the 3-yr ARS pilot program were reared on eggs of the Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier). Production techniques for AGM eggs are detailed in another section of this monograph.

After collection, the AGM eggs were held at 13°C to retard development. Although AGM eggs held for up to 10 days at 13°C can be successfully parasitized, all eggs used for parasite production were held under refrigeration for no more than 72 h.

When needed, AGM eggs were returned to 27°C, passed through a #30 standard sieve screen, and weighed. Then one side of a piece of opaque white acrylic plastic (20X46X0.16 cm) was lightly and uniformly coated with water mist generated by a modified Brahma® insecticide fogger (B and G Co.,

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[1/] Hymenoptera:Trichogrammatidae

[2/] Mention of a proprietary product does not constitute an endorsement by the United States Department of Agriculture. This research was done in cooperation with the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843.

Plumsteadville, PA) filled with tap water. The fogger was modified to operate with a foot-operated, automatic shut-off switch (Fig. 1-A). Immediately thereafter, prepared AGM eggs were poured onto the misted surface of the plate. Then the eggs were rolled back and forth over the plate until the misted surface was completely coated with eggs. Excess eggs were removed by inverting the plate over a 1 m^2 sheet of white butcher paper (Fig. 1-B). The plate was then set aside and the unattached eggs were collected again and weighed. With this technique, 15.0 + 1 g of eggs/plate were attached. A natural adhesive on the AGM eggs, activated by the moisture, caused the eggs to adhere sufficiently to allow handling of the plate without egg loss. After parasitism, they were removed easily without damage by pushing a soft-bristle paint brush (ca. 20 cm wide), held diagonally to the plate, across the surface. The displaced eggs were caught on a sheet of paper (Fig. 2-A).

Oviposition Unit Construction. The oviposition unit was constructed of 1.3 cm thick cabinet-grade plywood and 0.6 cm thick tempered Masonite®. This unit measured 23.7X27.2X94.7 cm and was so constructed as to form 10 insect-proof shelves each measuring 2X21.2X94.7 cm. The plywood sides were grooved to accept nine 0.6X22.2X94.7 cm pieces of Masonite.

Indentations (27X47 cm) on both sides of the unit, 2 cm from one end, were left uncovered. During assembly, all joints were glued and nailed and the ends planed flush. The exposed edges of all shelves at both ends and side indentations were then covered with adhesive-backed, closed-cell, foam weatherstripping (0.6X0.3 cm). Two glass covers (26.8X46.8 cm) were cut and edged from 0.5 cm clear-crystal, plate glass and fitted to the side indentations of the unit. The glass was held in place by turn latches at each of the four corners. The latches were screw-adjusted so the glass would uniformly compress the weather stripping ca. 0.1 cm. The two end covers (23.7X27.2X1.3cm) were secured with two rubber bands, each attached diagonally across the cover to screw posts on the sides of the unit (Fig. 3).

Ten additional accessory shelves of acrylic plastic (14.2X21.2X0.16 cm) were glued in place at the end opposite the glass sides. Pieces of balsa wood 0.3X0.3X14.2 cm had first been glued to the sides of each shelf so as to position these shelves parallel with and ca. 0.8 cm above the bottom of each shelf (Fig. 4). These accessory shelves were used to hold the parasitized eggs that produced adults for subsequent parasitization ("sting stock"). This arrangement allowed both new and expended "sting" stock" to be introduced into and removed from the unit without interfering with the introduction and and removal of the egg plates.

Oviposition Unit Operation. During operation the oviposition unit was centered on a line bisecting the length of a waist-high table (0.8X2.1 m). At each end of the table, two vertical 20 w, cool-white fluorescent tubes with ballasts were spaced ca. 17 cm apart and supported by a simple wood stand. The tubes faced away from the unit and were centered and aligned parallel at a distance of ca. 97 cm from each glass cover. These lights were controlled by time clocks set for alternate 2:2 h L:D regimes (Fig. 3-B). Light was thus provided on alternate sides of the unit at 2 h intervals. Additionally, pieces of white poster board ca. (0.8 m^2) were erected at each end of the table. Light from the tubes, reflected off the poster board toward the unit, was further reduced and dispersed with pieces (ca. 0.8 m^2) of polystyrene light-diffusion louvers (American Louver Corp., Skokie, IL 60077) (1.2 cm thick) that were covered on both sides with 18-mesh fiberglass screen. One of these pieces was placed vertically and parallel with the production unit, ca. 15 cm away from the light units on the production unit-side of the table (Fig. 3-C). This arrangement gave a uniform light intensity of ca. 160 Lumens/m^2 at the glass covers. The table was draped with black muslin supported on a wooden frame to a height of 0.8 m. The drapes effectively excluded normal laboratory lighting. The production unit was serviced by temporarily folding back the muslin cover.

FIG. 1. Application of Sitotroga cerealella eggs to plastic plate. A. Mist-coating plastic plate with misting device. B. Attaching eggs to plastic plate.

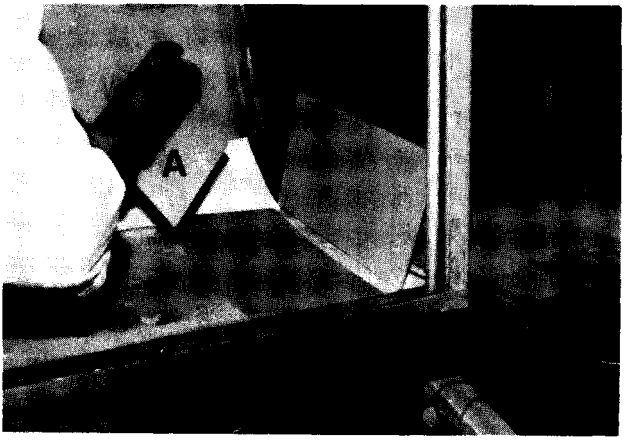

FIG. 2. Removal of Sitotroga cerealella eggs from plastic plate. A. Brushing eggs from plate. B. Paper containers for oviposition stock on organdy-bottomed tray.

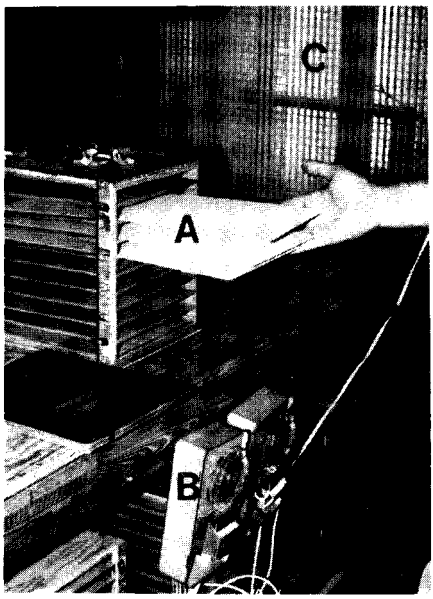

FIG. 3. Placement of unparasitized eggs into oviposition unit. A. Egg plates and "light" end of oviposition unit. B. Time clocks. C. Light arrangement.

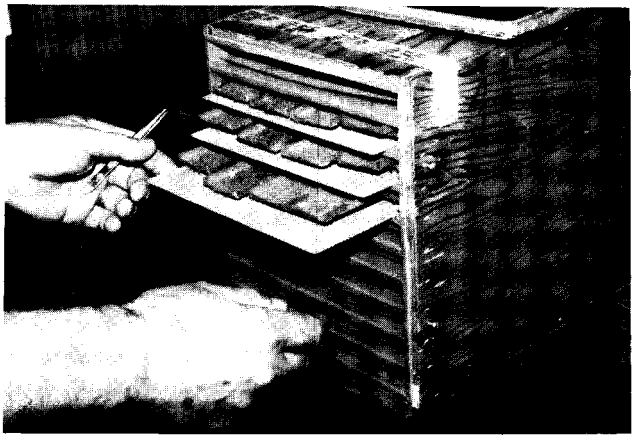

FIG. 4. Removal of parasitized eggs from "dark" end of oviposition and arrangement of oviposition stock on accessory shelves.

In daily operation of the unit, required numbers of AGM egg plates were prepared and the door at the "light" end of the unit then was removed and the prepared plates quickly inserted, one to each shelf. As the plates were inserted, the previous day's plates were pushed into the covered "dark" end of the unit; the door was then replaced. Within 1 h, the positively phototrophic T. pretiosum that were carried to the "dark" end on the previous day's egg plates moved to the light where the fresh unparasitized eggs were located. The door on the "dark" end of the unit then was removed, the egg plates removed, and the eggs that had been exposed to the parasites for 24 h were removed by brush as previously described.

After each day's parasitized eggs had been brushed from the plates, the total weight of these eggs was determined. An aliquot (20% by weight) of the eggs removed from each plate was then placed in contruction paper holding containers (4.2X15.2X0.6 cm). The filled containers were placed on a organdy-bottomed tray (45X45X2.2 cm) and labeled with the Julian date when the eggs were parasitized. These parasitized eggs were kept for later use. The remaining parasitized eggs were loosely distributed on the organdy of the tray (Fig. 2B) and the tray was shelved in an environmental chamber operated at 27° +1°C and 75±5% RH in total darkness.

There are ca. 50,000 eggs/g of S. cerealella when they are 0-24 h old. However, after they are parasitized and then held at 27°C, there is a measurable weight loss virtually hourly until the adult parasite emerges. Thus, any weight relationships made after the eggs are parasitized, such as determining the 20% reserved portions of "sting" stock, must be done by reweighing rather than basing it on initial total egg weight.

On day 8 postparasitization, the paper containers of "sting stock" that were ca. 1.5 days from adult emergence were removed from the environmental chamber and placed on the accessory shelves, one container/shelf. After 4 days, the containers were removed from the unit and replaced with fresh 8-day-old material. Thus, there was a constant rotation of "sting stock" from 8- to 12-days-old through the unit. Virtually all parasites that were held at 27°C had emerged by day 12. Since parasitized eggs were placed into the unit before and removed after adult emergence, manipulation of the "sting stock" was simplified. Generally, the removal of expended "sting stock", insertion of fresh "sting stock", and removal of the egg plates was accomplished at the same operation.

As the unit was essentially 10 independent units, it could be operated at any level. To increase the production level, it was only necessary to reserve more 20% portions of "sting stock". This allowed any given production level to be increased 5-fold ca. every 10 days.

Because of the accumulation of frass and dead parasites on the glass, the unit was cleaned weekly. This was accomplished by alternately removing each glass cover when it was in the dark phase, brushing off dead parasites from the exposed shelf edges, and then replacing and adjusting a clean glass cover. The inside of the cage was essentially self-cleaning due to the regular movement of egg plates through the unit. The rearing area was maintained at 27±1°C and 75±5% RH.

Quality Control. Production quality control involved daily monitoring of % parasitism, % adult emergence, and the sex ratio of emerged adults. This was accomplished by taking a small sample of eggs after their removal from the egg plates and counting out six subgroups of ca. 200 eggs under the microscope. After counting, each sample was isolated in capped and labeled plastic cups (30 ml) and held under laboratory conditions.

Six days after parasitization, the samples were examined again and counted to determine % parasitism. Parasitized eggs turn grey-black after 4-5 days; unparasitized eggs either hatch, remain white, or turn orange. After counting, each sample was returned to the container and held until day 12 post parasitization when all adult emergence had occurred. The samples were frozen overnight at -10°C and then the adults were counted to

determine % adult emergence. Of the six samples, two were selected and the sex ratio determined by examing the emerged adults.

RESULTS AND DISCUSSION

During a 7-wk period over three consecutive years, a mean of 21,145,000 eggs/day of S. cerealella was subjected to parasitism by T. pretiosum (Table 1).

TABLE 1. Mean Daily Utilization (by Wk) of Grams of Sitotroga cerealella Eggs for Production of Trichogramma pretiosum (50,000 eggs/g).

Wk of[1/]	1981	1982	1983
June 20	479.9	447.6	311.1
27	470.7	376.1	422.4
July 4	477.7	411.8	385.5
11	449.3	438.7	436.1
18	448.4	265.3	456.2
29	439.9	437.1	448.6
Aug 1	453.6	428.3	396.7[b/]
$\bar{x}$	459.9	400.7	408.1

a/+ Two days/yr.
b/Release program abandoned and production reduced.

Daily quality control studies of these eggs indicated that a mean of 79.6% of these eggs was parasitized and 93.9% of the parasitized eggs produced adults with a mean sex ratio of 1.71♀:1.0♂ (Table 2). Therefore, during

TABLE 2. Mean Daily % Parasitism, Adult Emergence, and the Sex Ratio by Week of Trichogramma pretiosum Produced on Eggs of Sitotroga cerealella.[a/]

Wk of[b/]	Parasitism			Adult emergence			Sex ratio ♀:♂		
	1981	1982	1983	1981	1982	1983	1981	1982	1983
June 20	80.3	72.8	83.9	95.1	93.4	98.2	1.40:1	1.78:1	1.85:1
27	79.8	72.0	86.2	91.2	93.2	95.4	1.42:1	1.98:1	1.88:1
July 4	82.9	69.3	86.6	92.9	93.7	95.0	1.46:1	1.71:1	2.22:1
11	86.3	72.8	89.1	91.0	88.4	97.0	1.24:1	1.85:1	1.99:1
18	80.7	64.7	87.1	95.7	93.9	95.5	1.32:1	1.83:1	1.78:1
29	81.9	71.1	87.1	96.5	92.6	97.3	1.59:1	1.62:1	1.51:1
Aug 1	81.7	70.4	85.5	92.9	85.9	96.4	1.62:1	2.11:1	1.77:1
$\bar{x}$	81.9	70.4	86.5	93.6	91.6	96.4	1.44:1	1.84:1	1.86:

a/As determined by daily quality control samples.
b/± 2 days/yr.

peak production for the three yr period, ca. 13,465,000 parasites were available each day for field release after the 20% portions of oviposition stock were reserved in the Laboratory. This was a production efficiency for release of 63.7% (T. pretiosum available for field release from S. cerealella eggs exposed).

This level of production required that three of the previously described production units be operated. In operation, it was found that two production units could be stacked on one table, thus requiring only two tables. It appears a single production unit can routinely produce 4-5 million T. pretiosum/day for field release. A cursory analysis of production costs during the three years showed that the expense of producing T. pretiosum at the production location was $.0178/thousand parasites (Table 3).

TABLE 3. Cost Analysis of Trichogramma pretiosum Based on Daily Production During 1981-1983.

Cost of 25m^2 rearing area @ $543,00/m^2	$13,575.00	
Depreciate 20 yrs (7300 days) daily cost		$1.86
Cost of four production units @ $150.00 ea.	600.00	
Cost of other equipment	15,000.00	
	$15,600.00	
Depreciate 10 yrs (3650 days) - daily cost		4.28
Cost of Sitotroga cerealella eggs[a]		
21,145,000 @ $.00655/1000		138.50
Labor - 6 h/day @ $10.00/h		60.00
Utilities @ $25.00/day		25.00
Maintenance @ $3650.00 yr ($10.00 day)		10.00
		$239.64
Cost of T. pretiosum/1,000	$239.64 / 13,465[b]	$0.0178

[a] Mean eggs utilized/day; cost derived from "Effective mass production of eggs of the Angoumois grain moth, Sitotroga cerealella". Southwest. Entomol.

[b] Derived from $\bar{x}$ S. cerealella eggs utilized/day x mean % parasitism less 20% reserved for oviposition stock (in 1000's).

LITERATURE CITED

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