Rearing egg parasitoids for use as biocontrol agents.

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Introduction

Wasp egg parasitoids of the genus *Trichogramma* are more commonly used as inundative biological control agents than any other natural enemy (Stinner, 1977). Large numbers of *Trichogramma* are required for field release programs. The target host is often too difficult and expensive to rear in sufficient numbers to mass rear parasitoids. Factitious hosts are generally used due to their ease of rearing and low rearing cost when compared to the target pest. Two factitious host insects are commonly used in western countries to mass rear *Trichogramma*, viz. the Mediterranean flour moth, *Ephestia kuehniella* Keller, and the Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier).

This paper presents techniques used for rearing egg parasitoids on AGM eggs in an experimental facility at the QDPI Division of Plant Protection entomology laboratories in Toowoomba. These techniques have been adopted by commercial interests so that egg parasitoids can be reared in sufficient numbers for use by farmers for pest management in the future.

Materials and Methods

AGM larvae were reared on wheat using techniques adopted from those described by others (Morrison, 1985b; Laing and Eden, 1990).

Host rearing

Chemically free wheat was used to rear AGM larvae. All wheat was heated in an incubator at 65°C for 24 hours prior to use to kill any insects or mites that may be present in the grain. The sterilised grain was treated with a miticide (dicofol) 15 ml/L at a rate of 600 ml per 5 kg of wheat to prevent infestation by mite pests. The treated grain was then placed in a sealed plastic container and held in a refrigerator (approx. 4°C) for three days to restore the grain to a favourable moisture content of approximately 15% (Morrison, 1985b).

AGM larval rearing cribs (50x30x2 cm) were constructed of a galvanised sheet metal frame (24 gauge) bent in a "U" shape. Wire mesh (4 strands/cm) was rivetted onto the metal frame to contain the grain. Each crib held 2.5 kg of

wheat. Wire ties were placed through the mesh sides to maintain the wheat at a uniform thickness of approximately 2 cm.

The cribs were held in hangers to collect emerging moths (Figure 1). The top and bottom of each hanger were made from galvanised sheet metal (24 gauge). The top (40x22x2.5 cm) was flat and contained paired hooks 30 cm apart to hang cribs vertically. Each hanger held two cribs, and the paired hanging hooks for cribs were 10 cm apart. The bottom of each hanger (40x22 cm) was funnelled to the lid (6 cm Ø) of a 600 ml glass collection bottle. The top and bottom of each hanger were slightly flanged (0.5 cm) so that a clear plastic covering could be attached by rubber bands (Superior size 107) to contain the cribs. Two squares (10x10 cm) of muslin cloth were taped on opposite sides of the plastic covering to provide ventilation.

Hanging racks (150 cm long and 120 cm high) constructed from 2x2 cm RHS steel supported four hangers (see Figure 1). The legs of each rack were placed in oil filled jars to prevent infestation by predatory mites and ants. Tanglefoot was spread between the hanging hooks for each hanger on the rack to reduce the likelihood of pest contamination between hangers.

The treated grain was infested with enough AGM eggs to produce two eggs per kernel of wheat. This required approximately 1.2 g of AGM eggs per kilogram of wheat (there were 28,300 kernels of wheat/kg, and 50,400 AGM eggs/g), or 3 g AGM eggs per 2.5 kg crib. The eggs were washed in a 10% formalin solution for 5 minutes and rinsed in running tap water for 5 minutes prior to infestation to kill mites and psocids. After rinsing, the eggs were placed in 250 ml of water in a small plastic bottle and gently poured uniformly over a horizontally placed crib so that the water passed through the crib and the AGM eggs adhered to the grain.

AGM eggs could be stored at 5±1°C (50±5% R.H.) for up to five days iand still maintain high levels of egg hatch (> 75%). After this, egg hatchability declined (Figure 2).

Following infestation the cribs were placed 20 cm apart on wooden horizontal storage racks to allow the AGM eggs to hatch, and the larvae to penetrate the grain and develop. After three weeks the cribs were transferred to hangers and hung vertically to collect AGM moths as they emerged from the grain.

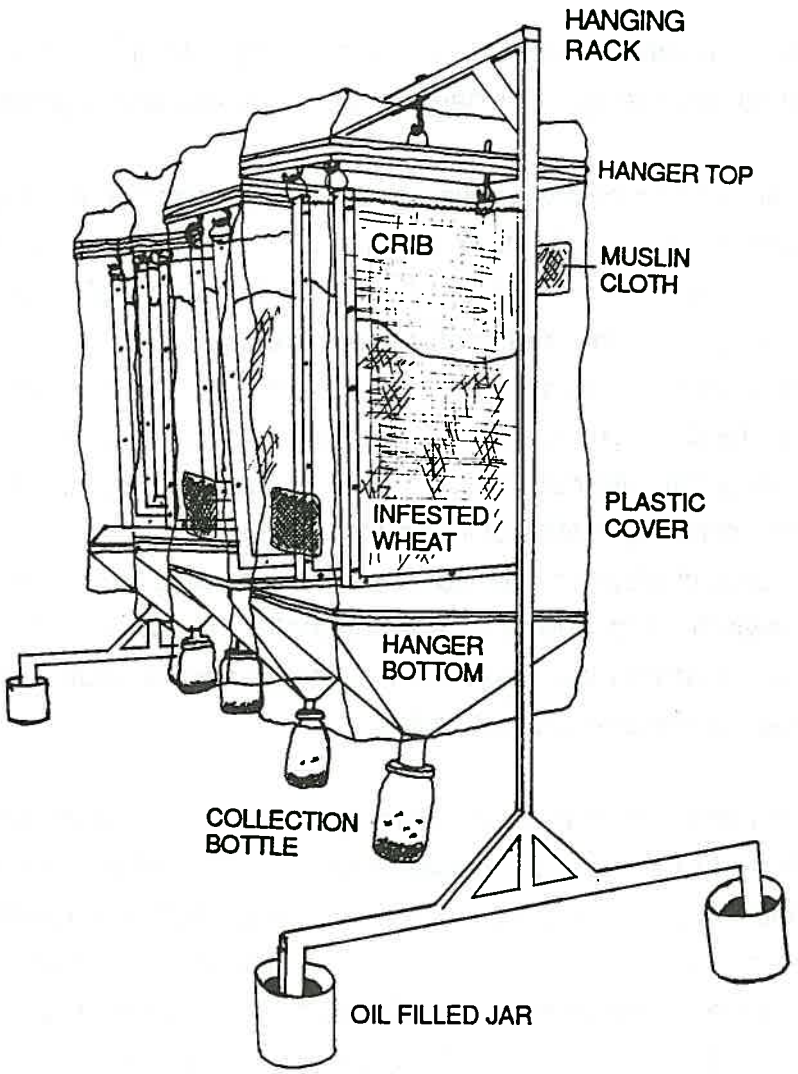

FIGURE 1: *Sitotroga cerealella* (AGM) rearing unit.

Temperature was maintained at 25±2°C and relative humidity at 70±10% in the AGM rearing room. Lights were used when the room was occupied by working staff (usually 6 hours maximum per day).

Adult AGM commenced emerging approximately 24 days after infestation. Moths naturally fell into collection bottles at the bottom of each hanger and were collected daily for four weeks. Used wheat was dumped and the cribs were washed and sterilised in an autoclave.

Moths were placed into stainless steel wire mesh (9 strands/cm, 28 SWG, 0.9 mm aperature) cylinders (20 cm long, 20 cm Ø) with plastic ends. A threaded steel rod (0.6 cm Ø) held the plastic ends against the wire mesh. A hole (6 cm

Ø) was drilled in one end of each cylinder to allow collection bottles to be emptied into the cylinder daily, and was sealed with a plastic stopper.

The oviposition cylinders were placed in an enclosed unit (113x50x43 cm). A hinged 0.6 cm thick clear acrylic door (113x42 cm) at the top of the unit allowed the cylinders to be placed on to two steel rods, one of which was connected to a geared motor that rotated the cylinders 4 times/minute. A vacuum cleaner was connected to a metal tube (7.5 cm Ø) containing two inlets (19x0.3 cm) located 0.3 cm from the top of cylinders placed in the unit. The vacuum cleaner removed moth scales from the top of the cylinders without drawing moths or eggs (at the bottom of the cylinders) from the unit. The vacuum cleaner and motor were connected to a timer with a manual over ride switch, and operated automatically for two minutes every hour. The manual over ride was used for five minutes when moths were first added to a cylinder to remove excess scales.

The tumbling of the moths dislodged the eggs, which fell through the wire mesh and onto plastic collection trays (50x35 cm) at the bottom of the unit. A second vacuum system continually drew a slight airstream over the collection trays to withdraw any minute moth scales falling out of the cylinders. AGM eggs were collected daily and sieved through a series of stainless steel mesh screens (9 strands/cm, 13 strands/cm, and 33 strands/cm). The finest screen separated mite (*Blattisocius keegani* Fox) eggs from AGM eggs.

Parasitoid rearing

After collection, AGM eggs were held at 7±1°C to retard development. AGM eggs held for up to 28 days at this temperature could still be used to rear parasitoids (Figure 3). When needed, AGM eggs were weighed and sprinkled uniformly through a stainless steel wire screen (16 strands/cm) onto a white plastic sheet (37x22.6x0.2 cm) misted with distilled water. A natural adhesive on the eggs stuck them to the plastic when they contacted moisture. After drying, eggs could be easily removed with a soft paint brush held diagonally to the plastic sheet and moved downwards in short, gentle strokes.

Parasitoids were reared in a Morrison type unit (Morrison, 1985a). The unit (76.2x54x25 cm) contained 10 shelves. The sides were made from 1.0 cm thick clear acrylic sheets (75x52.7 cm), and the top, bottom and ends were made from 0.6 cm thick black acrylic. The top and bottom (75x25 cm) were

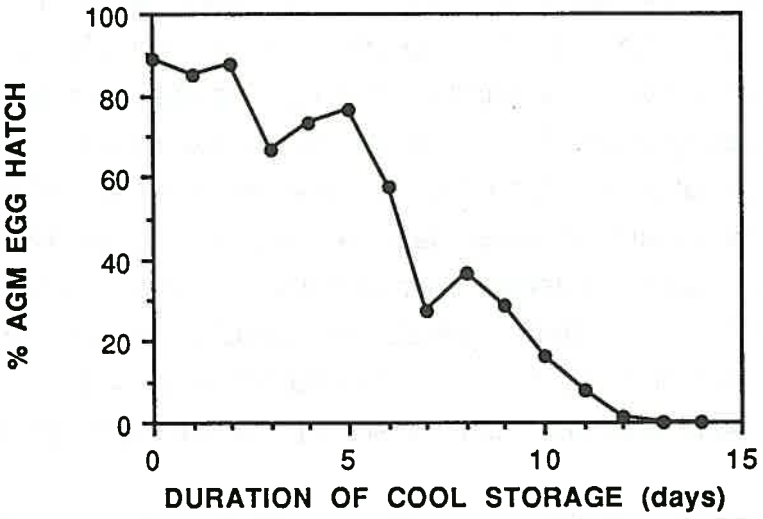

FIGURE 2: The effect of cool storage (5±1°C) on hatching of AGM eggs.

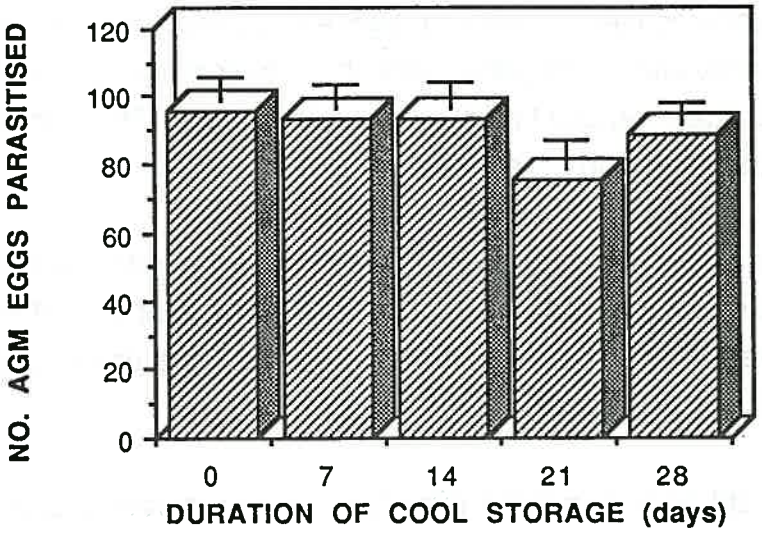

FIGURE 3: The effect of cool storage (7±1°C) of AGM eggs on parasitism by *Trichogrammatoidea bactrae* Nagaraja. Five female parasitoids were allowed to parasitise AGM eggs for 24 hours at 25±2°C and 70±10% R.H.. Values represent the mean ± standard error of 10 replicates. Data were analysed by analysis of variance and were not significantly different.

glued to the sides with 'Weld On #16' acrylic glue. Nine removable black acrylic shelves (75x24x0.3 cm), each 5 cm apart, were fited into routed channels (0.3 cm wide and 0.5 cm deep) in the clear acrylic sides. All black acrylic was sanded with fine sand paper to give a dull, non-reflective, lustre. Ventilation holes (2.5 cm Ø) were drilled in the clear acrylic sides between each shelf channel and 17.5 cm from each edge. The ventilation holes were covered with fine stainless steel mesh (47 strands/cm, 44 SWG, 0.125 mm aperature) to prevent parasitoid escape while allowing ventilation. Hinged, removable black acrylic doors (54x25 cm) attached by case catches were fitted to both ends of the unit. One half of each clear acrylic side was painted with flat black paint to create a unit consisting of a 'dark' end and a 'light' end.

During operation, parasitoid pupae ('sting stock') were placed in the dark end of the unit to provide approximately one parasitoid per 5 AGM eggs. AGM eggs stuck to white plastic sheets were placed in the light end of the unit. Emerging parasitoids were positively phototactic and moved to the light end of the unit where they encountered unparasitised AGM eggs. The sting stock were removed from the dark end after 24 hours of parasitisation, and a new AGM egg sheet was inserted into the light end pushing the previous day's egg sheet to the dark end. The parasitoids then moved from the dark end to the light end and again encountered unparasitied eggs. This process could be repeated for the life of the parasitoids (usually 4-5 days).

The unit was operated in a constant temperature room (25±2°C, 70±10% R.H.) with windows providing natural lighting. The light end of the parasitoid rearing unit faced the windows and the natural light entering the unit was sufficient to attract the parasitoids.

Parasitised AGM eggs turn black after four days (unparasitised eggs are white or red, depending on their stage of development) and adult wasps emerge after another six days at 25°C. Parasitised hosts can be removed from the egg sheets with a soft paint brush, weighed, and stuck to paper cards with a water soluble glue (Aquadhere) for use in field release programs or as sting stock to increase parasitoid numbers. Parasitised host eggs weigh slightly less than unparasitised hosts (54,900 and 50,400 eggs per g respectively). Weights can be used to determine parasitoid numbers as one parasitoid emerges per parasitised AGM egg. Samples of parasitised AGM eggs should

be regularly taken and the emerging parasitoids sexed and counted to determine sex ratios.

Discussion

Egg parasitoids are not difficult to rear. The key to success is having a supply of AGM eggs to rear parasitoids. The system described here returned a maximum of 3.0 g of eggs for every g used to infest the grain. Morrison (1985b) and Laing and Eden (1990) report returns of 10.9 g and 6.5 g respectively. Further research is required to identify areas in the AGM rearing procedure that can be improved.

The techniques described here can be used to develop commercial insectaries. The cribs could be made larger to hold more grain, and hangers could be modified to accomodate more than two cribs. It is important to maintain the temperature of the cribs below 35°C to prevent the induction of AGM larval aestivation which results in erratic moth production (Morrison and Hoffman, 1967). This is best achieved by ensuring that the cribs are designed so that the thickness of the wheat never exceeds 2 cm, and by providing some ventilation in the hangers for heat dissipation. If crib temperature is likely to exceed 35°C the cribs can be dipped for five seconds in a water bath to cool them 15 days after infestation (Morrison and Hoffman, 1967; Laing and Eden, 1990). Alternatively, a ventilation fan can be used to dissipate excess metabolic heat (Morrison, 1985b).

Hygiene is extremely important when rearing AGM. The moth scales can be hazardous to human health and staff should always wear face masks or respiratory hoods (Racal Dustmaster MkII). It is important to dispose of old grain 6-7 weeks after infestation. This reduces the likelihood of the insectary being invaded by the straw itch mite, *Pyemotes herfsi* Oudemans, a voracious predator of moths and personnel.

The above techniques can be used to develop an insectary for mass rearing egg parasitoids on AGM eggs. Further information can be obtained from Brad Scholz at QDPI Toowoomba, telephone 076 314200.

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