A Review of the Scientific Literature and Methods for Production of Factitious Hosts for Use in Mass Rearing of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) in the Former Soviet Union, the United States, Western Europe and China[1]

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Abstract *Trichogramma* spp. are important biological control agents for lepidopterous pests. Insectaries for mass rearing them, using factitious hosts, have been constructed in many countries. Selection of factitious hosts is based on the simplicity of their mass production, mechanization of rearing processes, and cost of production compared to that of utilizing target pest eggs. Scientific literature and personal experiences with the techniques used for production of factitious hosts for *Trichogramma* spp. in the former Soviet Union, the United States, Western Europe, and China are presented.

Key Words *Trichogramma*, factitious hosts, mass rearing, mechanization of rearing processes.

Trichogramma spp. (Hymenoptera: Trichogrammatidae) are the most widely studied, mass reared and released parasitoids in the world (King 1993). In recent years, over 32 million ha of agricultural and forest land have been treated with *Trichogramma* spp. annually (Li 1994). Insectaries for mass rearing of *Trichogramma* using factitious hosts have been constructed in many countries (Table 1). A variety of factitious host eggs are currently used for rearing *Trichogramma*. In the countries of the former Soviet Union, Eastern Europe, and North and South America, *Sitotroga cerealella* (Olivier) (Angoumois grain moth) eggs are widely used. In Western Europe, *Ephestia kuehniella* Zeller (Mediterranean flour moth) eggs are used. In Southeast Asia, *Corcyra cephalonica* (Stainton) (rice moth) eggs are used. In China, *Antheraea pernyi* (Guérin-Méneville) (oak-silkworm), *Samia cynthia ricini* Boisduval (eri-silkworm), and rice moth eggs are used (Fadeyev and Greenberg 1985). Wide use of factitious host eggs for mass rearing *Trichogramma* is based on the relative simplicity

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Table 1. Primary countries using factitious hosts for mass rearing *Trichogramma* spp.*

Country	Principal crops protected	Area protected (1,000s ha)	Target pests	*Trichogramma* species released	Factitious hosts
Bulgaria	corn, sugar beet, cabbage, vineyards, plum, apple, forest	41	*Ostrinia nubilalis*, *Mamestra brassicae*, Tortricidae	*maidis*, *evanescens*, *dendrolimi*	*Sitotroga cerealella*
China	corn, sugarcane, vegetables, sugar beet, cotton, forest	2,100	*O. furnacalis*, *Dendrolimus* spp., Tortricidae, *Pieris* spp.	*dendrolimi*	*Antheraea pernyi*, *Corcyra cephalonica*, *Samia cynthia ricini*
Columbia	cotton, sugarcane, soybean, sorghum, vegetables	240	*Heliothis*, *Diatraea* spp.	*pretiosum*	*Sitotroga cerealella*
France	corn	18.5	*O. nubilalis*	*brassicae*	*Ephestia kuehniella*
Germany	corn	7	*O. nubilalis*	*evanescens*	*Sitotroga cerealella*
India	sugarcane, cotton	14	*Chilo* spp., *Helicoverpa armigera*	*chilonis*, *japonicum*	*Corcyra cephalonica*
Mexico	cotton, sugarcane, vegetables, sorghum	1,000	*Helicoverpa zea*, *Diatraea* spp.	*semifumatum*, *pretiosum*	*Sitotroga cerealella*
Philippines	corn	12	*O. furnacalis*	*evanescens*	*Corcyra cephalonica*
Peru	cotton, sugarcane	200	*Helicoverpa zea*, *Diatraea* spp.	*pretiosum*, *exiguum*	*Sitotroga cerealella*
Switzerland	corn	4	*O. nubilalis*	*brassicae*	*Ephestia kuehniella*
USA	cotton, corn, vegetables	81	*H. zea*, *O. nubilalis*	*pretiosum*	*Sitotroga cerealella*
Former USSR	corn, cotton, sugar beet, vegetables	16,500	*O. nubilalis*, Noctuidae	*pintoi*, *evanescens*	*Sitotroga cerealella*

* Modified from data reported in Olkowski and Zhang (1989), Greenberg (1992), *Trichogramma* news (1992,1995) and Li (1994).

and comparative low cost of their production when compared to eggs of the target pest.

The feeding conditions of larval *Trichogramma* have a considerable influence on adult vitality. Insufficient quality or quantity food can cause considerable modification in metabolism, behavior and other vital functions of this parasitoid which can, in turn, influence its effectiveness in the field (Greenberg 1991). The correct selection of a factitious host can significantly improve the potential for efficient production of high quality *Trichogramma* for field release.

During the last decade considerable knowledge and experience in the production of factitious host eggs for use in mass rearing of *Trichogramma* has accumulated. The goal of this paper is to review practical and methodological problems for the production of these factitious hosts.

Countries of the Former Soviet Union

During the last decade, technology for mass rearing *Trichogramma* on Angoumois grain moth eggs has been adopted in the countries of the former Soviet Union. The mechanized process, which includes a number of specialized units, devices, and equipment, allows for commercial production of *Trichogramma.* At present approximately 800 mechanized units are functioning. One unit is able to produce 4 to 5 million individuals daily (Abashkin et al. 1987, Niconov et al. 1991, Nordlund and Greenberg 1994).

Processes for production of Angoumois grain moth. The process for Angoumois grain moth production was described by Abashkin et al. (1987, 1988), Greenberg et al. (1983, 1991), Nordlund and Greenberg (1994), and Startchevsky et al. (personal communication[6]) (Fig. 1).

Barley containing about 20,000 kernels/kg is used for Angoumois grain moth production. Use of small kernels (24,000 to 26,000 kernels/kg) produces small moths, which oviposit small eggs that result in low quality *Trichogramma* (i.e. low fecundity and searching activity). Use of large kernels (16,000 to 18,000 kernels/kg) is not efficient, because only 70 to 75% of their contents are utilized by the Angoumois grain moth larvae.

The grain is disinfected to prevent seed germination and to kill pests and parasites or predators of Angoumois grain moth. Chemical disinfection involves treatment of the stored grain with methyl bromide (40 g/m^3 at a temperature not lower than +10°C for 2 to 3 days). Grain is thermally disinfected with steam at 105 to 110°C for 60 to 90 sec, or by dipping the grain into hot water (90 to 95°C) for 40 to 60 sec. Use of hot water for grain disinfection is preferable to chemical or steam disinfection because it softens the grain facilitating invasion and development of Angoumois grain moth larvae and is not biohazardous. Use of methyl bromide may also have negative influences on moth growth. When steam is used for disinfection of the grain, moisture loss is rapid and the grain becomes hard, which can also negatively impact the development of larvae.

[6]A 1995 report by I. P. Startchevsky, A. I. Gontcharuk and A. N. Kilimnik. Technical Proposition on the Organization of Phytophagous and Entomophagous Complex Production. Biotechnics. Odessa, Ukraine to E. G. King, USDA, Subtropical Agricultural Research Laboratory.

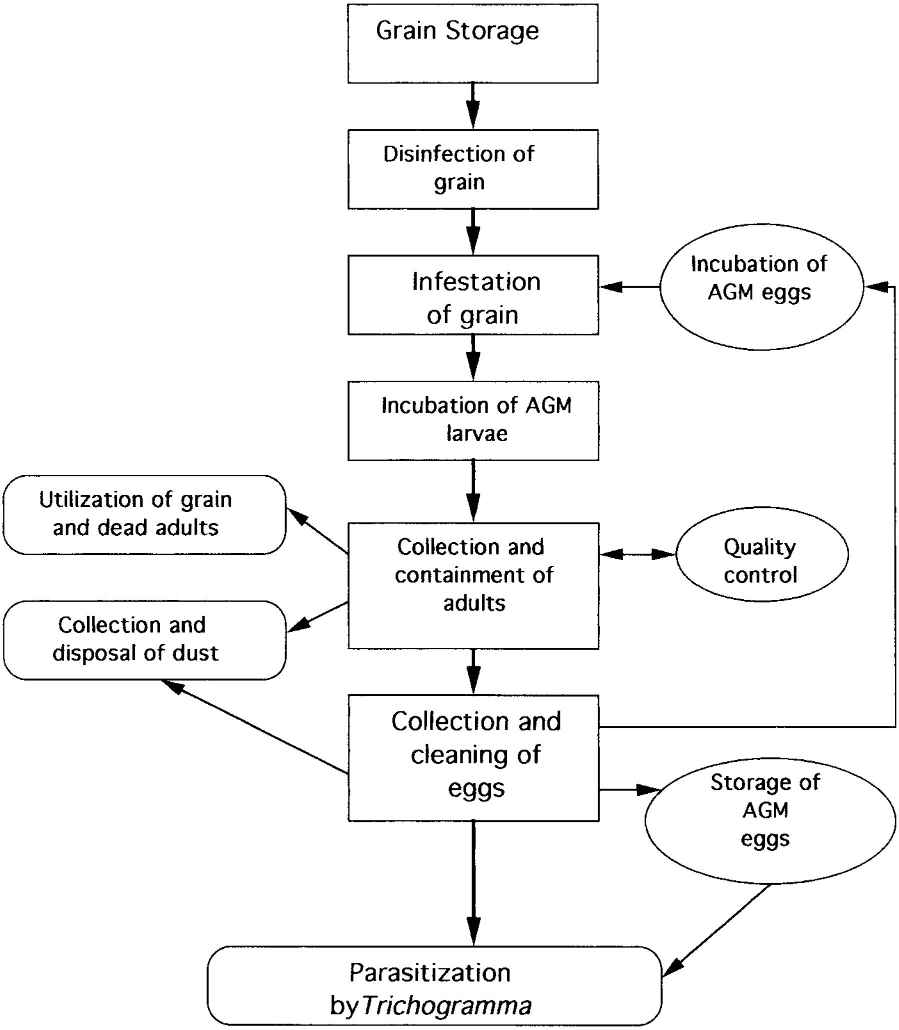

Fig. 1. The process for mass rearing Angoumois grain moth (AGM) used in the former Soviet Union (Greenberg 1991).

Grain to be infested with eggs should have a moisture content of 15 to 16%. Excessively moist grain can be dried by ventilation. Potassium permanganate ($KMnO_4$) can be added to the water used for moistening the grain at the rate of 0.1 g/l to prevent the development of mold.

Angoumois grain moth eggs from 48 to 72 h old are best for use in grain infestation. Prior to infestation eggs are placed in an environmental chamber at optimal conditions for embryo development (24 ± 1°C and 85 ± 5% RH). Just prior to egg hatch, they are evenly dispersed over the grain (1 g eggs per 1 kg grain). Larvae can penetrate the grain to a depth of 4 to 5 cm. The grain is neither mixed nor moistened during eclosion (1 to 2 d) and larval penetration of the grain (3 to 4 d). Optimal conditions for grain invasion are 24 ± 1°C, 80 ± 5% RH, and full darkness for the first day. An 8:16 (L:D) photoregime is established on day 2. These conditions ensure that 90 to 95% of the grain will be infested.

Larval development creates metabolic heat so that 7 to 8 d after infestation the temperature in the grain may increase by 8 to 10°C. The intensity of heat generation depends primarily on the level of infestation. At an infestation level of 90 to 95%, the grain temperature may rise to 35 to 38°C. To maintain an optimal temperature of 25 ± 1°C and 15 to 16% moisture, the grain is mixed, humidified, and held in a thin (2.5 to 3.0 cm) layer. The room is maintained at <20 ± 1°C and 75 to 80% RH. Intense heat generation lasts for 12 to 15 d then decreases as pupation occurs.

Under the above conditions, moth emergence begins approximately 30 d after infestation. Premature or delayed emergence indicates a disruption of the rearing regime which usually results in a decrease of the number of quality of insects obtained. Peak emergence occurs 3 to 5 d after emergence begins. During the first 18 d of emergence, the sex ratio is approximately 1:1, then the proportion of males increases, reaching a maximum on day 23 (80%). Because 90 to 95% of adults emerge during the first 18 d and the percentage of males increases after day 18, adult collection is terminated on that day (Paliy and Greenberg 1981).

When held at 24 to 25°C, 80 to 85% RH, and darkness, females will oviposit for 4 to 5 days. A female oviposits 25 to 31% eggs of her total egg complement during the first day, 29 to 41% during the second day, 23 to 28% during the third day, 6 to 12% during the fourth day, and 3 to 4% during the fifth day (Greenberg et al. 1981). From 0000 to 1200 h, the adult activity is low, with minimum oviposition occurring between 0600 to 0800 h. From 1300 till 0100 h the adults oviposit 60 to 68% of the daily production with peak oviposition occurring between 1600 to 1700 h (up to 30% of the daily collection) (Greenberg and Paliy 1981).

After collection, the eggs are cleaned, weighed, and packaged in paper packets (up to 50 g each), Petri dishes, or in a bulk layer approximately 1.5 to 2 cm deep. Eggs for use in grain infestation are held at 3 ± 1°C and 85 ± 5% RH for no more than 5 d. Eggs for use in *Trichogramma* production are held at the above conditions for up to 10 d. There is also a method for long-term storage (up to 1 year) of Angoumois grain moth eggs, at −196°C in liquid nitrogen, for use in *Trichogramma* rearing. Eggs that have been stored in liquid nitrogen are thawed in 44 to 45°C water, after which they are dried on hydrophilic plates for 20 to 30 min at 25 to 30°C. In practice, 63 to 65% of the eggs stored in this manner for 6 to 18 months were successfully parasitized by *Trichogramma* spp. with an adult emergence rate of 88 to 92%, of which 50 to 60% were females (Gennadiev et al. 1987).

Female fecundity and egg size are critical quality control indices for Angoumois grain moth. The number of eggs produced and their size are correlated with the size of the female. Acceptable female adults have an abdomen that is at least 6.4 mm long and weighs at least 6.7 mg. Females that are at least this large produce eggs more than 0.59 mm long and 0.28 mm wide. Their potential fecundity exceeds 140 eggs/female while the realized fecundity is approximately 90 eggs/female (Greenberg et al. 1983).

Spent barley from moth production can be used as a substrate for production of useful microorganisms or, after a thermal treatment, for cattle and poultry food. Dead adults and eggs not suitable for *Trichogramma* production may serve as food for the production of predaceous entomophages (i.e., chrysopids, coccinellids), fish food in aquaria, or for fry in pisciculture, birds, or insects in zoos. These materials are stored in liquid nitrogen or frozen in plastic bags.

The above procedures will produce 1,000 to 1,200 g of Angoumois grain moth eggs per 100 kg of grain utilized. Approximately 10% of these eggs must be returned to Angoumois grain moth production. The remainder can be used for *Trichogramma* rearing or other purposes.

Equipment for mass production of Angoumois grain moth. Three different production systems were developed in the former Soviet Union. Unit 1215 was designed by "Agropribor", which was in the Ministry of Agriculture of the former USSR (Moscow, Russia) and the former All-Union Institute of Plant Protection (St. Petersburg, Russia). Unit 1428 was designed by "Agropribor" and the former All-Union Institute of Biological Control (Kishinev, Moldova). Unit 1682 was designed by Institute of "Biotechnica" Ministry of Agriculture of the former USSR (Odessa, Ukraine) and former All-Union Institute of Biological Control. These production units were previously described (Anonymous 1980, Greenberg et al. 1983, 1991, Klochkova and Moiseiv 1988, Startchevsky et al. personal communication[6], Vobly 1990, Vobly and Gontcharuk 1991). Insectaries in the former Soviet Union use these production units with the number of units in an insectary ranging from 1 to 16, depending on the production level required.

The grain for moth rearing is stored in metal bins (10 to 20 tons) in all three of the systems and is transported for disinfection by gravity (units 1215, 1428) or pneumatic conveyer (unit 1682). On unit 1215 the grain is disinfected by autoclaving at 105 to 110°C and 1.0 to 1.2 technical atmospheres for 1 to 2 min. On unit 1682 the grain is treated with constant steam delivered to a rotating perforated barrel (50 to 100 sec at 92 ± 2°C) (Fig. 2). This device can treat approximately 180 kg of grain per hour. On unit 1428 a pan conveyer loaded with grain is plunged into hot water (90 to 95°C) for one min. This device can treat approximately 200 kg of grain per hour.

On units 1215 and 1428 approximately 20 to 25 kg of disinfected grain is placed in 1150 × 740 × 50 mm tray by gravity flow. The trays are then moved to shelving units by a wheeled cart. The shelving units measure 4916 × 930 × 1176 mm and hold 65 trays.

On unit 1682, disinfected grain is placed in a block of perforated rearing cassettes (9 cassettes/block with 3.5 kg grain/cassette) in 25 to 30 mm layers. The blocks are placed on the rack (12 blocks of cassettes with 378 kg of grain per unit) (Fig. 3). In all three systems the grain is infested with Angoumois grain moth eggs by hand and development continues in place until adult emergence begins.

When adult emergence begins, the grain from the trays on units 1215, 1428 is placed in blocks of 7 perforated rearing cassettes. The block of grain-filled cassettes is then moved to an adult collection room by wheeled cart and electric elevator. The adult collection room contains 10 (11,030 × 2,145 × 600 mm) adult collection boxes which are connected by a common tube (12 m long and 0.25 m diam) and a block of air filters. Each collection box can contain 14 cassettes (150 kg of infested grain). Adults 0 to 24 h old are collected in an insect collector by air flow. On unit 1215, the adults are dispersed into rectangular oviposition cages (608 × 302 × 40 mm) with a measuring device (12 to 15 adults per cm^2), and cages are then placed in a controlled environment chamber for oviposition. Eggs are collected by hand then cleaned with a separator. Separation of eggs from scales and trash is based on their different

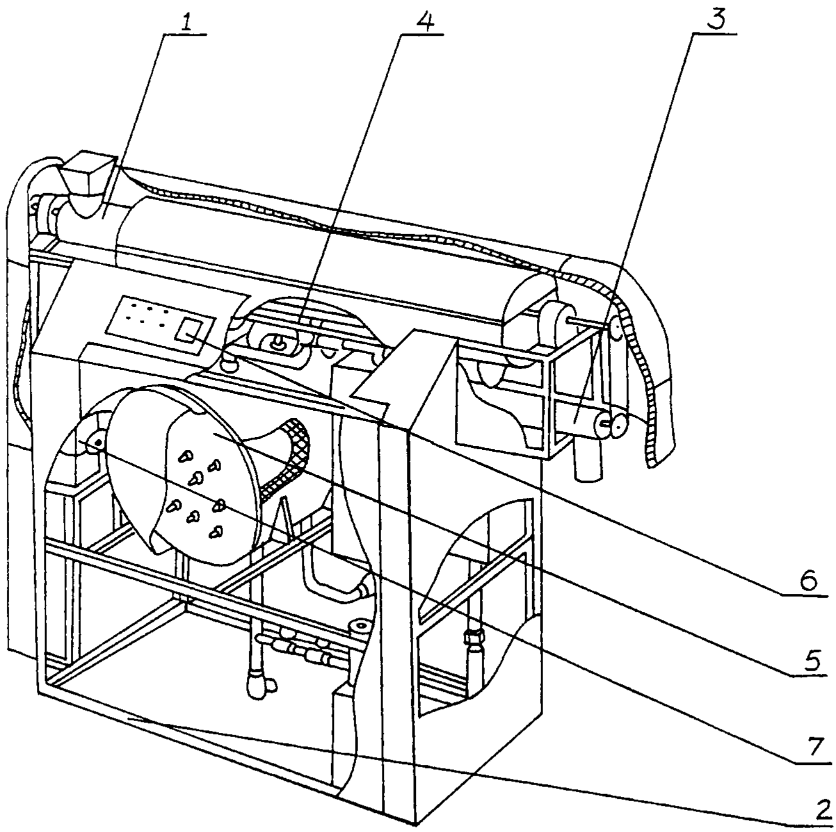

Fig. 2. Steamer for treatment of grain prior to infestation with Angoumois grain moth eggs with Screw drive grain conveyer (1); Frame (2); Distribution screw of grain (3); Distribution collector of steam (4); Steam generator (5); Control panel (6); Drive mechanism provides rotation distribution screw and collector (7).

specific gravities and is accomplished by regulated air flow. On unit 1428, screened, cylindrically-shaped oviposition cage combine adult maintenance (40 to 60 individuals per cm^2) and egg collection. Adults in oviposition cages are placed in a controlled environment chamber where the cages are automatically rotated. Eggs, which are oviposited on the screen, are removed by air pressure and a roller and fall into trays mounted under the cages. The eggs are then taken to an air flow hood and cleaned using a sieve and a separator.

On unit 1682, as adult emergence begins, 4 blocks of rearing cassettes with infested grain are moved to one of 18 adult emergence chambers (Fig. 4). Adults fall into a cone in the bottom of the adult emergence chamber and pass to the adult accumulator. Adults are collected in oviposition cages (approximately 200/cm^2) with an adult collector (Fig. 5). The cages are then transferred to a special vacuum-ventilation system (Fig. 6) for adult maintenance and egg collection. Besides main-

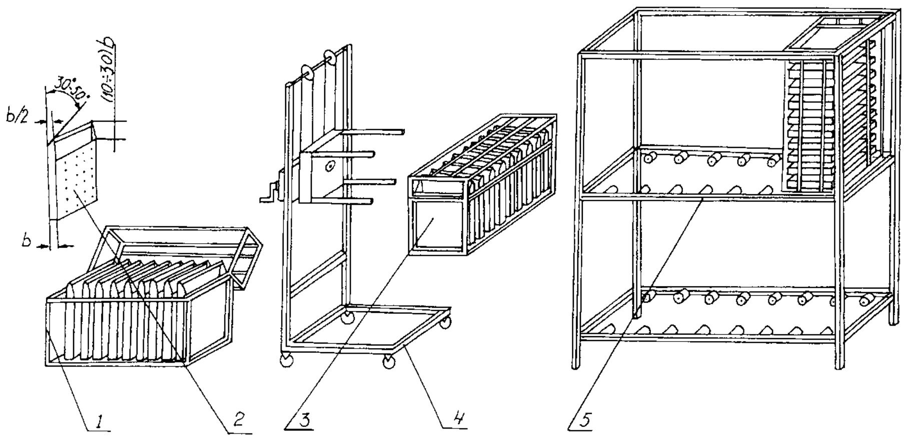

Fig. 3. Angoumois grain moth larval rearing system for line 1682 from the former Soviet Union with Rearing cassettes block frame (1); Perforated rearing cassette (2); A block of perforated rearing cassettes (3); Cart (4); Rack (5).

tenance of optimum hygrothermic conditions, additional ventilation through the stacks of adult collectors is provided. Egg collection and cleaning methods are the same as described for unit 1215. The eggs are collected in a tray and the scales removed by air flow. Contaminated air is passed through a filter then exhausted into the atmosphere.

Unit 1682 allows for the mechanized removal of production wastes (Fig. 7). Blocks of rearing cassettes with spent grain are taken out of the adult emergence chamber by wheeled cart, and transported to a collector into which the spent grain is dumped. The spent grain is then moved by air pressure into a cyclon-separator and separated into fractions. The large particle fraction goes to a storage container and the small particle fraction (which includes moth scales, Angoumois grain moth adults and dust) goes into the dust collector. The filtered air is exhausted to the atmosphere.

The technological characteristics of the different production unit systems are given in Table 2.

The United States

The production system for Angoumois grain moth eggs in USA, where it has been the most common factitious host for *Trichogramma* spp. rearing, was described by Morrison and Hoffman (1976), Morrison and King (1977), King et al. (1978), King and Morrison (1984), and Morrison (1985, 1988). Wheat, which averages 28,000 grains/kg and contains 13 to 15% of protein, is commonly used for Angoumois grain moth production. Both the wheat and production equipment are heat treated at 66°C for 24 h before being taken into the production area. This eliminates insects and microorganisms and prevents germination of the grain. When cool, 9.1 kg of wheat are placed in a perforated (10 mesh/2.54 cm) crib (2 × 61 × 122 cm) made of steel hardware cloth. The grain should have a moisture content of 15%. Ten grainfilled cribs are placed into a horizontally-positioned, wheeled production module (63.5 × 122 × 183

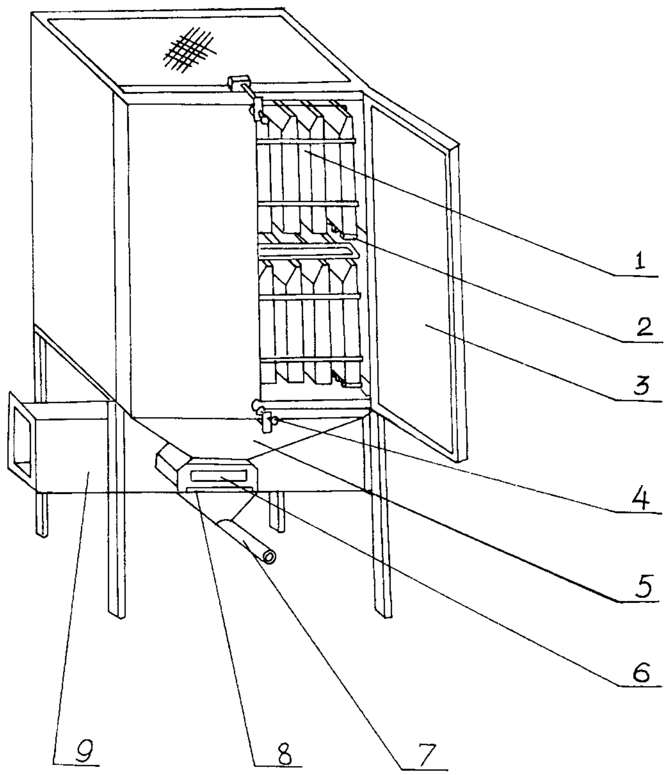

Fig. 4. Angoumois grain moth adult emergence chamber for line 1682 from the former Soviet Union with Block of rearing cassettes (1) (each chamber holds four blocks of nine rearing cassettes); Guide roller (2); Door (3); Clamp (4); Adult collection cone (5); Adult accumulator (6); Adult collection nozzle (7); Damper (8); Air-dust (9).

cm) which includes a system for moistening and ventilating the grain. The grain is infested with eggs (24 to 48 h old) that have first been disinfected in a 10% formalin solution for 10 min and rinsed in fresh water for 5 min. The eggs are then mixed with water (100 g eggs/5 liter water) in a vessel (the average number of eggs in 1 g of egg mass ranges from 48,000 to 52,000 as described by Marston and Ertle [1973]). The vessel is placed on a magnetic stirrer at a level above the highest crib in the production module. This egg solution (approximately 500 ml of solution/crib) is applied (by gravity) uniformly over the top of horizontally positioned cribs with a simple infestation wand made of galvanized pipe (0.6 cm inside diam).

After infestation the modules are maintained in a horizontal position for 12 d as the eggs hatch and the larvae penetrate the grain. The modules are then placed in a vertical position and a ventilation fan is placed on top of the unit to dissipate the metabolic heat generated by the developing larvae. Cooling is necessary because aestivation is induced in immature Angoumois grain moth if temperatures exceed

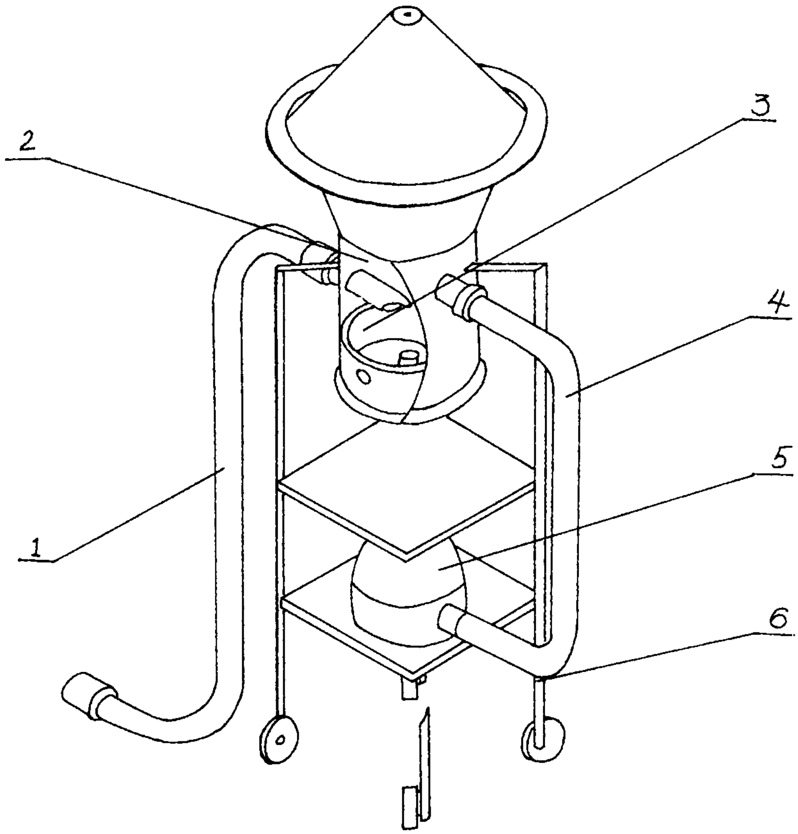

Fig. 5. Angoumois grain moth adult collection system used for line 1682 from the former Soviet Union with Suction hose (1); Air flow separation chamber (2); Oviposition cage (3); Exhaust pipe (4); Vacuum fan (vacuum source) (5); Frame (6).

35°C. The thin layers of grain combined with constantly moving ambient air over the cribs is sufficient to keep grain below 30°C. Immersing a grain-filled crib in water for 5 sec 15 d after infestation, draining the water, and replacing it in the module can also reduce the grain temperature to acceptable levels.

Adult emergence begins approximately 22 d after infestation, and adults are collected each 24 h for 18 d. The adults are blown into a muslin collection bag placed over the collecting cone of the module by air from the ventilation fan. The adult weight is determined and 500 g of adults (approximately 125,000 individuals) are placed in oviposition frames (40 × 70 × 3.8 cm) yielding an adult density of approximately 45/cm^2. The oviposition frames are covered with nylon net (7.6 strand/cm) on one side and 13 strand/cm plastic screen on the other. When loaded, the frames are placed (7.6 strand/cm side down) on a 100 × 75 cm pan. The pans are then placed on a horizontal storage rack for 3 d. Eggs are collected daily by brushing and shaking

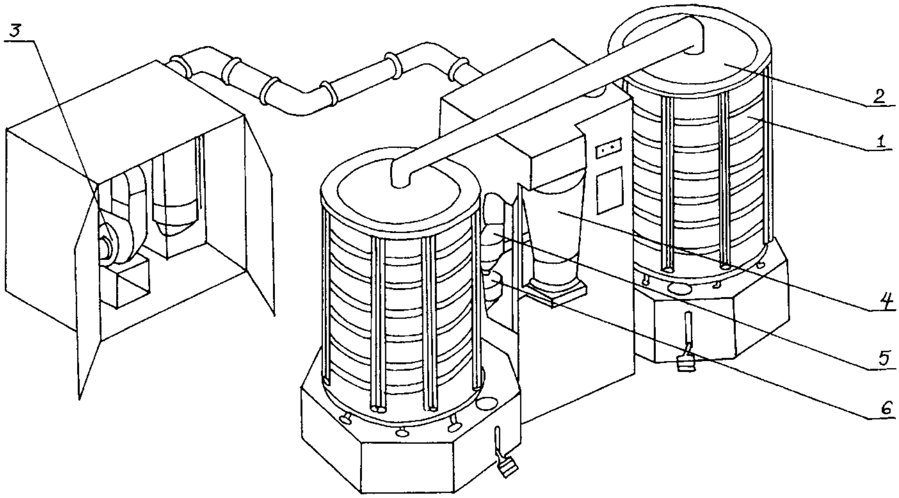

Fig. 6. Vacuum-ventilation system for Angoumois grain moth adult maintenance and egg collection used for line 1682 from the former Soviet Union with Oviposition cage (1); Turret (or ring mount) (2); Fan (3); Egg collector (4); Air flow separation chamber (5); Vessel for collecting of Angoumois grain moth eggs (6).

frames over the pan. Cleaning of the eggs is accomplished with a standard sieve series (nos. 20, 30, 40, 50, 60) and a catch pan which is placed on an automatic sieve shaker (for approximately 5 min). After this, insect parts in the #20 sieve and in the pan are discarded; eggs in the #30 sieve are held separately for use in grain infestation. Eggs are placed in open-top containers, dated, and stored at 13°C for up to 10 d. The eggs in the other sieves are combined and poured through a horizontally moving air stream from a ventilation fan to remove any remaining dust. Eggs used for *Trichogramma* production are refrigerated (3 ± 1°C) for no more than 72 h. For long-term storage eggs are placed in sealed vials, held at 10°C for at least 24 h, then placed in liquid nitrogen (−196°C) for 21 d. Once parasitized, these eggs produced parasitoid pupae at approximately one-half the rate (43.0%) of pupae produced on fresh eggs (82.1%). Adults reared from the treated eggs appeared normal, mated successfully, parasitized fresh eggs, and had a sex ratio similar to parasitoids produced from fresh eggs.

The Angoumois grain moth rearing areas are maintained at 26 ± 2°C, 75 ± 5% RH, and lights are on only when workers are present. Each production module required 11 m^2 of floor space.

A kilogram of wheat produced 600,000 Angoumois grain moth eggs (or 12 g); the adult sex ratio was 1:1, and the actual fecundity was not less than 60 eggs/female. A cost analysis of the system showed the production cost of eggs was $0.00655/1000 (0.00239 man-hr).

Western Europe

Both Angoumois grain moth and Mediterranean flour moth are used as factitious hosts for mass rearing of *Trichogramma* spp. in Western Europe.

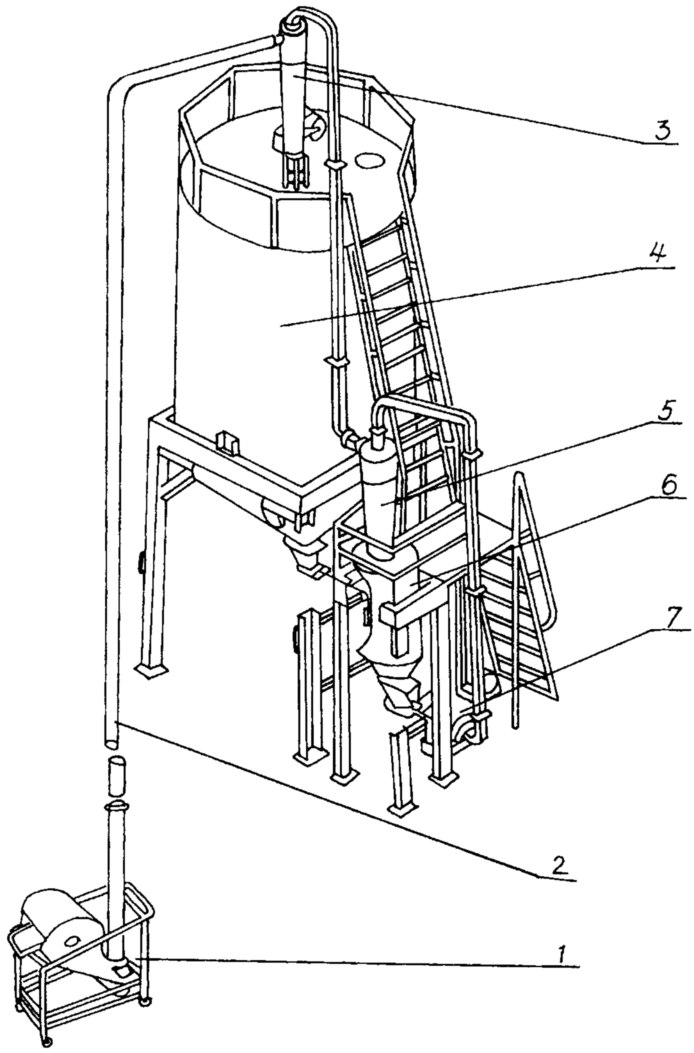

Fig. 7. System for removal and collection of waste products used for line 1682 from the former Soviet Union with Grain waste collector (1); Pipe for transporting grain waste (2); Cyclon-separator for separating large particle fractions of grain waste (3); Storage container for the large fractions of grain waste (4); Dust cyclon-separator for separation of dust from small fractions of grain waste and moths (5); Accumulator for small particle fraction of grain waste and moths (6); Exhaust fan (7).

Table 2. The basic characteristics of Angoumois grain moth mechanized production units in the former USSR.

Characteristics (per unit)	Type of units 1215	1428	1682
Amount of grain/unit, kg	1,750	1,500	2,000
Total power consumption, kwt	25	35	30
Average water consumption (including that used for sanitary cleaning equipment), m3 (kl)/24h	1.5	1.5	1.5
Working area (floor space) required, m2	160	160	200
Production output: eggs, g/kg of grain	8.0	8.0	9.4
Labor expenditure, man-h/100,000 eggs	0.266	0.182	0.14

Mass rearing of Angoumois grain moth. A method of moth production was developed by Hassan (1981, 1994) in Germany. Wheat or barley is mixed with water (100 ml water/kg), heated to 70°C for 6 h, then cooled. Grain (6 kg) is poured into 100 × 50 × 2 cm containers that are open at one end and covered on both sides with metal screen. The grain is infested by placing the container in a horizontal position and distributing 4.8 g of eggs on the surface. The container is held horizontally at approximately 27°C and 70% RH for 10 d for egg hatch and larval infestation. The container is then suspended in a vertical position in an adult collection unit. The adult collection unit (106 × 103 × 200 cm) holds 16 grain containers and is essentially a cage with a funnel at the bottom. The walls of the cage are covered with nylon gauze. Adults emerge from the grain and collect in a 1-liter plastic bottle at the end of the funnel.

A semi-automatic egg-collection unit was developed to collect and clean the eggs. The adults are poured by hand from the plastic bottle into cylindrical cages that are fitted into the unit. Once in the unit, the cylindrical cages are rotated for 10 min every 3 h. During the rotation the outer walls are swept with built-in brushes and air flow is used to remove the moth scales. The cleaned eggs fall through the bottom openings and are collected in a tray. Weekly infestation of 12 kg of grain produces approximately 3.5 million eggs/wk or 5.8 g eggs/kg of grain.

Mass rearing of Mediterranean flour moth. A partially mechanized system for Mediterranean flour moth production is used in France and Switzerland (Daumal et al. 1975, 1985, Bigler 1986). In this system fine-ground wheat, a mixture of wheat (40%) and corn (60%) flour, or wheat semolina which may be enriched with 1 to 2% Brewer's yeast is used as the larval diet. It is poured onto a tray (75 × 25 × 4 cm) and infested with 0.55 g of Mediterranean flour moth eggs (1 g = 34,000 to 38,000 eggs [Bigler et al. 1987]) per kg diet. After infestation, strips of corrugated cardboard (2.0 mm wide) are rolled into bundles to form large numbers of pupation cells (2.0 × 0.35 mm) then placed on the top of the diet. Rearing conditions of 20 to 25°C and 70 to 75% RH are maintained until larvae develop to the third stadium; then conditions of 15°C and 70% RH are maintained until the pupal stage. This system was developed because third instars exhibit intensive feeding and cannibalism behavior and produce increased metabolic heat. Once the larvae enter the cardboard cells and pupate (95%), the enclosed pupae are removed from the diet and placed into emergence cages. Daily emerged adults are anesthetized with carbon dioxide and collected with a vacuum. Adults are then placed into the oviposition cage where mating and oviposition occur. Eggs are collected in a small channel after passing through a fine wire grill (d = 3.5-4.0 mm). Egg cleaning is accomplished by a built-in ventilation system that operates constantly. When used for *Trichogramma* rearing, sterilization of the Mediterranean flour moth eggs by UV light is generally used to prevent cannibalism and to prolong the storage capability of the eggs (40 to 45 d at 3°C and 90% RH).

This improved system for Mediterranean flour moth egg production has increased the efficiency of egg production from 3.1 g eggs/kg of diet in 1979 to 7.6 g eggs/kg of diet in 1985. Also, labor costs were reduced approximately 4 times, from 3,220 Swiss Franks (161 man-h)/1 kg eggs to 780 Swiss Franks (39 man-h).

The People's Republic of China

The rearing or collection of factitious hosts for use in mass rearing of *Trichogramma* spp. in the People's Republic of China was described by Coulson et al. (1982), Lu Qi (1988), Li (1982), and Olkowski and Zhang (1989). Personal observations by the authors during visits to China (1979, 1989, 1992) also are included.

Oak silkworm cocoons are collected in northeastern China and sold to biological control stations throughout the country. The cocoons are field-collected and transferred to a storage facility in November and removed in March. Facilities for host cocoon storage consisted of 3 rooms, each approximately 36 m^2. The roof of the building is framed with oak and covered with steel. The ceiling is covered with a soil layer 1 m deep which, in turn, is covered with rice hulls in the attic. In winter the rice hulls are removed which allows the soil to freeze. Before the spring thaw, the rice hulls are replaced to keep the soil frozen throughout the spring and into the summer. About 25,000 kg of oak silkworm cocoons (100 cocoons per kg) can be stored within each room of the storage facility.

A machine was developed for separation of the oak silkworm female cocoons on the basis of size and weight. Accuracy of separation is greater than 70%. A screen is used to eliminate the smaller of the male cocoons. However, size is not a reliable diagnostic tool. Female cocoons are usually heavier than 8 g, and male cocoons are usually lighter than 8 g. Therefore, a balance mechanism is used for the second phase of separation. The balance mechanism appeared to be a metal blade which flexed under the weight of the female cocoons.

An incubation room is used to accelerate adult moth emergence. The cocoons are placed either in rows on shelves, into cells covered with coarse screen on the bottom, or hung from the ceiling on string. The cocoons are held at 28°C and 70% RH for 20 d. Emerging moths accumulate on the edges of the shelves or cells and are brushed into a running water channel, which delivers the moths to a collector. When the cocoons on string are suspended in double rows from the ceiling, the emerged moths are handpicked off the string and placed in a basket for transfer to the egg extractor.

The egg extractor first coarsely grinds up the virgin oak silkworm females. Then, the mass is water-washed through a screen which retains the larger body parts. The eggs sink to the bottom of a water-filled catch basin while lighter debris is decanted in the overflow. The eggs are then removed and transferred to a centrifuge with a radius of approximately 15 cm and spun at 1480 rpm. The eggs are dried for approximately 10 min in 2-kg batches. The centrifuge has a large conical core which ensures that the eggs are on a peripheral cloth. A newly-developed extractor can squeeze about 20,000 females/day, yielding 120 to 130 kg of eggs. Oak silkworm females produce up to 200 eggs each (~32 eggs/g). This device replaces more than 20 workers and provides enough eggs to produce 280 million *Trichogramma* per day (each host egg can produce an average of 70 *Trichogramma* adults). Oak silkworm female abdomens or cocoons can be stored at 3 to 5°C for up to 30 d. Eggs can be stored for 1 to 9 months in liquid nitrogen and defrosted in water at 30°C. When liquid nitrogen-stored eggs are used for rearing *T. dendrolimi* Matsumura and *T. confusum* Viggiani, there are no significant differences in percent parasitism or percent adult emergence when compared with fresh eggs (Ma Huai-Yi 1988).

Eri silkworm cocoons are collected in southern and central China and sold to production facilities. Each female moth can produce 300 to 500 eggs. Eri silkworms are reared on leaves of castor (*Ricinus communis*), cassava (*Manihot utilissimi*), or *Evodia meliae* folia. The Guangdong Plant Protection Institute has used an artificial diet with a high leaf content successfully for rearing eri silkworm. This larval diet consists of cornmeal, fishmeal, leaves of plant (castor and cassava), and ground seaweed (in place of agar). Eri silkworm reared on the artificial diet can be used to produce more species of *Trichogramma* than those reared on natural diet, because the egg shell from females reared on the artificial diet is thinner. This makes it possible for *Trichogramma* spp. with shorter ovipositors to oviposit into them. Eri silkworm eggs can also be stored at 1 to 3°C for 30 d or for 120 d in liquid nitrogen.

The rice moth can be produced year-round in all areas of China. This factitious host is reared on rice bran or a diet of wheat bran (90%), soybean (5%), and corn flour (5%). The diet must be heat treated at 65°C for 25 h. One kilogram (total weight) of diet is infested with about 3,000 host eggs. Adult emergence begins about 45 d after infestation (at 28°C, 70% RH, and a 24 h scotophase) and continues for 1.5 months. Adult collection is very time consuming. They are neither strongly geotactic nor phototactic. Therefore, a lid of two layers of hardware cloth 3 to 4 cm apart and open at the ends, is placed on the boxes. Freshly-emerged moths climb from the diet into the space between the hardware cloths. The lid is then held vertically above a polyethylene funnel and shaken. The moths fall into a 1-liter metal canister at the bottom of the polyethylene funnel. The bottom of the canister has a cloth sleeve from which the excess diet is removed. A large funnel for removing moths from 7 lids at one time is also available for use. Fresh rice moth eggs can only be stored for a short period of time (15 d at 1 to 3°C). Due to cannibalistic larvae and to prolong storage, rice moth eggs are sterilized with UV radiation (10 min, 30w, at 14 cm distance from the light source) or frozen for 24 h. They can be stored for 45 to 60 days at 1 to 3°C after these treatments. A kilogram of rice bran results in the production of approximately 10 g (about 200,000 eggs) of rice moth eggs.

Summary

This discussion concentrated on the production of factitious hosts for use in the mass rearing of *Trichogramma* spp. Emphasis was placed on mass rearing of Angoumois grain moth and Mediterranean flour moth because these hosts are used in most commercial *Trichogramma* rearing facilities. Though the rearing of factitious hosts involves the use of a number of automated mechanical processes, the cost of the final

product remains high. When factitious hosts are used for *Trichogramma* rearing about 78% of the space and cost is required for the production of these hosts (Abashkin et al. 1988).

Thus, it is clear that the use of artificial diets for the *in vitro* rearing of *Trichogramma* would dramatically reduce the production costs of these valuable parasitoids. Increased production would occur because of both increased opportunities for automation and reduced space requirements. The estimated cost of 100,000 *Trichogramma* adults produced on the Chinese artificial diet is approximately $0.06. This is substantially less expensive than the estimated $2.43/100,000 it costs to produce *Trichogramma* on the Angoumois grain moth eggs/wheat diet used in the United States (Olkowski and Zhang 1989).

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