METHODS AND RECOMMENDATIONS FOR MASS REARING OF THE NATURAL ENEMIES OF THE SUGARCANE BORER 
(Diatraea spp) (Lepidoptera: Crambidae)

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ABSTRACT

The sugarcane borer (Diatraea spp) is considered to be one of the most serious insect pests of sugarcane crops in many sugarcane producing areas of the world.

Biological control has been the most promising means of fighting this pest on account of its efficiency and relatively low cost. The traditional method is restricted mainly by the difficulty of mass rearing of parasitic flies (Tachinidae).

This paper suggests a methodology and relays information that will make possible mass rearing of the tachinid parasites of the sugarcane borer using larvae of Galleria mellonella L. and Achroia grisella F. as hosts. It also suggests utilisation of simple portable incubators heated by the heat generated by the larvae for rearing the flies on sugarcane farms. It discusses a technique for large scale production of Galleria eggs for rearing Trichogramma spp, which, in some regions, is considered an efficient enemy of Diatraea saccharalis F. It describes in detail rearing method for Galleria mellonella and Achroia grisella, in the laboratory, on a diet formulated by the author, and of Lixophaga diatraeae Towns., on these larvae.

INTRODUCTION

Sugar production is seriously impaired, from the time the sugarcane is planted to the time when it is processed, by the sugarcane borer, Diatraea spp. Studies have shown that there is a sharp decline in sugar production when sugarcane attacked by this pest is utilised. It is estimated that for a certain percentage infestation of the crop there is a corresponding decrease in industrial production of sugar. Considering that, for example, in certain regions of the north-east of Brazil, the rate of infestation is over 50%, the importance of this pest cannot be over-emphasised. Up to the present, no control method has been sufficiently successful to be adopted as standard practice although biological control has been the most promising.

The USDA Sugarcane Insects Investigations Laboratory, in Louisiana, was the pioneer institution working on biological control of Diatraea spp. Charpentier et al have made an historical review of work in the United States, which was initiated with the introduction of the Cuban fly, Lixophaga Diatraea Towns. in the period 1915 to 1918. Clausen discusses the successes and failures associated with the biological control of the sugarcane borer in the United States, and comments on its limiting factors and promising aspects. In Brazil, biological control of the sugarcane borer was first suggested by Almeida & Souza, in 1936, but the method was only established in 1951, with the intro-
duction of *L. diatraeae* by Gallo. Ardy et al. working in Cuba on a survey of natural infestation and biological control of the sugarcane borer, suggested the possibility of utilising larvae of *Galleria mellonella* L. reared on Haydok’s diet as hosts of *L. diatraeae*. In July 1973, at the meeting of the Brazilian Entomological Society, Gallo et al. reported on the rearing of *Lixophaga* to the pupal stage on *G. mellonella* using a diet and method proposed by the author. They also suggested the possibility of rearing other parasites such as *Metagonistylum minense* Tns. and *Paratheresia claripalpis* (Wulp). At the same meeting, Mendonça Fo presented an historical review of sugarcane borer control in Brazil including a detailed description of the method of rearing the parasitic flies on larvae of *Diatraea* spp. He also offered suggestions for mounting a laboratory for artificial rearing of natural enemies. Guagliumi discusses the inconvenience of chemical control of sugarcane borer under Brazilian ecological conditions and emphasises biological control. He also describes several species of *Diatraea*.

**FIGURE 1.** Cocoon and imago of *Galleria mellonella* L.

The great importance of the matter at present, along with the encouragement provided by the Sugar and Alcohol Institute have led the author to report on a method and offer some recommendations for rearing natural enemies of *Diatraea* spp on eggs and larvae of *G. mellonella* and *A. grisella*.

**MATERIALS AND METHODS**

*Rearing of Galleria mellonella and Achroia grisella*

The method proposed is based on information reported in the literature by several investigators who have worked with *G. mellonella* and also on the
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The author's experiments. The same method may be used for rearing both *G. mellonella* and *A. grisella*. Greater emphasis is given to *G. mellonella* because this species presents characteristics which are more favorable to the objective proposed, such as a calmer behavior, larger size and shorter biological cycle.

**Size, mm**

<table>
<thead>
<tr>
<th>Species</th>
<th><em>D. saccharalis</em></th>
<th><em>G. mellonella</em></th>
<th><em>A. grisella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation, days</td>
<td>4-9</td>
<td>4-6</td>
<td>4-6</td>
</tr>
<tr>
<td>Larval period, days</td>
<td>40</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Pupal period, days</td>
<td>9-14</td>
<td>6-10</td>
<td>5-10</td>
</tr>
<tr>
<td>Complete cycle, days</td>
<td>53-60</td>
<td>34</td>
<td>42</td>
</tr>
</tbody>
</table>

For additional details on the biology, behavior and feeding habits of the species under study, see Guerra.12

Two methods are suggested for obtaining eggs. The first is based on the method utilized by Marston & Campbell.14 For egg deposition, utilise a chamber consisting of a glass tube, approximately 18 cm long and 10 cm in diameter, having the internal walls lined with a layer of sugar crystals. One end of the tube should be sealed with a closely woven cloth (cotton organdy) and over this cloth a plastic screen fastened to the tube with a metal ring. The purpose of the cloth is to hold the eggs when the sugar is diluted. Approximately 10 males and 10 females are placed in the container, which is then stored in a dark place for 3 days, at 32°C (±2°C) and 70% (±10%) relative humidity. To obtain virgin females, it is recommended that 1 pupa, in its cocoon, be placed into each tube. After 3 days dilute the sugar with distilled water. This causes the eggs to be deposited in the bottom of the tube, where they are held by the cloth. The eggs which remain clinging to the walls may be brushed down to the bottom with a soft brush (marten fur). After that, the cloth containing the eggs is removed and taken to the oven to be dried. When the eggs are dry they may be easily brushed off into a vial where they are stored for 4 days at 30°C, 8 days at 25°C and 30 days at 18°C as recommended by Duthy et al.2

Thirty mg represents approximately 1,100 eggs.

The second method suggested for collecting eggs is as follows: Into each 10 cm × 2 cm vial, introduce one male and one female *Galleria* together with a strip of thick black paper, folded and stapled in such a way as to form a narrow loop for egg laying. In this method, the eggs adhere strongly to the paper.

The eggs should be placed in an incubator nucleus for hatching. This nucleus consists of a small plastic box (9 cm × 6 cm × 2.5 cm), into which a honeycomb has been introduced. The honeycomb cells which are opposite to those in which the eggs will be laid, are filled with the diet. The empty honeycombs used to prepare the nucleus should be heat-treated, according to a process recommended by Cantwell and Smith,9 consisting of exposure to 48°C (±1°C) for 80 minutes. The quantity of eggs per nucleus should be around 1,000, or 30 mg and they should be distributed at random in the honeycomb cells after which the vial is sealed with a piece of cotton organdy or similar material. The nucleus, prepared in the manner described above, is placed in the incubator at 32°C (±2°C) and kept there until the larvae grow to approximately 10 mm, which usually takes about 12 days. For safety, the nuclei should be placed on a holder immersed in water. This prevents escape of the newly hatched larvae, protects the nuclei against ant invasion and improves humidity. The use of nuclei assures the availability of large numbers of larvae, especially...
because it prevents escape of newly-hatched insects which are extremely active. Another advantage is ease of handling and transportation.


Twelve days after hatching, the larvae will have reached the 4th stage, with an average size of 10 mm. At that time the nuclei should be opened and the material (4 or 5 honeycombs) should be transferred to a rearing tray previously supplied with the diet. Aluminum trays, of an adequate size to fit into the incubator, usually 37 cm × 25 cm × 3.5 cm, with galvanised screen covers fixed with screws or hinges, are recommended. The trays are easily handled and provide better aeration, an important factor for quick development of the larvae. Several diets are proposed for rearing G. mellonella. The author suggests the one which he developed himself, in view of its low cost and also because it promotes growth of the larvae in approximately 18 days, larval productivity above 95%, and larval weight of 300 to 350 mg. These results are much higher than those obtained by Marston & Campbell14 when they compared 9 diets utilised in rearing Galleria. The composition and preparation of the recommended diet are described by Guerra.12

Once the screen cover is tightly sealed, the tray is taken to the incubator under the conditions of temperature and humidity quoted above. The incubator interior should be kept dark since the larvae do not tolerate light. After 18 to 20 days they will have reached maximum growth and may be inoculated.

For small-scale rearing in bottles, 2 cm of the diet should be placed in the bottom of the bottle and covered with a thin layer of melted wax for protection. The eggs are placed on the protecting layer to hatch and this provides a place
for the newly-hatched larvae to move around freely. A protecting ring made with vaseline is placed around the top of the bottle to prevent escape of the larvae. Two or 3 hundred eggs per bottle are recommended. After being prepared as described above, the bottles are placed in the incubator and stored there until the larvae are used. Usually they will have reached maximum size and weight after twenty days. If necessary, the larvae may be stored for up to a year provided that they are anesthetised with CO₂ and kept at 15 °C and 60% relative humidity. Immature larvae should not be stored with the cocoons or mature larvae without them since the insects may become cannibalistic, especially under these storage conditions (Duthy et al). When it is necessary to remove the cocoons in order to release the pupae, this can be done by dipping them into a mixture of 500 ml of sodium hypochlorite solution (5% chlorine) and 500 ml of 5% sodium carbonate solution. By segregating the pupae it is possible to rear males and females separately. Mating can then be effected at an appropriate time when eggs are required.

Rearing of Lixophaga diatraeae Townsend

Rearing L. diatraeae in captivity is a well known but time consuming process. Many rearing techniques have been tested, using the larvae of Diatraea spp as hosts. The utilisation of larvae of G. mellonella has only recently been suggested and, for this reason, the following information, which is also applicable to the rearing of Tachinidae on larvae of A. grisella, is given.

The tachinid pupae used to initiate rearing may be obtained from some institution where this activity is carried out, or they may be reared from parasitised Diatraea collected on sugarcane or corn in the field. The pupae are

FIGURE 3. Cages for rearing Lixophaga diatraeae Townsend.
placed on humid filter paper, in a petri dish containing washed clay. The dish containing the pupae is placed in a rearing cage and kept at a minimum temperature of 25 C. The cages are cylindrical, and have a removable bottom and a cloth sleeve that permits the hand to be inserted for easy manipulation inside the cage. The bottom is covered with a layer of bagasse which has previously been boiled and then dried in the sun or in an oven. As the flies emerge, a wad of cotton soaked in a mixture of 10\% honey and distilled water should be kept in the center of the cage. At this stage, care should be taken (1) not to let the cotton wad touch the cage screening to prevent fungus development, and (2) to change the cotton wad daily since failure to do this might endanger the life of flies due to fermentation of the honey. The cage should frequently be sprayed with distilled water to assure a high percentage of relative humidity.

In the initial phase of rearing, when the number of individuals handled is small, it is a good practice to introduce one male and one female into vials soon after emergence, and expose them to direct sunlight to induce earlier copulation. At a later date when the number of flies permits this practice, all individuals that emerge on a certain day should be introduced into a separate cage. This practice permits control of the gestation period of the fly. The gestation period of *L. diatraeae* is 8 days, but it is sometimes necessary to wait a few more days until the larvae are sufficiently strong to penetrate the *Galleria* larvae.

Exposure to direct sunlight encourages copulation. If this practice is not possible, a light should be kept near the cage. Eight days after mating some flies should be tested, to determine if they are sufficiently developed for inoculation, by moistening the internal walls of a small glass tube and introducing a female into it. If the flies are ready for inoculation they will expel the small larvae when they make an effort to release their wings which are stuck to the dampened walls. Once the possibility of utilising the larvae is determined, the flies are killed by smashing their heads, and then transferred to a watch glass dampened with physiological serum (7.5 g of NaCl in one liter of distilled water) to prevent drying out of the larvae. A longitudinal cut is made in the abdomen of the fly to release the larvae. This material is washed with physiological serum and, with the aid of a small brush, transferred into a petri dish containing approximately 50 *G. mellonella* larvae. Two flies are sufficient for this number of larvae. As the host larvae move along the dish they are parasitised and a short time after inoculation commences no parasite larvae can be seen in the dish. The host larvae should then be transferred to a rearing tray to which some diet should be added. The tray should be covered with a strip of thick black corrugated paper, and then the screen cover should be replaced. The trays should be kept in a dark place at a temperature above 25 C, but not too high since temperature of 34 and 35 C caused the death of 88 and 100\% of the young *Lixophaga* larvae.

The *Galleria* larvae lodge in the corrugated paper and this facilitates the operation of opening the cocoons to look for the fly pupae that were not formed freely at the bottom of the tray or on the surface of the diet. To release fly pupae formed inside the *G. mellonella* cocoon, straight point, micro-dissection scissors should be utilised or the paper strips should be immersed in the hypochlorite solution described above.

A larger number of pupae is obtained when each larva is inoculated
FIGURE 4. Trays with *L. diatraeae* Towns reared over *G. mellonella* L. Larvae as hosts.

FIGURE 5. Detail showing *L. diatraeae* leaving *G. mellonella* larvae.
separately and isolated in a vial. A 350 mg larva may receive from 5 to 6 fly larvae. Some diet should be introduced into the vial.

The pupae are recovered into petri dishes containing washed clay, isolated with moistened filter paper. The material thus prepared is placed inside a rearing cage and kept at a temperature of not less than 25°C. As explained before, it is a good practice to spray distilled water on the cages several times a day to assure satisfactory relative humidity conditions.

When it is necessary for the flies to be carried to a distant place, it is recommended that they be transported in the pupal stage. The farmer who is interested in utilising biological control may obtain the pupae from a specialised laboratory for emergence, mating and adult release on the farm. The pupae should be carried in a field incubator or emergence box. The incubator is a small box (50 cm × 30 cm × 26 cm) for ease of transport. The top consists of a fixed part with a central opening and a moving part that facilitates handling inside the box. Resting on the larval nest there is a glass capsule covered by a funnel; the tube of the funnel passes through the opening of the incubator cover. Over the opening should be placed a glass chimney, with the top end closed by a net. The uniform temperature necessary for emergence of the flies will be maintained by the Galleria colony developing inside the incubator.

For establishment of *G. mellonella*, a niche should be prepared with pieces of honeycomb, without honey but containing the diet. Over this niche some cards with eggs should be placed. To initiate rearing, nuclei may be obtained from specialised producers. Two weeks after installation of the incubator it may be used.

In due course the pupae will be placed inside the glass capsule. Circular cards should be used to separate the several layers of pupae. Each card should have an opening in the center as an outlet for the flies to reach the funnel. Passing through the funnel they will reach the glass chimney, where sunlight will induce copulation. A cotton wad soaked in a solution of honey should be placed in the chimney to feed the flies.

The adults can be released each day. For protection of the flies against environmental agents they should be kept for approximately 1 week in a
rearing cage and only released when they are about to release their larvae. Care should be taken during the incubator operations to prevent the *G. mellonella* from escaping. It should be kept in mind that this species constitutes a serious pest in apiaries.

**Rearing parasites from eggs (Trichogramma spp)**

*G. mellonella* is a very fertile species, producing over 2,000 eggs per female. This is a favorable characteristic for multiplication of egg-parasites such as *Trichogramma* spp which if correctly reared and handled, may be very helpful in controlling the sugarcane borer.\(^5,13\) When the eggs to be used for this objective are obtained by using a deposition chamber, they should be fixed to a card to ensure easier handling and a higher yield. The technique may be that of Flanders\(^8\) for rearing *Trichogramma* spp on eggs of *Sitotroga cerealella*. The card should be of an adequate size and it should be brushed with shellac. While the shellac is damp, the *Galleria* eggs are distributed evenly over the surface. After the shellac is dry, the card should be shaken to eliminate the eggs which did not adhere and should then be placed in an adequate container along with another card containing parasitised eggs from which adults of *Trichogramma* spp are about to emerge. Each day the card should be replaced by another containing eggs to be parasitised.

The genus *Trichogramma* exhibits strongly positive phototropism when newly emerged, but light does not exert any influence on egg-laying. Some strains reproduce easily in complete darkness but, as a general rule, maximum egg-laying occurs under daylight conditions. Duration of the life cycle of *Trichogramma* varies with temperature from 6 days at 32 C to 80 days at 10 C.

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**REFERENCES**


MÉTODO Y RECOMENDACIÓN PARA CRIA MASAL DE ENEMIGOS NATURALES DEL BARRENADOR DEL TALLO DE LA CANA DE AZÚCAR

(Diatraea spp) (Lepidoptera: Crambidae)

Milton de Souza Guerra

RESUMEN

El barrenador del tallo de caña de azúcar (Diatraea spp.) es considerada una de las plagas mayores en cañaverales de muchas regiones productoras de azúcar en el mundo.

El control biológico ha sido la medida de combate más efectiva para esta plaga por su eficiencia y economía. Por los métodos tradicionales el proceso es limitado, principalmente por las dificultades de cria masal de los parásitos, en el caso de las moscas Tachinidae.

Este trabajo sugiere una metodología y transmite informaciones que tornan posible la cria masal de tachinídeos parásitos del barrenador del tallo de caña de azúcar usando como hospedero larvas de Galleria mellonella L. o de Achroia grisella F. Sugiere también el uso de incubadoras rústicas de tipo portátil, calentado por el propio calor producido por las larvas para criar estas moscas en estabecimientos de cultivos de caña de azúcar. Es abordado una técnica para la producción numerosa de huevos de Galleria mellonella para cria de Trichogramma spp., considerado en ciertas regiones un eficiente enemigo de la Diatraea saccharalis F. Se describe minuciosamente un método de cria masal de Galleria mellonella y Achroia grisella en laboratorio, sobre dieta formulada por el propio autor y de la mosca Lixophaga diatraea Towns, sobre las referidas larvas.