Biocontrol of *Chilo sacchariphagus* (Lepidoptera: Crambidae) a key pest of sugarcane: Lessons from the past and future prospects †

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ABSTRACT

Biocontrol of sugarcane stemborers using *Trichogramma* releases is a common strategy used in numerous countries, and the efficacy of such releases has been proven in most cases. On Reunion Island, the key stemborer *Chilo sacchariphagus* and its egg parasitoid *Trichogramma chilonis* have been the subject of intensive research for 10 years. From the identification of the best candidate for biocontrol to mass production and experimental releases in sugarcane fields, improvement has been constantly made through the years, with successful results. In this paper, we report some of the important outcomes, problems encountered and questions that have arisen during the field experiments (Reunion) and laboratory rearing activities (Reunion and France). Behind promising results from an eco-friendly technology that is used worldwide, one of the current debates that remain is the cost of such biocontrol to the growers. Since 2008, a new research and development project has been conducted to optimise the technology of field releases as well as to increase the efficacy of mass production and storage at cold temperature. To deal with profitability and quality and establishing a new industrial process for *Trichogramma* production, a partnership has been formed with a French company, BIOTOP, which has extensive experience in biocontrol of the maize stemborer *Ostrinia nubilalis* in France.

Keywords: biological control, *Chilo sacchariphagus*, field releases, mass production, parasitoids, Reunion Island, sugarcane, stemborers, *Trichogramma chilonis*

Introduction

Chilo sacchariphagus, a key pest of sugarcane

In many regions, sugarcane is the target of insect pests and some of them are very damaging. On Reunion Island, which is a French department, the two major pests are the white grub, *Hoplochelus marginalis* (Coleoptera: Scarabaeidae), introduced from Madagascar in the seventies and the spotted stemborer, *Chilo sacchariphagus*, originally from Java. Control of the white grub by the entomopathogenic fungus, *Beauveria brongniartii*, has been successful, but the spotted stem borer remains problematic.

In the past few years the problem has been increasing due to the adoption of a susceptible cultivar (R579) which is often more productive than the resistant one, R570, if not attacked. Stalk and internodes bored by larval stages result in productivity loss (tonnes cane per ha) of 30% in case of severe infestations (Goebel *et al.*, 1999). Today, it is estimated that at least 10 000 ha of sugarcane (40% of the overall sugarcane area in Reunion) are at medium or high infestation risk.

Because chemical treatments are generally ineffective, expensive and, at the present, none are registered for use, biological control represents a good option that combines environmental preservation and biodiversity conservation.

Why use Trichogramma as a biocontrol agent on sugarcane?

Trichogramma spp. (Hymenoptera: Trichogrammatidae) are extremely tiny egg parasitoids widely used on sugarcane and other crops globally to control moth borers of economic importance. They are characterised by wings covered by hairs layered in radiant lines. Once mass-produced and released, the tiny parasites seek out and destroy eggs of caterpillar pests, such as sugarcane borers, codling moths, cotton bollworms, corn borers, spruce budworms and many others (Hassan, 1993; Li *et al.*, 1994). The result is a living, biological 'insecticide' that strikes only the target pest with no risk to other natural enemies, human health or the environment.

The interest in these parasitoids as biocontrol agent is evident because they kill the pest at the most critical stage (the egg) before the damage occurs. In France, there is a good example of the efficacy of *Trichogramma brassicae*, which is currently used to control the European corn borer, *Ostrinia nubilalis*, on 20% of the maize crop area (more than 100 000 hectares) (Frandon and Kabiri, 1999). Futhermore, the low cost of production has encouraged the commercialisation of rearing *Trichogramma*.

10 years of research and continuous improvement of field releases

Findings made during two critical periods were compiled: Period 1 (2000 to 2004) when preliminary inventory and experiments were conducted in the field and Period 2 (2005 to the present) which mainly focused on the improvement of the quality of production and *Trichogramma* releases. All research phases were supported by funding through the European Union and the Ministry of Agriculture in France.

The importance of identifying the best Trichogramma candidate for field releases

In 2000, a thorough inventory of egg parasitoids on the island showed that only one species was present in sugarcane fields: *Trichogramma chilonis* Ishii. Rather than introducing additional egg parasitoids, it was decided to use the local parasitoid which was probably introduced from Indonesia, as was its host. However, further observations concluded *C. sacchariphagus* egg batches were not sufficiently parasitised by the local species to be an effective control measure (Goebel, 1999; Rochat *et al.*, 2001). It was then decided to use inundative releases to increase parasitism.

Before implementing such releases, the first step was to select the best candidate through laboratory experiments. To assess the potential of the parasitoid, the functional response of three *T. chilonis* strains (St. Benoît, St. Joseph, and St. Pierre, corresponding to three different climatic conditions, from humid to dry) was tested with *G. mellonella*, a facticious host, and one strain (St. Benoît) with *C. sacchariphagus* host eggs. The functional response is defined as the relationship between the number of prey consumed by a predator/parasitoid and prey density. The shape of the functional response (type II or III) based on logistic regression, attack coefficients and handling times (Th) led to the conclusion that the behaviour of all three strains with *G. mellonella* host eggs exhibited a type III response (Reay-Jones *et al.*, 2006). The St. Benoît *T. chilonis* strain had a significantly shorter estimate of handling time than the St Pierre strain ($P < 0.05$).

In addition, the functional response with *C. sacchariphagus* host eggs was also a type II with the St. Benoît strain. Lastly, more *T. chilonis* wasps from this locality developed from the larger *C. sacchariphagus* host eggs (2.9 per egg) relative to *G. mellonella* (1.1 per egg). From these results, it was decided to use the St. Benoît strain for further evaluations.

Trichogramma releases: timing and application rates are essential

The rationale behind our biocontrol project conducted in Reunion was to set up inundative releases in sugarcane fields at the beginning of the crop cycle because it corresponds to the oviposition period of the borer which occurs on 1 to 4 months old cane (Tabone *et al.*, 2002; Tabone and Goebel, 2005). Field experiments commenced in 2001-02 in two locations, Sainte-Marie (SM: humid area/north east part of the island) and Savannah (SAV: dry area/west part of the island). 100 000 *T. chilonis* per hectare per week were released, during a 4 month-period.

Two-hundred release points/ha were also set up at this time to ensure a good distribution of the parasitoids. The results from these first experiments showed that the control of the pest from

Table 1. Effect of *Trichogramma* releases on damage levels and stalk mass at harvest at Sainte-Marie & Savannah, Reunion Island (2003, Variety R 579)

Treatment	% INB (harvest)		Stalk weight (g)		Cane yield (Tc/ha)	
Sites	SM	SAV	SM	SAV	SM	SAV
Control	22.3 a	12.4 a	1390 b	1.16 b	138	93.3
Treated plots	9.1 a	5.6 a	1720 a	1.34 a	175	107.9
CV%	47.1	39.9	5.7	5.9		
F value	6.42	7.05	26.80	12.17		
P	0.0851	0.0766	0.0140	0.0398		

SB = Stalks bored; INB = Internodes bored; CV = coefficient of variation.
Statistical results are from an ANOVA (SAS Institute). For each variable, the means followed by the same letters (a,b) are not significantly different (P > 0.05, Student-Newmans-Keuls test).

Table 2. Effect of *Trichogramma* treatments on damage levels and stalk mass at harvest at Sainte-Marie, Reunion Island (2005, variety R579)

Treatment	%INB (harvest)	Stalk weight (g)	Cane yield (Tc/ha)
Control	33.1 a	1.78 a	115
T 80	30.7 b	2.21 b	132
T 100	25.4 c	2.43 c	150
CV%	52.8	32.9	
F Value	40.5	122.64	
P	0.0001	0.0001	

SB = Stalks bored; INB = Internodes bored; CV = coefficient of variation; T80 = 80 000 *Trichogramma* per ha.
Statistical results are from an ANOVA (SAS Institute). For each variable, the means followed by the same letters (a,b) are not significantly different (P > 0.05, Student-Newmans-Keuls test).

Table 3. Biological parameters of *T. chilonis* after storage at 3°C for 9 weeks

Parameters	Values	Statistical test vs control
Fecundity (Mean number of eggs per female ± S.E)	67 ± 5	ns (Chi-2 test, $p < 0.05$)
F1 progeny emergence	87%	ns (Chi-2 test, $p < 0.05$)
Sex-ratio (% females)	80%	ns (ANOVA, $p > 0.05$)
Mortality at 7 days	6%	ns (ANOVA, $p > 0.05$)
F2 progeny emergence	82%	ns (ANOVA, $p > 0.05$)

a total of 16 releases significantly increased the cane yield by 15 to 20% depending on the location. In field releases set up later in 2003 (Table 1), the results showed a financial gain estimated at between 600 and 1400 € /ha (Soula *et al.*, 2003; Barreault *et al.*, 2005).

The efficacy of *Trichogramma* releases was again confirmed during a field trial in 2005 where the recommended release rate of 100 000 *Trichogramma*/ha was opted for instead of 80 000 (Table 2).

Between 2007 and 2009, two additional experiments confirmed and validated optimisation and simplification of release methods, particularly the reduction of release points per hectare from 200 to 100 (where cane yield increased by 23%) (Marquier *et al.*, 2008). Furthermore, the experiment in 2007 did not show any difference between 100 and 50 release points. These findings are being tested in a new experiment set up in 2009 with the following treatments:

- Untreated control (UTC);
- Treatments 'Reference' (16 weekly releases of 100 000 *T. chilonis* per ha with 100 release points per ha for 4 months);
- Treatments 'Period' (12 weekly releases at the same rate for 3 months);
- Treatments 'Optimisation' (8 fortnightly releases of 200 000 *T. chilonis* per ha with the density of 50 release points per ha for 4 month).

The data are not fully collected yet, but the low level of infestation observed could hinder us from seeing a significant effect among the treatments. In future experiments, we will continue to reduce the number of releases (the ideal is 5 or 6) while ensuring that the efficacy of borer control will not be compromised.

Predation by ants: how to manage it?

On Reunion, the importance of predation of *C. sacchariphagus* eggs by ants (mainly *Pheidole megacephala*, the big-headed ant) has been reported as an essential component of the natural control of this pest (Goebel *et al.*, 1999), but ant predation is better known on other stemborer species (Teran, 1980; Bonhof *et al.*, 1997).

Under these circumstances of the predation by ants, control with *T. chilonis* is best when the releases is targeted on young cane where the oviposition peak of the borer occur and the natural predation by ants is very low. When cane's age is around 6-12 months, probably due to a build up of ant colony, natural predation of *C. Sacchariphagus* is quite significant, making the use of *T. Chilonis* redundant or wasteful.

To decrease this negative impact, new dispensers with tiny holes to prevent ants from penetrating and feeding on parasitised eggs are being tested. These dispensers are produced by our private partner, Biotop.

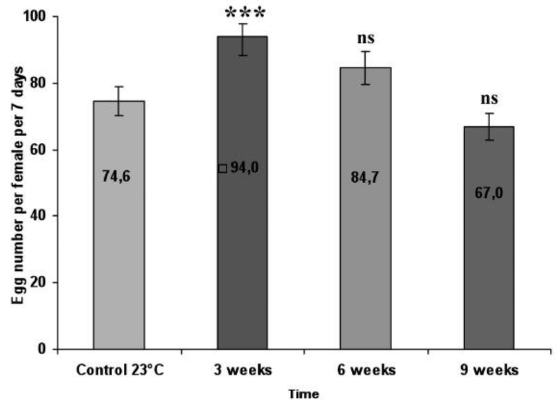
Initial results on diapause and storage at cold temperature

To apply a biocontrol strategy on a wider scale, there is a critical need to decrease the costs of insect production and field releases. Delaying emergence of *Trichogramma* spp. is critical for commercial production, and cold storage has been widely studied and used for this purpose (Voegelé *et al.*, 1986; Pitcher *et al.*, 2002; Ventura-Garcia *et al.*, 2002; Özder, 2004; Rundle *et al.*, 2004; Tezze and Botto, 2004), including its use on *T. chilonis* (Farid *et al.*, 2001; Shirazi, 2006). The technique has many advantages, including reducing overall costs, optimising the organisation and increasing production capacity. The storage capacity of *Trichogramma* will undoubtedly allow better management of staff and premises devoted to production. Finally, by reducing the number of progenies per year the risk of having a genetic drift is minimised. For the field, the possibility to cold store *Trichogramma* with delayed emergence will also facilitate delivery to farmers and reduce the number of releases.

At the end of 2008, a new research program began to determine optimal conditions (T°, RH, photoperiod, developmental stage) for arresting development by diapause or inducing quiescence of *T. chilonis*. Initial efforts allowed us to store the *T. chilonis* strain from Reunion for 2 months in a state of quiescence without affecting biological performance in the laboratory (Table 3 and Figure 1). The egg numbers per female were not different at 6 and 9 weeks (Student's t- test, $p > 0.05$), but fecundity was significantly higher after 3 weeks of storage at 3°C than the control (Student's t- test, $p < 0.001$) (Figure 1).

However, it is essential to determine if cold storage affects the efficacy of *Trichogramma* in the field, which will be an important step for our project in conjunction with our local partner FDGDON (Fédération Départementale de Défense contre les organismes nuisibles).

Figure 1. Fecundity of *T. chilonis* after storage at 3°C and 70 – 80% RH for 7 days (sample used for the Control, n = 32 females; 3 weeks, n = 34; 6 weeks, n = 32; 9 weeks, n = 32)



***Trichogramma* production: How to optimise biocontrol and the supply?**

The production of *T. chilonis* has improved through the years by our main partner FDGDON on Reunion. From 2000 to 2004, a small production unit was first set up for experimental needs using the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) as host.

In 2008, FDGDON reached its capacity in production of *G. mellonella* with 8 million eggs and 2 million of *T. chilonis* produced per month. This production is very costly and time consuming and the parasitism level on Galleria eggs is often variable.

In 2009, *Galleria mellonella* (Gm) was replaced by the Mediterranean flour moth *Ephestia kuehniella* (Ek), a widely used host for commercial production of *Trichogramma* spp. Further laboratory tests comparing the quality of *T. chilonis* produced on these two factitious hosts concluded that *E. kuehniella* was superior in terms of fecundity per *T. chilonis* female (Ek: 31 parasitised eggs; Gm: 26) average parasitism (Ek: 70%; Gm: 30%) and emergence rate (Ek: 92%; Gm: 55%).

However, rather than starting a new production site locally with FDGDON, we began to produce *Trichogramma* in France with our industrial partner Biotop. In 2009, experiments were conducted from consignments (cool boxes) sent to FDGDON, in Reunion on a regular basis.

The *E. kuehniella* eggs were then conditioned in appropriate dispensers and released in the fields. Having a company specialised in biological control in the project has become a necessity to apply *Trichogramma* releases on a wider scale.

This will also guarantee the quality of production and field releases of beneficial insects at an affordable price to farmers.

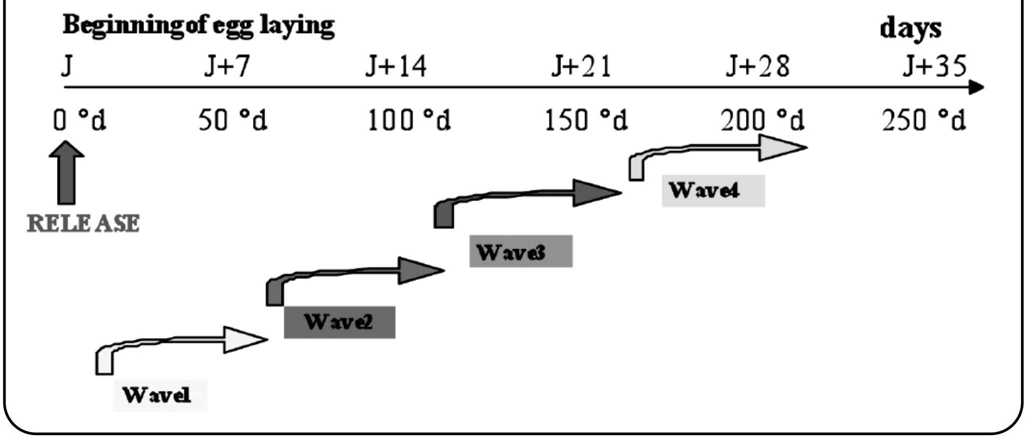
Biotop, a company based in France, is a subsidiary of the InVivo group, which plans to develop the appropriate technology for Réunion through our project. Biotop has around 20 years experience on improving the use of *Trichogramma brassicae* to control the European corn borer, *Ostrinia nubilalis*.

This biocontrol strategy has gradually become widespread in France where the control of the pest was pursued by this means in 2008 on some 120 000 ha of maize. Good pest control resulting, convinced many farmers to adopt biological control in place of chemical control.

Biotop has successfully developed a technology based on very efficient preparation and packaging plus delayed emergence of *Trichogramma* in fields (Figure 2). The parasitised eggs are prepared in special dispensers, made with cardboard sheets, which protect eggs from predators and rain or irrigation during several weeks.

These preparations use different stages of *T. chilonis* larvae (basically 4 stages) in order to successively release waves of this parasitoid, providing a long duration of beneficial activity. It has been possible to reduce the number of applications in corn fields from three to only one, with the same efficacy. In addition, the dispensers are easily supported by the plants and it is possible to treat 5 ha in one hour walking in the fields.

Figure 2. An example of the ultra-delayed release waves in dispensers implemented by Biotop for the control of the European corn borer *O. nubilalis* in France



In France, the cost of a *Trichogramma* application approximates a chemical application, including the mechanisation, of about 35€/ha. For this project, the goal is to use the same release technology with an expectation of controlling the pest with six applications, at a cost to the farmer ≈ 200€/ha. Considering the financial gain due to biological control, this price is affordable for sugarcane farmers.

Discussion: Which biocontrol strategy for the sugarcane industry in the future?

Based on the promising results obtained and the experience accumulated during this project, we are confident that biocontrol using *Trichogramma* spp. will be a success story and a realistic strategy for growers to reduce borer infestations. However, *Trichogramma* is just one of the components of biocontrol and certainly not a panacea. From our experience in Reunion, we have learned that a biocontrol program needs proper research following strict protocols and requires constant technical improvement. The failure of borer control using *Trichogramma* spp. in the 1960s and 1970s was partly due to lack of research on parasitoids themselves (species, bionomics and efficacy), but also lack of quality control of mass production (Goebel, 1999). During this period, biological programs often introduced exotic parasites and released them without evaluating (in some cases) their impact on pests (Goebel, 1999). All these facts have led to a negative image of biocontrol with *Trichogramma* spp. and other parasitoids and loss of interest for this strategy (Tabone *et al.*, 2002). Nevertheless, many countries, such as Indonesia (Java) and India are using this parasitoid as the main component of their biocontrol strategy. Indonesia is still producing millions of *Trichogramma* in association with the sugar factories while India has seen small farmers taking over the production and release of *Trichogramma* wasps in their own fields.

Another example is Brazil which has succeeded in controlling *Diatraea saccharalis* using two parasitoids: *Cotesia flavipes* and *Trichogramma galloi* (Botelho *et al.*, 1999). This example is noteworthy as it is an example where key parasitoids are used in concert. It demonstrates that it is preferred to have additional pressure on stemborer populations. It is an interesting option to consider for Reunion. As we have seen, predation should also be considered as a component of the system.

Research and development activities should continue to improve biocontrol in all its components: quality control, cost reduction, conditioning, packaging, efficacy and adoption by growers. We have started to understand the induction mechanisms of quiescence and/or diapause of *T. chilonis* in laboratory conditions. If successful, this research offers the possibility of storing the parasitoid for long periods without loss of viability.

In the era of GMOs and the concern of the environmental impacts of such technologies, biological control remains a credible alternative for ecologically, sound-based pest management.

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