Biological control of the sugarcane stem borer Chilo sacchariphagus (Lep:Pyralidae) in Réunion Island

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BIOLOGICAL CONTROL OF THE SUGARCANE STEM BORER *CHILO SACCHARIPHAGUS* (LEP: PYRALIDAE) IN RÉUNION ISLAND: CURRENT AND FUTURE STUDIES ON THE USE OF *TRICHOGRAMMA* SPP.

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Abstract

One of the most serious insect pests of sugarcane in Reunion Island is the spotted stem borer *Chilo sacchariphagus* Bojer (Lepidoptera: Pyralidae). Collaborative work between CIRAD and INRA has led to a biological control programme with the exotic egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae).

Experience with exotic biological control agents in Réunion suggests that they have not had a significant impact on the populations of *C. sacchariphagus* in the field. Nevertheless, adaptation over long periods may allow such agents to be used in a revised biological control programme. Recent observations confirm the key role of *Trichogramma* spp. in biological control of this stem borer, but parasitism levels appear to be lower than previously reported. Current research including morphological and biological studies on *Trichogramma* spp. are presented and the value of inundative releases in sugarcane are discussed.

Introduction

Species of the hymenopteran genus *Trichogramma* occur worldwide on a broad range of hosts in many crops. Research on the use of this egg parasitoid in biological control started in the early 1900s. The past 20 years have seen considerable use of this parasitoid on a large scale, particularly on corn, sugarcane, cotton, fruit trees and vegetables in more than 30 countries (Li, 1994). Today, *Trichogramma* spp. are the most widely used parasitoids in biological control (King, 1991). In sugarcane, *Trichogramma* spp are considered to be effective egg parasitoids of many lepidopterous stem borers (Metcalfe and Brenière, 1969; Nagarkatti and Nagaradja, 1977; Browning and Melton, 1987; Pham *et al*., 1995).

In the Indian ocean islands, attempts to control stem borers, mainly the spotted stem borer *Chilo sacchariphagus* with exotic parasitoids started in the 1940s (Greathead, 1971). However, variable results were obtained (Brenière *et al*., 1966; Etienne, 1974). For example, in Mauritius and Reunion Islands, introduction and large scale releases of parasitoids, mainly originating from India, did not control *C. sacchariphagus*, despite the successful acclimatisation of species such as *Trichogramma chilonis* Ishii and *Cotesia flavipes* Cameron (Hymenoptera, braconidae) in sugarcane fields (Moutia, 1952; Etienne, 1971; Williams, 1983). These inconsistent results showed the difficulty of acclimatization of exotic parasitoids in the specific conditions of islands with patchy local climates, such as Reunion. Paradoxically during these years of mass-releases, there was a lack of ecological studies on *C. sacchariphagus* and its indigenous parasitoids and predators. Moreover, no accurate information is available concerning the parasitisation rate of borer eggs by *Trichogramma* species and other egg parasitoids.

In Réunion, under natural conditions, Vercambre (1993) indicated that parasitism of *C. sacchariphagus* eggs ranged from 80 to 90%. Brenière (1965) found similar results in an extensive study on *T. chilonis* in Madagascar.

However, in Réunion, results were based on irregular surveys and biases were introduced because visual estimations of parasitism were influenced by leaf density and the black colouration of parasitised eggs being more visible than unparasitized eggs.

To date, *C. sacchariphagus* remains a major pest of sugarcane in Réunion Island, causing significant yield loss, particularly in the variety R579 where 10-30 tons cane/ha can be lost (Goebel, 1999). Integrated protection is currently implemented on the basis of new results obtained from experiments and farm surveys. In this respect, varietal resistance appears to be an effective strategy of controlling the stemborer (Goebel *et al.*, 2000).

A new biological control programme using *Trichogramma* spp. began in 2000. This important programme encompasses different steps from field and laboratory research to technology transfer. It includes choice of suitable species and the selection of strains to improve field performance (higher fecundity, survivorship and more efficient parasitism). The selected strain should also have qualities suitable for mass rearing. Technology transfer includes examining release density per hectare, methods of conducting releases (manual or mechanical), weather assessment during releases, timing of releases, and integration with other methods of pest or weed control and existing natural control (e.g. predation by ants). This paper examines current progress in the use of *Trichogramma chilonis* as a suitable biological agent to control the spotted stemborer in Réunion.

Level of parasitism by *Trichogramma chilonis* Ishii and the importance of egg predation

During 1996 and 1997, regular surveys were carried out at two sites on Réunion (Savannah and Sainte-Marie) on the susceptible variety R579. Fields ranging in area from 0.15 to 0.25 ha were surveyed on commercial farms. Five plots of 60 m^2 were ob-

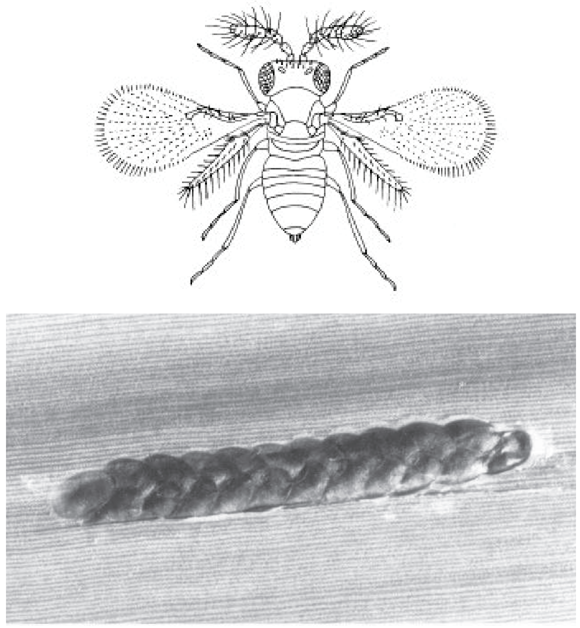

Figure 1. ***Trichogramma chilonis*** **Ishii (from Williams, 1983) and parasitized eggs of** ***Chilo saccharíphagus*****.**

served every 10 days in each field. All the sampled plants were carefully inspected for *C. sacchariphagus* egg masses. Parasitism was noted when black-coloured eggs were found in egg masses (Figure 1). Observations stopped when the vegetation became too dense. In the same plots, predation was also recorded on eggs brought from laboratory cultures and placed directly in the field every week.

Results (Table 1) showed a peak of *Trichogramma* activity in November, December and January. This corresponds to the main period of egg laying of the stemborer and coincides with the rainy season as well as the beginning of the crop growth period (3-4 months old), when borer attack can be high. However, the percentage of egg parasitism remained half that observed by Vercambre (1993) at both sites (Table 1).

Importance of predation of eggs by ants was observed and appeared to interfere with parasitism. In the two surveyed sites (Savannah and Sainte-Marie), when sugarcane was six months old, 70% and 100% respectively of the eggs were attacked by ants (Goebel, 1999). Trap catches and regular observations in the plots revealed that *Pheidole megacephala* F. (Hymenoptera : formicidae) was the major predatory species. This result shows the importance of timing of releases which must take place before ant predation occurs in the fields. To ensure the highest efficiency, the releases of *Trichogramma* should be conducted during the window period of low predation by ants.

Revised programme with *Trichogramma spp.*

The first phase of the new programme was to survey egg parasitoids in the sugarcane agrosystem (Figure 2). The parasitoids were collected in the field, from May to December 2000, at 10 sites selected around the island to get a wide range of climatic and cropping conditions. At each site, 8 plots about 200 eggs of the greater wax moth *Galleria mellonella* (Lepidoptera, Pyralidae), a factitious host of *Trichogramma*, or about 75 eggs of *C. sacchariphagus*, were placed in the field. Egg-traps were renewed weekly with fresh eggs. Each *Trichogramma* strain collected from different geographic areas was reared separately and kept in the laboratory as original material for different bionomics studies. A sample was sent to INRA, for specific determination (collaboration with B. Pintureau, INRA-Lyon and F. Vanlerberghe, INRA-Antibes) .

Trichogramma was collected in nine of the ten sites (Figure 2). However, the mean percentage parasitism of *Galleria* eggs was surprisingly low (0-3%) compared with previous results from direct counts of *C. sacchariphagus* eggs (Goebel, 1999). However, *G. mellonella* eggs may be less attractive than those of *C. sacchariphagus* in the field, underestimating parasitoid efficacy. In future field experiments, only *C. sacchariphagus* eggs will be used.

Molecular and morphological characterisation of *Trichogramma* spp.

Trichogramma species collected in the fields were identified by the molecular means and morphological traits.

Molecular identification. For molecular studies of insects several methods are available (Isoenzymes, RFLP, RAPD, etc.). Here the Random Amplified Polymorphic DNA (RAPD) technique is applied. It allows the differentiation of species by comparison

Table 1. Percentage of ***C. sacchariphagus*** **eggs parasitized by** ***Trichogramma*** **spp. in 1996/97 at two different sites.**

	Sainte-Marie			Savannah		
Months (age of crop)	No. egg masses	No. eggs	% parasitism per egg mass	No. egg masses	No.e ggs	% parasitism per egg mass
Sept-96 (2)	-	-	-	2	44	13,1
Oct-96 (3)	15	371	35,8	7	188	16,3
Nov-96 (4)	15	372	49,4	13	309	38,2
Dec-96 (5)	17	390	39, 2	17	384	40,5
Jan-97 (6)	24	549	40,1	8	198	24,3
Feb-97 (7)	7	163	30,3	3	80	33,3
Mar-97 (8)	4	92	31,8	4	105	20,3
Apr-97 (9)	3	72	0	1	28	0
Total	85	2009	38,2	56	1326	32,4

Counting periods : Sainte-Marie: 9/10/96-29/04/97; Savannah: 26/09/96-15/04/97

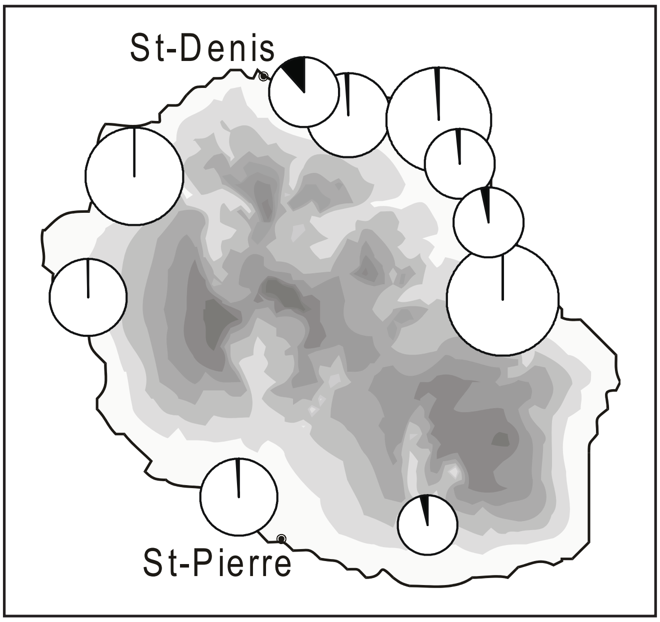

Figure 2. Localities on Reunion island of the 10 sites where egg parasitoids of *C. sacchariphagus* have been trapped and observed parasitism rates (pie charts – the size of the circles is proportional to sampling intensity).

of molecular patterns of the individuals, and their identification when the pattern matches the pattern of reference species. Results in Figure 3 show that all strains from different localities on Réunion have the same pattern, compared with other *Trichogramma* strains from INRA. This method is also used to monitor quality of the *Trichogramma* produced in industrial processes.

Morphological characterisation. This method is usually used for the identification of known species and the description of new species. It requires the examination of male genitalia (Voegelé and Pintureau, 1982 ; Pinto and Stouthamer, 1994) (Figure 4). Morphometric criteria (e.g. length of tibia, length of antennal hair) as well as characteristics such as the sex ratio of

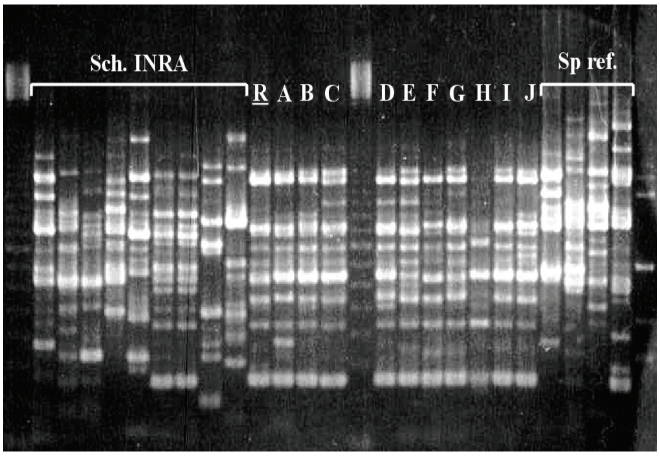

Figure 3. Molecular pattern of 10 strains of *Trichogramma* collected in sugarcane (2000): electrophoresis of amplified DNA fragments obtained with the primer Bioprob® N7. R : standard pattern of *T. chilonis* from Réunion, A - J : pattern of tested individuals collected from field. Sch. INRA : different strains of *Trichogramma* spp. from INRA-Valbonne ; Sp ref. : other reference species (Process and photograph by P. Chavigny, INRA-Antibes).

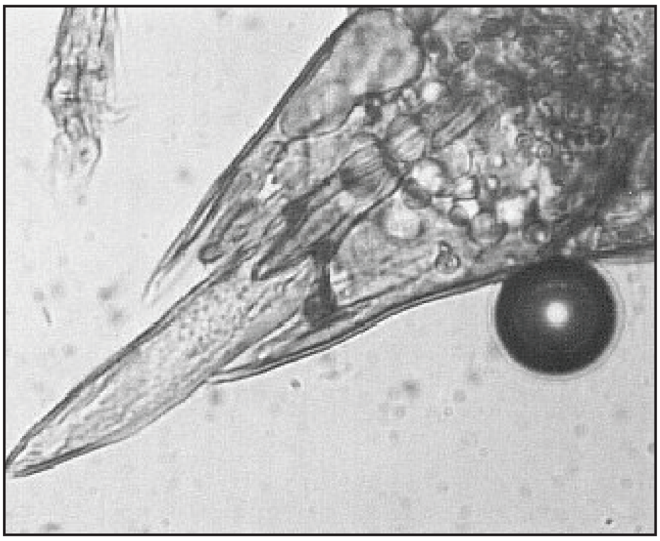

Figure 4. Aspect of genital ampoulae of *T. chilonis* male (photograph by E Fernandez/B Pintureau).

the population, the colour and the general aspect of the individuals can also be used to complete or to confirm the identification.

On the basis of these methods, all the examined strains from Réunion were determined as *T. chilonis* (Figures 3 and 4). All biotypes identified by INRA will be assessed locally for parasitism and the most suitable one will be mass reared for inundative releases in the field.

Future *Trichogramma* research and development

In the context of Reunion, where numerous exotic parasitoids were introduced and released, different steps for the use of a suitable strain of *Trichogramma* are required. Consequently, research on *Trichogramma* strains associated with biology and population dynamics is particularly important.

The inventory of egg parasitoids in sugarcane will continue in 2001 at different ecological sites. Simultaneously, studies on population dynamics of *T. chilonis* will commence. For these experiments parasitoids will be collected, preferably with *C. sacchariphagus* eggs rather than *G. mellonella* eggs for reasons previously stated. Moreover, *C. sacchariphagus* eggs are more resistant to field conditions than eggs of the factitious host and consequently remain suitable and attractive for longer during the exposure period.

Further studies on population dynamics of *T. chilonis* will focus on the susceptible variety R579, at two different sites, where numerous studies have already been done. The period of trapping will be completed for all ages of cane and the best trapping system will be used.

The main objective of this study will be to investigate the response of *T. chilonis* to different densities of *C. sacchariphagus* eggs (density-dependence). This information is essential to determine the ability of *T. chilonis* to control the borer in the field. In addition, at each site, all the ecological parameters will be recorded to investigate their impact on population dynamics of insects.

Predation will be monitored to confirm the results obtained in 1996/97 (Goebel, 1999). The presence of *Pheidole megacephala* must be taken into account in the timing of *Trichogramma* releases. This natural control could even be used to improve overall control of *C. sacchariphagus.*

The technology for mass rearing of *Galleria* moths to produce high quality *Trichogramma* has already been transferred to a professional organisation, which has many contacts with the farmers. This method will need to be improved, particularly in the case of mass releases (possibly for a future commercial use). Nevertheless, small scale production of *Trichogramma* will allow the testing of different factors linked to the methods of release, quantity of *Trichogramma*/ha, time of releases, packaging of the parasitoids, and indicators to assess efficacy of the releases. These practical studies will be conducted in partnership with farmers.

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