BIOLOGICAL CONTROL OF *CHILO SACCHARIPHAGUS* (LEPIDOPTERA: CRAMBIDAE) IN MOÇAMBIQUE: THE FIRST STEPS

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Abstract

In 1999, it was confirmed that *Chilo sacchariphagus* Bojer was attacking sugarcane at Açucareira de Moçambique, Mafambisse, and in 2001 from Companhia de Sena, Marromeu. In 2000, Açucareira de Moçambique requested that the South African Sugar Association Experiment Station investigate a classical biological control programme against this exotic borer using *Xanthopimpla stemmator* Thunberg. To this end, during March and June 2001, intensive pre-release surveys for this borer and possibly already established parasitoids were completed. No pupal parasitoids were recorded, and only 1.3% parasitism of the larval population was found. However, many egg batches were parasitised by *Trichogramma bournieri* Pintureau and Babault.

Because of the absence of pupal parasitoids in these collections, it was deemed safe to release *X. stemmator*, as it was likely that no native species would be displaced. Releases were planned to coincide with the *C. sacchariphagus* pupal peak expected during July /August 2001. One thousand mated female parasitoids were released in five selected sugarcane fields, in batches of 200 at fortnightly intervals over the two-month period. When post-release surveys were completed in October 2001, population reductions of between 31% to 90% in *C. sacchariphagus* larval and pupal numbers were recorded in all the release fields, when compared to control fields.

In the light of these findings, it seems that a classical biocontrol programme against *C. sacchariphagus* using *X. stemmator* in Moçambiquan sugarcane is beneficial. The way forward is discussed, as well as an augmentation biocontrol programme using *T. bournieri.* The possibility of importing and introducing larval parasitoids of *C. sacchariphagus* is proposed.

Keywords: biological control, *Chilo sacchariphagus*, *Xanthopimpla stemmator*, *Trichogramma bournieri*, Moçambique, sugarcane

Introduction

Lepidopteran stalk borers are generally considered to be the most injurious insect pests of sugarcane, maize and sorghum in sub-Saharan Africa (Polaszek, 1998). However, all stalk borers in Africa, with the notable exception of *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) (the spotted maize stalk borer) and *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae) (the spotted sugarcane stalk borer), are indigenous to the continent (Conlong, 2001a).

Chilo sacchariphagus is south east Asian in origin (Kuniata, 1994). It has, however, become a major pest of sugarcane in the Indian Ocean islands of Madagascar, Mauritius and Reunion, and is thought to have been introduced into Mauritius from Java in 1850 (Williams, 1983). It was recorded from Acucareira de Moçambique, Mafambisse in Moçambique (34º 10'E; 19º 20'S) in 1999 (Way and Turner, 1999), although its presence on this sugar estate was reported in unpublished reports as

early as 1989 (van Rensburg *et al.,* 1989). This is its first confirmed record in continental Africa, where it poses a substantial threat to re-establishing sugar industries in Moçambique, and to sugar industries in surrounding countries of Tanzania, Zimbabwe and South Africa. In 2001 a further confirmation of its presence was obtained from Companhia de Sena (18º17'S; 35º57'E; 6-11 amsl), at Marromeu on the Zambezi River in Moçambique (Conlong, 2001b).

In its area of origin it is well controlled by numerous parasitoids (Cheng, 1994; Kuniata, 1994). It has been the target of an intensive biological control programme for many years in Mauritius (Williams, 1983; Ganeshan and Rajabalee, 1997) and Reunion (Vercambre, 1993; Goebel, 1999). At Mafambisse Sugar Estate (6410 ha), losses due to this borer are estimated at between 14000 and 35000 tons of cane per ha per year (Way and Turner, 1999). In Reunion Island, losses of 30 to 40 tons of cane have been measured in heavy infestations (Goebel, 1999).

The life cycle and biology of *C. sacchariphagus* is well known and has been described fully by Williams (1983), Cheng (1994), Kuniata (1994), Way and Turner (1999) and Goebel (1999). It differs from the biology of *Eldana saccharina* Walker (Lepidoptera: Pyralidae), in that it oviposits predominantly on abaxial and adaxial surfaces of green leaf blades, whereas *E. saccharina* hides its eggs in the dead trash and leaf sheaths in the lower third of a sugarcane plant. Larval peaks generally occur during early cane growth (from 3 to 7 months). All larval stages are found in all internodes from bottom to top of the stalk, depending on period of attack and time of survey (Goebel, 1999).

Larvae ready to pupate cut an exit hole in the stalk rind, and then pupate in the boring, or in the leaf sheath near it (Williams, 1983; Kuniata, 1994). They do not spin a silken cocoon before pupation. The insect is multivoltine, breeding throughout the year, with 3 to 4 generations per annum. In Reunion, life tables studies show that *C. sacchariphagus* is more likely to develop large populations in the lowlands of the island, where the temperature exceeds 20°C nearly all year round (Goebel, 1999).

Because it is a pest exotic to Africa, attacking only sugarcane, it is regarded as a good target for classical biological control. It seemingly has not spread out of sugarcane into other crops or wild hosts in Moçambique, nor elsewhere in Africa. Control of it in this country is thus of utmost importance, and would save national and international agencies considerable capital if it is brought under control at this stage. The extremely rapid spread of *C. partellus*, since it was first found in Malawi in 1930 (Tams, 1932) and resultant heavy crop losses it has caused since, serves as a current reminder of what an uncontrolled exotic stalk boring pest of graminaceous crops can do in Africa (Overholt *et al.,* 1994).

In 1987, a consignment of the pupal parasitoid *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) was imported from Mauritius into South Africa as a new-association parasitoid against *E. saccharina.* Since then, it has continuously been reared and released against *E. saccharina* in the South African sugar industry (Conlong, 1994). It was successfully introduced from Sri Lanka into Mauritius to control *C. sacchariphagus* in sugarcane (Jepson, 1939). From Mauritius it was successfully introduced into Reunion against *C. sacchariphagus* in sugarcane (Caresche, 1962). Attempts have also been made to introduce it from Mauritius into Madagascar, where it probably did not establish (Caresche and Breniere, 1962; Appert, 1973).

A laboratory stock, free of hyper-parasitoids was thus readily available from SASEX for release in Moçambique. As *X. stemmator* has a wide geographic range in its native habitat (Moore and Kfir, 1996), it was felt that it should readily adapt to Mozambican conditions**.** It was proposed that this be the first step in a classical biocontrol programme against *C. sacchariphagus*.

Materials and Methods

Release area description and Xanthopimpla stemmator *releases*
Açucareira de Moçambique has been heavily infested by *C. sacchariphagus* for many years. In 2000, heavy rains just before the completion of the milling season exacerbated the infestation. Many hectares of cane due for harvesting before mill closure were left unharvested because of field inaccessibility. This led to major infestation build-ups in stand-over cane, with up to 3 generations of borer being produced from these old fields. The management therefore requested that *X. stemmator* be introduced into the estate as a matter of urgency, to bring some level of control to its natural host, *C. sacchariphagus*.

In early June 2001, permission was received from the Departemento de Sanidade Vegetal of the Republic of Moçambique to release *X. stemmator* against *C. sacchariphagus*. Plans were set in place to determine suitable release sites on the estate. Criteria used to determine the suitability of a release site were:

- A *C. sacchariphagus* population of greater than 10 individuals per 100 stalks.
- The presence of pupae and large instar larvae in the population of borers sampled.
- The sugarcane to remain in the field for at least two months after the last release in a particular field.
- The proximity of younger sugarcane in the vicinity of the release field to allow the released parasitoids opportunity to move onto *C. sacchariphagus* in these fields, in this way allowing the population of released parasitoids to establish on the estate once initial release fields were harvested.

Five fields were found suitable. Areas of high infestation by *C. sacchariphagus* were identified in each field, and demarcated for releases of *X. stemmator.* The first release of *X. stemmator* took place on 7 July 2001, followed by releases on 19 July, 2 and 16 August 2001. On each occasion, 1000 mated adult females were shipped to Açucareira de Moçambique. Two hundred of these females were released into each chosen field at each shipment date. Females ranged in age from two days old to 16 days old on the date of shipment.

In order to determine possible field establishment of *X. stemmator*, post-release survey was planned 2 months after the last release of females. This allowed offspring from any released females to have had time to parasitise further feral *C. sacchariphagus* pupae. To this end, fields that received releases were surveyed in the same manner as described below for indigenous parasitoid surveys.

Indigenous parasitoid and release site surveys
As no basic biological work or surveys for local natural enemies attacking *C. sacchariphagus* had ever been completed at Açucareira de Moçambique, initial visits were planned to put these in place. It was important to determine if indigenous parasitoids were present, and, if so, the impact they were having on *C. sacchariphagus* populations. In this way, the potential environmental impact of the introduction of a new parasitoid could be assessed. Basic surveys were also completed to determine if there were suitable life stages (i.e. pupae) of *C. sacchariphagus* present on the estate to provide hosts for *X. stemmator* to parasitise and establish on when these parasitoids were released. Screening for indigenous parasitoids of all life stages of *C. sacchariphagus* found during subsequent visits were continued, so that a reliable database could be built up.

When permission to release was given, identified fields were surveyed at two corners. One hundred stalks were taken at each corner by positioning four field inspectors 25 steps apart along the field margin. The inspectors were asked to collect a stalk of cane every five steps along a row until they had collected 25. This process sampled approximately one hectare of sugarcane. Harvested stalks were brought to the field margin, and number of internodes counted. The stalks were divided into three sections (top, middle and bottom), each with an equal number of internodes, and each section dissected. Number of damaged internodes were recorded, as well as the stage and number of immature *C. sacchariphagus* found in each section. These were placed singly into vials containing diet medium, and the vials numbered. Vials were sent to the South African Plant Protection Research Institute Quarantine Laboratory where they were screened for adult borer and/or parasitoid emergence. All individuals emerged were identified (unless otherwise stated) by the Biosystematics Division of the Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa, where all voucher specimens are housed.

Results

Indigenous parasitoids and suitability of the estate for release of X. stemmator.
An initial visit to the estate in March 2001, following the request to release *X. stemmator,* was made to an old stand-over field (Field 364, variety N19, 18 months old) with a known population of *C. sacchariphagus.* Damaged stalks were sought and dissected for 90 minutes by four people. Table 1 summarises the population structure of the *C. sacchariphagus* found.

Table 1. The population structure, in March 2001, of the immature life stages of *C. sacchariphagus* found in Field 364 containing 18 month old N19 sugarcane at Açucareira de Moçambique.

Life stage	Number	Percentage of total population
Eggs	1 batch	4.0
3rd instars	6	24.0
4th instars	4	16.0
5th instars	10	40.0
6th instars	3	12.0
Pupae	1	4.0
TOTAL	25	

Parasitism of larvae and pupae was not evident. However, all eggs in the *C. sacchariphagus* egg batch found were parasitised. Many of the larvae and pupae were in the middle to bottom of the stalks, and in some cases there were more than one large larva per boring. In one instance five large larvae were found in a boring.

A younger sugarcane field (Field 612, Variety NCo376, five months old) was visited to find more egg batches. This was successful, as four people found 30 egg batches in a two-hour search. Of these, 29 were fully parasitised (i.e. all the eggs in the batch were parasitised). In addition, young larvae were found in the whorl of young sugarcane plants, and some medium size larvae were found in stalk tips.

Parasitoids emerging from parasitised egg batches were sent to INRA, Antibes, France (Entomology and Biological Control Unit), who identified them using morphological characteristics as *Trichogramma bournieri* Pintureau and Babault (Hymenoptera: Trichogrammatidae). Voucher specimens are housed at the Natural History Museum in Paris, France.

Xanthopimpla stemmator *pre-release surveys and indigenous natural enemies*
Populations, and population structure of *C. sacchariphagus* on the estate was thus determined as suitable for *X. stemmator* releases. To select release sites, more intensive surveys took place in June 2001. Table 2 outlines the results of these surveys. The shaded fields indicate sections chosen as release sites for *X. stemmator*.

Table 2. Results of *Chilo sacchariphagus* surveys completed in June 2001, in sugarcane fields considered suitable for the release of *Xanthopimpla stemmator* at Açucareira de Moçambique.

Field no.	% stalks damaged	Internodes		No. *C. sacchariphagus* immature stages/100 stalks				
		Mean no.	% damaged	Small larvae	Medium larvae	Large larvae	Pupae	Total
294 (A)	66.0	15.8	9.8	2	7	18	2	29
294 (B)	60.0	14.3	8.3	2	11	24	1	38
353 (A)	56.0	15.5	6.7	4	4	8	0	16
353 (B)	57.0	16.5	6.3	1	1	5	0	7
811 (A)	39.0	16.9	4.6	3	1	7	0	11
811 (B)	56.0	14.6	8.5	3	2	8	0	13
812 (A)	52.0	16.8	6.7	8	7	8	0	23
812 (B)	66.0	14.4	9.1	1	9	9	1	20
2101 (A)	62.0	13.4	9.4	5	6	18	2	31
2101 (B)	72.0	16	10.4	3	5	15	2	25
7212 (A)	53.0	15.0	7.1	0	4	7	0	11
7212 (B)	59.0	15.5	7.5	1	6	6	1	14

These had pupae present at the time of survey, and higher populations of medium and large larvae, which would have been at the pupal stage during the adult female *X. stemmator* release period.

While it was common to find parasitised egg batches on the green leaf blades in most of the fields sampled, very little larval, and no apparent pupal parasitism was evident. Pathogen mortality was, however, evident. Mortality was always recorded from large instar larvae. Many dead larvae were fluid filled bags, symptomatic of *Bacillus thuringiensis* infection. Bioassays will however have to be completed to confirm identity. The entomopathogenic fungus, *Beauveria bassiana*, was found infecting three larval cadavers. A solitary unknown parasitoid emerging from some large larvae was subsequently identified as belonging to the hymenopteran family Ichneumonidae. It cannot be identified further until more field material becomes available.

Of the 240 larvae and pupae found, a total of 15 (6.3%) were dead at the time of collection. These were killed either by a pathogen (5.0%) or by an insect parasitoid (1.3%), indicating that pathogen induced mortality may be an important mortality factor of larval stages of *C. sacchariphagus*.

Xanthopimpla stemmator *post-release surveys and indigenous natural enemies*
All sugarcane surveyed in the post-release surveys of October 2001 was between 11 and 12 months old, and as in past surveys, most stalk damage was concentrated in the top third of the stalk. With the exception of Field 353, all living immature *C. sacchariphagus* stages were found only in the top third of the stalk. In the control section of Field 353 a single pupa and three larvae were found in the middle third. In the release section, only two empty pupae were found in the lower third of the stalks sampled. Two empty pupae were found in the top third of cane stalks exhibiting the typical 'opercular' opening made by a pupal parasitoid emerging from it, rather than the 'split' seen when an adult moth emerges. Parasitoid identity however could not be verified.

A total of 91 immature life stages were found. Of these four were empty pupae, one a live pupa, and the rest larvae, comprising 24 sixth, 21 fifth, 18 fourth and 23 third instars. It was thus evident that the sampling period coincided with low pupal numbers.

It appeared as if a moth peak had passed. This was supported by searches for *C. sacchariphagus* eggs. At least six hours was spent, with 12 people looking for eggs in four to six-month-old sugarcane. Only four batches were recovered, and none were parasitised. The presence of many sixth instar larvae indicated an imminent moth peak. The fields, however, were due to be harvested by November 2001, so infestation from expected moths would not occur. No parasitised larvae or pupae were found in October 2001 surveys.

Impact of Xanthopimpla stemmator *releases*
Four of the original five fields selected for release were used (Fields 294, 352, 812 and 2101). The fifth release was moved from field 7212 to field 728 by the estate. As this field had no previous stalk borer survey history, it was decided to use as a control field the last standing field of sugarcane being cut for seed, and into which no parasitoid releases had been made (field 371). This field was the same age, and was planted to the same variety as field 728. It meant, however, that no data were available for pre-release levels of *C. sacchariphagus*. Nevertheless, when *C. sacchariphagus* populations obtained from the first post-release survey in these two fields are compared (Figure 1), it is clear that far fewer *C. sacchariphagus* were found in the release site (6 per 100 stalks) compared to the control site (28 per 100 stalks), a 78% reduction.

A shortage of seed cane on the estate had forced the harvest of all but the release sections of fields 294 and 2101. This meant that no comparison of post-release survey results between release and control sections could be made, as was done for fields 728 and 371. However, comparisons could be made of populations of *C. sacchariphagus* found in release and control sections of pre-release surveys in June, and the remaining release section in the post-release survey. Figures 2 and 3 summarise these results.

It is clear from both Figures 2 and 3 that populations of *C. sacchariphagus* were considerably reduced in release sections of both fields after *X. stemmator* was introduced. A reduction from 37 to 8 *C. sacchariphagus*/100 stalks (78%) was recorded in Field 294 (Figure 2), and from 31 to 11 (64.5%) in Field 2101 (Figure 3).

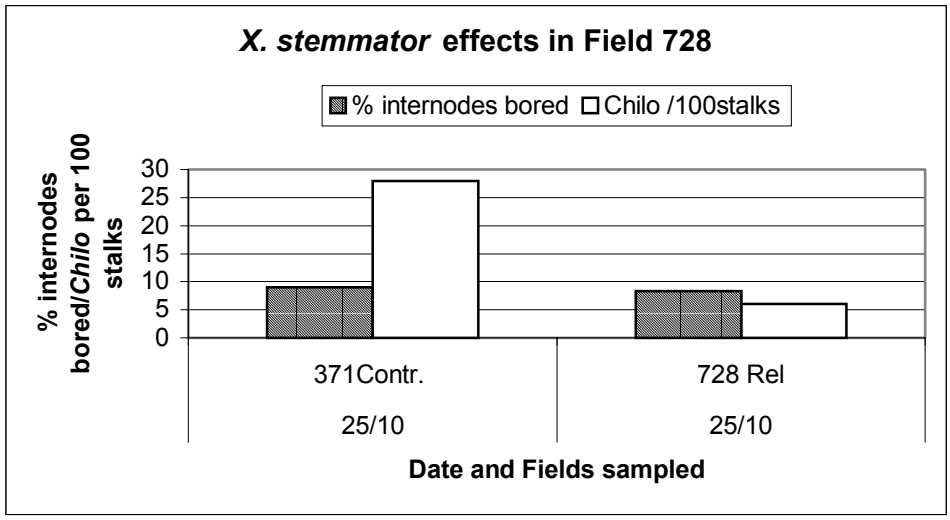

Figure 1. Results of the first post-release survey of Field 728, which had *X. stemmator* released into it; and Field 371, which served as the control field.

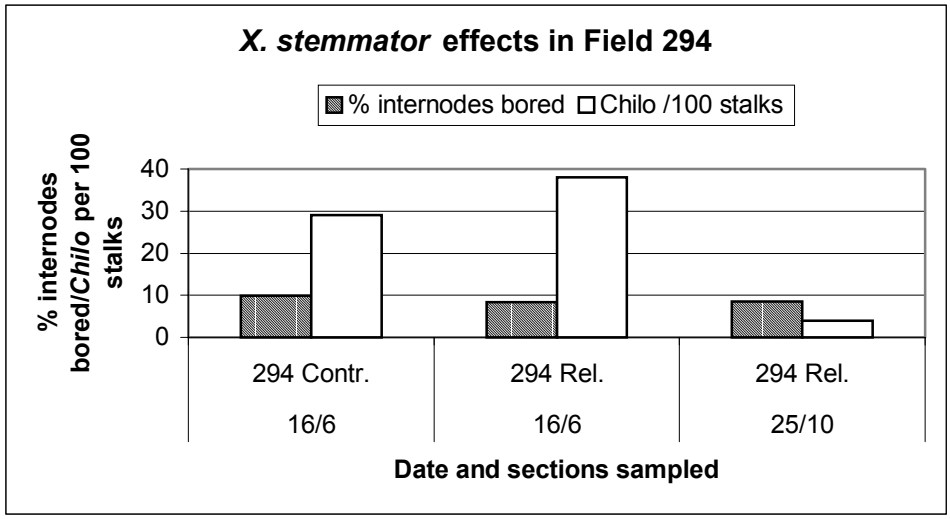

Figure 2. Results of the pre-release survey of release and control sections of Field 294, and the first post-release survey of the remaining release section.

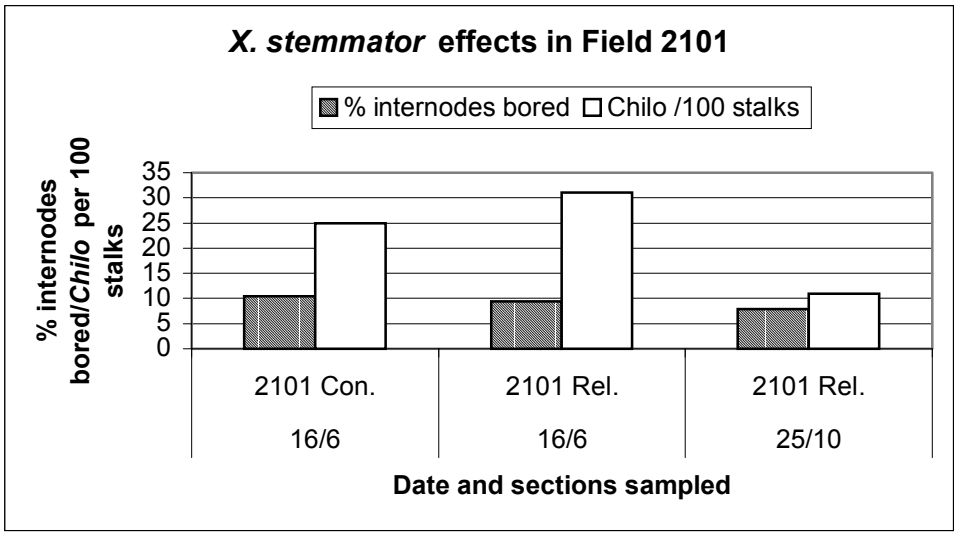

Figure 3. Results of the pre-release survey of release and control sections of Field 2101, and the first post –release survey of the remaining release section.

Where both control and release sections were surveyed after the release of *X. stemmator*, similar reductions in release sections in Fields 353 (16 to 11°*C. sacchariphagus*/100 stalks; 31.3% reduction) and 812 (20 to 2; 90% reduction) were recorded (Figures 4 and 5 respectively). However, over the same time period, populations of *C. sacchariphagus* increased from 6 to 24 in the control section of Field 353 (Figure 4). The *C. sacchariphagus* populations recorded in the post-release surveys of the control (24 *C. sacchariphagus*/100 stalks) and release sections (11/100 stalks) (Figure 4) showed a 54.2% reduction between the two.

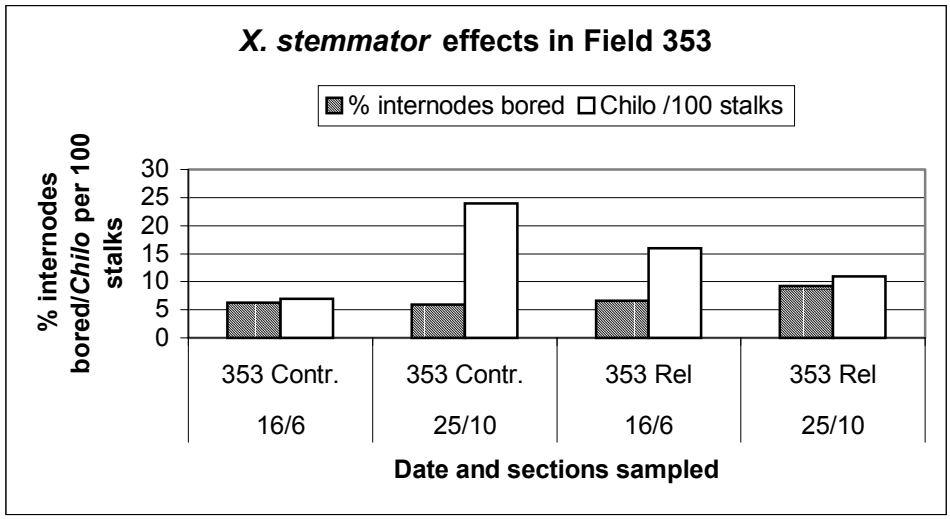

Figure 4. Results of pre- and post-release surveys of release and control sections of Field 353.

In Field 812, there was a reduction in *C. sacchariphagus* populations in the control section between the pre-release (23/100 stalks) and post-release (5/100 stalks) survey. It was, however, not as marked as the reduction in the release field for the same period (Figure 5). The post-release survey showed an 80% reduction in *C. sacchariphagus* populations between the control section (5/100 stalks) and the release section (1/100 stalks) (Figure 5).

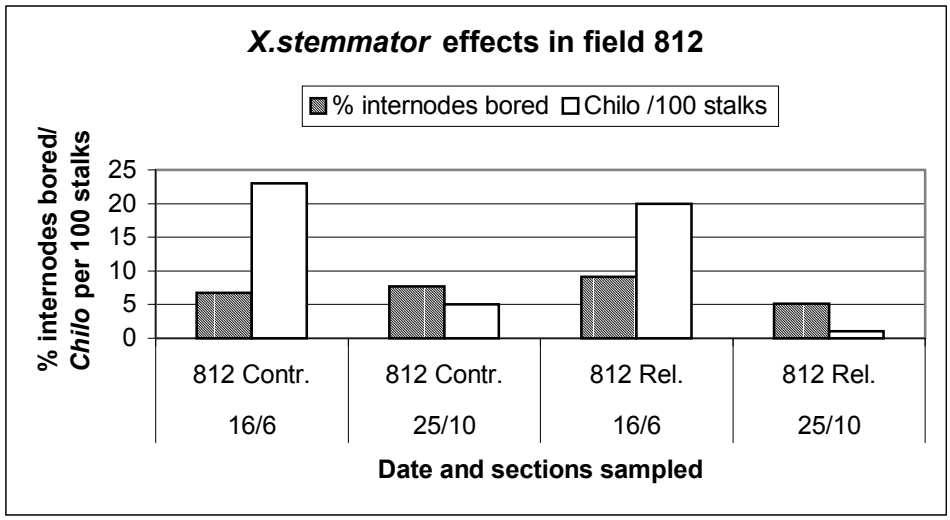

Figure 5. Results of pre- and post-release surveys of release and control sections of Field 812.

Discussion

Indigenous parasitoids

Very few indigenous larval parasitoids have so far been collected from *C. sacchariphagus* individuals found at Açucareira de Moçambique (less than 1% parasitism of the total larvae found). In addition, no pupal parasitoids have emerged from *C. sacchariphagus* collected at this estate. It is thus apparent that at this stage, indigenous larval and pupal parasitoids do not form part of a community with *C. sacchariphagus* in sugarcane in Moçambique, and as such leaves a void niche that can be filled by *X. stemmator*.

This is not so with egg parasitoids, however. At Açucareira de Moçambique many parasitised egg batches of *C. sacchariphagus* have been collected. So far, only one parasitoid species has been identified from these, *T. bournieri.* This species was reared from *C. partellus* eggs collected in 1982 from maize in Djomani on the island of Ngazidja in the Comores (Bournier, 1993). Bonhof (2000) collected the same species from *C. partellus* eggs from maize in coastal Kenya. Here egg parasitism was regarded as the most important mortality factor of eggs, above predation. Although the egg parasitoid was not identified, Gonçalves (1970) recorded 60% egg parasitism on *C. partellus* eggs. Cugala (2001) similarly recorded high egg parasitism by an unidentified egg parasitoid in his surveys in Moçambique.

It is thus apparent that the egg parasitoid niche is not void, being filled by at least one species of parasitoid, *T. bournieri.* This opens up further biological control opportunities that will be discussed below.

Classical biocontrol with X. stemmator

The preliminary results presented in this paper are very encouraging. In all release sections on Açucareira de Moçambique, populations of *C. sacchariphagus* were lower during October 2001 post-release surveys than those measured in pre-release surveys of June 2001. Reductions of 31.3% were measured in field 353, 64.5% in 2101, 78% in 294 and 90% in 812. Where control sections were left standing for the October survey, the reduction in *C. sacchariphagus* populations between control and release sections at the time of surveys varied from 54.2% in field 353, to 66.7% in field 812. In field 728, where no pre-release survey was completed, *C. sacchariphagus* populations were 78.6% lower than in a nearby field (371) of the same variety and age, but which had not received releases of *X. stemmator.* No live pupae were found during surveys to screen for *X. stemmator* emergence, but many of the empty pupae found showed signs of parasitoid emergence rather than adult moth eclosion.

In all release fields, internodes bored remained at between 5 and 10%. At this stage no real reduction in internodes bored would be expected, even between release and control sections. This is because *X. stemmator* is a pupal parasitoid. They are thus attacking an insect stage once it has fed and caused damage. Should the parasitoid be effective, less internode damage could be expected in ratooning or newly planted fields in the next season, as fewer pest adults would have emerged, following parasitism of their pupae. These would lay fewer eggs, which in theory would produce fewer larvae to re-infest new sugarcane plants.

Classical biological control of *C. sacchariphagus* thus seems possible using its pupal parasitoid *X. stemmator*. Establishment of this parasitoid on *C. sacchariphagus* still needs confirmation. A further release of *X. stemmator* should be considered in February/March 2002 and again in July/August to impact further on existing *C. sacchariphagus* populations.

Augmentation of T. bournieri *populations*

The collection of parasitised *C. sacchariphagus* eggs during the first visit to the estate (Table 1) and subsequent visits, and the identification of the egg parasitoid (*T. bournieri*) are very important in the context of future biological control of this borer in sugarcane in Moçambique. If this is the only egg parasitoid impacting on the populations of *C. sacchariphagus*, then mass rearing it for augmentative releases on the estate becomes a real possibility. Even though *C. sacchariphagus* is multivoltine, in Reunion there are periods when eggs are more abundant in fields than at other times (Goebel, 1999). As Reunion is on similar lines of latitude as Açucareira de Moçambique, it is reasonable to assume that life cycle parameters of this borer will be similar. To this end, augmentation of the existing *T. bournieri* populations with mass reared individuals could be completed from October through to late December, and possibly also from April to end of May (Goebel, 1999).

Importations of other classical biocontrol agents

Consideration should in the future be given to the importation and release of parasitoids of other life stages of *C. sacchariphagus*, which do not compete with *X. stemmator.* This would provide further suppression of *C. sacchariphagus* populations. In Madagascar for example, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was released against *C. sacchariphagus* in sugarcane. Parasitism increased each year for 6 years after the release, and then leveled off at about 60% (Betbeder-Matibet and Malinge, 1967). This would be a very good second parasitoid candidate to consider for importation.

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