EVALUATION OF EGG PARASITOIDS IN THE BIOLOGICAL CONTROL OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

By D. E. CONLONG and H. HASTINGS

South African Sugar Association Experiment Station, Mount Edgecombe

Abstract

A research programme for the biological control of the pyralid sugarcane borer *Eldana saccharina* Walker is outlined briefly. *Trichogramma australicum* Girault, *Trichogramma* sp. and *Trichogrammatoidea eldanae* Viggiani are the egg parasitoids used in experimental inundative releases, and the scelionid egg parasitoid *Telenomus applanatus* Bin & Johnson is used in small-scale experimental inoculative releases. Consideration is given to the behaviour of the parasitoids in relation to the oviposition habits of the target host. Preliminary results of parasitoid releases are summarized and their implications for future research are discussed.

Introduction

The current invasion of Natal sugarcane by the borer *Eldana saccharina* Walker began about 14 years ago when an isolated outbreak occurred at Hluhluwe (Carnegie[4]). Since then the pest has become established in the warmer coastal regions of the industry (Atkinson *et al*[2]). Eldana is an endemic species, normally resident in wetland sedges (Cyperaceae). Screening larvae collected during surveys of natural host plants in Natal has revealed the existence of seven larval parasitoids as well as a few pathogens and parasitic nematodes. The larvae of eldana are easy to find in the field, but the eggs are not, since they are laid mostly in small batches which are concealed amongst dead leaf material on the host plant (Atkinson;[1] Carnegie & Smaill;[5] Waiyaki;[17, 18] Walker [19]). Consequently, very few feral eggs, and no egg parasitoids, have been found in natural host plants in Natal.

When many thousands of eldana larvae, collected during surveys of Natal sugarcane, were screened, no parasitoids of any importance were found, neither were any parasitoids found in the few egg batches that were recovered during a series of intensive searches for feral eggs in sugarcane.

The study of endemic parasitoids of eldana is complementary to the other part of the research programme, which is an attempt to obtain local or imported parasitoids of eggs or larvae which will either establish themselves on eldana in sugarcane when they are released in relatively small numbers (inoculative releases), or which will parasitize eldana when they are released over a small area in large numbers (inundative mass releases). In inoculative releases, the aim is to achieve long term pest suppression through infield reproduction of the parasitoid species, while in inundative releases the aim is to cause an immediate and direct mortality of the pest population with no expectation of long term suppression (eg Stinner,[16] van den Bosch *et al*[20]).

Some species of parasitoids will be better suited than others to a specific objective; and even parasitoids which are known to be well suited to their target organisms have failed in certain biological control projects because the basic mechanics of introduction and release methods have not received sufficient attention (DeBach,[7] DeBach & Huffaker,[8] van den Bosch *et al*[20]). In the present programme of testing various parasitoids of eldana in the field, an attempt has been made to use release methods that take into account as many basic ecological requirements of the parasitoids and their target host as possible. The methods of field releases developed for four hymenopteran species of egg parasitoids and procedures for monitoring their impact on eldana in the field are described, and some preliminary results are discussed briefly.

Procedures

Three of the four parasitoid species used in the programme were collected from eldana eggs on maize in West Africa: *Trichogrammatoidea eldanae* Viggiani, an unnamed species of *Trichogramma* and a scelionid, *Telenomus applanatus* Bin & Johnson. The fourth, *Trichogramma australicum* Girault, is a parasitoid of the oriental cane borer, *Argyroploce schistaceana* Snellen. The present culture was imported from Taiwan after initial laboratory tests showed that this species readily attacked eldana eggs *in vitro*.

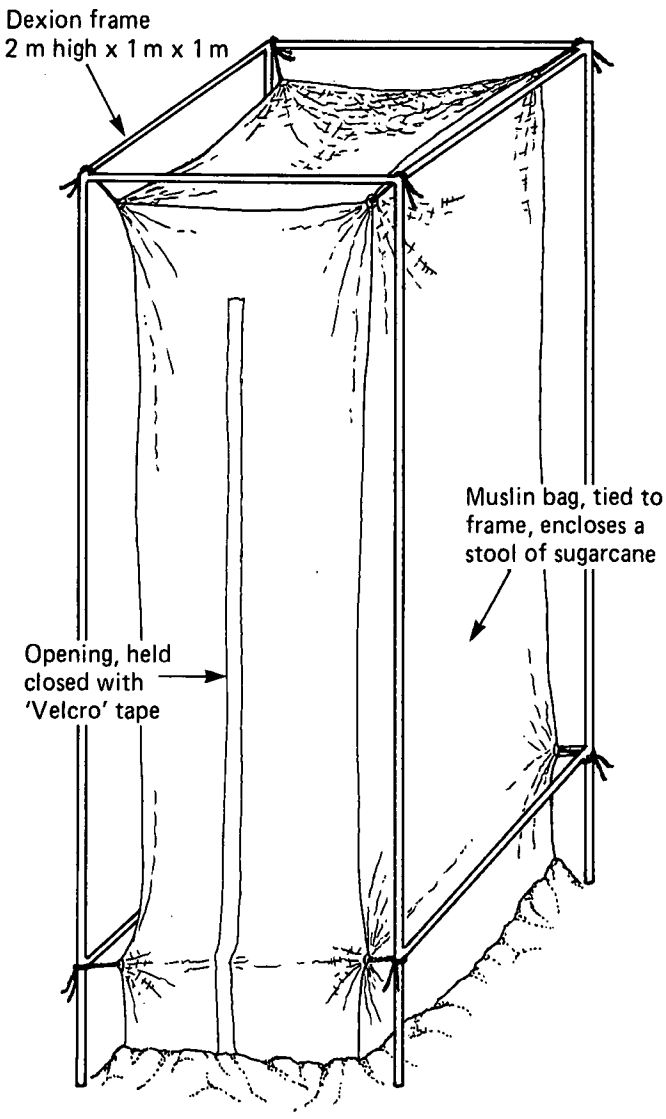

FIGURE 1 Small cage used for initial releases of trichogrammatids.

A small inoculum of each trichogrammatid species was obtained and was enlarged in accordance with availability of host eggs. Initially, small cultures were maintained on the eggs of the potato tuber moth *Phthorimaea operculella* (Zeller). The parasitoids produced were released into small cages (Figure 1) in the field in order to assess their ability to locate and parasitize naturally laid eldana eggs in sugarcane. At the same time, a mass culture of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller) was used to rear larger numbers of parasitoids, some of which were released from central points in sugarcane fields. The aim was to assess their parasitizing ability, dispersal, and general ability to survive conditions encountered in the field. When the *A. kuehniella* culture eventually came into full production, experimental inundative releases were made in several hectares of sugarcane.

The scelionid parasitoid *T. applanatus* refused eggs from all available alternate hosts (*P. operculella, Sitotroga cerealella* Olivier, *Galleria mellonella* L., *A. kuehniella*) and was therefore reared exclusively on eldana eggs. Because the number of eldana eggs from the culture was limited, relatively low numbers of this parasitoid were reared and released; thus the strategy for release was inoculative rather than inundative. This method of release was considered worth attempting with a host-specific egg parasitoid, such specificity having been implicated as important for successful colonization (Doutt & DeBach,[10] Huffaker & Messenger,[12] Huffaker *et al*[13]).

The oviposition habits of eldana on maize in West Africa were found to be different from those on sugarcane. On maize, eggs were usually found in the hirsute margins of green leaf sheaths of the cob and stem and occasionally in loose folds of dry leaf sheaths. In sugarcane the most common site was under dry leaf sheaths where eggs were concealed in tight folds and cracks and even in intracellular spaces of dead leaf material. This cryptic form of oviposition occurs in Natal sugarcane (Atkinson[1]) and has been reported in sugarcane in East Africa by Waiyaki,[17,18] and Walker.[19]

The general inaccessibility of the eggs is perhaps the most important factor to consider when egg parasitoids are used for the biological control of eldana. To be successful, the parasitoid must have the capacity to search extensively under dry leaf material. It is uncertain whether the egg parasitoids being used at Mount Edgecombe will search successfully in Natal sugarcane because, in their natural environment they all parasitize eggs in relatively exposed positions. The eggs of *A. schistaceana*, the original host of *T. australicum*, are laid openly on the midribs of green cane leaves. The three parasitoid species from West Africa were collected from exposed eldana eggs on maize.

Cryptic oviposition by eldana in sugarcane has necessitated the development of methods to find egg masses in the field, and to produce artificial infestations of eggs so that the efficacy of egg parasitoids can be assessed.

Surveys for feral eggs are conducted about once every six weeks in fields in which egg parasitoids are released. All dry leaf material from samples of cane stalks is thoroughly searched for all the life stages of eldana, which are then collected and screened. Ten labourers can efficiently search about 300 stalks in eight hours. The highest rate of recovery of feral egg masses has been approximately five for every 100 cane stalks searched. When sufficient data have accumulated, the trends of each of the life stages will be analysed and, in this way, the expected number of eggs will be compared with the actual number recovered so that an index of the efficacy of the present method of searching for feral eggs can be developed.

The excessive effort required to retrieve feral eggs from cane fields necessitates the provision, by artificial means, of eldana eggs for monitoring parasitoid performance in the field. There are two methods of doing this. One method is to enclose eldana moths from the laboratory culture in mesh cages over patches of cane or other host plants. Gravid female moths will oviposit naturally in these conditions. Parasitoids are released into these cages immediately after the moths have oviposited. Alternatively, when the moths have oviposited, the cages are removed and the consequent high density of eggs is exposed in a general field release of the parasitoids. A few days after the releases, cane stalks which had been caged are collected, searched, and any egg masses found are retrieved and screened for parasitism. Some caged stalks may be left to provide an inoculum of naturally parasitized eggs for the reproduction of parasitoids in the field.

The second method entails placing laboratory-laid eggs in the field in a way which simulates natural conditions. For field presentation these eggs are prepared by placing 60 mm pieces of dry cane leaf ('trash pieces') in an oviposition chamber with gravid eldana moths. After eggs have been laid on them, pairs of trash pieces, with eggs between them, are stapled together (Figure 2). In experimental parasitoid releases, these trash pieces may be placed in positions where eldana normally oviposits, eg between the dry leaf sheath and the stalk. The eggs are later retrieved and screened to assess rates of parasitism.

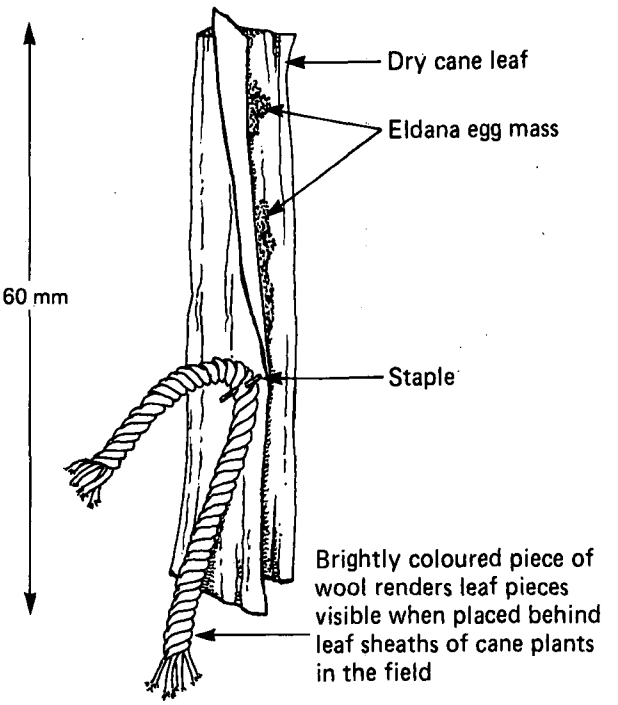

FIGURE 2 Pieces of trash containing eldana eggs, used for monitoring performance of egg parasitoids in the field.

Methods

Small-scale trichogrammatid releases

At the inception of the biological control programme, between 500 and 1 000 trichogrammatids from small, incipient cultures were occasionally released into cages covering single stools of cane. The cages were constructed from nylon mesh, fine enough to contain the trichogrammatids and were suspended inside a metal frame (Figure 1). Batches of ten male and ten female eldana moths were placed in the cage on two successive days. On the second and third days, cards containing 250 to 500 eggs from which parasitoids were emerging, were pinned to the cane stalks in the cage. Seven days after the first parasitoids were released, the cage was removed and the cane was carefully cut. All the eldana eggs which were found were screened for parasitoids.

The aim of the above releases was to determine whether the parasitoids were able to find and parasitize naturally laid eldana

eggs, particularly those situated under leaf sheaths. *Trichogramma* sp. (Ivory Coast), *T. australicum* and *T. eldanae* were all found to possess this ability and were therefore used in the next phase of observations which consisted of preliminary releases in the open field.

As the mass culture of *A. kuehniella* was built up, trichogrammatids became available for release into the fields in batches of 500 to 8 000, which were considered sufficient for open field releases, provided they were released from a single point.

The three trichogrammatid species were released once a week, each at a different site. A one-metre wooden post marked the middle of the cane field, the point at which the parasitoids were released. Four rows of seven posts each radiated from the central release point, each at a right angle to its neighbour. The posts in each row were positioned at 0,5; 1; 1,5; 2; 3; 4 and 6 m from the central release point. At each point, up to three pieces of trash containing eggs were stapled to the leaf blades of the cane or slipped between the dry leaf sheath and the stalk. Where the posts did not coincide closely with cane plants, ie in the interrows, the trash pieces were stapled to the posts themselves, at top, middle and bottom. The cards with the emerging parasitoids were stapled to the central post. Five days after the release the trash pieces were retrieved and labelled according to the positions from which they were collected. After a further two days, the egg masses were examined and the extent of parasitism was assessed.

During the summer of 1982/83 many releases of the three trichogrammatid species were carried out and in all cases their performance was similar. The results were as follows:

- when 1 000 or more parasitoids were released, parasitized eggs on trash pieces were commonly found up to 2 m from the release point at all placements and occasionally up to 6 m from the release point;
- if fewer than 1 000 parasitoids were released, parasitized eggs were rarely found;
- the overall parasitism of eggs on the pieces of trash rarely exceeded 10% in any release;
- in the majority of releases, more eggs on pieces of trash attached to the posts were parasitized than those placed on cane stalks;
- there was no difference between the extent of parasitism of eggs on trash pieces placed under leaf sheaths, and those placed on leaf blades;
- approximately 20% of all egg masses placed in the field were eaten by predators: the highest level of predation occurred where the trash pieces containing the eggs were placed under leaf sheaths;
- in periods of strong prevailing winds, more eggs on pieces of trash down wind of the release point were parasitized than those further up;
- high rainfall and low temperatures seemed to reduce parasitism.

Inundative releases of trichogrammatids

When the *A. kuehniella* culture began producing about a million eggs per day, the cultures of the three trichogrammatid species were expanded in the mass rearing apparatus of Morrison *et al.*[15] The card carrying the parasitized *A. kuehniella* eggs was cut into small rectangular pieces, each containing about 1 000 parasitized eggs which were used for releases (Conlong *et al*[6]).

Cane fields in which eldana numbers were high were used as the release sites. In each of these fields a grid of release points five metres apart was established over an area of approximately one hectare. At each of the release points a plastic shelter, under which the parasitoid release card was placed, was suspended from a fence-wire stand, at a height of 300 to 400 mm above ground level (Figure 3).

At the time of each parasitoid release, pieces of trash containing eggs were placed in the lowermost leaf sheaths in cane rows, midway between each release point. They were recovered seven days later and screened for parasitoids. At four to six week intervals during the release season, surveys were made for feral eggs and larvae. Samples of 100 stalks were taken from within the release block, and from equal sized blocks surrounding the release block, which were regarded as controls.

The programme of inundative releases is still in its infancy and few results are available. Eggs on trash pieces have been parasitized, but at a low rate. No feral eggs have been found. There have been no significant differences in the number of larvae recovered from release and control blocks.

Inoculative releases of Telenomus applanatus

The high degree of host specificity of *T. applanatus* is considered to be an advantageous characteristic in terms of its potential for colonizing eldana in the field. This characteristic has, however, been a disadvantage in initial releases, because the parasitoid can be reared only on eldana eggs, which are available in relatively low numbers; therefore only 4 000 to 8 000 *T. applanatus* can be released per week. In an attempt to offset this disadvantage, a method was developed in which many eldana eggs were provided at the release point. About 50 gravid female moths were enclosed in a large cage over mature cane (Figure 4) at the release site. Additional eggs were supplied on pieces of trash. To provide the parasitoid with the maximum opportunity to establish itself, fields with high eldana populations were chosen as release sites. In these release-fields, only half the cane was cut at any one time, in order to enhance the chances of survival of any parasitoids that might have become established.

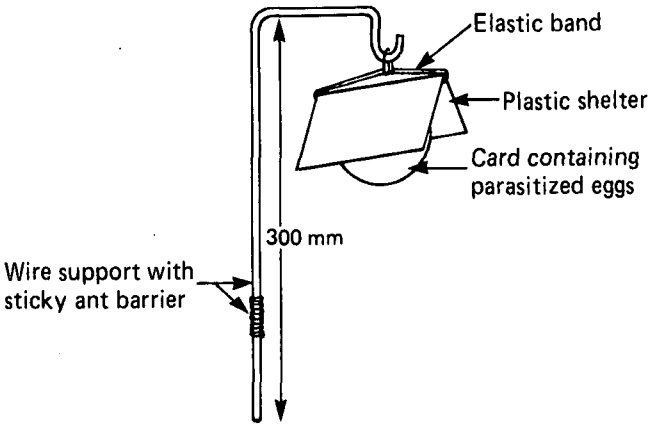

FIGURE 3 Plastic shelter on wire support for mass release of egg parasitoids.

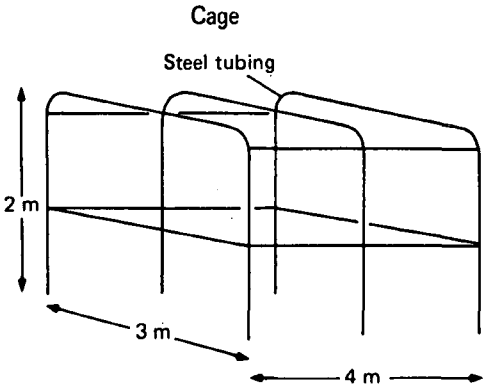

FIGURE 4 Large cage used for releasing *T. applanatus*.

At each release, an average of 6 500 parasitized eldana eggs on cards from the *T. applanatus* culture were attached to a small wooden frame under a plastic rain cover. Half of the cards contained emerging adult parasitoids while the other half contained eggs from which parasitoids would emerge the following day: there was thus a steady emergence of adults over two or three days. Four potted maize plants were positioned equidistant from one another, each one metre from the release point. In the four spaces between the maize plants, single, newly cut, mature cane stalks were suspended from the roof of the cage and secured so that their butts were a few inches above the ground. A sticky insect barrier was smeared on all supporting wires to exclude crawling predators. Between five and ten pieces of trash containing eggs were placed on each of the eight plants surrounding the release point.

They were also placed at the top, middle and ground level of cane plants growing inside the cage, and on cane plants immediately surrounding the cage. The emerging parasitoids were thus provided with an abundance of eldana eggs in a variety of situations, including an encirclement one metre from the release point; a high density in the 12 square metres of the cage; a few in trash pieces in the cane surrounding the cage; and naturally distributed feral eggs.

Seven days after the release, all trash pieces were collected from the field and brought to the laboratory. Five cane stalks were cut from within the cage and were searched thoroughly for eldana eggs. Approximately once every six weeks, 300 cane stalks were sampled from the hectare of cane surrounding the cage and searched for feral eldana eggs. All egg masses which were retrieved were screened for parasitoids and any that were recovered were used to start cultures for future field releases.

In a series of 11 releases, three yielded no parasitized eggs. In the others, a very low proportion of parasitized eggs (less than 5%) were recovered only from the trash pieces one metre from the release point. Near negative results such as these make it necessary to decide whether to: continue with current release methods; initiate experiments to determine the factor limiting the parasitoid's performance in the field; continue field releases, but use a less elaborate method; or discard the parasitoid as being useless. The general literature on biological control provides no guidelines on precisely when to abandon a parasitoid, unless a limiting factor is determined, and this requires intensive and time consuming experimentation. Occasionally, long after a parasitoid release project has been abandoned owing to apparent failure, parasitoids have been unexpectedly discovered in the former release area (van den Bosch *et al*[20]). There is therefore an element of indetermination in the release and monitoring of some parasitoids. In these cases, the best that can be done with limited time and labour resources is to release, in a suitable environment, realistic numbers of the parasitoid as often as practicable over a long period. The target host must be regularly sampled and screened, so that the rate of colonization can be determined. This course of action has been taken with *T. applanatus*. Since May 1983 weekly releases have been made over a 16-point grid by the same method as described for the mass release of trichogrammatids. At each release the cards are divided equally between the 16 points and, to date, the rate of recovery has been low. Since the beginning of this series of field releases only two feral egg masses, out of 40 which have been recovered, were parasitized.

Discussion

DeBach *et al*[9] warned of the 'inadvisability of trying to predict the success of natural enemies before they are actually tested in the field'. An important fact regarding the biological control of eldana is that, at present, there is no known parasitoid anywhere in Africa that is particularly effective against any stage of the insect in sugarcane.

There are certain larval parasitoids in West African sugarcane which parasitize eldana at a fairly high rate (Betbeder-Matibet,[3] Jerath[14]) and a programme is under way for some of these to be imported and tested. At present, the hope of reducing eldana by biological means lies in discovering an effective larval parasitoid. Because of the cryptic oviposition habit of eldana in sugarcane, the prognosis for egg parasitoids is not as good as it is for larval parasitoids. Any endemic egg parasitoid collected from eldana eggs in sugarcane anywhere in Africa would be regarded as a good potential agent for applied biological control; but none has been found, despite extensive searches. For example, Waiyaki[18] collected 11 350 eldana eggs from sugarcane in Tanzania and none was parasitized. Nevertheless, it is considered to be worthwhile to test thoroughly some of the more likely egg parasitoids from other sources; for example egg parasitoids such as *T. australicum,* which are effective against other lepidopteran borers in sugarcane in the Orient. If, as was the case with *T. australicum*, these parasitoids readily attack eldana eggs *in vitro,* then it is worth trying them on eldana in sugarcane because they are adapted to this environment. There are also egg parasitoids which inhabit eldana eggs on plants other than sugarcane, notably maize in West Africa. Such parasitoids have the ability to find and parasitize eldana eggs, but may be less successful in sugarcane. The efficacy and potential of such parasitoids on eldana in sugarcane in Natal can be determined only by subjecting them to well-structured field tests, and by accumulating as much knowledge as possible about their ecology and behaviour.

Conclusion

The procedures of experimental testing, releasing and monitoring the egg parasitoids of eldana in sugarcane are demanding and laborious and the results obtained after a great deal of work are most often negative. The implementation of effective biological control of eldana is clearly a long term project, of which the methods and preliminary results of some of the initial steps have been discussed.

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