

REARING PARASITIDS AND THEIR HOSTS FOR THE BIOLOGICAL CONTROL OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

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Abstract

At Mount Edgecombe, four species of egg parasitoids and two species of larval parasitoids are reared in a programme for the biological control of the sugarcane borer *Eldana saccharina* Walker. The rearing methods employed for each host species and each parasitoid are described. Some new techniques have had to be developed and others have been modified to meet the particular demands of local conditions. The host insect cultures include *Eldana saccharina*, *Sesamia calamistis* Hamps., *Galleria mellonella* L., *Phthorimaea operculella* (Zell.) and *Anagasta kuehniella* (Zell.). The parasitoids which are reared on one or another of these host insects include the hymenopteran egg parasitoids *Trichogramma* sp. (ex Ivory Coast), *T. australicum* Girault, *Trichogrammatoidea eldanae* Viggiani, *Telenomus applanatus* Bin & Johnson; the hymenopteran larval parasitoid *Goniozus* sp., and the dipteran (tachinid) larval parasitoid *Descampsina sesamiae* Mesnil.

Introduction

Biological control programmes commonly comprise a sequence of operations, entailing a search for beneficial organisms in the 'home range' of the pest; importing and rearing them in the laboratory under quarantine conditions; mass propagating them before releasing them in the field; and then evaluating their impact on the pest population (DeBach³, van den Bosch *et al*⁶). This sequence of operations has been applied in the programme of research on the biological control of the borer *Eldana saccharina* Walker. At Mount Edgecombe, procedures have been developed for rearing the egg parasitoids *Telenomus applanatus* Bin & Johnson (Scelionidae), *Trichogrammatoidea eldanae* Viggiani, *Trichogramma australicum* Girault and *Trichogramma* sp. (Trichogrammatidae), and the larval parasitoids *Goniozus* sp. (Bethyidae) and *Descampsina sesamiae* Mesnil (Tachinidae).

All these parasitoids can be reared on an appropriate life stage of *eldana* but, because this insect is expensive to rear in large numbers, other lepidopterous species which are easier to rear and which reproduce more quickly, are used as alternative hosts in the laboratory. The three trichogrammatid species are reared on the eggs of the Mediterranean flour moth *Anagasta kuehniella* (Zell.) and are released at a rate of several million per week. *T. applanatus*, on the other hand, will parasitize only the eggs of *eldana* and production of this parasitoid is thus limited to several thousand each week.

The larval parasitoid *Goniozus* sp. is reared exclusively on *eldana* since it will not parasitize *Galleria mellonella* L. or *A. kuehniella* which are common alternative hosts for a variety of parasitoids of stem borers. The tachinid fly *D. sesamiae* survives better on the sugarcane borer *Sesamia calamistis* Hamps. than on *eldana*, so *S. calamistis* is used as the main host in the laboratory.

Host Insect Cultures

Eldana

Atkinson¹ described a system for the rearing of *eldana*. Since then, the methods have been modified slightly. Batches of newly

hatched larvae are introduced daily into the culture on five days of the week and they are reared on an artificial diet medium in sterile glass jars. Twenty-five newly hatched larvae are put into each jar where they remain for the duration of the larval stage which, at 25°C, is about 28 days. Larvae usually pupate in burrows in the diet medium. The cocoons are separated from the diet medium and the pupae are removed from the cocoons by hand. The pupae are then placed in a box with gauze sides where eclosion takes place. The pupal stage lasts approximately ten days at 27°C. Newly emerged moths are placed daily in oviposition boxes which are lined with paper towelling onto which the eggs are laid. The egg masses are clipped out of the paper every day and are sealed in a transparent plastic bag where they remain at 27°C until they hatch about five days later. Considerable mortality may occur at this stage because the larvae are cannibalistic. Thus the newly emerged larvae are transferred into rearing bottles soon after eclosion.

The *eldana* culture supports a variety of research projects: eggs are used to culture *T. applanatus* and occasionally, some trichogrammatids. They are also distributed at the sites where parasitoids are released so that parasitization rates can be monitored, and are used in studies on egg predation and plant resistance to *eldana*. Early instar larvae are used in insecticide screening and plant resistance studies. Older larvae are used to culture *Goniozus*, *D. sesamiae* and other parasitoids, and are also placed in host plants where the performance of parasitoids in the field is monitored. Moths are used in studies on pheromone production and oviposition habits.

To cater for varied and often unpredictable demands, an excess of all the life stages of *eldana* are produced in the culture. Each week about 500 rearing jars are inoculated with a total of 12 500 first instar larvae and, with a survival rate of about 70%, 8 750 adults can be produced. However, each week, between 1 000 and 4 000 larvae and pupae are used for *Goniozus* cultures and for other projects. The remainder, which survive to adulthood, are used for experimental purposes, or to produce eggs for re-seeding the culture, or to support the egg parasitoid cultures, or for other projects.

Sesamia calamistis

The method of rearing *S. calamistis* is similar to that for *eldana* but, because this insect does not grow satisfactorily on the diet medium used for *eldana*, various other recipes have been tried. Adequate growth rates and survival have been obtained on a modified diet (Jackai & Raulston⁶, Nagaraga¹³). However, putrefaction of the diet medium is a severe problem which limits the production of *S. calamistis* larvae.

It was found that handling the newly hatched larvae of *S. calamistis* caused about an 80% mortality rate. To avoid this, batches of 20 eggs were glued to a card and placed 5 cm above the surface of the diet medium. When the larvae emerge after five days (27°C), they move down onto the diet medium and burrow into it. After approximately 14 days, most containers are heavily infected with fungus and the larvae must be transferred to new medium. Batches of 20 larvae are placed in each new container where they remain until they pupate. Pupae are extracted from the medium by hand and are transferred to a

jar with a gauze lid where eclosion occurs after 10 days at 27°C. Newly emerged moths are placed in containers in which paper towelling is provided as a substrate for oviposition. Development from the egg to the adult takes about 35 days under the above culture conditions.

Late instar larvae are produced in this culture for rearing the tachinid *D. sesamiae*. Before it was decimated by bacterial/fungal infections, the culture produced a surplus of approximately 700 larvae per week. To maintain this production, 1 500 eggs were placed in rearing jars each week: 1 350 late instar larvae were obtained, 700 of which were used for the parasitoid culture. The remaining 650 individuals were allowed to develop into adults which produced eggs for re-seeding the culture.

Galleria mellonella

The greater wax moth *G. mellonella* is a standard laboratory animal with simple rearing requirements (King *et al.*⁷). The larvae, which are similar in size to those of *eldana*, can be produced fairly cheaply in large numbers. They are used as host insects in the rearing of a number of tachinids (King *et al.*⁷). Larvae from a small culture at Mount Edgecombe are tested as hosts for parasitoids to be used against *eldana*. Neither of the larval parasitoids *D. sesamiae* or *Goniozus* sp. will parasitize *G. mellonella*.

Phthorimaea operculella

The potato tuber moth *P. operculella* provides a steady, reliable supply of eggs on which the three trichogrammatid species are reared. The size of the culture is regulated by the number of potato tubers which are infested. Twice a week, batches of 50 tubers are infested by covering them with filter papers on which there are eggs which are about to hatch. After about a week, by which time the larvae have emerged and bored into the tuber, the egg papers are removed. The larvae develop in the tubers within about 21 days at 25°C and then leave the tuber to pupate. In so doing, they fall through the mesh shelves on which the potatoes are placed, into a tray containing sterile sand. The larvae pupate under the sand. Twice a week the sand is sieved to remove all the pupae which are then placed in aluminium pans.

After three to four days the moths emerge and, since oviposition occurs at night, filter papers are placed every evening on the gauze covering the opening of the container. The moths lay their eggs through the gauze onto the filter paper. Every morning the filter papers carrying the eggs are sorted for use either in the trichogrammatid cultures or for re-infesting the tuber moth culture. This culture provides a steady supply of a few hundred thousand eggs each day for small-scale trichogrammatid cultures.

Anagasta kuehniella

Sufficient eggs for mass rearing trichogrammatids can be obtained from species of moth which infest stored grain products, eg *Sitotroga cerealella* (Olivier) and *A. kuehniella*. At the inception of the biological control project at Mount Edgecombe in 1980, *S. cerealella* was mass cultured according to the method of Morrison and Hoffman.¹¹ The culture was successful until it was invaded and annihilated by the pteromalid larval parasitoid, *Habrocytus cerealellae* (Ashmead), which proved difficult to control because the mesh-sided racks allowed the parasitoids free access. A further disadvantage was that the copious amounts of dust, formed from loose cuticular scales from the moths, had allergenic effects on laboratory personnel. *A. kuehniella* rather than *S. cerealella* was therefore used as a host insect and was reared in containers which excluded detrimental organisms such as mites and parasitoids, and which enabled loose scales to be extracted mechanically. An added advantage of using *A. kuehniella* is that its eggs are larger than those of *S. cerealella* and larger parasitoids are produced. These

are thought to be more robust and more efficient than their counterparts reared on eggs of *S. cerealella* (Lewis *et al.*,⁸ Marston & Ertle¹⁰).

The culture was designed to produce about one million eggs per day. In a small pilot culture it was found that each female produced 48 eggs per day, therefore 20 833 females were required to produce 1 million eggs. The male:female ratio is assumed to be 1:1 and, as in any mass culture of stored-grain moths, the mortality can be up to 30% (losses result from infertile eggs and adults and the death of larvae, pupae and adults in rearing procedures). To offset these losses, 70 000 eggs (a surplus of about 30 000) are put into the culture each day. The larvae are reared on a dry mixture of semolina, maize meal and brewer's yeast. The 70 000 larvae require approximately 5 kg of diet medium per day to develop to adulthood (Ullyett & van der Merwe¹⁵). Each day the requisite quantity of eggs is mixed thoroughly with the diet medium, and this mixture is spread in a larval rearing tray (Figure 1).

The temperature is maintained at 24°C: after 40 to 45 days, the larvae pupate in sites provided by corrugated cardboard strips which are stacked in rows (see Figure 1) on the surface of the diet medium. The mature larvae pupate in the holes of the cardboard and, when the moths begin to emerge, the cardboard stacks are removed and put into emergence boxes (see Figure 2). Each cardboard stack is left in the moth emergence box for 13 days which, at a temperature of 25°C, is the duration of the pupal stage of *A. kuehniella*.

When the moths are collected from the emergence box each day, they are narcotized with carbon dioxide. The unconscious moths fall to the floor of the box from where they are collected and transferred into an oviposition chamber (see Figure 3).

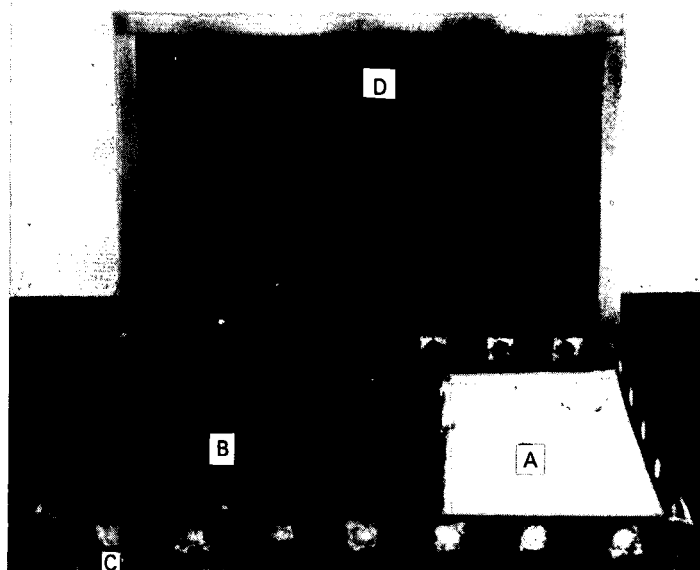


FIGURE 1 Rearing tray for larvae of *A. kuehniella*.

- A Diet medium.
- B Cardboard strips for pupation.
- C Aeration holes covered with gauze on either side.
- D Lid with foam for sealing.

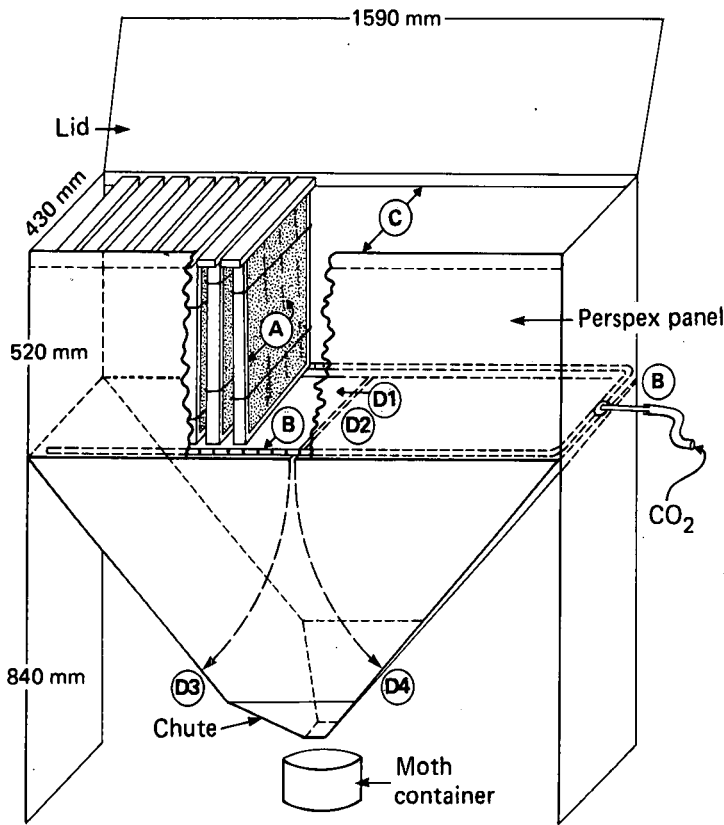


FIGURE 2 Emergence box for moths.

- A. Cardboard stacks (with emerging moths) from rearing boxes (Fig. 1) are tied in moveable wooden frames which are suspended on rabbits (C) in emergence box.
- B. Moths are narcotized with CO₂ which is led in via a manifold.
- C. When moths are unconscious the trapdoors D1 and D2 are lowered to positions D3 and D4. The moths slide down the chute into the container below.

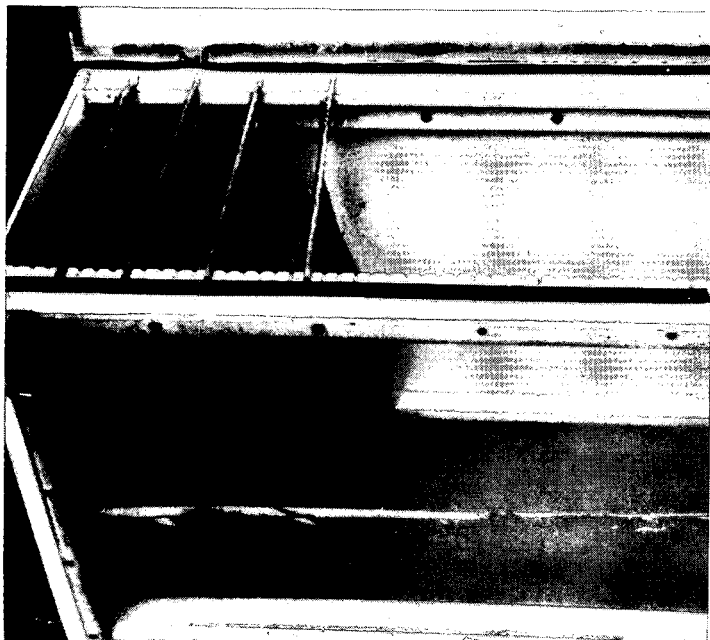


FIGURE 3 Oviposition chamber showing gauze perches.

The oviposition chamber contains vertically suspended mesh sheets on which the moths perch. As the eggs are laid they drop through the mesh floor of the chamber into the drawer beneath. They are then removed from the debris by sieving through a series of meshes, and every day two grams of eggs are used to inoculate a fresh batch of medium so that the rearing cycle is maintained. The remainder of the eggs is used for mass rearing trichogrammatids.

The present culture was built up from a series of small inocula and took about three months to come into full production. The daily rate of egg production later became erratic mainly as a result of a heavy mite infestation and inoculations with eggs which were infertile (probably because they were stored at temperatures which were too low). This method of culturing *A. kuehniella* is nevertheless capable of sustaining a yield of one million eggs per day.

Parasitoid Cultures

Egg parasitoids

The four species of egg parasitoid are reared in small *in vitro* cultures, the sizes of which are limited by storage space. The daily production of each of these cultures varies between 5 000 and 20 000 parasitoids. *In vitro* culturing is essential for establishing cultures from small amounts of feral material. Trichogrammatids produced from these cultures are used either in small-scale experimental releases, or as inocula in the mass rearing system.

Fresh eggs of eldana or an alternative host such as *A. kuehniella* or *P. operculella*, which are glued to filter papers, are placed in the culture bottles which contain the adult parasitoids. The number of eggs placed in each bottle each day is determined according to number of adult parasitoids, and the size of each culture can be regulated by the number of eggs put into each bottle. Adult parasitoids emerge in the bottles over three to four days. Batches of fresh eggs are supplied daily until the adults begin to die. The newly parasitized eggs, which turn black after a few days, are sorted according to their age and all cards bearing eggs of the same age are reared together. The time taken for them to develop is closely associated with the ambient temperature; 10 to 12 days at 25°C for trichogrammatids and 14 days at 27°C or 19 days at 25°C for *T. applanatus*.

Trichogrammatids are mass produced by a method adapted from Morrison *et al*¹² which is based on the positive phototropic behaviour of trichogrammatids. Large numbers of eggs are presented to optimum numbers of adult parasitoids with the aim of obtaining maximum parasitism of eggs.

Fresh eggs of *A. kuehniella* which are due to be parasitized are glued onto pieces of cardboard cut to fit the rearing apparatus. Three evenly spaced lines 1 mm thick are drawn down the length of the card, to be used as markers to estimate the number of parasitized eggs. Between 120 000 and 140 000 eggs are sprinkled onto the glued surface of each card.

The eggs on the card are sterilized by exposing them to UV light for one hour before they are placed in the illuminated half of a mass rearing unit (Figure 4). Seeding cards to which the parasitized eggs are attached are placed in the darkened half of the apparatus (Figure 5). When they emerge, the parasitoids move towards the illuminated side of the rearing box where they encounter fresh eggs. Two fluorescent tubes, one on either side of the apparatus, switch on and off alternately every two hours. This ensures that the parasitoids move over the entire egg card, giving rates of parasitism between 80 and 95%. Once an egg has been parasitized, it is ignored by other trichogrammatids, and superparasitization of eggs does not occur. This mass rearing apparatus can produce up to 665 000 parasitoids per day.

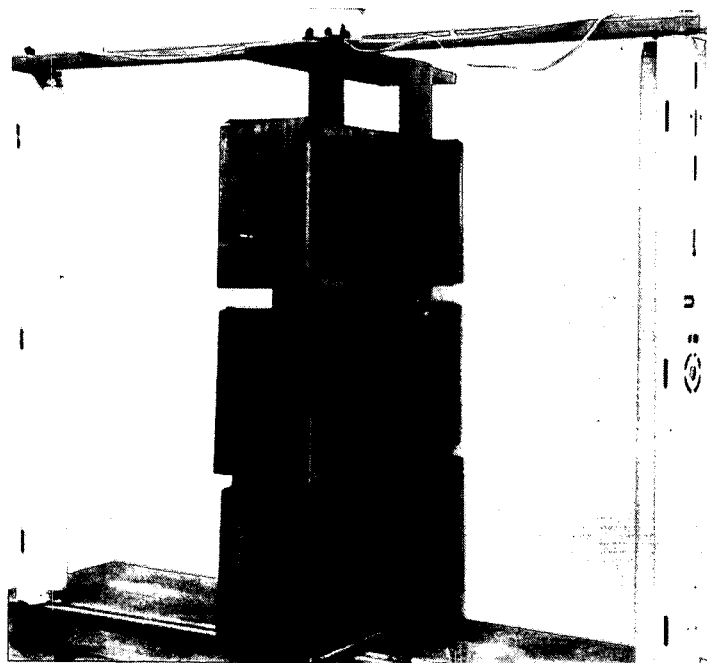


FIGURE 4 A series of units used in the mass production of trichogrammatids. Light sources are also shown.

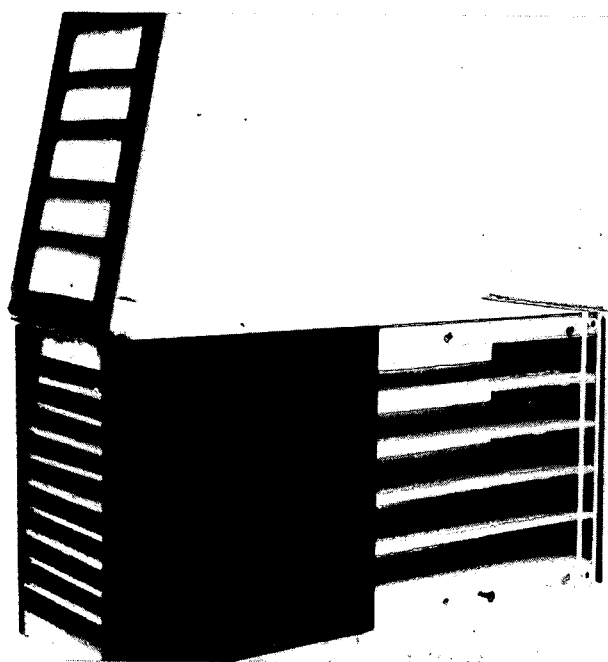


FIGURE 5 A mass rearing unit for trichogrammatids showing darkened sides and seeding shelves.

After 24 hours, the rearing box is opened at the illuminated end and a new card carrying fresh, unparasitized eggs is pushed into the shelf. By this action, the 'old' card is pushed into the 'dark side'. The box is closed and left for an hour, during which time the adult trichogrammatids move from the dark to the light. Next, the dark side is opened and the 'old' card is removed. At the same time, the appropriate seeding card is replaced, and thus a supply of new adults to the 'light side' is maintained.

The rate at which the parasitoids develop is influenced by temperature. Thus parasitoid emergence can be regulated to suit requirements for field releases. The release of the parasitoids so produced is described by Conlong and Hastings².

Larval parasitoids

Goniozus sp.: In March 1982 the parasitic wasp *Goniozus* sp. was found on late instar eldana in the flowering heads of *Cyperus papyrus* at Kosi Bay. Since then *Goniozus* has been found in all coastal papyrus searched in Natal, as far south as Lake Inseze (Figure 6).

Samples of *Goniozus* collected in the field were used to start a laboratory culture at Mount Edgecombe. The rearing method was initially based on that of Girling³ and Ly⁹ but it has been modified to suit the requirements of the local species.

Each day, the newly emerged adults are aspirated from their containers and are placed together for at least four hours in a glass jar so that they can mate. Four fifth-instar eldana larvae are placed in artificially bored sections of cane in a glass jar, together with two newly mated *Goniozus* females. The female lays eggs on the dorsal and lateral surfaces of an eldana larva which she has stung and temporarily immobilized. When the *Goniozus* larvae hatch, they attach themselves to their host and ingest its body juices. An average of eight individuals develop on each late stage eldana larva. The ratio of males to females is 1:5. Males usually emerge before the females and a male may bite open other cocoons in the batch and mate with the females before they emerge. The larval stage lasts about two weeks at 25°C. The female *Goniozus* remains in the eldana burrow with her offspring until they pupate. Thereafter, the female searches for another host larva and immediately parasitizes it. In her lifespan of about 15 to 30 days, the female can parasitize up to three host larvae. At present the culture at Mount Edgecombe can produce a maximum of 500 adults per generation for experimental field releases.

Descampsina sesamiae: The method for rearing the parasitic fly *D. sesamiae* was developed by Nagarkatti and Rao¹⁴ and comprises three stages: rearing and mating the adults; dissecting the gravid females and artificial inoculation of the hosts; rearing the parasitized hosts and recovering the parasitoid puparia.

The survival of the parasitoid in the laboratory culture is much higher on *S. calamistis* than it is on eldana. It is not known whether this is due to differences in compatibility between the parasitoid and the two hosts, or to some effect of the artificial rearing system. Late instar *S. calamistis* larvae are used in most of the laboratory rearing.

Adults are kept in small cages and are provided with water and a diet of sucrose, enzymatic yeast and raisins. For successful mating to occur, the female must be a few hours old and the male must be at least 24 hours old. To induce copulation, pairs of flies are placed in test tubes and gently agitated in bright sunlight. If the age difference between the pair is correct, copulation may commence within 20 seconds. The greater the age difference the more difficult it is to induce mating and if the difference is too great mating cannot be induced. Pairs *in copula* are removed from bright sunlight and are not disturbed until they separate. Mated females are kept individually in small gauze boxes. After a gestation period of seven to ten days, the female is placed in distilled water and the abdomen is split open and the maggots are removed with a single-haired brush. Before the host larvae are inoculated, they are sterilized by shaking them for a few seconds in 2% formaldehyde and then in distilled water. Each inoculated larva is placed in a vial with a piece of sugarcane stalk which has been soaked in streptomycin sulphate. As the fly puparia emerge, they are transferred to an emergence box.

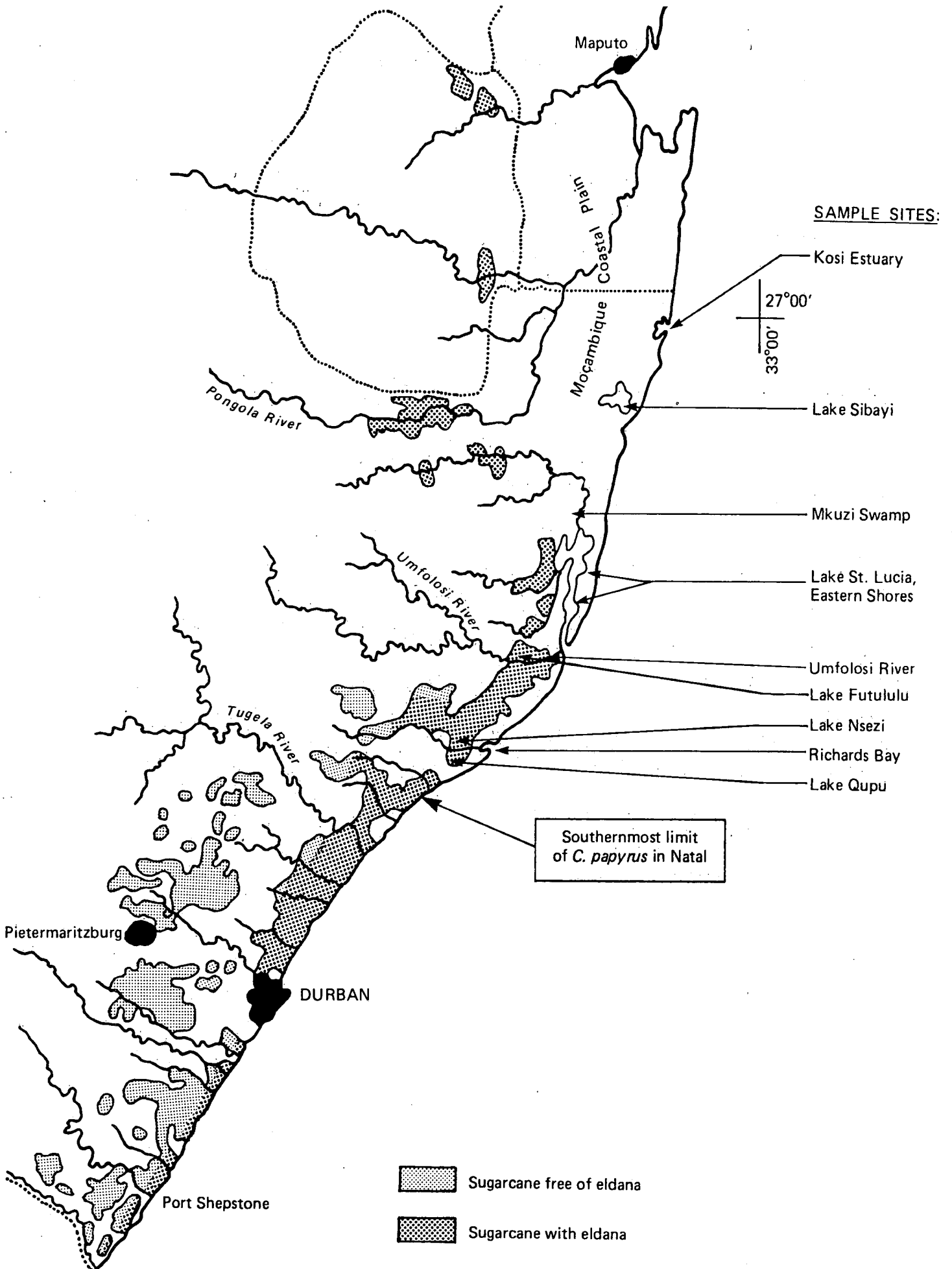


FIGURE 6 Locations of *Cyperus papyrus* wetlands in Natal that have been sampled for parasitoids of eldana. To date, *Goniozus* has been found at all except the southernmost site.

The survival rates of the various stages are given in Table 1. From these data it is possible to assess the number of flies produced in the culture so that experimental field releases can be planned. The number which can be produced is limited by the time required to inoculate the larvae and to induce the adults to mate, and also by the availability of *S. calamistis* larvae. This host is difficult to culture in large numbers and the *D. sesamiae* culture has to be bolstered with *S. calamistis* larvae collected in the field. Six maggot-producing females are obtained for every 100 larvae which are inoculated. This is a very low rate of production for a laboratory culture where the aim is to produce parasitoids for field releases.

TABLE 1

Average survival rates of various life stages of *D. sesamiae* laboratory reared on *S. calamistis* (based on data from 20 generations)

Life stage of <i>D. sesamiae</i>	% survival at each life stage	Numbers of flies surviving at each life stage, per 100 inoculated host larvae
Infestation of <i>S. calamistis</i> by maggots of <i>D. sesamiae</i>	33	33,00
Adult emergence from successfully parasitized <i>S. calamistis</i>	84	27,72
50% of adults are female	50	13,86
Females successfully mated	69	9,56
Mated females that produce viable offspring (maggots)	60	5,74

Conclusion

It is often stressed in the literature that in most cases where a parasitoid has established itself, the releases have been heavy and repeated (DeBach & Bartlett⁴, van den Bosch *et al*¹⁶). The nature of each target organism will determine what rearing operations are required to produce natural enemies for substantial releases. The host cultures for egg and larval parasitoids described in this paper have been established to provide a base for research on the biological control of eldana over the next few years. This research, which is still in its early stages, will follow a number of courses: testing egg parasitoids in inoculative and inundative mass releases; testing currently available

larval parasitoids (*Goniozus sp. Descampsina sesamiae*); importing and testing certain larval parasitoids which are effective against sugarcane borers in other continents (eg *Allorhogas sp.*); studying endemic parasitoids of eldana in wild host plants, and testing some of these against eldana in sugarcane; and screening eldana larvae in local sugarcane in case an endemic parasitoid becomes established.

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