IMPROVED LABORATORY REARING OF *ELDANA SACCHARINA* (LEPIDOPTERA : PYRALIDAE) AND ITS INDIGENOUS PARASITOID *GONIOZUS NATALENSIS* (HYMENOPTERA : BETHYLIDAE)

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Abstract

The development of a 32-cell (multicell) plastic tray and the formulation of an insect diet suitable for both *Eldana saccharina* Walker growth and successful *Goniozus natalensis* Gordh parasitism are outlined. A method of presenting the eldana reared in the multicell trays to *G. natalensis* is discussed. The new and original rearing systems are compared in terms of labour requirements, contamination control, reduction in insect damage, and improved insect production.

Introduction

Several attempts have been made to rear *Goniozus natalensis* Gordh on *Eldana saccharina* Walker economically. The best results were obtained when 4 suitably aged larvae were presented, in borings in sugarcane setts 60 mm in length, to two *G. natalensis* females in a 1 ℓ glass jar (Conlong *et al.*[2]). Mean larval parasitism of 18% was obtained, the mean use of the *G. natalensis* females being about 36% (Conlong *et al.*[3]). When large-scale rearing was initiated the glass rearing jars were replaced by 700 ml plastic jars in order to reduce costs, and to make washing easier. Results obtained were comparable with those of the 1 ℓ glass jars.

Although the method of rearing with sugarcane setts in plastic jars was the most economical in terms of both host and parasitoid use, alternative rearing methods were sought in order to:

- produce eldana larvae more suitable for presentation to *G. natalensis*. In the method of mass rearing in glass jars, eldana larvae are subject to high mortality as a result of cannibalism and disease. Contamination can result in the entire contents of a jar being lost. On average only 40 to 50% of the larvae originally inoculated (approximately 25 per jar), are suitable for presentation to *G. natalensis*
- reduce the handling of eldana larvae. The larvae reared in glass jars need to be harvested and placed in sugarcane setts before presentation to *G. natalensis*. Many deaths occur at this step of the rearing process. This procedure is also laborious and expensive
- replace sugarcane as a rearing substrate as it is time-consuming to prepare; has a limited life in the laboratory and cannot support the entire life-cycle of *G. natalensis*; is prone to fungal contamination resulting in mortality of both eldana larvae and *G. natalensis* progeny, and the quality varies considerably due to the weather
- reduce handling of *G. natalensis* progeny. After 2 weeks the *G. natalensis* cocoons need to be removed from the sugarcane setts. At this stage of the life-cycle, the parasitoids are most easily handled. Splitting of the sugarcane setts and removal of the cocoons does however result in high mortality (up to 50%). This step in the rearing procedure is also expensive in terms of time and labour requirements. The harvested cocoons are stored in vials which must be emptied in order to collect the emerging adults, resulting in repeated handling, which again could lead to damage of the parasitoids
- improve levels of parasitism by *G. natalensis*
- reduce the amount of rearing equipment required, and make use of disposable containers where possible to limit the amount of cleaning required.

This paper documents the development of techniques designed to reduce or eliminate these shortcomings.

Rearing developments

Ideally the parasitoids should be exposed directly to the eldana larvae in the diet in which they are developing, thus eliminating the need for sugarcane setts and for handling of the larvae. The diet medium should not encumber *G. natalensis* at any stage in its life-cycle.

Assessment of eldana diet and rearing

The diet medium developed by Atkinson[1] for eldana (Table 1) and dispensed into 1 ℓ glass jars is an unsuitable substrate for *G. natalensis* activity. Because of its high moisture content, eldana larvae in the medium do not produce easily accessible borings. A 15% increase in fibre content (dried, finely crushed sugarcane) resulted in a diet which desiccated rapidly in the glass jars, causing retarded larval development.

Table 1

The formulation and quantities of diets used for eldana growth and *G. natalensis* parasitism studies

Ingredient	*Eldana saccharina* diet	*Sesamia calamistis* diet	Modified *Sesamia calamistis* diet
Dried crushed sugarcane	22 g	115 g	250 g
Ground chickpea	120 g	69 g	100 g
Glucose	20 g		
Casein	12 g	17 g	17 g
Brewer's yeast powder	12 g	17 g	17 g
Ascorbic acid	4 g	3 g	3 g
Sorbic acid	2 g	1 g	
Ferric citrate		0,06 g	0,06 g
Calcium lactate		1 g	1 g
Tri-sodium citrate		2 g	2 g
Citric acid		2 g	2 g
Sodium chloride		0,6 g	0,6 g
Multivitamins		2 g	2 g
Agar	10 g	10 g	10 g
Water	1 000 ml	1 000 ml	1 000 ml
Nipagin (methyl paraben)	1,6 g		
Sodium propionate		9 g	9 g
Streptomycin sulphate		0,5 ml	1 ml
Dithane		0,2 g	0,2 g
Benomyl	0,03 g		
Formaldehyde 40%	1,2 ml	2,3 ml	3,5 ml
Methanol	50 ml	35 ml	35 ml

When dispensed into 20 ml plastic flip-cap vials and inoculated with single medium-to-large sized eldana larvae, this diet desiccated at a slower rate and the larvae produced well-defined borings and copious amounts of frass. Single *G. natalensis* females were introduced into the vials when the eldana larvae had attained a size suitable for parasitism.

Rearing of parasitoids in eldana diet

Examination of the vial contents after 2 weeks revealed that 66,7% (n = 30) of the larvae had been parasitised by *G. natalensis*, but the parasitoid eggs shrivelled up after failing to develop.

Modified eldana diets were then tested. Single ingredients were omitted in the hope of finding one which might have been exerting an inhibitory effect on the hatching of parasitoid eggs. Successful parasitism (development to cocoon stage) of 8,3% (n = 49) was eventually obtained with the exclusion of the preservative sorbic acid.

However, the percentage of larvae with unhatched parasitoid eggs was still relatively high (29,2%).

Alternative diet medium

It was then decided to test the diet developed for *Sesamia calamistis* Hamps (Table 1). As with the eldana diet, sorbic acid had an inhibitory effect on the development of *G. natalensis* offspring, with a high level (42,9%; n = 50) of unsuccessful parasitism recorded. When sorbic acid was omitted from the diet, successful parasitism occurred at a level similar to that in the eldana diet (8,2%; n = 49) while 44,0% had unhatched parasitoid eggs. It was found that the level of parasitism could be improved by increasing the amounts of dried crushed cane, chickpea, and antimicrobials (to compensate for sorbic acid) in the diet (Table 1). The maximum parasitism obtained from a single diet was 40,0% (n = 25).

Alternative rearing containers

Suitable rearing containers were not available in South Africa. Samples were obtained of a 32-cell (multicell) plastic tray being used successfully in the United States. These are custom-made for the rearing of lepidopterous larvae. The trays were tested for eldana and *G. natalensis* rearing and initial results were encouraging. A local manufacturing company was approached and a tray was designed for eldana rearing purposes. It consists of 32 cavities, each with a volume of approximately 12 ml.

Diet preparation

A 30 ℓ high-speed foodmixer was modified at the Experiment Station for the bulk preparation of eldana diet. It is fitted with a pump and metering unit for automatic dispensing of diet into the multicell trays. The diet is prepared in 15 ℓ batches and 8 ml is dispensed into the cells, 4 cells at a time.

Inoculation of diet with eldana

To place the first instar larvae into each cell of the multicell trays and to keep them in the cells before the tray has been sealed has proved to be a problem. This has been overcome by the development of a corn-cob grit inoculator (Davis & Oswalt[4]). First instar larvae are mixed with corn-cob grits in a known ratio, and are then dispensed onto the surface of the diet in each cell. In this way larvae can be dispensed quickly, and do not readily move out of the grits.

Different ratios of larvae and corn grits have been tested so that each cell in the multicell tray will contain at least one eldana larva (Table 2). Agitation of the larvae and corn-cob grits is important both before and during the inoculation process to ensure a random dispersion of the larvae.

Table 2

Summary of the number and dispersion of first instar eldana larvae obtained in the 32-cell multicell trays when the corn-cob grit inoculator was used

No of larvae expected per multicell tray	Cell occupation		No of larvae		Larval dispersion in cells								Inoculation method
					1		2		3		4		
	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD	
32	77,2	–	77,2	–	100,0	–	0	–	0	–	0	–	Hand
35	61,6	16,5	86,6	29,2	70,8	11,5	23,2	11,4	5,6	5,4	2,0	4,7	Rough grits
40	76,6	8,3	121,0	27,4	59,8	18,4	43,4	22,6	7,0	7,3	3,3	5,2	Rough grits
45	75,0	16,3	115,9	34,2	42,3	21,0	29,4	12,3	18,0	15,0	2,2	2,8	Rough grits
40	66,8	12,7	108,8	45,1	68,6	7,9	17,9	5,9	8,8	7,6	2,1	2,8	Fine grits
45	83,8	13,5	140,0	42,2	54,8	13,0	32,8	7,6	8,4	4,2	2,6	3,7	Fine grits

Newly hatched larvae were then inoculated into the vials for further testing of diets. Larvae proved to be more suitable for parasitism when the entire larval period was spent in the diet. The highest level of successful parasitism obtained from a diet was 55,8% (n = 163), with a further 22,9% of the eldana larvae carrying unhatched parasitoid eggs. The level of parasitism was sufficiently high to warrant the use of the diet in large-scale rearing despite a substantial percentage of unsuccessful parasitism. This diet is now routinely used in the rearing of eldana.

With the vial system of rearing, optimum use is made of the eldana larvae, as only one is required per *G. natalensis* female (1 : 1 host-parasitoid ratio). However, the use of vials is not feasible in a large-scale rearing programme. They are not disposable because of the costs involved, and they need permanent ventilation. In addition, problems are experienced with dispensing the diet into the vials, and with cleaning them after use.

Table 3

Preliminary assessment of eldana larval survival in the different rearing containers and media

Rearing container	Number assessed	Number of 1st instar inoculated per container	Mean total pupae/larvae per container recovered	% survival
Glass jar, no vermiculite	75	20	11,5	57,5
E. saccharina diet	130	25	12,3	49,2
Glass jar, vermiculite	70	20	12,6	63,0
E. saccharina diet	25	25	12,9	51,6
Plastic multicell tray, *S. calamistis* based diet	74	32	24,7	77,2

The multicell trays are covered with plastic foodwrap and each cell is ventilated by perforating the wrap. In the United States, a perforated heat-seal mylar sheet is used to close the tray (F.W. Davis pers.comm), but this product is not locally available.

Eldana development in multicell trays

The numbers of eldana larvae surviving in the multicell trays have been assessed and compared with results from the glass rearing jars. The survival rates in the multicell trays and jars are summarised in Table 3.

Parasitoid rearing in multicell trays

Several methods of presenting eldana to *G. natalensis* in multicell trays have been attempted (Table 4). The highest *G. natalensis* female use was obtained in 4 ℓ shallow food containers, each holding a single multicell tray. This method is now exclusively used for *G. natalensis* rearing.

The *G. natalensis* females are introduced into the rearing container via a funnel inserted into an aperture in the lid. The ventilation holes in the plastic foodwrap cover are enlarged to facilitate entry of the parasitoids into the cells of the multicell trays.

The entire rearing process takes place in the plastic container. The *G. natalensis* progeny are left undisturbed in the multicell trays to complete their development. Newly emerged *G. natalensis* adults leave the cells and accumulate within the plastic container from which they are readily collected.

In the multicell trays an average of 5,0 *G. natalensis* females were obtained per parasitised eldana larva.

The sugarcane culture in comparison initially produced an average of 6,6 females per parasitised larva, but this figure later decreased to 4,6 (Table 5).

Table 4

Initial results of methods used to present eldana to *G. natalensis* females

	Method	Result		Problems
		Number of multicell trays presented	% female *G. natalensis* use	
1	Multicell trays with plastic foodwrap cover removed, placed in plastic bag and sealed. *G. natalensis* introduced through hole in bag in 1 *G. natalensis* to 2 *E. saccharina* ratio. Two bag sizes tested:			Lack of ventilation. Escape of *E. saccharina* into plastic bag. Escape of *E. saccharina* and *G. natalensis* through plastic bag. Yeast contamination
	a) slightly larger than multicell tray	16	4,5	
	b) twice size of multicell tray	17	7,3	
2	*G. natalensis* introduced into individual cells of multicell tray through plastic foodwrap cover	4	15,3	Escape of *E. saccharina* and *G. natalensis* through plastic foodwrap cover of multicell tray
3	*G. natalensis* introduced into multicell tray as per method 2. When *G. natalensis* entered diet plastic foodwrap cover removed and tray covered with inverted unused multicell tray. These were stapled or taped together	8	11,8	*G. natalensis* moved to adjacent cells and escaped from tray. Inadequate ventilation
4	Multicell trays cut up containing 2 to 8 *E. saccharina*. Cut cells placed in 700 ml plastic jars. 1 *G. natalensis* to 2 *E. saccharina* ratio used	128	40,7	Logistical problems with bottles and cutting of multicell trays
5	Multicell trays placed in shallow 2 ℓ or 4 ℓ plastic containers. 1 *G. natalensis* to 2 *E. saccharina* ratio used.			Initial problem of yeast contamination and inadequate ventilation.
	a) 2 ℓ container + 2 multicell trays	2	16,4	Too crowded
	b) 2 ℓ container + 1 multicell tray	119	31,1	
	c) 4 ℓ container + 2 multicell trays	31	15,6	
	d) 4 ℓ container + 1 multicell tray	93	41,8	

Table 5

An initial comparison of the multicell tray/plastic container method of rearing *G. natalensis* and the sugarcane piece/plastic jar method

No vessels inoculated		Number of *G. natalensis* females inoculated		Number of *E. saccharina* larvae parasitised		% *G. natalensis* female use		Total female *G. natalensis* offspring		No females per parasitised *E. saccharina*	
Trays	Jars	Trays	Jars	Trays	Jars	Trays	Jars	Trays	Jars	Trays	Jars
17	313	220	626	113	190	51,4	30,4	615	588	5,4	3,1
9	700	103	1 400	55	561	53,4	40,1	281	2 925	5,1	5,2
47	640	446	1 280	223	480	50,0	37,5	1 096	2 043	4,9	4,3
7	1 000	73	2 000	44	434	60,3	21,7	163	1 952	3,7	4,5
10	1 056	121	2 112	39	623	32,2	29,5	180	2 718	4,6	4,4
1	478	8	956	8	211	100,0	22,1	37	1 446	4,6	6,9
17	512	223	1 024	77	80	34,5	7,8	373	430	4,8	5,4
18	380	221	760	75	114	33,9	15,0	431	573	5,7	5,0
4	567	31	1 134	18	163	58,1	14,4	74	515	5,1	3,2
12	533	81	1 066	46	147	56,7	13,8	328	656	7,1	4,5
142	6 179	1 527	12 358	698	3 003	45,7	24,3	3 578	13 846	5,1	4,6

Cannibalism and predation in multicell trays

One hundred and fifty-one multicell trays have been examined for the effects of cell occupation by more than one eldana larva. Of the cells examined, 14,4% were found to contain between 2 and 6 eldana larvae, and 16,4% of these were successfully parasitised. Only one batch of *G. natalensis* cocoons (0,6%) was damaged by an eldana larva occupying the same cell. Similarly, a low level of cannibalism (1,0%) of eldana larvae and pupae occurred in the multicell trays.

Contamination by micro-organisms of diet in the multicell trays

In initial trials inadequate ventilation in both 2 and 4 ℓ plastic food containers caused condensation and contamination of the diet by yeasts and other fungi, resulting in a reduction in levels of parasitism. Improved ventilation resulted in an increase in female *G. natalensis* use from 19,7% (n = 64) to 49,6% (n = 91).

Yeast contamination of the diet as the larvae matured was traced to the presence of active yeast cells in the brewer's yeast powder in the diet. Autoclaving prior to incorporation into the diet has now largely eliminated the problem. Parasitism by *G. natalensis* did not appear to be adversely affected by the yeast contamination, but the autoclaving treatment resulted in an increase of 7,1% in female *G. natalensis* use (Table 6).

Table 6

Assessment of *G. natalensis* female use in multicell trays containing autoclaved and unautoclaved brewer's yeast powder

Treatment	Number multicell trays presented to *G. natalensis*	Number female *G. natalensis* inoculated	Number *E. saccharina* larvae parasitised	% female *G. natalensis* use
Unautoclaved	186	2 640	794	30,1
Autoclaved	65	629	234	37,2

The cause of sporadic outbreaks of the fungus *Aspergillus* sp. is presently under investigation. Hygiene during preparation and inoculation of the multicell trays may be an important factor.

Conclusions

The multicell method of rearing both host and parasitoid in the same container has eliminated many of the problems experienced with the sugarcane-based culture. By avoiding the handling of both the eldana larvae and *G. natalensis* cocoons, considerable savings of host and parasitoid material, time and labour have been made. Mortality rates of both hosts and parasitoids have been drastically reduced, with cannibalism and the spread of contaminants in the diet being controlled to a large extent by the culturing of the larvae in isolated units.

Many refinements of this improved rearing method are envisaged with the aim of producing an optimal diet with a minimum of infection by micro-organisms and a high degree of suitability to *G. natalensis*. Attempts are under way to adapt the multicell rearing approach to other parasitoids presently reared on eldana. These include the dipterous larval parasitoid *Paratheresia claripalpis* Wulp, and the hymenopterous pupal parasitoid *Xanthopimpla stemmator* Thunb.

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