

LABORATORY REARING OF *PAREUCHAETES INSULATA* (LEPIDOPTERA: ARCTIIDAE), A BIOLOGICAL CONTROL AGENT OF *CHROMOLAENA ODORATA* (ASTERACEAE)

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Abstract

Since 2001, *Pareuchaetes insulata* Walker has been reared in the Insect Unit of the South African Sugar Association Experiment Station for release at selected sites in KwaZulu-Natal, by the Department of Water Affairs and Forestry's Working for Water programme. To date, more than 650 000 *P. insulata* larvae have been supplied to Working for Water for release against *Chromolaena odorata*, an extremely invasive alien plant. This paper describes the rearing methodology used to produce *P. insulata*, and the monthly production over the period 2001 to 2002.

Keywords: *Pareuchaetes insulata*, Arctiidae, *Chromolaena odorata*, biological control, mass rearing, release

Introduction

Chromolaena odorata (L.) King and Robinson (Asteraceae) is the most serious alien invasive plant in the subtropical regions of southern Africa. The South African sugarcane belt is located in this region, and consequently many sugarcane farms are infested with *C. odorata*. A biological control initiative against *C. odorata* was launched in 1988 in South Africa. Since then, several biological control agents have been imported and tested against *C. odorata* (Zachariades *et al.*, 1999). Two agents have been released without apparent establishment. *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae), a defoliator, was released in 1989 and 1998, and its relative, *Pareuchaetes aurata aurata* (Butler), was released between 1990 and 1993 (Zachariades *et al.*, 1999; Conlong, 2000).

The Weeds Division of the Agricultural Research Council's Plant Protection Research Institute (ARC-PPRI) approached the South African Sugar Association Experiment Station (SASEX) to rear *P. aurata aurata* in 1992 (Conlong and Way, 2000). Releases were carried out over a one-year period. Rearing of *P. aurata aurata* was terminated because no establishment was evident at any of the release sites (Conlong, 2000). *Pareuchaetes insulata* Walker was imported by the Weeds Division of the ARC-PPRI from Florida, United States of America, and approved for release in 1999 (Zachariades *et al.*, 1999). After failed attempts to rear this insect in their laboratories at Tzaneen, in the Northern Province of South Africa, the ARC-PPRI in 2000 requested that SASEX rear *P. insulata* in their Insect Unit at Mount Edgecombe, KwaZulu-Natal Province, for mass release against *C. odorata*. The Insect Unit provides an environmentally controlled multiple-room facility, which allows for efficient mass rearing of insects (Conlong, 1992). These multiple rooms allow for separation of clean and dirty operations, sterilising operations and workflow patterns that result in high production rates of healthy insects (Conlong and Way, 2000). The Insect Unit received the first consignment of *P. insulata* from the ARC-PPRI in February 2001.

Colony establishment commenced immediately and, by April 2001, excess individuals were available for release. The insects were supplied to the Department of Water Affairs and Forestry (DWAF), for use in their Working for Water (WfW) programme, which performed the release operations.

Materials and Methods

Host plant preparation

Chromolaena odorata was collected daily from areas surrounding Mount Edgecombe, KwaZulu-Natal (29°42'S, 31°02'E, altitude 96 m). Leafy stalks of good quality were cut into lengths of approximately 20 cm. Blocks of floral Oasis® measuring 10 x 5 x 5 cm were soaked in water to which 5 ml Milton® per litre had been added. Five to six *C. odorata* stalks were pressed into the Oasis to form bouquets. Freezette trays® (4 L) were sterilised by spraying with D-germ® and drying with paper towel. A single *C. odorata* bouquet was placed into each sterilised tray.

Rearing of Pareuchaetes insulata

Larval inoculation

Newly enclosed first instar larvae of *P. insulata* were inoculated onto the bouquets of *C. odorata* at a stocking rate of 40 larvae per tray. After inoculation, the tray was sealed with a nylon gauze lid. Two hundred first instar larvae were inoculated per day. Inoculated trays were placed in larval growth rooms maintained at a temperature of $26 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5\%$, and with an 8 h light:16 h dark photophase. Light was provided by fluorescent tubes of colour W58/840.

During larval development, fresh bouquets were provided for the larvae at least every four days until pupation took place. The stocking rate for each tray was gradually reduced at each successive instar to a final stocking rate of 10 sixth instar larvae per tray, consequently increasing the number of trays in the growth room.

Pupation

The number of pupae collected daily was recorded, as was the date of pupation. Fifty pupae were retained for the laboratory culture by transferring them into a plastic 9 cm diameter round petri dish containing autoclaved vermiculite. The petri dish was placed in a 30 x 30 x 30 cm emergence box constructed from clear Perspex, and provided with netted sleeves for access. Excess pupae were maintained until adults emerged. These adults were released.

Adult emergence and oviposition

Adults from the stock of laboratory pupae were counted and sexed, and the date of emergence was recorded. They were then placed into a mating box (as described for the emergence box) at a stocking rate of five females and five males per box, for mating and oviposition. Sponges (3 x 3 x 3 cm) soaked in a 1:1 honey:water solution were provided as food for the adults, and replaced on a daily basis. One *C. odorata* bouquet was supplied per mating box, for oviposition by the females. Bouquets were replaced daily, and eggs batches collected from the previous day's bouquets. The position of the egg batches and the number of eggs laid were recorded on a daily basis until the adults died. Leaves, with egg batches attached, were placed in plastic 9 cm petri dishes and held in the same larval growth room as the inoculated trays. Dates of oviposition, female mortality and emergence of first instar larvae were recorded.

Preparation of field release material

Large plastic release boxes measuring (100 L), with stainless steel gauze (mesh size 0.1 mm) windows (26 x 16 cm and 49 x 21 cm) on all four sides and a solid lid were filled with *C. odorata* bouquets. Excess first instar larvae were placed into the release boxes at a stocking rate of 1000 per box, and supplied to WfW for release in selected areas of KwaZulu-Natal.

Results and Discussion

Life cycle data

Although *P. aurata aurata* had been successfully mass reared in South Africa (Conlong and Way, 2000), and *P. pseudoinsulata* in Ghana (Zachariades *et al.*, 1999; Braimah and Timbilla, 2000), *Pareuchaetes insulata* had never before been mass reared (Strathie-Korrûbel and Zachariades, 2000). It was thus important to collect as much life cycle data as possible during the rearing of *P. insulata*, so that quality control parameters could be set and production estimates formulated. Life cycle data are given in Table 1.

Table 1. Life cycle data for *P. insulata* determined from the laboratory rearing of this species by the Insect Unit at the South African Sugar Association Experiment Station (from Parasram, 2002).

Life stage	Range (days)			Survival rate (%)
	Min	Max	Average	
Egg	5	5	5	94.7
Larvae	21	32	27.6	92.2
Pupae	8	12	9.6	78.4
Male moth	2	13	7.2	93.6
Female moth	4	10	7.4	93.6
Total cycle			49.5	
Egg per batch	1	187	28.6	
Fecundity per female	80.4	452.7	336	

Production for field release

The main aim of maintaining the colony of *P. insulata* was to provide reliable numbers of high quality insects for field release (Singh and Moore, 1985). Table 2 summarises the monthly production of the laboratory colony of *P. insulata* for its own maintenance, and the supply of larvae for field releases.

Table 2. Laboratory production of *P. insulata* and numbers of larvae released.

Month	No. larvae inoc	No. pupae	% pupation	No. pupae lab stock	Emergence			% emerge	Sex ratio (F:M)	No. larvae released
					Females	Males	Total			
March 2001	1 800	1 422	79	-	-	-	-	-	-	
April	3 000	2 457	81.9	762	387	302	689	90.0	1.5:1	12 452
May	4 400	3 613	82.1	1 150	586	386	972	86.1	1.9:1	19 266
June	4 200	3 300	78.6	1 050	551	345	896	85.3	1.8:1	24 683
July	4 400	3 364	76.5	1 100	516	327	843	76.6	1.7:1	26 728
August	4 400	3 450	78.4	1 100	529	333	862	78.4	1.8:1	27 489
September	3 800	3 024	79.6	950	531	289	820	86.3	2.0:1	19 447
October	4 600	3 574	77.7	1 150	604	326	930	80.9	2.0:1	15 892
November	4 400	3 439	78.2	1 100	605	314	919	83.5	2.1:1	31 018
December	3 600	2 944	81.8	900	504	277	781	86.8	1.9:1	23 060
January 2002	4 400	3 572	81.2	1 100	535	344	879	79.9	1.7:1	37 621
February	3 800	2 957	77.8	950	471	321	793	83.5	1.8:1	27 816
March	4 000	3 016	75.4	1 000	504	297	801	80.1	1.8:1	31 696
April	4 400	3 673	83.5	1 100	558	326	884	80.36	1.9:1	31 439
May	4 400	3 513	79.8	1 100	474	383	857	77.9	1.4:1	47 104
June	3 800	2 721	71.6	950	478	290	768	80.84	1.7:1	40 751
July	4 600	3 396	73.8	1 150	572	329	907	78.95	1.9:1	44 954
August	4 200	3 340	79.5	1 050	423	294	717	68.29	1.5:1	42 795
September	3 000	2 556	85.2	1 000	453	254	707	70.70	2.1:1	45 232
October	4 400	3 667	83.3	1 150	445	313	758	65.91	1.9:1	40 718
November	4 200	3 152	75.0	1 050	399	232	631	60.10	1.9:1	38 750
December	3 400	982	28.8	700	276	182	458	65.42	1.7:1	45 056
Total	88 480	67 213		21 856	10 524	6 544	17 075			673 967
Average			73.6					77.8	1.8:1	

Conclusion

The above shows that a laboratory colony of *P. insulata* can be maintained in the insect unit, using the methods described, to provide material for large scale releases. From April 2001 to December 2002, SASEX provided a total of 673 967 *P. insulata* for release against *C. odorata* in selected areas of KwaZulu-Natal. The release phase of this project is described elsewhere in these proceedings.

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