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M88/DAQ22L
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DAQ22 (Spare)

COTTON RESEARCH COUNCIL

RG 20/1/92

FINAL REPORT

PROJECT TITLE: DAQ22L AN EVALUATION OF THE POTENTIAL OF EGG PARASITES FOR THE CONTROL OF HELIOTHIS IN COTTON.

RELATED PROJECT: DAQ39L EGG PARASITES FOR HELIOTHIS CONTROL IN COTTON.

PERIOD: July 1985 - June 1988.

ORGANISATION: Queensland Department of Primary Industries.

LOCALITY: Toowoomba.

PROJECT SUPERVISOR: Dr. P. H. Twine.

RESEARCH STAFF: Mr. B.C. Scholz.

SUMMARY

Heliothis armigera and *H. punctigera* are serious pests of cotton in Australia. Control of *Heliothis* is currently dependent on insecticides. Levels of resistance to several of the major chemical groups of insecticides is sufficient to threaten the long term use of them for *Heliothis* control. This project evaluated one alternative non-chemical form of *Heliothis* management that may have potential to be used alone or in combination with other techniques - egg parasitoids. In the past, field trials with egg parasitoids in Australia have involved releasing an introduced species that was readily available from overseas research institutions. This project was the first attempt to evaluate native egg parasitoids for *Heliothis* control in cotton.

Six species of egg parasitoids were collected from field crops in Queensland. Of these, four species were new records for the State. Laboratory colonies of egg parasitoids were established so that the potential of each species for use as a biocontrol agent against *Heliothis* could be evaluated.

Laboratory and glasshouse studies found significant differences in *Heliothis* parasitism levels, temperature adaptations, host-plant adaptation, host handling behaviour, host acceptance behaviour and host searching efficiency between different species of egg parasitoids.

Pre-release evaluation studies clearly indicated that two species of egg parasitoids (*Trichogramma carverae* and *Trichogrammatoidea bactrae*) have potential for *Heliothis* management and should be evaluated in field trials in the future.

INTRODUCTION

The cotton bollworm (*Heliothis armigera*) and the native budworm (*H. punctigera*) are serious pests of field, horticultural and ornamental crops in Australia. The annual loss to the economy caused by *Heliothis* in Queensland

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was recently estimated at averaging \$73 million, with the annual cost to the cotton industry averaging \$14 million (McGahan *et al.*, 1989).

To date, insecticides are the only effective means of controlling *Heliothis* on cotton in Australia. *H. armigera* resistance to the synthetic pyrethroids, however, is now widespread in the major cropping areas of eastern Australia (Daly, 1988).

The combined effects of insecticide resistance, legislation, increased public concern, and higher insecticide costs suggests that insecticides will not remain the sole pest management tool for the cotton industry in the future. Supplementary control tactics must therefore be developed and integrated into existing management strategies. Natural enemies represent one such supplementary tool. Egg parasitoids, in particular, are ideal for biocontrol programmes because they are comparatively easy to mass rear, store and transport.

Egg parasitoids of the genus *Trichogramma* are frequently used as inundative biological control agents of lepidopterous pests. Inundative biocontrol involves releasing large numbers of natural enemies to overwhelm a pest population and maintain pest density below that causing economic damage. In Australia, field trials with the introduced *Trichogramma pretiosum* (an American species) provided inadequate control of *Heliothis* populations on cotton (Twine and Lloyd, 1982). *T. pretiosum* was released because it was readily available and no attempt was made to evaluate the suitability of this species before field release.

There are approximately 130 species of *Trichogramma* in the world, yet all American and Australian field trials with egg parasitoids against *Heliothis* have involved *T. pretiosum*. No attempt has been made to evaluate other species of egg parasitoids.

The research presented in this report is the first attempt to evaluate native egg parasitoids as biocontrol agents of *Heliothis* in cotton.

RESEARCH OBJECTIVES

- i) Supplement existing information on the identification, abundance and distribution of native egg parasitoids of *Heliothis*.
- ii) Develop suitable laboratory rearing techniques for egg parasitoids and maintain several colonies.
- iii) Study the biology and ecology of native egg parasitoids to develop criteria that enable the selection of a candidate species that has the greatest potential as a biocontrol agent of *Heliothis* in cotton.

RESULTS

Field Surveys of Native Egg Parasitoids: Egg parasitoids of *H. armigera* and *H. punctigera* were collected from field crops on the Darling Downs, Biloela, Kingaroy and Emerald. Six species of egg parasitoids were collected (Table 1). Of these, only *Trichogramma australicum* and *Telenomus* sp. have been recorded before (Twine, 1973), and they accounted for 6% and 2% of all parasitoids collected respectively. *Trichogrammatoidea bactrae* is a

new record for Australia, previously being known from the Oriental Region (Nagaraja, 1978). *T. bactrae* was the most abundant species collected, accounting for 89% of all egg parasitoids. *Trichogramma carverae* belongs to the subgenus *Trichogrammanza* which is endemic to Australia. This is the first record of *T. carverae* in Queensland, previously being recorded in Victoria and South Australia (Oatman and Pinto, 1987). *T. carverae* was reared from 3% of parasitized *Heliothis* eggs. There were also two new species (one *Trichogramma* and one *Trichogrammatoidea*) collected, but these accounted for only 4 of 4039 parasitized *Heliothis* eggs collected and were considered rare.

TABLE 1.

The species of egg parasitoids reared from *Heliothis* eggs collected from field crops during 1985-1988.

FAMILY	SPECIES
TRICHOGRAMMATIDAE	<i>Trichogramma australicum</i> <i>Trichogramma (Trichogrammanza) carverae</i> <i>Trichogramma</i> sp. (new species) <i>Trichogrammatoidea bactrae</i> <i>Trichogrammatoidea</i> sp. (new species)
SCELIONIDAE	<i>Telenomus</i> sp.

Laboratory colonies of each species were established so that their biology could be studied and their biocontrol potential evaluated.

Natural Levels of Egg Parasitism in Field Crops: Natural levels of *Heliothis* egg parasitism varied from 0.0% in chickpeas to 75% in sweet corn (Table 2). In cotton 17% of all eggs collected were parasitized, but this level varied from zero to 79% depending on the time of the season and incidence of insecticide application (Figure 1).

TABLE 2.

Natural egg parasitism levels recorded in Queensland field crops 1985-88.

FIELD CROP	NO. SAMPLES COLLECTED	NO. EGGS COLLECTED	% EGGS PARASITIZED	SAMPLE RANGE (%)
linseed	1	37	0.0	--
sweet corn	1	63	74.6	--
chickpea	2	118	0.0	--
navy bean	1	171	43.3	--
sunflower	4	340	8.8	0.0-29.8
pigeon pea	4	566	1.2	0.0 - 3.6
maize	6	1804	37.6	8.3-50.6
sorghum	7	2589	46.9	32.6-77.0
cotton	68	11572	17.2	0.0-78.9
TOTAL	94	17260	23.4	

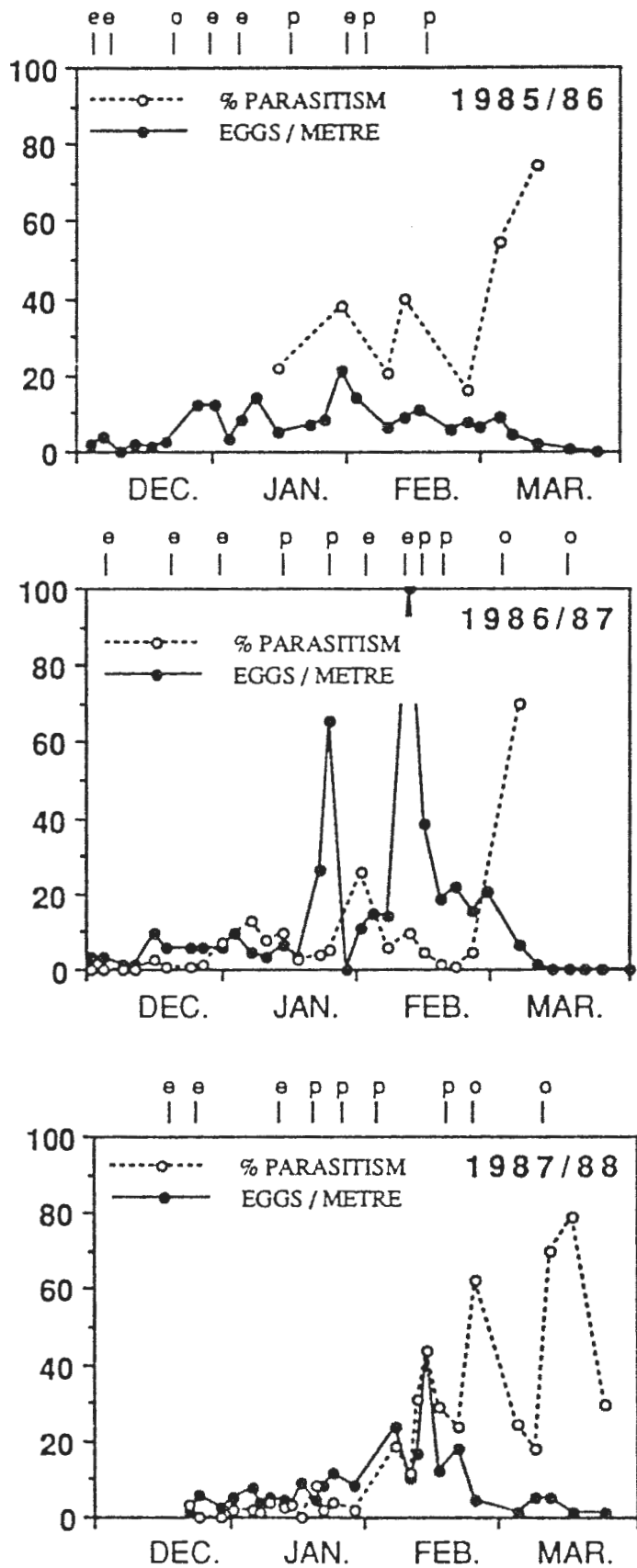


FIGURE 1: The variation in the number of *Heliiothis* eggs per metre and the percentage of egg parasitism in siokra cotton sampled on the Darling Downs for three seasons from 1985/86 to 1987/88. The spray intervals and insecticides used are shown: e = endosulfan, p = synthetic pyrethroid, o = organophosphate.

Laboratory Parasitism Levels: *T. carverae* parasitized significantly more eggs per lifetime than all other species studied (Table 3).

TABLE 3.
Laboratory parasitism levels of egg parasitoids.

SPECIES OF EGG PARASITOID	NUMBER OF REPLICATES	NUMBER OF HOSTS* PARASITIZED	FEMALE LIFESPAN (days)
<i>T. australicum</i>	25	35.7 ± 3.5 b	11.4 ± 1.2 a
<i>T. carverae</i>	22	57.2 ± 4.8 a	10.0 ± 0.9 a
<i>Trichogramma</i> sp.	23	36.9 ± 3.3 b	9.7 ± 0.8 a
<i>T. bactrae</i>	22	22.2 ± 3.0 c	4.6 ± 0.3 b
<i>Trichogrammatoidea</i> sp.	21	31.8 ± 5.2 b	5.8 ± 0.6 b
<i>Telenomus</i> sp.	8	18.8 ± 5.8 bc	8.3 ± 1.8 a

* Hosts = *Heliothis armigera*. Values represent means ± standard errors. Column means followed by the same letter are not significantly different for P<0.05. Laboratory conditions: 25°C, 75%R.H..

Telenomus sp. was difficult to rear on *H. armigera* eggs and the laboratory colony of this species died before completion of the experiment.

Temperature Adaptations: The rate of development of each trichogrammatid parasitoid colony was studied at different constant temperatures from 10-36°C (R.H. 75%). Pradhan's (1946) temperature model was fitted to the data and significant differences were found between species (Table 4).

TABLE 4.
Developmental equations and temperature thresholds .

SPECIES OF EGG PARASITOID	PRADHAN EQUATION *	TEMPERATURE THRESHOLDS (°C)	
		LOW	HIGH
<i>T. australicum</i>	$D = 16.124 e^{-0.00611(33.93-T)^2}$ a	14.5	34.1
<i>T. carverae</i>	$D = 13.530 e^{-0.00633(31.82-T)^2}$ b	12.3	31.9
<i>Trichogramma</i> sp.	$D = 13.039 e^{-0.00665(31.15-T)^2}$ c	12.4	31.2
<i>T. bactrae</i>	$D = 11.978 e^{-0.00658(30.27-T)^2}$ d	10.4	30.3
<i>Trichogrammatoidea</i> sp.	$D = 11.005 e^{-0.00793(29.54-T)^2}$ cd	14.3	29.5

* After Pradhan (1946); D = rate of development (%/day) at temperature T; equations followed by the same letter are not significantly different for P<0.05. Linear regression was used to estimate lower and upper temperature thresholds; all equations were significantly correlated for P<0.05.

T. bactrae developed quickest at temperatures below 25°C, and *T. australicum* at temperatures above 25°C. *T. carverae* was the best generalist species. A higher proportion of *T. carverae* successfully parasitized *H. armigera* eggs at temperatures above 30°C (Figure 2) and this is an important characteristic when selecting a candidate species for use in Australia where summer temperatures can exceed 30°C.

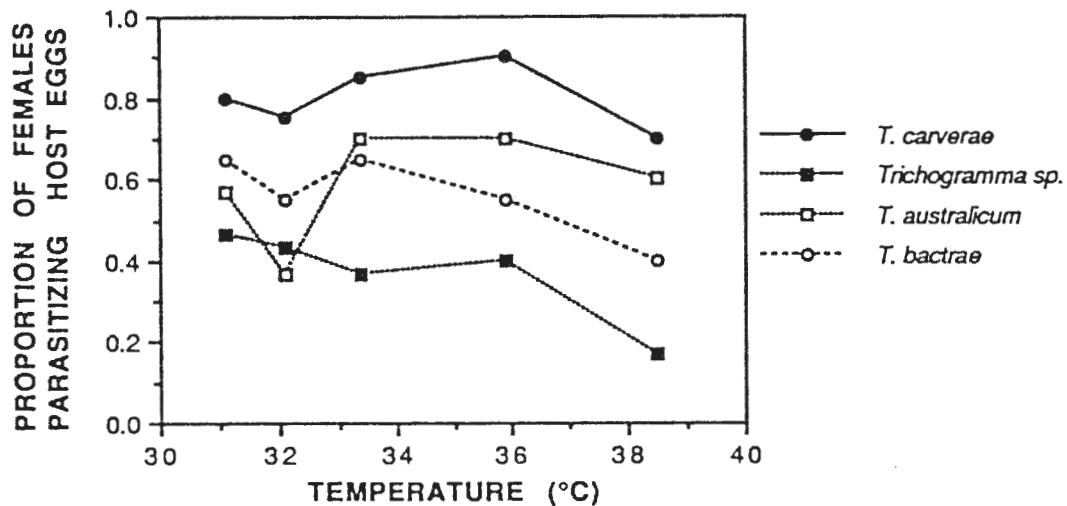


FIGURE 2: The effect of high temperatures on the parasitization activity of egg parasitoids. Egg parasitoids were exposed to *Heliothis armigera* eggs for 2 hours at each temperature.

Host Species Preference: The number of *H. armigera* and *H. punctigera* eggs parasitized by egg parasitoids during 24 hours was compared. There was no significant difference in the host species preference for all species of parasitoids (Table 5), indicating that all have potential to control both *Heliothis* pest species.

TABLE 5.
Host species preference.

SPECIES OF EGG PARASITOID	NUMBER OF HOST EGGS PARASITIZED	
	<i>Heliothis armigera</i>	<i>Heliothis punctigera</i>
<i>T. australicum</i>	7.6 ± 0.6	7.3 ± 0.7
<i>T. carverae</i>	10.6 ± 0.8	10.5 ± 1.0
<i>Trichogramma sp.</i>	9.8 ± 0.8	11.2 ± 1.1
<i>T. bactrae</i>	5.3 ± 0.6	5.4 ± 0.6
<i>Trichogrammatoidea sp.</i>	5.5 ± 0.9	6.4 ± 0.7

There was no significant difference between hosts for each species of egg parasitoid.

Host-Plant Adaptation: The levels of *H. armigera* egg parasitism on eight different field crops was assessed in a glasshouse. There was no parasitism recorded on chickpeas for all species of parasitoids. *T. bactrae* parasitized significantly more hosts on cotton (50.3% and 45.6% for siokra and DP90 respectively) than all other field crops (Figure 3). *T. bactrae*, *Trichogramma* sp. and *T. carverae* displayed potential for *Heliothis* management on cotton. *T. australicum* parasitized significantly fewer *Heliothis* eggs on all field crops indicating that it would be a poor candidate for field release.

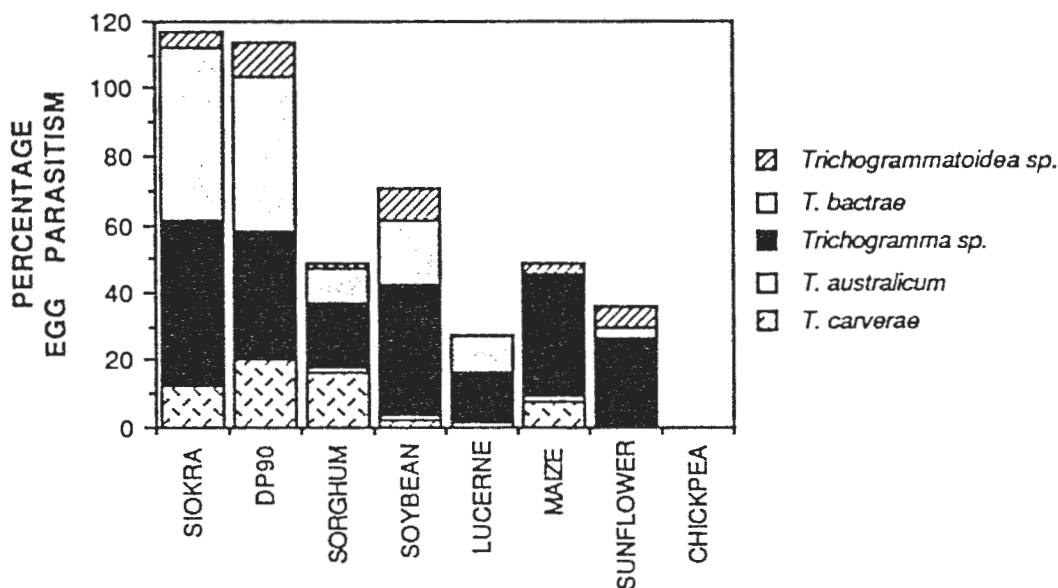


FIGURE 3: The levels of *Heliothis armigera* egg parasitism on eight different field crops. Values represent the means of four replicates. Parasitoids were released in a glasshouse at a rate equivalent to 7.5 females per plant.

Host Handling Behaviour: The host handling behaviour of egg parasitoids consisted of drumming, drilling and ovipositing. In some instances parasitoids were observed feeding on exudate from the host egg at the ovipositional puncture site. Significant differences in the drumming, drilling, ovipositing and total host handling times were found between parasitoid species. *T. carverae* displayed a significantly lower total host handling time (141 secs.) (Figure 4) than all other species, indicating that it is the quickest species at parasitizing cotton bollworm eggs.

Host Searching Efficiency on Cotton: The host searching efficiency of four species of egg parasitoids on siokra cotton was assessed by recording the proportion of cotton plants that contained parasitized *H. armigera* eggs 1-4 days after parasitoids were released in a glasshouse. There were significant differences between the host searching efficiencies of parasitoid species on days 1-3, but not on day 4. *T. carverae* was recovered from 58% cotton plants on day one, followed by *Trichogramma* sp. (43%), *T. bactrae* (35%) and *T. australicum* (10%) (Figure 5). *T. australicum* displayed the poorest host searching efficiency on each day. Host searching efficiency was significantly greater on day one than any other day for all parasitoid species. The results

indicate that *T. australicum* would be a poor candidate for *Heliothis* control in cotton.

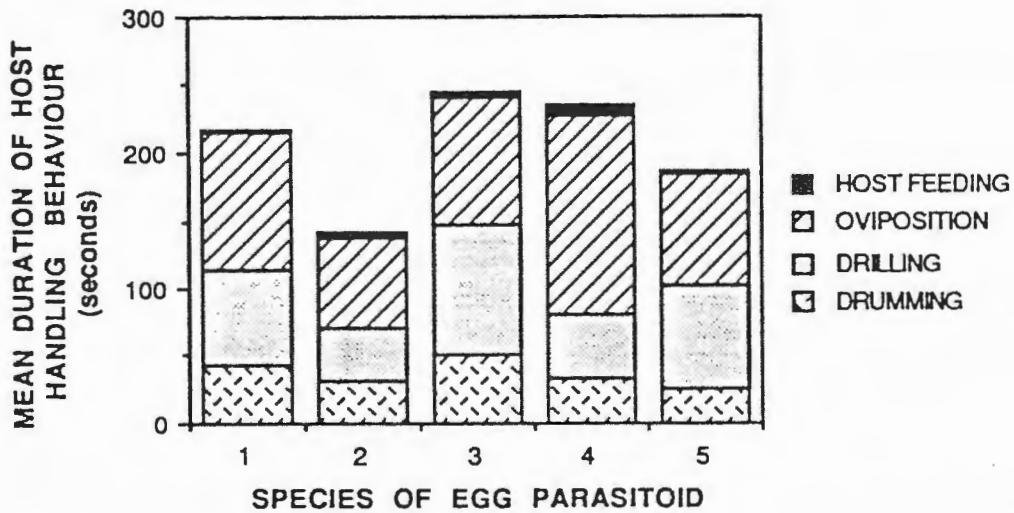


FIGURE 4: The duration of each host handling behaviour by five species of egg parasitoids: 1 = *T. australicum*, 2 = *T. carverae*, 3 = *Trichogramma sp.*, 4 = *T. bactrae*, 5 = *Trichogrammatoidea sp.*. Host = *Heliothis armigera*.

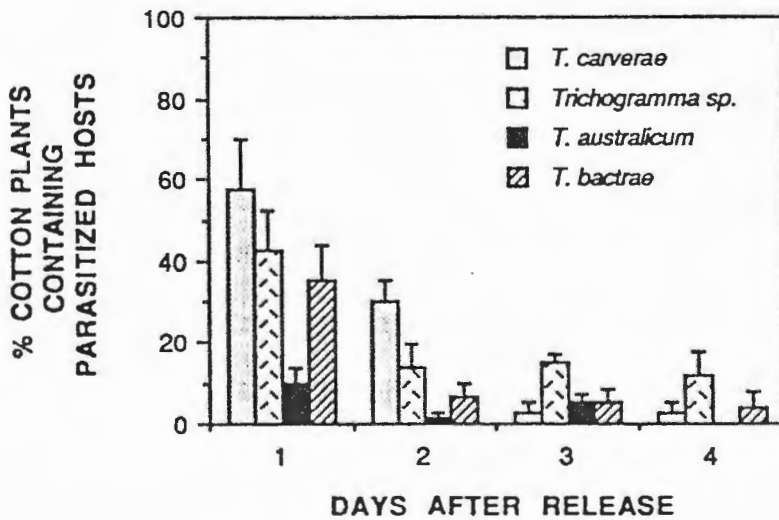


FIGURE 5: The host searching efficiency of four species of egg parasitoids on siokra cotton. Values represent the means \pm standard errors of four replicates. Parasitoids were released at a rate equivalent to 1.25 females per plant.

Host Acceptance Behaviour: The host acceptance behaviour of parasitoids was observed in the laboratory. Inexperienced *T. carverae*, *Trichogramma sp.* and *T. bactrae* required significantly fewer encounters with *H. armigera* eggs before accepting their first hosts than *T. australicum* (means

= 1.2, 2.7, 4.2 and 17.9 encounters respectively). Inexperienced *T. australicum* have poor *H. armigera* host acceptance, explaining the poor performance of this species following glasshouse releases (Figures 4 and 5).

DISCUSSION

The results highlighted variation in the biology of different species of native egg parasitoids. Significant differences in laboratory parasitism levels, temperature adaptations, host-plant adaptation, host handling behaviour, host acceptance behaviour and host searching efficiency were found between parasitoid species.

The scelionid, *Telenomus* sp., could not be continually reared on *Heliothis* eggs in the laboratory. This species would be difficult to mass rear and is therefore a poor candidate for use in a biocontrol programme.

Trichogramma australicum was the only trichogrammatid egg parasitoid collected that has been previously recorded in Queensland. Although *T. australicum* was adapted to high summer field temperatures (Figure 2, Table 4), it displayed the poorest host acceptance behaviour and host searching efficiency on cotton (Figure 5).

Trichogramma (Trichogrammanza) carverae, *Trichogramma* sp. and *Trichogrammatoidea bactrae* all have potential to control *Heliothis*. All of these species are new records for Queensland, and this highlights the importance of field surveys when establishing laboratory colonies of candidate species.

T. carverae parasitized significantly more eggs than all other species (Table 3). It also displayed the best host searching efficiency (Figure 5) and host acceptance behaviour. The results suggest that this species has the best potential as a biocontrol agent of *Heliothis*, and it should be evaluated in field trials.

T. bactrae was the most abundant species collected during the field surveys (occurring in 89% of collections) and displayed satisfactory host acceptance behaviour and host searching efficiency, suggesting that it is also worthy of field evaluation.

Ultimately, proof of an egg parasitoid's effectiveness at controlling *Heliothis* is its performance in the field. Six species of egg parasitoids were collected during this project (Table 1) and it would be difficult to mass rear this number of species for field evaluation. This project has demonstrated the value of pre-release evaluations when developing a biological control programme. Pre-release evaluations represent the most effective method of screening species when selecting a candidate for field release, enabling time savings and, more importantly, selection of the species with the greatest potential for field releases.

ACHIEVEMENT OF RESEARCH RELATIVE TO OBJECTIVES

All project objectives were achieved. The parasitoid surveys undertaken during this project revealed four new species records for Queensland, including two new species.

Suitable laboratory rearing techniques for egg parasitoids were developed quickly and as many as eleven colonies of parasitoids were reared simultaneously. Prof. John Pinto (University of California) provided specialist taxonomic identifications of representative colonies.

Laboratory and glasshouse studies of candidate egg parasitoid colonies highlighted significant differences in the suitability of different species for field trials against *Heliothis* in cotton. The next step in the development of a biocontrol programme is the construction of a parasitoid mass rearing facility, and field testing of the most promising species.

RECOMMENDATIONS FOR FUTURE RESEARCH

The procedure for developing a biological control programme of *Heliothis* using native egg parasitoids is shown in Figure 6. The research presented in this project satisfies steps 1-3 of the procedure. Future research should be directed at steps 4-6 of the procedure (Figure 6).

The results of this project have indicated that *T. carverae* and *T. bactrae* have potential as biocontrol agents of *Heliothis*, and these species should be mass reared and evaluated in field trials.

An essential prerequisite for field trials is the development of a mass rearing facility. This involves the construction of i) a host mass rearing unit, and ii) a parasitoid mass rearing unit. *Trichogramma* are commonly mass reared on eggs of the Angoumois Grain Moth (AGM), *Sitotroga cerealella*. It is also essential to continually monitor the quality of egg parasitoids mass reared in this way to ensure that their ability to parasitize *Heliothis* eggs is not adversely affected by the rearing procedure.

In order to conduct field trials it is also essential to develop suitable storage techniques for egg parasitoids so that adults emerge within four hours of parasitized AGM eggs being placed in the field.

Considerable research is then required to understand how to best utilize egg parasitoids as biocontrol agents of *Heliothis*. Details of how parasitoid release timings and release rates affect *Heliothis* population levels are poorly understood, and need thorough investigation.

The research presented in this report indicates that egg parasitoids have potential for *Heliothis* management, it is now necessary to determine how to effectively utilize these natural enemies and incorporate them into pest management strategies.

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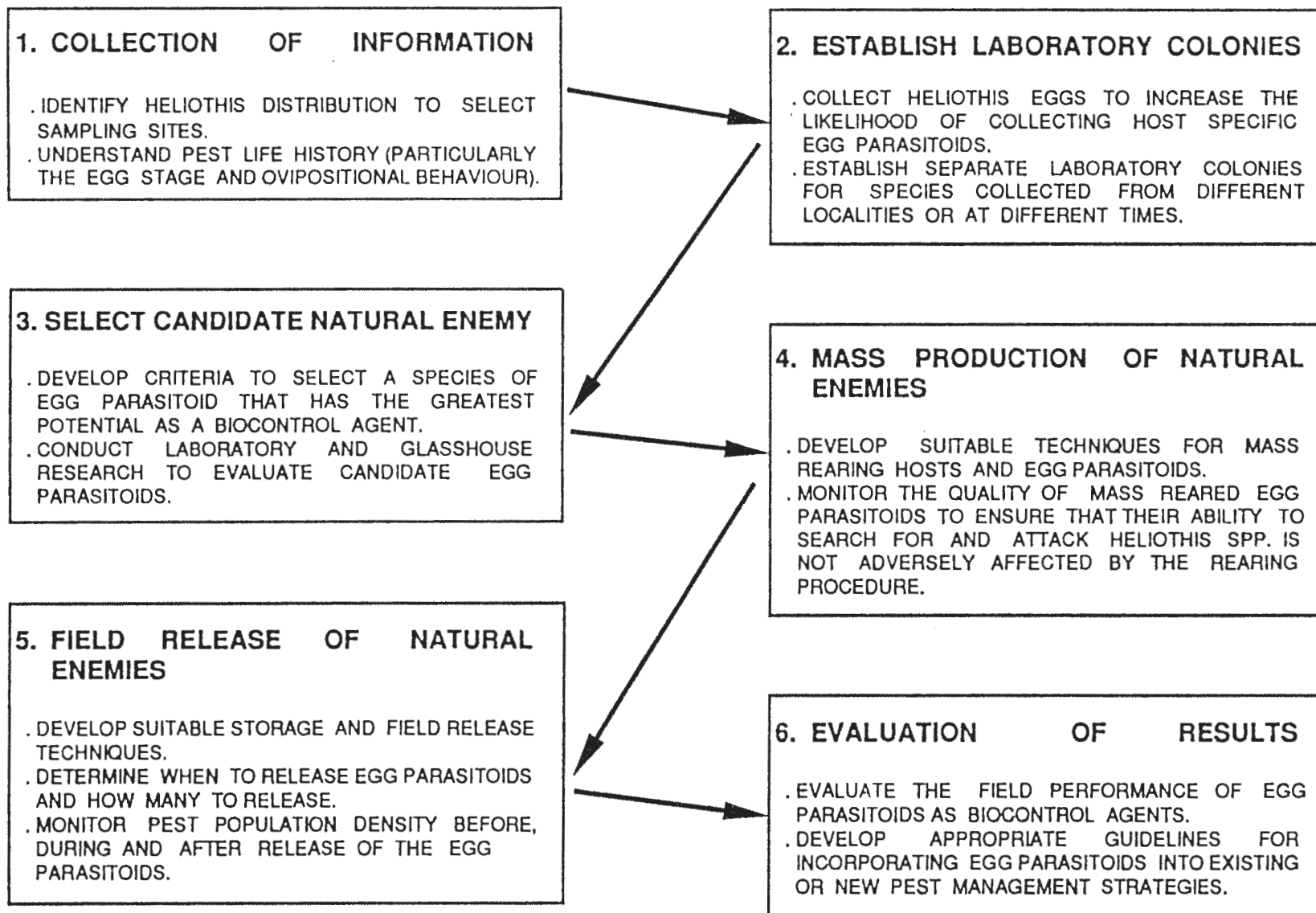


FIGURE 6: The procedure for undertaking an applied biocontrol programme of *Heliothis* using native egg parasitoids.

Oatman, E.R. and Pinto, J.D. (1987). A taxonomic review of *Trichogramma* (*Trichogrammanza*) Carver (Hymenoptera: Trichogrammatidae), with descriptions of two new species from Australia. *J. Aust. ent. Soc.* 26, 193-201.

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PUBLICATIONS (copies attached)

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Scholz, B.C. (1988). Selection of egg parasites for the biocontrol of *Heliothis*. *Proc. 4th Aust. Cotton Conf.*, Surfers Paradise, Queensland, 17-18 August, 1988. pp. 107-114.

PUBLICATIONS IN PREPARATION

- i) Field surveys of *Helicoverpa* spp. egg parasitoids in Queensland.
- ii) Pre-release evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton. 1. Parasitism and progeny production.
- iii) Pre-release evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton. 2. The effect of temperature on development and parasitism levels.
- iv) Pre-release evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton. 3. Host preference.
- v) Pre-release evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton. 4. The effect of host plant on parasitism levels.
- vi) Pre-release evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton. 5. Host acceptance.
- vii) Pre-release evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton. 6. Host searching efficiency.
- viii) The selection and evaluation of egg parasitoids for the management of *Helicoverpa* spp. in Australian cotton.