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Cotton Research & Development Corporation

FINAL REPORT

Project Title:

Evaluation of *Microplitis* and exotic parasitoids for biological control of *Helicoverpa* spp.

Project Number:

DAQ 48C

Research Organisation:

Queensland Department of Primary Industries

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Summary

Helicoverpa (=Heliothis) punctigera (Wallengren) and *H. armigera* (Hübner) are two of the most destructive insect pests of field and horticultural crops in Australia. *Helicoverpa* management relies almost entirely on insecticides and other problems associated with this dependence include secondary pest outbreaks, environmental contamination, human health hazards and insecticide resistance. Investigations into the use of larval parasitoids for inoculative, augmentative and/or inundative releases were warranted. Central to this issue was the need to have parasitoid production systems. Development of satisfactory culturing techniques would allow experimentation with native larval parasitoids, as well as allow researchers to consider a classical biocontrol approach, where exotic agents could be cultured and released.

The objectives of this project were to evaluate the potential of larval parasitoids, principally *Microplitis*, for use in biological control programs against *Helicoverpa* spp. and to introduce and monitor the establishment of exotic larval parasitoids of *Helicoverpa* spp.

Significant improvements were achieved in the culture and production of host insects, particularly *H. armigera*. Four other noctuid species - *H. punctigera, Neocleptria punctifera* (Walker), *Chrysodeixis argentifera* Guenee and *Spodoptera litura* (F.) - were also maintained using improved procedures developed for *H. armigera* and modified where necessary for each species. Recovery of *H. armigera* exceeded the target of 80% and production costs were significantly reduced over previous culture methods.

Several strains of *M. demolitor* Wilkinson were maintained in culture to allow taxonomic studies to be carried out. Although *M. demolitor* shows substantial morphological variability, the genetic data support the notion that all populations sampled were representatives of a single biological species. *M. demolitor* was included in a complete revision of Australian and New Guinean Microgastrine Braconids (Austin and Dangerfield in press).

Rearing methods were developed for *M. demolitor* which showed great promise for producing large numbers of parasitoids for use in field release experiments. However infection by *Streptococcus faecium* Orla-Jensen caused major parasitoid production problems during the period November 1991 to March 1993. As a consequence of the *S. faecium* contamination, some of the proposed experimental activities were curtailed.

Two exotic larval parasitoids were introduced and inoculative releases commenced in Queensland during November 1991. *Cotesia kazak* (Telenga) and *Hyposoter didymator* Thunberg adapted readily to the rearing methods developed for *M. demolitor*. A total of 33 releases were made throughout Queensland during the 1991/92 and 1992/93 summer cropping seasons. As at 30 June 1993 neither exotic species had confirmed establishment (overwinter), although post-release surveys recorded successful recoveries of parasitised larvae 1-2 weeks after release at a number of sites.

All three parasitoids were reared successfully in *H. punctigera, H. armigera, N. punctifera* and *C. argentifera*. *H. didymator* was the only parasitoid that developed in *S. litura*. The value of having evaluated these alternative hosts was realised when *S. faecium* severely limited production of *H. punctigera, H. armigera* and *N. punctifera*. Production was ultimately resumed using *C. argentifera* to rear *M. demolitor* and *C.*

kazak and *S. litura* to rear *H. didymator*. *C. argentifera* and *S. litura* were unaffected by *S. faecium*.

The performance of *M. demolitor*, *C. kazak* and *H. didymator* against *H. armigera* was evaluated on several crop hosts. Parasitism was low (0-11.8%) for all parasitoid species on chickpea. Moderate to high levels of parasitism (25.7-85.0%) were recorded on sorghum, sunflower, cotton, soybean and pigeonpea. Establishment releases should be directed against *Helicoverpa* spp. infestations on the major summer crops - sorghum, sunflower, cotton and soybean - rather than against the first spring generation infesting chickpea. Releases of larval parasitoids into chickpea are unlikely to enhance parasitism levels during the first spring generation of *Helicoverpa* spp.

Introduction

H. punctigera and *H. armigera* are two of the most destructive insect pests of field and horticultural crops in Australia. A 1989 estimate of the cost of chemical control of *Helicoverpa* spp. in Queensland was $27.8M (McGahan *et al.* 1991). Damage to unprotected crops and residual damage where protection is incomplete may approximately double the cost of *Helicoverpa* infestation. *Helicoverpa* management relies almost entirely on insecticides and other problems not costed include secondary pest outbreaks, environmental contamination, human health hazards and insecticide resistance.

Future *Helicoverpa* management is unlikely to reside with any one control method. Since pyrethroid resistance was discovered in Australia in 1983, there has been increased emphasis towards understanding the *Helicoverpa* life systems, with the purpose of identifying aspects which may enhance our prospects for future pest management. Progress has been made in such fields as biological control, microbial control and plant resistance.

Investigations into the use of larval parasitoids for inoculative, augmentative and/or inundative releases were warranted. Central to this issue was the need to have parasitoid production systems. Development of satisfactory culturing techniques would allow experimentation with native larval parasitoids, as well as allow researchers to consider a classical biocontrol approach, where exotic agents could be cultured and released.

Objectives

To evaluate the potential of larval parasitoids, principally *Microplitis*, for use in biological control programs against *Helicoverpa* spp.

To introduce and monitor the establishment of exotic larval parasitoids of *Helicoverpa* spp.

Materials and Methods

The research proposal was

- to collect and maintain laboratory colonies of *Microplitis* spp. attacking *Helicoverpa* spp. throughout their distribution in eastern Australia.
- to investigate the taxonomy of *Microplitis* spp. to determine how many species are involved.

- to investigate rearing methods which will facilitate production of large numbers of larval parasitoids.
- to investigate host range, host preference and biological features of *Microplitis*.
- to investigate field release methods and monitor parasitism levels following releases of *Microplitis*.
- to introduce and maintain laboratory colonies of exotic larval parasitoids.
- to monitor establishment of exotic larval parasitoids following their introduction and field release.

Results

Maintanence of host and parasitoid cultures

Culture methods for *Helicoverpa* spp. in Australian institutions have essentially used techniques referred to in Teakle and Jensen (1985). By this method larvae were reared on artificial diet in 28 mL disposable cups. While this method is satisfactory for maintainance of small laboratory populations, it involves considerable labour and material input which make production on a large scale very costly. Production methods for larval parasitoids of *Helicoverpa* spp. were also developed in USA (Powell and Hartley 1987).

Although basic rearing procedures were available from the literature, progress in this project was greatly enhanced when Dr D Murray visited south-eastern USA during May-June 1991 to examine progress with the production and use of larval parasitoids for the biocontrol of *Helicoverpa*. This study tour was funded jointly by Cotton R&D Corporation (CRDC) and Grains R&D Corporation (GRDC). Several of the techniques observed in USA were incorporated to improve our rearing procedures. This project was supported by concurrent studies on culture methods for larval parasitoids of *Helicoverpa* spp. funded jointly by RIRDC and Grainco (see Appendix I).

Five species of noctuids were held in culture. Their origins were as follows:-

H. armigera	sorghum	Darling Downs
H. punctigera	chickpea/cotton	Darling Downs
N. punctifera	weeds	Inland Australia
C.argentifera	lab culture	per Dr R Teakle, Brisbane
S.litura	cotton	Kununurra
	ex-light trap	Toowoomba

Nine cultures of larval parasitoids were also maintained. These were six strains of *M. demolitor*, two strains of *C. kazak* and one strain of *H. didymator*. Details of the six strains of *M. demolitor* maintained at Toowoomba were:-

Host Insect	Host Plant	Location	Collection Date
1. *H. armigera*	cotton	Darling Downs	Feb 1990
2. *H. punctigera*	weeds	Winton	Feb 1990
3. *H. armigera*	sorghum	Narrabri	Mar 1991
4. *H. punctigera*	weeds	Diamantina Lakes	May 1991
5. *H. punctigera*	weeds	Great Victoria Desert	Sep 1991
6. *H. armigera*	sorghum	Biloela	Nov 1991

Collections from Winton, Diamantina Lakes and Great Victoria Desert were made in collaboration with the Heliothis Inland Research Group which carried out regular surveys of potential *Helicoverpa* spp. breeding areas in inland Australia.

Rearing Procedure and Colony Maintenance

Progress in developing our rearing procedures for hosts was based on regular quality assessments as determined by percentage pupal recovery from rearing trays and mean pupal weights of recovered pupae. All assessments were based on *H. armigera* and *H. punctigera*. Quality assessments for parasitoids were not carried out routinely because of serious disease problems.

A separate report on the methods of production of the five host species and three parasitoid species is in preparation and will include a complete list of suppliers of materials and equipment. A copy will be provided to CRDC and GRDC when it is completed. To avoid unnecessary duplication, details will not be included in this final project report. Each of the five hosts was maintained successfully using the techniques developed in the course of this project. Improved techniques resulted in lower production costs while not compromising biological attributes. Our target of 80% *H. armigera* pupal recovery from 126 cell rearing trays was consistently achieved during the latter part of the project when disease had been controlled (Figure 1). Recovery of *H. punctigera* was lower than for *H. armigera* . The lower recovery of *H. punctigera* (about 60%) resulted from the smaller grid size used (308 cells/tray) and the greater spread in *H. punctigera* larval development times. Production efficiency was nevertheless high for *H. punctigera*. Pupal size using the rearing trays was maintained at or near that obtained when larvae were reared individually in 28 mL cups (Table 1 and Figure 2). Smaller *H. armigera* pupae were produced from the smaller cell grids. Grid size was found to have no effect on *H. punctigera* pupal weights.

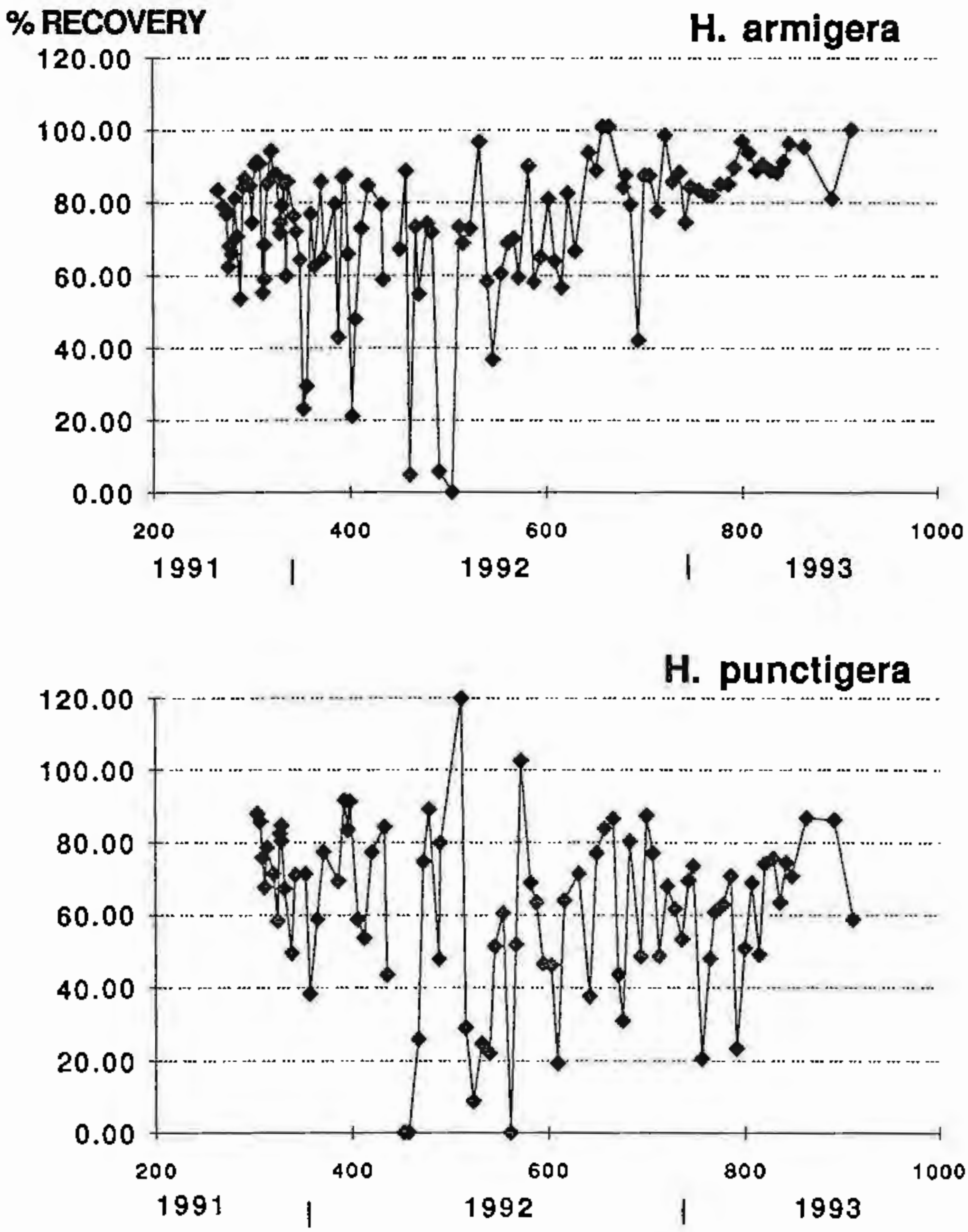
Table 1. Mean weight (mg) of *H. armigera* and *H. punctigera* pupae when reared in 28 mL cups, and 126 and 308 cell trays.

Species	28 mL Cups	126 Cell Trays	308 Cell Trays
H. armigera	460 ± 4	433 ± 11	354 ± 5
H. punctigera	330 ± 4	313 ± 7	324 ± 10

Cost of dietary ingredients used per rearing unit was reduced substantially by changing from 28 mL cups to multi-cellular trays. Cost of diet was estimated at 1.61 cents per cup, 1.02 cents/cell for 126 cell trays and 0.42 cents/cell for 308 cell trays. Disposable diet cups cost 4.18 cents each. The costs for the re-usable trays and inserts is $10.37 (comprising $2.01 for the stainless steel tray, $4.29 for the aluminium grid insert and $4.07 for the perforated aluminium cover) with depreciation expected over 5 years. These metal components were washed and autoclaved between uses. Substantial efficiency gains were achieved by pouring diet into and collecting pupae from multicellular trays.

Parasitoid production was initially promising using *H. armigera* as host. Recovery as high as 315 parasitoid pupae per 308 cell tray was achieved before disease drastically reduced survival of both hosts and parasitoids.

Figure 1. Percentage recovery of *H. armigera* and *H. punctigera* pupae from multi-cellular rearing trays from September 1991 to June 1993.



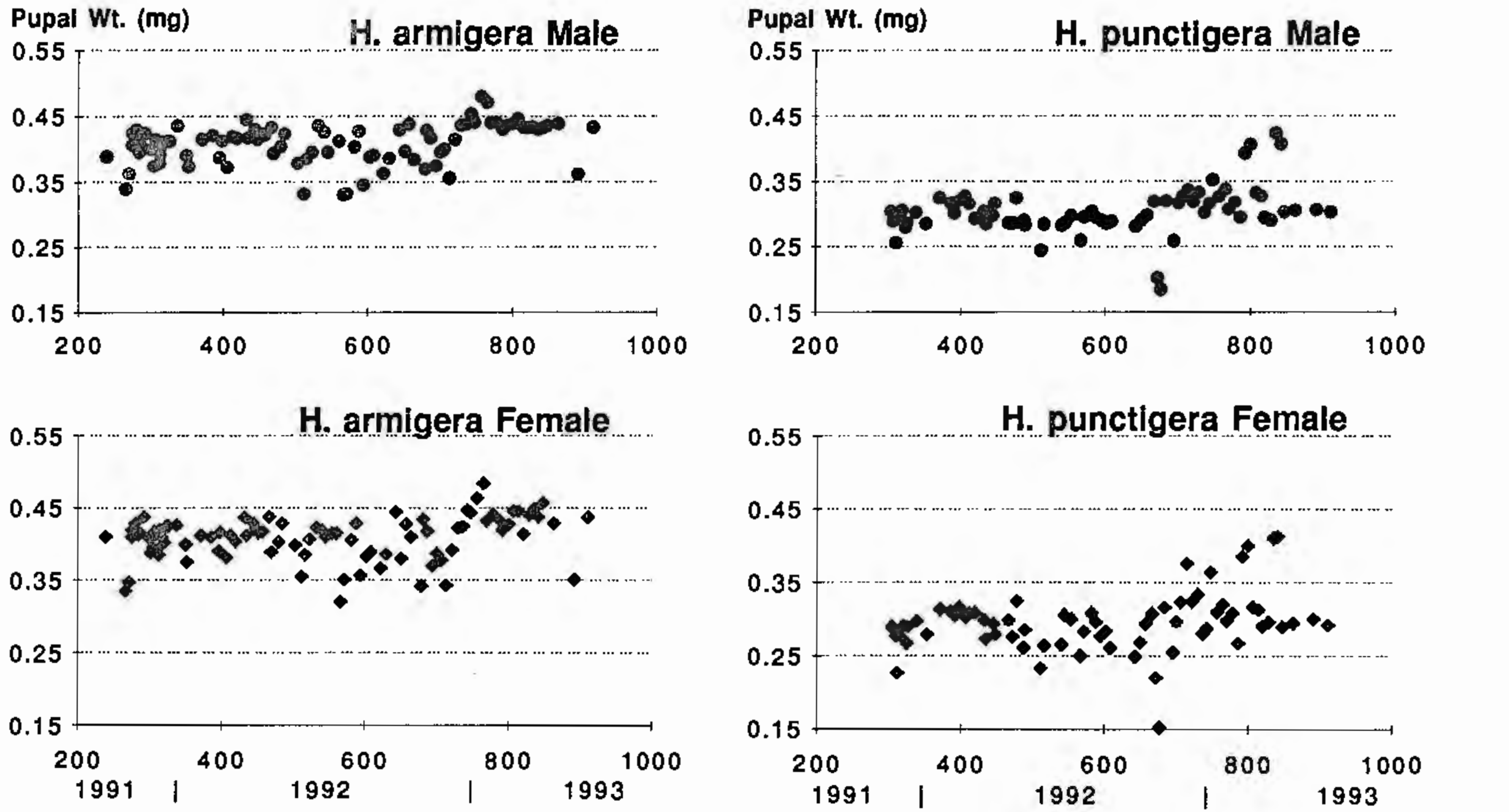


Figure 2. Mean weight (mg) of batches of male and female pupae of *H. armigera* and *H. punctigera* reared in multi-cellular trays.

Streptococcus Infection

The bacterium *S. faecium* was isolated in pure culture from diseased *Helicoverpa* spp. larvae and adults of each of the parasitoids. The source of *S. faecium* infection was thought to be honey fed to adult parasites. During November 1991, the usual supply of honey was exhausted because of the drought. Honey from a different supplier was used. This honey was later found to be contaminated with *S. faecium*. Sensitivity testing showed that the strain of *S. faecium* was resistant to the antibiotics streptomycin, neomycin, apromycin, furazolidone, penicillin, ampicillin and cloxacillin, had intermediate sensitivity to erythromycin and lincospectin, and sensitivity to tetracycline, erythromycin and cotrimoxazole. Tetracycline was added to food provided to wasps and host moths in an effort to reduce infection. This measure was not successful in eliminating disease from the insect cultures.

Injection of *S. faecium* into third instar *H. armigera* larvae resulted in death of larvae 2-4 days later. During oviposition *S. faecium* was transferred to hosts and larvae infected in this way died before parasitoid development was completed. *S. faecium* infected larvae first appeared oily on the outside before finally collapsing and discharging the entire body contents. These symptoms were similar to those reported for *Helicoverpa* nuclear polyhedrosis virus (NPV). However, scanning electromicrograph (SEM) examinations carried out by Mr H Prior, QDPI Animal Research Institute, Yerongpilly, failed to find virus particles. After some months, *S. faecium* caused massive losses in cultures of *H. armigera*, *H. punctigera* and *N. punctifera*, sometimes resulting in total losses in rearing trays. Such occurences suggested contamination also occurred via ingestion. *C. argentifera* and *S. litura* were not affected by *S. faecium*.

Sections of parasitoid abdomens viewed under a light microscope showed high incidence of internal contamination by *S. faecium*. Infection of wasps also reduced their longevity.

Most success in overcoming the problems of bacterial contamination resulted from the adoption of improved laboratory hygiene, and the use of autoclavable and/or disposable components in rearing units.

<u>Taxonomy of *Microplitis* spp.</u>

Five strains of *M. demolitor* were sent to Dr A Austin, Department of Crop Protection, University of Adelaide, for taxonomic studies. Although *M. demolitor* shows substantial morphological variability, the genetic data support the notion that all populations sampled were representatives of a single biological species (Austin, Dangerfield and Adams 1993). Dr Austin has recently completed a systematics revision of Australian and New Guinean Microgastrine Braconids (Austin and Dangerfield in press). The importance of this taxonomic study to the concurrent biocontrol program can not be stressed enough. *M. demolitor* can be cultured and released with confidence knowing that we are dealing with a single species attacking *Helicoverpa* spp., and not several species as had been initially suggested.

Importation of exotic larval parasitoids

Shipments of the exotic parasitoids *C. kazak* and *H. didymator* were received from Western Australia during February/March 1991, in cooperation with Dr Phil Michael, Western Australian Department of Agriculture. These *Helicoverpa* spp. parasitoids were introduced to Western Australia in 1983 (Michael 1989) and are now established.

C. kazak was also reimported from New Zealand with the cooperation of Dr Peter Cameron, DSIR New Zealand. All introductions and sharing of costs were done in collaboration with Dr Peter Ridland, Victorian Department of Agriculture and Rural Affairs, Burnley. *C. kazak* from New Zealand were reared for one generation in quarantine to exclude pathogens and secondary parasitoids using facilities made available by Queensland Department of Lands, Alan Fletcher Research Station, Sherwood.

Host preference

All three parasitoid species were reared successfully in *H. armigera*, *H. punctigera*, *N. punctifera* and *C. argentifera* (Table 2). *N. punctifera* and *C. argentifera* are recorded as new laboratory hosts. *H. didymator* was the only parasitoid that developed in *S. litura*. The duration of larval development of all three parasitoid species was significantly longer when reared in *N. punctifera* and significantly smaller adult *M. demolitor* and *H. didymator* were produced from *N. punctifera* than from each of the other hosts. For all parasitoid species, the pupal development times were mostly independent of the duration of larval development. While *H. armigera* and *H. punctigera* are the targeted field hosts, *C. argentifera* will be used for laboratory rearing of *M. demolitor* and *C. kazak* because it is best suited for laboratory culture. It needs to be determined whether the use of factitious hosts will impact on the success of the field release program.

Host plant effect

The effect of host plant on parasitism of second instar *H. armigera* larvae by *M. demolitor*, *C. kazak* and *H. didymator* was investigated in a series of glasshouse experiments. For *M. demolitor*, parasitism was low (0% and 11.8%) on chickpea (Figure 3). Moderate to high levels of parasitism (32.9% to 75.4%) were recorded on soybean, cotton, and sorghum. For the exotic parasitoids, parasitism was lowest on chickpea (5.4% for *H. didymator* and 11.8 % for *C. kazak*) (Table 3). Higher levels of parasitism (50.1-85.0% for *H. didymator* and 25.7-55.3% for *C. kazak*) were recorded on sorghum, sunflower, cotton, soybean and pigeonpea. Establishment releases should be directed against *Helicoverpa* spp. infestations on the major summer crops - sorghum, sunflower, cotton and soybean - rather than against the first spring generation infesting chickpea. Sorghum and sunflower are preferred release crops because parasitism levels are high and disruption by insecticide sprays is less likely. The results also suggest that releases of larval parasitoids into chickpea are unlikely to enhance parasitism levels during the first spring generation of *Helicoverpa* spp.

Field release and parasitism levels

Experiments planned to evaluate *M. demolitor* under field conditions were severely curtailed by the production problems caused by *S. faecium*. On 17 January 1992, a single release of *ca* 200 female *M. demolitor* was made to supplement natural parasitism in a 1 ha unsprayed raingrown cotton plot near Dalby (Murray *et al*. 1992). Larval

Table 2. Comparison of larval and pupal development periods, host size 24 h prior to parasitoid emergence and length of hind tibia (mean ± SE) for three larval parasitoids reared in various noctuid hosts at 26 ± 1°C.

Host	Duration of larval development (days)		Duration of pupal development (days)		Host weight 24 h prior to parasitoid emergence (mg)	Length of hind tibia (mm)	
	Male	Female	Male	Female		Male	Female
Microplitis demolitor							
H. armigera	8.1 ± 0.2 b (18)	8.5 ± 0.1 b (24)	4.4 ± 0.1 b	4.7 ± 0.1 b	31.24 ± 0.61 b	1.05 ± 0.01 a	1.11 ± 0.01 b
H. punctigera	7.3 ± 0.2 c (22)	7.3 ± 0.1 c (38)	5.1 ± 0.1 a	5.2 ± 0.1 a	35.89 ± 0.80 a	1.07 ± 0.01 a	1.13 ± 0.01 a
N. punctifera	10.4 ± 0.9 a (7)	9.9 ± 0.4 a (11)	4.7 ± 0.2 ab	4.8 ± 0.1 ab	21.68 ± 0.75 d	1.01 ± 0.01 b	1.04 ± 0.01 d
C. argentifera	8.3 ± 0.2 b (21)	8.3 ± 0.2 b (26)	4.6 ± 0.1 b	5.2 ± 0.1 a	28.62 ± 0.61 c	1.05 ± 0.01 a	1.08 ± 0.01 c
Cotesia kazak							
H. armigera	7.6 ± 0.2 ab (22)	8.2 ± 0.2 ab (15)	5.3 ± 0.2	5.4 ± 0.2	16.40 ± 0.93 c	0.85 ± 0.01	0.91 ± 0.01
H. punctigera	7.5 ± 0.1 b (25)	8.0 ± 0.1 b (40)	5.6 ± 0.3	5.6 ± 0.1	20.67 ± 0.50 ab	0.86 ± 0.01	0.93 ± 0.01
N. punctifera	8.2 ± 0.4 a (17)	8.8 ± 0.3 a (14)	5.2 ± 0.1	5.4 ± 0.2	21.93 ± 1.08 a	0.87 ± 0.01	0.92 ± 0.01
C. argentifera	7.1 ± 0.1 b (10)	7.8 ± 0.3 b (16)	5.3 ± 0.2	5.6 ± 0.1	18.71 ± 0.70 bc	0.85 ± 0.01	0.91 ± 0.01
Hyposoter didymator							
H. armigera	8.7 ± 0.1 b (34)	9.0 ± 0.0 (2)	6.7 ± 0.2 a	6.5 ± 0.5	35.78 ± 0.82 a	1.74 ± 0.01 a	1.77 ± 0.05
H. punctigera	8.1 ± 0.1 cd (37)	8.0 ± 0.0 (2)	6.5 ± 0.1 abc	7.0 ± 0.0	33.06 ± 0.59 b	1.73 ± 0.01 a	1.83 ± 0.01
N. punctifera	10.9 ± 0.5 a (17)	No data (0)	6.7 ± 0.2 ab	No data	28.97 ± 0.90 c	1.68 ± 0.01 b	No data
C. argentifera	8.3 ± 0.1 bc (52)	9.0 ± 0.0 (4)	6.3 ± 0.1 c	6.3 ± 0.3	34.86 ± 0.55 ab	1.72 ± 0.01 a	1.73 ± 0.02
S. litura	8.0 ± 0.0 d (36)	8.0 ± 0.0 (2)	6.4 ± 0.1 bc	7.0 ± 0.0	34.86 ± 1.04 ab	1.74 ± 0.01 a	1.69 ± 0.07

Means for a parasitoid species within a column not followed by the same letter differ significantly ($P<0.05$, ANOVA).

No letters indicate no significant difference ($P>0.05$).

Number in brackets is the number of individuals in the data set.

Figure 3. Percentage parasitism (and standard error bars) of *H. armigera* by *M. demolitor* on different crop hosts in two glasshouse experiments. In each experiment, parasitism levels with the same letters were not significantly different (P>0.05).

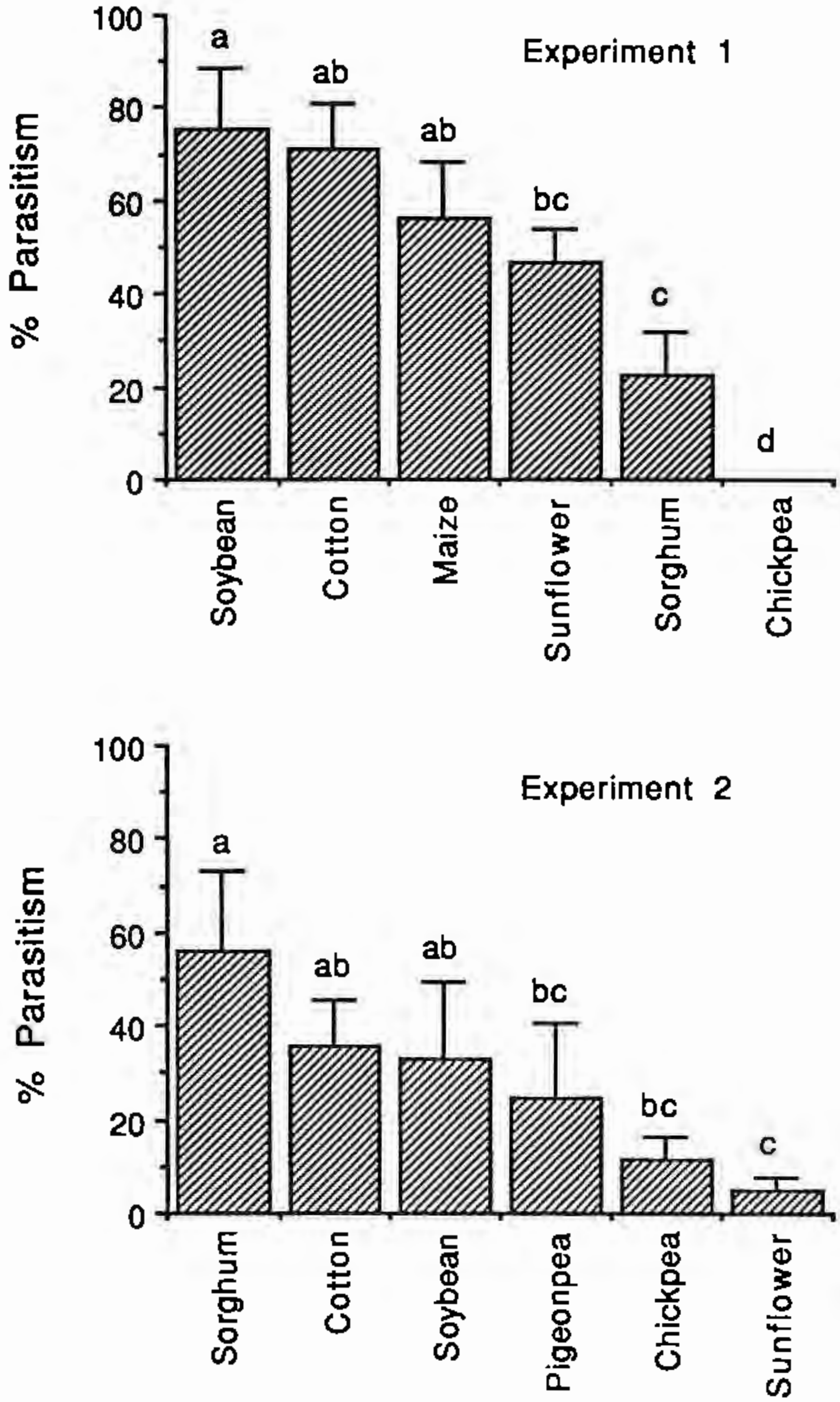


Table 3. Recovery of *H. armigera* larvae, parasitoid survival, parasitism and number of eggs per parasitised host for *H. didymator* and *C. kazak* on different crop hosts.

Crop	Sunflower	Soybean	Cotton	Sorghum	Pigeonpea	Chickpea
H. didymator						
% Larval recovery	97.5 ± 1.7 a	82.5 ± 9.7 a	89.2 ± 4.7 a	62.5 ± 12.0 b	94.2 ± 1.5 a	95.0 ± 2.2 a
% Wasp survival	97.2 ± 2.8 a	86.1 ± 8.0 ab	97.2 ± 2.8 a	75.0 ± 13.4 ab	66.7 ± 9.6 b	5.6 ± 3.5 c
% Parasitism	85.0 ± 2.7 a	82.1 ± 6.4a	64.7 ± 13.3 ab	57.6 ± 7.7 b	50.1 ± 3.9 b	5.4 ± 2.0 c
No. eggs per parasitised host (range)	2.8 ± 0.1 a (1-8) n=101	2.1 ± 0.2 bc (1-4) n=83	2.2 ± 0.2 bc (1-7) n=70	1.6 ± 0.2 cd (1-5) n=45	2.2 ± 0.3 b (1-8) n=57	1.3 ± 0.3 d (1-2) n=6
C. kazak						
% Larval recovery	91.7 ± 3.8	81.7 ± 1.7	85.8 ± 2.7	86.7 ± 3.1	83.3 ± 6.0	90.8 ± 4.0
% Wasp survival	69.4 ± 10.0	46.7 ± 9.4	58.3 ± 7.1	55.6 ± 7.0	47.2 ± 10.9	38.9 ± 5.6
% Parasitism	54.8 ± 11.7 ab	26.6 ± 6.3 bcd	44.5 ± 9.8 abc	55.3 ± 13.8 a	25.7 ± 8.3 cd	11.8 ± 7.3 d
No. eggs per parasitised host (range)	1.2 ± 0.1 (1-3) n=62	1.6 ± 0.3 (1-4) n=26	1.7 ± 0.2 (1-5) n=45	1.7 ± 0.3 (1-7) n=58	1.4 ± 0.2 (1-4) n=25	1.8 ± 0.7 (1-2) n=12

Means within rows not followed by the same letter differ significantly (P<0.05, ANOVA).
No letters indicate no significant difference (P>0.05).
n = number of larvae dissected

parasitism increased to 68% twelve days after release, but the relative contribution of naturally occuring and released *M. demolitor* was not determined as they could not be separated. During a second peak of larval activity, parasitism declined despite an abundance of suitably sized larvae in the crop. This outcome may have been influenced by an *Ascovirus* infecting *Helicoverpa* larvae. The inadvertent transmission of virus diseases by parasitoids, and the complex interactions which can take place, highlight the difficulty of integrating some biological options into a management program.

During 1993, parasitism was monitored in a 0.5 ha unsprayed raingrown cotton plot at Kupunn, near Dalby. Naturally occuring parasitism by *M. demolitor* increased from 15.5% to peak at 50% two weeks later. This level of parasitism failed to reduce the infestation below economically damaging levels.

Release and establishment of exotic larval parasitoids

A total of 33 inoculative releases were made throughout Queensland during 1991/92 and 1992/93 summer cropping seasons. A complete list of release sites is given in Table 3. Released parasitoids totalled *ca* 1500 *C. kazak* females and *ca* 3000 *H. didymator* females. Production and release of introduced parasites were substantially lower than planned because of *S. faecium* infection. Recovery of parasitised hosts 1-2 weeks after parasitoid release was made at 50% of release sites in 1991/92 and 16% of release sites in 1992/93. One of the factors contributing to the lower positive recoveries in 1992/93 was that many of the releases were made into lucerne because the drought had severely curtailed plantings of summer grain and oilseed crops. Larval samples from lucerne suffered high mortality from virus which resulted in host death before parasitoid development was completed. It is not known to what extent the release of *S. faecium* contaminated parasitoids influenced field recovery. As at July 1993, there was no evidence of permanent establishment (overwinter survival) of either introduced species at any release site.

Discussion

Rearing and Production

At July 1993, production of all host species was considered satisfactory for the immediate demands of parasitoid production. In view of the requirements of research programs for 1993/94, and based on host suitability characteristics, production of *H. punctigera* and *N. punctifera* will not be continued. Future production of *M. demolitor* and *C. kazak* will be in *C. argentifera* and *H. didymator* in *S. litura*. *H. armigera* will still be maintained for experimental purposes. The effect of parasitoid rearing in non-*Helicoverpa* spp. hosts is an issue needing to be addressed.

Various techniques and equipment developed during this project have been passed on to other organisations/individuals in order to improve their host production programs.

The sex ratio of progeny of *M. demolitor* and *C. kazak* was satisfactory and near 1:1. However, the sex ratio of *H. didymator* was male biased, with 70-90% of progeny being males, depending on age of females used in stings. The sex ratio of progeny improved with older females. Similar problems with sex ratio have been encountered by other researchers culturing *H. didymator* (Harrington *et al.* 1993).

Table 3. Details of releases of the introduced larval parasitoids - *C. kazak* and *H. didymator*.

Location	Date	Crop	Parasitoid	No. females released	Recovery
Gatton	27/11/91	sorghum	*C. kazak*	150	Yes
Biloela	14/11/91	sorghum	*C. kazak*	100	Yes
St Ruth	30/12/91	sorghum	*C. kazak*	100	Yes
Formartin	17/1/92	cotton	*C. kazak*	100	Yes
Formartin	13/2/92	sorghum	*C. kazak*	50	Yes
			H. didymator	30	Yes
Formartin	27/2/92	cotton	*H. didymator*	25	Yes
Formartin	11/3/93	cotton	*H. didymator*	20	Yes
Kingsthorpe	6/3/92	pigeonpea	*H. didymator*	30	No
Emerald	1/4/92	sunflower	*H. didymator*	100	Yes
Emerald	3/4/92	sunflower	*H. didymator*	20	Not sampled
Gatton	14/4/92	lucerne	*H. didymator*	50	No
Formartin	15/4/92	sunflower	*H. didymator*	100	No
Emerald	14/5/92	dolichos	*H. didymator*	100	Not sampled
Gatton	15/5/92	lucerne	*H. didymator*	200	Not sampled
Diamantina	24/7/92	weeds	*H. didymator*	50	No
Lakes			*C. kazak*	100	No
Gatton	15/8/92	lucerne	*H. didymator*	100	No
Brookstead	14/10/92	lucerne	*C. kazak*	50	Yes
			H. didymator	150	Yes
Brookstead	21/10/92	lucerne	*H. didymator*	150	No
Brookstead	28/10/92	lucerne	*C. kazak*	100	No
Brookstead	25/11/92	lucerne	*C. kazak*	200	No
			H. didymator	100	No
Brookstead	2/12/92	lucerne	*H. didymator*	150	No
Forest Hill	2/12/92	lucerne	*H. didymator*	200	No
Gatton	2/12/92	lucerne	*C. kazak*	100	Yes
			H. didymator	300	No
Warra	11/12/92	cotton	*C. kazak*	100	No
			H. didymator	250	No
Brookstead	30/12/92	sunflower	*C. kazak*	200	Not sampled
			H. didymator	200	Not sampled
Warra	6/1/93	cotton	*H. didymator*	75	No
Kingsthorpe	20/1/93	pigeonpea	*H. didymator*	50	Not sampled
Dalby	3/3/93	cotton	*H. didymator*	50	No
Jondaryan	26/3/93	lucerne	*C. kazak*	50	No
			H. didymator	100	Yes
Gatton	7/4/93	lucerne	*H. didymator*	100	No
Jondaryan	7/4/93	lucerne	*H. didymator*	100	Not sampled
Gatton	22/4/93	lucerne	*H. didymator*	100	No
Gatton	29/4/93	lucerne	*C. kazak*	100	Not sampled
			H. didymator	90	Not sampled

Support for Other Institutions

Several institutions were supplied with insect material for experimental purposes (Table 4). Most of these requests were for eggs or pupae of *H. armigera*. A consignment of *M. demolitor* was exported to USDA Stoneville, Mississippi, USA to revitalise their colony originally imported in 1981. Colonies of *C. kazak* and *H. didymator* were supplied to the Victorian Department of Agriculture and Rural Affairs (DARA), Burnley. DARA actively collaborated with QDPI in the release of exotic agents into eastern Australia.

Table 4. List of Institutions/Groups supplied with cultures.

Australian:
University of Queensland, St Lucia - *H. armigera* and *H. punctigera*
Griffith University, Nathan - *H. armigera*
University of Southern Queensland, Toowoomba - *H. armigera*
University of New England, Armidale - *N. punctifera*
University of Adelaide, Adelaide - *M. demolitor*
CSIRO Cotton Research Unit, Narrabri - *H. armigera*
Victorian Department of Agriculture and Rural Affairs, Burnley - *C. kazak* and *H. didymator*
Stahmann Farms, Moree - *H. armigera*
International:
Ministry of Agriculture and Fisheries, Auckland - *S. litura*
USDA-ARS Southern Insect Management Laboratory, Stoneville, Mississippi, USA - *M. demolitor*
Natural Resources Institute, United Kingdom - *H. armigera*

Conclusions and recommendations

Larval parasitoids investigated in this project have a very important role in the management of *Helicoverpa* spp. *M. demolitor*, *C. kazak* and *H. didymator* all prefer small larvae for oviposition and parasitised hosts are killed before completing more than *ca* 10% of their potential feeding damage. There may be competitive interactions between these parasitoids in the field because of similar host preferences, but it will be some years before widespread establishment is achieved and these interactions, if present, become evident.

Performance of each of the introduced parasitoids against *Helicoverpa* spp. on summer crop hosts provided encouraging support for the continuation of establishment releases of these agents. Establishment has not been confirmed for any introduced species. With more favourable cropping seasons than have existed since 1990, the prospects for establishment of introduced parasitoids should improve.

If the introduced species become widely established and complement the mortality provided by the native *M. demolitor*, the resultant higher mortality of fourth instar *Helicoverpa* larvae may be sufficient to further reduce insecticide usage. It will be several years before widespread establishment across the major cropping regions is achieved. In order to assess the success of the initial project, there is a need for a continuing project to rear and release the introduced parasitoids, and follow-up monitoring of their establishment in cropping areas. This need has been realised by GRDC support for a new project which commenced in July 1993 - "Larval parasitoids for biocontrol of *Helicoverpa* spp." As part of this new project it is planned to consider the introduction of other exotic larval parasitoids. Application has already been made to import *Microplitis croceipes* (Cresson) from the USA. This larval parasitoid is very important in USA cropping systems, is specific to *Heliothis/Helicoverpa* spp., and has a preference for hosts slightly larger than those of *M. demolitor*, *C. kazak* and *H. didymator*. *M. croceipes* was introduced into New Zealand in 1987 and is now well established on *H. armigera*.

Proper field evaluation of either *M. demolitor* or the introduced agents was not possible because of the disease problems. Further experimentation with larval parasitoids will take place within the project "Integrated pest management in raingrown cotton" which is supported by CRDC. Within this project biological options will be assessed. These will include egg and larval parasitoids of *Helicoverpa* spp. and the use of *Bacillus thuringiensis* sprays. Integrating parasitoids into pest management systems requires basic knowledge of the effects of various insecticides on the life stages of the parasitoid. The effect of insecticides on egg and larval parasitoids must be investigated as a matter of urgency. Data on the effect of residues of the commonly used cotton insecticides on egg parasitoids are currently being generated. A project has been proposed within the Cooperative Research Centre for Sustainable Cotton Production to investigate the effect of insecticides on beneficial insects. Parasitoid production facilities at QDPI Toowoomba are well positioned to supply egg and larval parasitoids for these investigations.

The interactions between larval parasitoids and NPV are the subject of current investigations in collaboration with Dr R Teakle, QDPI Brisbane who has a GRDC project "*Helicoverpa* biocontrol on midge-resistant sorghum using parasitoids and a specific heliothis virus". This study is providing information on how parasitoids and NPV can be integrated into a pest management program. While this investigation is for sorghum, the results will be applicable to any crop to which NPV is applied. CRDC is funding research to develop genetically engineered viruses. Interactions between parasitoids and microbial agents must not be ignored.

The problems with parasitoid production resulting from *S. faecium* infection severely limited field activities and jeopardised the successful outcome to the project. Problems of this nature need to be taken into consideration when assessing the risks associated with projects that rely on insect production. While *S. faecium* is extremely pathogenic to *Helicoverpa* spp. and could be considered a candidate for microbial control, the pathogen has a broad host range, including hymenoptera. It is most improbable that authorities would approve its development and use.

Investigations are still warranted to study the induction and termination of diapause in *M. demolitor* as this mechanism offers scope for the manipulation of parasitoid development. Knowledge on how to manipulate pupal development would allow year-round production and storage until required for field release. This would be essential knowledge in the commercial production of these parasitoids.

The costs of larval parasitoid production have not been determined. However, it is considered that production relying on living hosts will be too costly to support inundative field releases (suggested release rates may require more than 2000 females per ha). Research needs to investigate the effectiveness of augmentative releases, as lower release rates could be economically viable. *In vitro* rearing methods are being investigated for *M. croceipes*, but successful production methods are still some years away. The use of artificial diets for parasitoid production offers the greatest hope for cost-effective production capabilities.

The demand for experimental cultures by other institutions indicates a potential role for a culture/rearing laboratory to provide insects by order, as has been established in USA. The present demand for insect cultures within Australia is not sufficient to justify full-time maintenance of culture facilities. It may be useful to operate such a facility in conjunction with other activities. There is a need to determine a costing structure for supply of insects.

Communication of Results

Videos

Segments on the research with larval parasitoids of *Helicoverpa* spp. were included in the following video releases:- The Cotton Report, The Australian Cotton Video and AgLink.

Presentations

Presentations were made on many occasions to field days, farm walks, NSW and Queensland crop consultant's meetings, grower meetings and Advisory Committee meetings. Media releases were also circulated to the Rural Press.

Publications

MURRAY, D. and RYNNE, K. (1991) - Microplitis versus heliothis: an inside story. *The Australian Cottongrower* 12:55-56.

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MURRAY, D.A.H. and RYNNE, K.P. - Effect of host plant on parasitism of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) by *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae). Submitted to *Entomophaga*

MURRAY, D.A.H., RYNNE, K.P., WINTERTON, S.L., BEAN, J.A. and LLOYD, R.J. - Effect of host plant on parasitism of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) by two introduced parasitoids. Ms submitted for internal review.

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APPENDIX I. STATEMENT OF CONTRIBUTIONS TO THE PROJECT

Year	Source of Funds			
	CRDC	GRDC	Research Organisation[1]	TOTAL
1990/91	17700	26940	179000	223640
1991/92	15908	23337	179000	218245
1992/93	17700	26325	179000	223025
TOTALS	51308	76602	537000	664910

1 - Queensland Department of Primary Industries (Based on 3.5 multiplier for annual salary components).

Funds were also received to support a concurrent project 'Culture methods for larval parasitoids of *Helicoverpa* spp.'

Year	Source of Funds			
	RIRDC	Industry[1]	Research Organisations[2]	TOTAL
1990/91	Nil	4400	13000	17400
1991/92	4950	4400	13000	22350
1992/93	5150	4500	13000	22650
TOTALS	10100	13300	39000	62400

1 - Initially Central Queensland Grain Sorghum Marketing Board, and later Grainco via the Grain Research Foundation.
2 - Queensland Department of Primary Industries