VG405
An IPM package for heliothis in vegetables

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Know-how for Horticulture™

VG405

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INDUSTRY SUMMARY

The heliothis caterpillar is a serious insect pest of vegetables in Australia. The larvae cause obvious feeding damage in vegetables, rendering them unsuitable for sale as fresh produce. Heliothis must be killed when they are eggs or small caterpillars to prevent noticeable damage in most vegetables.

Large heliothis caterpillars normally feed in sheltered sites (e.g. the heart of lettuce plants, or in corn cobs) and are virtually impossible to contact with chemicals. In addition, the caterpillars are resistant to many of the chemicals used against them.

Problems arise in vegetables when large larvae are prevalent. There are a number of possible reasons as to how this situation could arise, e.g. an egg lay may be undetected and a crucial insecticide application missed or poorly timed; or weather conditions may render spraying impossible; or poor application may result in poor pest kill. Resistance to chemicals may also be a factor.

The combined effects of insecticide resistance and increased public concern about the environmental safety of using chemical insecticides suggests that chemicals will not remain the sole pest management tool for the vegetable industry in the future. New control tactics must therefore be developed and integrated into existing management strategies. In some instance chemicals insecticides may need to be abandoned - simply because they don't work any more.

The objective of the research presented in this report was to evaluate new ways of killing heliothis that may be useful in an Integrated Pest Management (IPM) approach to pest management. IPM involves using a number of pest management tactics to manage pests, with no total dependence on a single tactic (e.g. chemical insecticides). The use of IPM practices is a more sustainable approach to pest management.

Two new tactics were investigated for this report. One involved using parasitic wasps that attack heliothis eggs, and the other involved using a viral pathogen that only kills heliothis caterpillars. These tactics were studied in lettuce and sweet corn.

The periodic release of wasps is of no value in lettuce because the plants do not provide enough shelter to protect the wasps from the high temperatures encountered during summer. Wasp releases in corn may have some potential, although encouraging natural populations to build up by avoiding the use of chemical insecticides is the best and cheapest practice, particularly if the crop is late-planted.

The results indicated that the virus has enormous potential for use in IPM programs in both lettuce and sweet corn. Not only does the virus kill caterpillars, it is safe on predators. The combined action of virus and naturally occurring predators/parasites killed 95% of heliothis in some trials. Additional research is needed to capitalise on the use of virus and natural enemies.

Researchers are currently obtaining data to assist the registration of virus in vegetable crops. Hopefully it will be available in the next 2-3 years.

TECHNICAL SUMMARY

Heliothis, *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (LEPIDOPTERA: NOCTUIDAE), are serious pests of field, horticultural and ornamental crops in Australia. The annual cost of heliothis to Queensland's primary producers is estimated to be $70 million, including over $17 million in horticultural crops. Both heliothis species are pests of lettuce, and only *H. armigera* is a pest of sweet corn. It is becoming increasingly difficult to manage *H. armigera* with conventional chemical insecticides due to resistance problems. Despite this and increasing public concern about the environmental safety of chemicals, insecticides continue to be the most widespread commercially used method of controlling heliothis on vegetables.

The objective of the research presented in this report was to evaluate heliothis management tactics that display promise for inclusion in Integrated Pest Management (IPM) strategies in sweet corn and lettuce. IPM involves using a number of pest management tactics to manage pests, with no total dependence on a single tactic (e.g. chemical insecticides). The use of IPM practices is a more sustainable approach to pest management.

Two new tactics were investigated for this report. One involved using the egg parasitoid *Trichogramma*, and the other involved using NPV - a viral pathogen that only kills heliothis larvae.

Inundative releases of *Trichogramma* against heliothis in lettuce were ineffective because the plants do not provide enough shelter to protect the wasps from the high temperatures encountered during summer. Wasp releases in corn may have some potential, although encouraging natural populations to build up by avoiding the use of chemical insecticides is the best and cheapest practice, particularly if the crop is late-planted.

A number of heliothis management options were evaluated in sweet corn. viz. chemical insecticides, NPV plus *Trichogramma*, *B.t.*, *Trichogramma* alone, and no action at all (control). The NPV + *Trichogramma* plots had the lowest cob damage (6%), followed by the *B.t.* plots (12%), *Trichogramma* alone (20%), the control plots (23%) and the chemically sprayed plots (53%). There was no evidence to suggest that the *Trichogramma* releases had any impact on heliothis egg mortality. However, there was a large natural population of *Trichogramma* present in all plots. The application of chemicals in some plots reduced the action of these wasps and lead to significantly more damage in those plots.

The results indicated that NPV has enormous potential for use in IPM programs in both lettuce and sweet corn. Not only does the virus kill caterpillars, it is safe on predators. The combined action of NPV and naturally occurring predators/parasitoids killed 95% of heliothis in some trials. Additional research is needed to capitalise on the use of virus and natural enemies. Researchers are currently obtaining data to assist the registration of NPV in vegetable crops. Hopefully it will be available in the next 2-3 years.

INTRODUCTION

BACKGROUND

Heliothis, *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (LEPIDOPTERA: NOCTUIDAE), are serious pests of field, horticultural and ornamental crops in Australia (Zalucki *et al.*, 1986; White *et al.*, 1995). The annual cost of heliothis to Queensland's primary producers is estimated to be $70 million (Adamson *et al.*, 1997). This includes over $17 million in horticultural crops. These figures are based on a residual pest damage of 5% and include pest management costs, i.e. 5% of the crop is still lost after pest management expenses. The average expected loss without pest management is estimated at over $260 million for Queensland, including $100 million in horticultural crops (Adamson *et al.*, 1997).

In sweet corn, heliothis lay eggs on the emerging silks. Small larvae (caterpillars) feed on the silks as they tunnel into the top of the cob. Larvae cause obvious feeding damage to cobs, rendering them unsuitable for sale as fresh produce. Heliothis must be killed at the egg or young larval stage to prevent cob damage. Once larvae are sheltered they are difficult to contact with insecticides. Larvae may seek shelter under silks or by burrowing into cobs. Only one of the two heliothis pests (*H. armigera*) attacks corn (Fitt, 1989). It is becoming increasingly difficult to manage *H. armigera* with conventional chemical insecticides due to resistance problems (Deuter, 1995).

In lettuce, heliothis lay eggs on the upper and lower leaf surface. The larvae eventually move or tunnel into the heart of the plant where they are sheltered and difficult to contact with insecticides. Infested heads are difficult to detect and may be packed with clean heads. The larvae are then free to move to other (clean) heads and cause more widespread feeding damage. Both *H. armigera* and *H. punctigera* are pests of lettuce. *H. punctigera* is not resistant to chemical insecticides, and can be managed by correctly timed and applied sprays. *H. armigera* is resistant to all of the chemicals commonly used in lettuce, and is becoming harder to manage.

To date, insecticides are the most widespread commercially used means of controlling heliothis on vegetables in Australia. There is now widespread resistance to the synthetic pyrethroids throughout eastern Australia (Daly, 1988). Heliothis has also developed resistance to endosulfan (Daly, 1992), and there is increasing environmental concern about this product (Pyke, 1996). The carbamate group of chemicals have been favoured for heliothis in recent years - methomyl (Lannate®) at first, and thiodicarb (Larvin®) more recently. However the incidence of resistance to this group is on the increase, and resistance to thiodicard imposes resistance to all carbamates (Howie, 1995; Gunning, 1996).

Problems arise in vegetables when large larvae are prevalent. There are a number of possible reasons as to how this situation could arise, e.g. an egg lay may be undetected and a crucial insecticide application missed or poorly timed; or weather conditions may render spraying impossible; or poor application may result in poor pest kill. Resistance to chemicals may also be a factor.

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 horticultural industry in the future. Supplementary control tactics must therefore be developed and integrated into existing management strategies. In some instance chemicals insecticides may need to be abandoned - simply because they don't work any more.

The objective of the research presented in this report was to evaluate heliothis management tactics that display promise for inclusion in Integrated Pest Management (IPM) strategies in sweet corn and lettuce. IPM involves using a number of pest management tactics to manage pests, with no total dependence on a single tactic (e.g. chemical insecticides). The use of IPM practices is a more sustainable approach to pest management.

Two new tactics were investigated for this report. One involved using parasitic wasps that attack heliothis eggs, and the other involved using a viral pathogen that only kills heliothis larvae.

Trichogramma WASPS

The parasitic wasps that attack heliothis eggs are scientifically known as *Trichogramma*, and they have been successfully used as inundative biocontrol agents of caterpillar pests throughout the world (Stinner, 1977; Wajnberg and Hassan, 1994). Inundative biological control involves releasing large numbers of natural enemies to overwhelm a pest population and maintain pest density below that level causing economic damage (Stehr, 1982). For *Trichogramma* this means releasing hundreds of thousands of wasps per hectare of crop.

Trichogramma and relatives are known as egg parasitoids, and have attracted worldwide interest because they kill pest insects before they hatch and cause damage. Parasitoids differ from true parasites in that the development of an individual always destroys its host (Doutt, 1959). Their action on a host population more closely resembles that of a predator than a parasite

Trichogramma are minute wasps - less than ½ mm long. The female *Trichogramma* inserts her ovipositor through the shell of a host egg (e.g. a heliothis egg) and lays her eggs. The *Trichogramma* eggs hatch and the wasp larvae consume the contents of the host egg as they develop. Metamorphosis occurs inside the host egg. The larvae eventually pupate and emerge as adult wasps. The entire life-cycle, from egg to wasp, takes approximately ten days at 25°C. The adult wasps feed on nectar and exudate from the puncture sites of parasitised host eggs and live for approximately five days.

Host egg colour is useful when assessing levels of egg parasitism. A heliothis egg that has been parasitised by a *Trichogramma* wasp turns black, having a uniform black shell, after four days at 25°C. The colour of unparasitised eggs changes with age also. One, two and three-day-old normal (unparasitised) eggs are white, brown and 'black' respectively. The 'black' colour of the three-day-old normal eggs is due to

the black head capsule of the developing larva that is visible through the clear egg shell (the egg shell of a parasitised egg is black) (see Plate 1).

Trichogramma have to be mass reared if they are used as inundative biocontrol agents. The Queensland Department of Primary Industries (QDPI) has been actively involved in the development of mass production techniques, and has provided assistance to companies that want to produce *Trichogramma* for sale to primary producers and the public. *Trichogramma* are currently available commercially from Bugs for Bugs at Mundubbera in Queensland.

Experimental work evaluating *Trichogramma* against heliothis in sweet corn has given mixed results. Some workers found that heliothis can not be managed by *Trichogramma* (Fletcher, 1935; Larrimer, 1935; Neil and Specht, 1990). However, research interest in *Trichogramma* persists because natural populations of egg parasitoids can have an impact on heliothis in unsprayed crops (Oatman, 1966). *Trichogramma* have also been successfully used against other insect pests (see Wajnberg and Hassan, 1994).

Previous Australian research demonstrated that releases of *Trichogramma* could be used to increase heliothis egg mortality in maize/sweet corn (Scholz, 1994; Scholz and Webster, 1994), but did not evaluate the effect of wasp releases on cob damage and larval presence at harvest. Scholz (1994) also found that the combination of natural populations of *Trichogramma* and applications of *B.t.* (a bacterial pathogen) could effectively manage heliothis in late planted sweet corn.

Trichogramma have potential to be utilised as biological control agents of heliothis. However, the level of heliothis control that can be expected from wasp releases is still unclear. Therefore, wasp releases were further studied for this report.

NPV - A Viral Pathogen

The viral pathogen that attacks heliothis is known as Nuclear Polyhedrosis Virus, or NPV. The use of viral insecticides have several advantages over chemical insecticides (Jones, 1994; Teakle *et al.*, 1996). NPV is a virus that only infects heliothis larvae, and it can be applied to a crop using conventional spray equipment. It does not affect beneficial invertebrates or vertebrates (including humans), and consequently it does not leave toxic residues. NPV can be self-perpetuating because dead infected larvae release virus particles into the environment, and healthy larvae may become infected by consuming plant material containing these particles. To date, there have been no problems with heliothis developing resistance to NPV in the field.

NPV is highly potent against newly hatched larvae, and is most effective when applied just prior to egg hatch so that the hatching larva ingests the virus as it chews through the egg shell. NPV has to be ingested to cause death. It is slow acting, usually taking 5-7 days to kill larvae. There is, however, reduced feeding by larvae during this time. Infected larvae typically move to the top of a plant as their body contents are dissolved by the virus (see Plate 2). Their body eventually ruptures releasing millions of virus particles into the surrounding environment. NPV is

sensitive to UV exposure and breaks down rapidly in sunlight - it is persistent in the field for about 2 days.

NPV was available in Australia during the early 1980's as a product called Elcar® (Teakle *et al.*, 1996), and showed promise for managing heliothis in cotton, sorghum and navy beans (Room, 1979; Teakle *et al.*, 1983; Rogers *et al.*, 1983; Teakle *et al.*, 1985). However its introduction coincided with that of the synthetic pyrethroids (and other chemicals) and it attracted little interest in the market place.

A new NPV product called GemStar® has recently become available for experimental evaluation and this product has shown promise against heliothis in cotton, sorghum and chickpea (Teakle, 1994; Murray *et al.*, 1996). GemStar® also has potential for use against heliothis in vegetables, so it was evaluated for this report.

Research Objectives

The project objective was to evaluate new IPM techniques against heliothis in lettuce and sweet corn. Evaluations of native *Trichogramma* and/or NPV against heliothis were carried out in experimental (lettuce and corn) and commercial (lettuce) plantings of crops.

Aspects of the biology of *Trichogramma* that affect their performance in the field were also investigated. These included laboratory and field studies evaluating different species of *Trichogramma*, and studies of some factors affecting the emergence of *Trichogramma* in the field (storage; release technique; high temperatures; predation).

A bioassay investigating the persistence of NPV on lettuce plants in the field was also conducted.

The research findings are discussed, and recommendations for future work are provided.

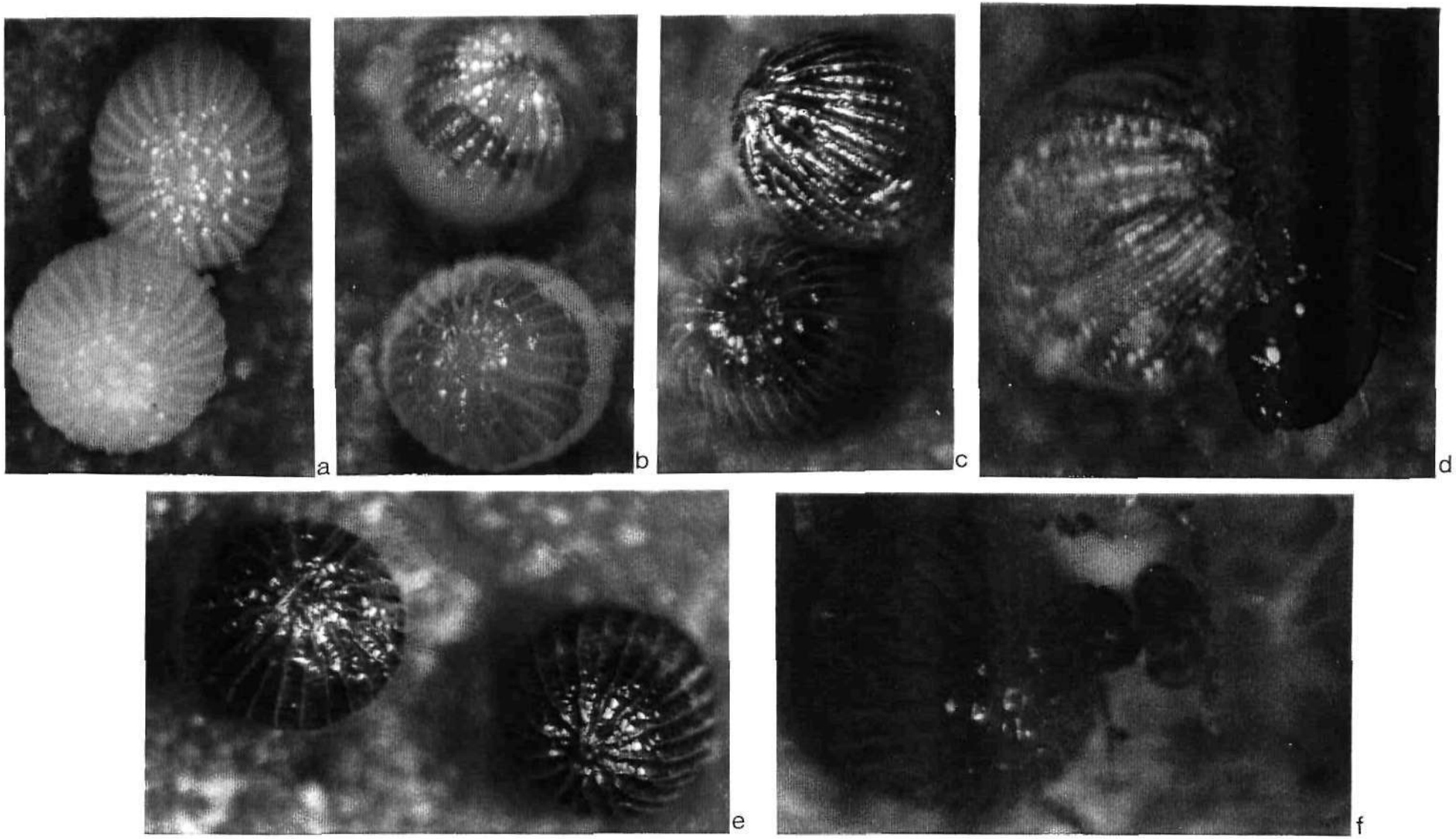
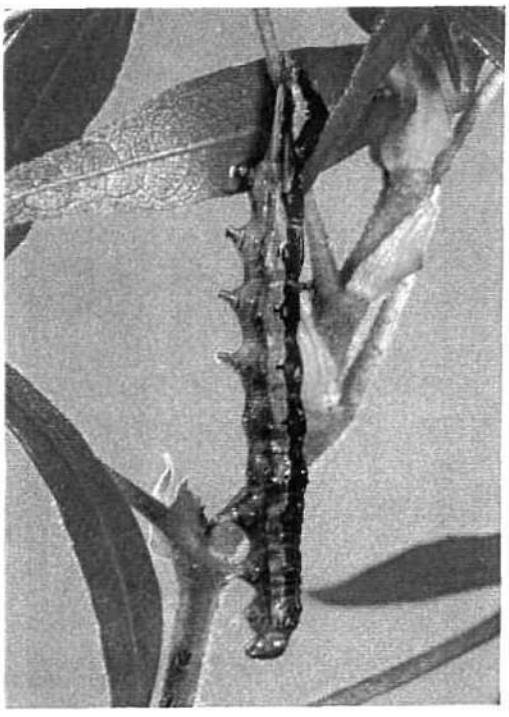


PLATE 1 : Healthy (unparasitised) and parasitised *Helicoverpa armigera* eggs. (A) Healthy one day old eggs. These eggs are pearly white in colour. **(B)** Healthy two day old eggs. Eggs at this stage are called brown, coloured, or 'red-ring'. **(C)** Healthy three day old eggs. This is called the 'black head' stage of egg development because the black head capsule of the developing larva is clearly visible through the clear egg shell. **(D)** Neonate larva emerging from an egg. **(E)** Four day old parasitised eggs. Parasitised eggs turn black four days after being parasitised by *Trichogramma*, i.e. the egg shell turns black, unlike (C) above where the black colouration is due to the neonate head capsule. Six days later one or more wasps emerge from the egg (F). The life-cycle of a Trichogrammatid wasp (from egg to adult emergence) takes approximately ten days at 25°C. The occurrence of black eggs in a crop indicates the presence of egg parasitoids. Magnifications: A, B, C, E - x 40. D, F - x 60.
Photos: B. Scholz.

A



B

PLATE 2 : Heliothis larvae infected with NPV. Infected larvae typically move to the top of a plant or plant structure (e.g. a corn cob) and 'hang' with their head down. Their body eventually ruptures releasing millions of virus particles. Insect skin and a brown stain on a plant structure indicates mortality due to virus. (**A**) A virus infected heliothis on sorghum - x 3. (**B**) A virus infected heliothis on cotton - x 2.5.
Photos courtesy of QDPI Photo Library.

MATERIALS AND METHODS

GENERAL LABORATORY PROCEDURES

Controlled Environments: Two insect rearing rooms and a constant temperature room were maintained at approximately 25 °C and 70% R.H.. A natural photoperiod (via windows) was used in the insect rearing rooms, and a 14:10 (L:D) photoperiod was used in the constant temperature room. Field collected heliothis eggs and larvae were always held in the constant temperature room.

Insect Rearing: Full details of insect rearing techniques are provided in Appendix 1. Heliothis (*Helicoverpa armigera* (Hübner)) were reared on a navy bean based diet (after Teakle and Jensen, 1985). Egg parasitoids were mass reared on eggs of the Angoumois grain moth (*Sitotroga cerealella* (Olivier)) using techniques similar to those of Morrison (1985) and Laing and Eden (1990).

Egg Cards: Heliothis egg cards were used to assess predation and parasitism in some field trials. Adult moths were placed in oviposition chambers where they laid eggs onto paper towelling (see Teakle and Jensen, 1985). Each card was made by stapling pieces of paper towelling containing *H. armigera* eggs (less than 24 hours old) to paper strips measuring 1.5 x 7 cm. There were at least ten eggs per card. The cards were stapled to the upper surfaces of leaves in the field. They were collected 48 hours later and held in a constant temperature room to assess parasitism.

Egg Collections: Naturally laid heliothis eggs were collected from crops on many occasions. A 6 mm Ø leaf punch (after Hoffman *et al.*, 1970) was used to collect leaf discs containing one or more eggs from lettuce and vegetative sweet corn. Each leaf disc was individually placed into a well in a plastic microtitre tray (6 mm Ø wells; 96 wells per tray). The tray was covered with clear plastic film or sticky tape to contain neonates and emerged parasitoids, and held in a constant temperature room until levels of parasitism could be determined.

Eggs on silking corn were collected by cutting off silks with secateurs and individually placing them into brown paper bags (13 x 25 cm). The paper bags were labelled, held in an esky, and returned to the laboratory where each silk was individually examined. All heliothis eggs were removed from silks using a fine paint brush (Sable 000) dipped in water, and individually held in microtitre trays. The egg colour was recorded for each egg collected from each silk, i.e. white, brown or black (parasitised). The eggs were kept in a constant temperature room until parasitism levels could be determined.

Microtitre trays were also used to evaluate the emergence of egg parasitoids. Parasitised *Sitotroga* eggs were individually placed into the wells using a fine paint brush (Sable 000) dipped in water. The trays were then covered with sticky tape and held in the field or laboratory to monitor the daily emergence of egg parasitoids.

Larval Collections: Field collected larvae were individually held in 28 mL plastic cups containing a small amount of navy bean diet. A tight fitting lid prevented larvae from escaping. A dissecting needle was used to punch 8-10 pin holes in each lid for

ventilation. Individual records of larvae could be kept by numbering each lid with a niko pen.

Trichogramma Release Rates: *Trichogramma* were released as pupae in parasitised *Sitotroga* eggs. The number of *Trichogramma* egg parasitoids released in trials was assessed by counting the number of parasitised *Sitotroga* eggs in a weighed sub-sample, i.e. to determine a number per gram. One wasp normally emerges from each parasitised *Sitotroga* egg. There are approximately 54,000 parasitised *Sitotroga* eggs per gram when almost all eggs are parasitised. However, it is best to assess a sub-sample of the release material because there may not be 100% parasitism. Individual parasitised eggs were held in microtitre trays (see above) to determine sex ratio and emergence. The number of wasps released can be calculated from the following formula:

$$\text{No. wasps Released (R)} = N \times W \times P$$

where N = the number of parasitised *Sitotroga* eggs per gram.
W = the weight of parasitised *Sitotroga* eggs released in grams.
P = the proportion of wasps emerged from the parasitised eggs.

The number of females released (only the females attack heliothis eggs) can be calculated by multiplying R above by the proportion of females determined in the sex ratio assessment.

Handling Adult Trichogramma: *Trichogramma* are positively phototactic (attracted to light). Adults seldom fly when released onto a laboratory bench, preferring to walk towards light. Individuals can be captured by placing the base of a clear gelatin capsule (Parke-Davis No. 100) over them, and waiting until they walk up its side. The wasp can then be contained by capping the capsule. Individuals can be transferred to other containers by removing the cap of the gelatin capsule and flicking the base with a finger.

LABORATORY INVESTIGATIONS

A number of laboratory studies were conducted to complement field work.

Lab Study 1: Species comparisons

The parasitism levels of different species/strains of egg parasitoids was studied to determine if the commercially available species of wasp (*Trichogramma* nr. *brassicae* Bezdenko) was a suitable heliothis biocontrol agent.

The number of *H. armigera* eggs parasitised, and the sex and number of progeny produced, by different species/strains of egg parasitoids was investigated in the laboratory. A newly emerged (less than 6 hours old), fed, mated female parasitoid was placed into a small glass vial (25 x 50 mm) containing approximately 100 *H.*

armigera eggs (<24 hours old) on paper towelling. Each female was exposed to the eggs for 4 hours.

The eggs were held in a constant temperature room until those that were parasitised produced progeny. The number of eggs parasitised, and the sex and number of emerged progeny were counted. There were 20 replicates for each of 14 species/strains of egg parasitoids collected (Table 1).

Lab Study 2: Short-term Storage of *Trichogramma* at Cool Temperatures

The life-cycle (egg-adult) of an egg parasitoid takes approximately ten days at 25°C. Adult emergence can be delayed by storing parasitised eggs at cool temperatures for short durations. This may be necessary when adult emergence does not coincide with a planned release date.

The variation in emergence of wasps stored at cool temperatures was investigated in the laboratory. Parasitised *Sitotroga cerealella* eggs were individually held in micotitre trays (50 eggs per tray, 4 replicates). They were covered with sticky tape and cold stored eight days after being parasitised at 25°C. The trays were stored in an incubator at approximately 8.7°C, 70% R.H. and 0:24 (L:D) photoperiod for 0 (control), or 1-7 weeks.

After storage the trays were transferred back to a constant temperature room (approx. 25°C) and checked for emergence daily. Control trays were not cold stored, but were checked daily to record emergence at 25°C. Data were obtained for two species of parasitoids, i.e. *Trichogramma pretiosum* Riley and *Trichogrammatoidea bactrae* Nagaraja.

Lab Study 3: Emergence of *Trichogramma* at High Temperatures

Egg parasitoids released as biocontrol agents are released as pupae in *Sitotroga cerealella* eggs. These eggs may land on the soil or on the plant, and may be exposed to high temperatures before they emerge. Laboratory experiments were designed to study the effects of short exposures to high temperatures on the emergence of *Trichogramma* wasps.

Paper cards containing wasp pupae in parasitised *Sitotroga cerealella* eggs were cut into small strips of 100-200 eggs and placed into small glass vials (50 x 25 mm) stoppered with a gauze covered lid (the gauze contained wasps that emerged and provided ventilation). The small glass vials were placed inside a sealed one litre glass jar containing a sodium chloride saturated salt solution that maintained the relative humidity at approximately 75% (Winston and Bates, 1960).

The wasp pupae were exposed to constant temperatures of 30, 34, 38, 44 and 51°C in a multichamber constant temperature incubator for either 1, 2, 4 or 6 hours. After exposure, they were held at 25°C for two weeks and the percentage adult emergence was assessed by counting the numbers of adult wasps and the number of host eggs (% emergence = (no. wasps/no. eggs) x 100). This procedure was repeated for three

species of wasp egg parasitoids (*Trichogramma* nr. *brassicae*, *Trichogramma pretiosum*, *Trichogrammatoidea bactrae*) following 0, 1, 2, or 3 weeks of cold storage (8.7°C) seven days after parasitisation.

Lab Study 4: Egg Predation

Predation of *Trichogramma* pupae (in parasitised *Sitotroga* eggs) and heliothis eggs was assessed in the laboratory. Adult predators were collected from sweet corn at Gatton during December 1996. Five species were collected, i.e. the red and blue beetle (*Dicranolaius bellulus* (Guérin-Méneville)), the two-spotted ladybird (*Diomus notescens* (Blackburn)), transverse ladybird (*Coccinella transversalis* Fabricius), the striped ladybird ((*Micraspis frenata* (Erichson)), and the pirate bug (*Orius* spp.).

The predators were held in a constant temperature room overnight before they were exposed to eggs. One predator was placed in a 9 cm Ø glass petri dish and exposed to either ten heliothis eggs (< 24 hours old) or 20 parasitised *Sitotroga* eggs on a filter paper. The numbers of eggs eaten at various times after exposure were recorded (2, 4, 24 hours after exposure). This procedure was repeated ten times for each predator/egg combination.

Lab Study 5: *Trichogramma* Release Techniques

The emergence of *Trichogramma* nr. *brassicae* from parasitised *Sitotroga* eggs was evaluated following different release techniques. The first release technique involved releasing a dry mixture of parasitised *Sitotroga cerealella* eggs and semolina (used as a bulking agent because of the small volumes of *Sitotroga* eggs normally released). The mixture was released from a hopper mounted on a tractor, and was dispensed via a seed drill into six outlets. Samples of the mixture were collected before passage through the hopper/seed drill, and samples were collected from each of the six outlets after passage through the hopper/seed drill. Parasitised *Sitotroga* eggs from each sample were individually held in micotitre trays (50 eggs per tray, 4 replicates). The trays were covered with sticky tape and stored in a constant temperature room until the adult wasps emerged. The proportion of wasps that emerged was then determined.

The second release technique evaluated liquid suspensions of parasitised *Sitotroga* eggs released from a pressurised hand held sprayer (the spray section was modified so that individual parasitised eggs would flow through the nozzle) or a mistblower (Stihl SR400). A water 'thickener' was used to disperse the parasitised *Sitotroga* eggs evenly throughout the suspension. Two thickeners were tested: 1) Instant Gel-It® at a rate of 40 g product/L, and 2) AquaKeep® at a rate of 2 g product/L. Parasitised *Sitotroga* eggs were mixed in a solution with each thickener and sprayed onto corn leaves. The leaves were then collected and leaf sections containing eggs were stored in ventilated 1 L glass jars in a constant temperature room until the wasps emerged. The levels of wasp emergence were then determined.

TABLE 1

Egg parasitoid strain backgrounds.

CODE	LOCALITY		SPECIES	DATE STARTED	CROP	HOST
BP6	Bundaberg (Bio-Protection)	S 24°52' E 152°21'	*Trichogramma* nr. *brassicae*	8 February 1995	tomatoes	*Sitotroga cerealella*
BP8	Elimbah (Bio-Protection)	S 27°01' E 152°57'	*Trichogramma* nr. *australicum*	15 November 1995	tomatoes	*Sitotroga cerealella*
JON4	Jondaryan	S 27°25' E 153°33'	*Trichogramma carverae*	30 March 1992	sorghum	*Helicoverpa armigera*
JON5	Jondaryan	S 27°25' E 153°33'	*Trichogramma funiculatum*	2 April 1992	sorghum	*Helicoverpa armigera*
JON6	Jondaryan	S 27°25' E 153°33'	*Trichogramma* nr. *australicum*	2 April 1992	sorghum	*Helicoverpa armigera*
JON7	Jondaryan	S 27°25' E 153°33'	*Trichogrammatoidea bactrae*	2 April 1992	sorghum	*Helicoverpa armigera*
KUN1	Kunnunurra	S 15°47' E 128°44'	*Trichogramma pretiosum*	6 Octoober 1994	sunflowers	*Helicoverpa armigera*
M1	Mulgowie	S 27°44' E 152°22'	*Trichogramma* nr. *brassicae*	13 December 1994	sweet corn	*Helicoverpa armigera*
MA4	Ma Ma Creek	S 27°38' E 152° 11'	*Trichogramma carverae*	14 April 1994	cauliflowers	*Helicoverpa* spp.
N2	Nandi	S 27°13' E 151°10'	*Trichogrammatoidea bactrae*	15 March 1995	cotton	*Helicoverpa* spp.
N3	Nandi	S 27°13' E 151°10'	*Trichogramma carverae*	15 March 1995	cotton	*Helicoverpa* spp.
PS1	Gatton (Pacific Seeds)	S 27°34' E 152°19'	*Trichogramma* nr. *brassicae*	18 January 1995	sweet corn	*Helicoverpa armigera*
RR1	Red Ridge	S 25°10' E 152°24'	*Trichogrammatoidea bactrae* (yellow form)	24 March 1993	tomatoes	*Helicoverpa* spp.
T1	Tallarook VIC.	S 37°06' E 145°06'	*Trichogramma* nr. *brassicae*	22 November 1990	peas	*Helicoverpa* spp.

LETTUCE TRIALS

Lettuce Trial 1

Inundative releases of *Trichogramma* nr. *brassicae* against heliothis on Seagreen lettuce were evaluated in a commercial crop situation at Cambooya on the Darling Downs (S 27° 45' E 151° 53'). The *Trichogramma* were purchased from a commercial supplier, and were released from a hopper mounted on a tractor. Parasitised *Sitotroga cerealella* eggs (containing wasp pupae) were mixed with semolina (wheat cereal powder) as a bulker when releasing. The mixture was dispensed via a seed drill into eight outlets that fed eight plastic hoses. These hoses deposited the mixture onto each row planting of lettuce.

The lettuce were planted in bays 16 rows wide and 120 m long, with approximately 50,000 plants/ha. The semolina/*Sitotroga* mixture was applied dry, usually early in the morning so that any dew on the plants would assist in sticking the parasitised eggs to the plants. Approximately 1 kg mixture was applied per hectare, and the release rates of parasitised *Sitotroga* eggs ranged from 100-900,000/ha. The trial coincided with the spring flight of *H. punctigera*.

Heliothis egg infestation was evaluated by counting the numbers of eggs on three consecutive plants in three consecutive rows, i.e. a grid of nine plants. This was undertaken at three random locations throughout the site, and mean egg infestation per plant was determined for the 27 plants sampled. Approximately 50 eggs were randomly collected from the crop on each sampling date. The eggs were individually placed into the wells of microtitre trays and held in a constant temperature room until the eggs hatched or turned black (indicating that they were parasitised). The trial was sampled nine times over a four week period.

Suction samples were taken on each sampling occasion to assess egg parasitoid numbers. A Stihl BG72 suction machine was used to collect the samples. A fine muslin bag was attached to the inlet tube of the machine using a velcro strap. Four samples of 50 consecutive plants were collected on each sampling occasion. The collection was immediately emptied into 70% alcohol in the field and taken back to the laboratory for sorting and counting under a stereo microscope.

The crop consultant who managed the *Trichogramma* releases provided samples of parasitised *Sitotroga* eggs that were stored in an ordinary refrigerator to delay emergence. Samples of 50 eggs were individually placed into the wells of a microtitre tray, covered with thin plastic film, and held in a constant temperature room. The trays were checked for wasp emergence at regular intervals and the wells containing wasps were recorded. This was completed for samples of parasitised *Sitotroga* eggs that had been cold stored for different lengths of time ranging from less than one week to four weeks.

Lettuce Trial 2

A replicated small plot trial was carried out in a commercial planting of lettuce at Cambooya to compare the levels of heliothis egg parasitism following releases of four different strains of egg parasitoids. Each plot consisted of 8 rows of Seagreen lettuce, with 16 plants per row length. The plants were 10-15 cm in diameter.

The *Trichogramma* were released as pupae (in parasitised *Sitotroga* eggs) by mixing 0.05 g of parasitised *Sitotroga* eggs with 5.0 g of semolina. This mixture was sprinkled by hand onto every plant in each plot. The plants were misted with water from a hand held atomiser to adhere the mixture to the leaves.

Samples of the parasitised *Sitotroga* eggs were held in microtitre trays to estimate the number of wasps produced per unit of parasitised *Sitotroga* eggs, their sex ratio, and the daily emergence pattern. The numbers of *Trichogramma* released per plot were estimated using these data and are given below.

The treatments were:

1) *Trichogramma pretiosum:* 1210 females/plot
2) *Trichogramma* nr. *brassicae* (strain BP): 1620 females/plot
3) *Trichogramma* nr. *brassicae* (strain QDPI): 1654 females/plot
4) *Trichogrammatoidea bactrae:* 1435 females/plot
5) Control - no egg parasitoids released.

The levels of heliothis egg parasitism were assessed by stapling egg cards onto the upper surfaces of lettuce leaves. The egg cards were stapled to leaves on every third plant in every row in a grid pattern. There were 48 cards stapled in each plot, and four replicates per treatment. They were collected after 48 hours and held in a constant temperature room until the eggs had either hatched or turned black (indicating that they were parasitised). The proportion of cards containing parasitised eggs was used to indicate *Trichogramma* activity in the crop. Two sets of cards were stapled to plants in the field and they were collected 2 and 3 days after wasp release respectively.

Lettuce Trial 3

A replicated small plot trial was carried out in a commercial planting of hail damaged lettuce at Cambooya to compare the levels of heliothis egg parasitism following releases of two different strains of egg parasitoids in fully grown lettuce plants. Each plot consisted of 8 rows of Seagreen lettuce, with 16 plants per row length. The plants were 30-40 cm in diameter.

The *Trichogramma* were released as pupae (in parasitised *Sitotroga* eggs) by mixing 0.2 g of parasitised *Sitotroga* eggs with 5.0 g of semolina. This mixture was sprinkled by hand onto every plant in each plot. The plants were misted with water from a hand held atomiser to adhere the mixture to the leaves.

Samples of the parasitised *Sitotroga* eggs were held in microtitre trays to estimate the number of wasps produced per unit of parasitised *Sitotroga* eggs, their sex ratio, and the daily emergence pattern. The numbers of *Trichogramma* released per plot were estimated using these data and are given below.

The treatments were:

1) *Trichogramma pretiosum:* 3695 females/plot
2) *Trichogramma* nr. *brassicae* (strain BP): 3324 females/plot
3) Control - no egg parasitoids released.

The levels of heliothis egg parasitism were assessed by stapling egg cards onto the upper surfaces of lettuce leaves. The egg cards were stapled to leaves on every third plant in every row in a grid pattern. There were 48 cards stapled in each plot, and four replicates per treatment. They were collected after 48 hours and held in a constant temperature room until the eggs had either hatched or turned black (indicating that they were parasitised). The proportion of cards containing parasitised eggs was used to indicate *Trichogramma* activity in the crop. The egg cards were stapled to plants in the field on the day of release, and collected 48 hours later.

Lettuce Trial 4

Nuclear Polyhedrosis Virus (NPV) was applied to a commercial lettuce planting in Toowoomba where sprays of chemicals had failed. The following levels of resistance were found in larvae collected from this site (results from Dr. Robyn Gunning, NSW Agriculture):

synthetic pyrethroids	80%
endosulfan	50%
thiodicarb	60%.

A commercially produced formulation of NPV, GemStar®, was applied to the infested lettuce on 26 March 1995, and again two days later. The first application was at a rate of 1100 ml/ha with 1% molasses at 1500 hours (moderate rain occurred in the evening). The second application was at 750 ml/ha with 1% molasses at 1500 hours.

Heliothis larvae were collected on March 26 (pre-application), 27 and 31. The larvae were individually placed into 28 mL diet cups and held in a constant temperature room until they had died or completed development. The cause of death was recorded for each larva.

Lettuce Trial 5

The effects of NPV (GemStar®), with and without the UV protectant Coax®, on heliothis larvae were assessed in a field planting of lettuce (variety Classic) at QDPI Gatton Research Station. The lettuce were planted on 5 February 1997 in raised beds 140 cm apart and 70 m long. There were two rows of lettuce in each bed, and seven beds in the trial. Twelve plots (10 x 3 m) arranged as randomised complete blocks were used to compare three treatments replicated four times. Each treatment plot was separated by a buffer plot (10 x 3 m). The treatments were: 1) NPV - applied at 741 mL/ha in a spray volume of 100 L/ha using a knapsack sprayer; 2) NPV + Coax - applied at 741 mL/ha with 1% Coax and 0.1% non-ionic wetting agent; 3) Control - sprayed with water + wetting agent.

There was no significant natural heliothis larval infestation at the site. Laboratory reared larvae (reared on a navy bean based diet (Teakle and Jensen, 1985)) were individually placed on lettuce leaves in the field before spraying. Two small larvae (3-7 mm long) were placed on each plant (approximately 2,400 larvae total). The virus was applied at 1500 hours.

Lettuce plants were destructively sampled 1 and 3 days after spraying (DAS). The larvae found on the plants were individually placed into 28 mL diet cups, returned to the laboratory and held in a constant temperature room. At least 25 larvae per plot were collected on each sampling day. These were inspected daily to record their fate, i.e. healthy, NPV mortality or other mortality.

Lettuce Trial 6

A bioassay study to determine the residual effects of NPV (GemStar®), with and without the UV protectant Coax®, on heliothis larvae was carried out in a field planting of lettuce (variety Classic) at QDPI Gatton Research Station. The lettuce were planted on 5 February 1997 in raised beds 140 cm apart and 70 m long. There were two rows of lettuce in each bed, and seven beds in the trial. Three unreplicated adjacent plots 10 x 9 m were treated as follows: 1) NPV - applied at 741 mL/ha in a spray volume of 30 L/ha using a hand held rotary cage atomiser; 2) NPV + Coax® - applied at 741 mL/ha with 1% Coax® and 0.1% non-ionic wetting agent; 3) Control - sprayed with water + wetting agent.

Leaf discs (45 mm Ø) were cut from sprayed plants in the field using a pastry cutter. Each leaf disc was placed into a 50 mm Ø plastic petri dish (Falcon #1006). One small (3-7 mm long) laboratory reared *H. armigera* larva was placed onto the upper (sprayed) surface of the each leaf disc, and a tight fitting lid contained each larva in the petri dish. After 24 hours the larvae were individually transferred to 28 mL diet cups and held in a constant temperature room. They were examined daily and their fate recorded.

SWEET CORN TRIALS

Corn Trial 1

A comparison of heliothis egg parasitism by two species of *Trichogramma* was assessed in replicated small sweet corn plots at Lowood (S 27° 28' E 152° 27'). The trial site was a 0.75 ha planting of sweet corn (Golden Sweet) that was tasselling. Each plot was 8 rows wide and 6 m long and 40 m apart. A randomised complete block design of three treatments with four replicates was used in this trial. The treatments were: (1) Release of *Trichogramma* nr. *brassicae* (the commercially available species); (2) Release *of Trichogramma pretiosum*; (3) Control - no *Trichogramma* releases.

The levels of heliothis egg parasitism were assessed by stapling egg cards onto the upper surfaces of corn leaves. The egg cards were stapled to leaves every metre in every row in a grid pattern. There were 48 cards stapled in each plot. They were collected after 48 hours and held in a constant temperature room until the eggs had either hatched or turned black (indicating that they were parasitised). The proportion of cards containing parasitised eggs was used to indicate *Trichogramma* activity in the crop.

The *Trichogramma* were released as pupae (in parasitised *Sitotroga* eggs) by mixing 0.2 g of parasitised *Sitotroga* eggs with 5.0 g of semolina. This mixture was sprinkled onto plants misted with water from a hand held atomiser every 0.5 m in every row.

Samples of the parasitised *Sitotroga* eggs were held in microtitre trays to estimate the number of wasps produced per unit of parasitised *Sitotroga* eggs, their sex ratio, and the daily emergence pattern. The numbers of *Trichogramma* released per plot were estimated using these data. Approximately 3,480 female *T. pretiosum* and 7,006 female *T.* nr. *brassicae* were released per plot.

Corn Trial 2

An assessment of heliothis egg parasitism following a release of *Trichogramma pretiosum* was assessed in replicated small sweet corn plots at Pacific Seeds plant breeding farm at Gatton (S 27° 34', E 152° 19'). The trial site was a 1.0 ha planting of sweet corn (mixed hybrids) that was tasselling. Each plot was 9 rows wide and 6 m long. All treatments were at least 20 m apart and positioned randomly throughout the trial site. *T. pretiosum* were released in six plots and six plots were left untreated (control).

The levels of heliothis egg parasitism were assessed by stapling egg cards onto the upper surfaces of corn leaves. The egg cards were stapled to leaves every metre in every row in a grid pattern (5 cards/row). There were 45 cards stapled in each plot. They were collected after 48 hours and held in a constant temperature room until the eggs had either hatched or turned black (indicating that they were parasitised). The proportion of cards containing parasitised eggs was used to indicate *Trichogramma* activity in the crop.

The *Trichogramma* were released as pupae (in parasitised *Sitotroga* eggs) by mixing 0.04 g of parasitised *Sitotroga* eggs with 5.0 g of semolina. This mixture was sprinkled onto plants misted with water from a hand held atomiser every 0.5 m in every row.

Samples of the parasitised *Sitotroga* eggs were held in a constant temperature room to record when emergence commenced. Approximately 1,836 *T. pretiosum* (males + females) were released per plot. Emergence commenced the day after the release. The percentage of egg cards containing parasitised eggs was determined for each plot.

Corn Trial 3

An assessment of small scale inundative release of *Trichogramma* was undertaken at the Pacific Seeds plant breeding farm at Gatton (S 27° 34', E 152° 19'). Plant breeders at Pacific Seeds were having difficulties managing heliothis in their nursery and breeding sites, and decided to abandon chemical applications and try using egg parasitoids. *Trichogramma* nr. *brassicae* were obtained from a commercial supplier and released weekly at a rate of approximately 200,000 wasps/ha. The crop was sown on 8 February 1996. Wasp releases commenced 21 DAS and ceased when the crop had finished silking (approximately 70 DAS). Pacific seeds staff carried out the releases according to supplier recommendations. This provided an opportunity to assess the results of a 'real world' attempt at using *Trichogramma* inundatively.

The release site was a 0.75 ha planting of mixed sweet corn hybrids. A nearby unsprayed planting of mixed maize varieties (0.35 ha) was used as a control site because no wasps were released there. The maize planting was approximately one kilometre from the sweet corn site.

The levels of heliothis egg parasitism were monitored weekly by stapling egg cards onto the upper surfaces of corn leaves. There were 96 cards stapled in the release site, and 45 cards stapled in the control site. They were collected after 48 hours and held in a constant temperature room until the eggs had either hatched or turned black (indicating that they were parasitised). The proportion of cards containing parasitised eggs was used to indicate *Trichogramma* activity in the crop.

Heliothis egg numbers were assessed weekly by counting the numbers of eggs (white or brown) on each of five consecutive plants at five randomly selected locations per site. These counts were averaged to obtain a mean number per plant for each site.

Suction samples were taken every week to assess predator and egg parasitoid numbers. A Stihl BG72 suction machine was used to collect the samples. A fine muslin bag was attached to the inlet tube of the machine using a velcro strap. A 20 m row of plants was sampled at four random selected locations per site. The collections were immediately emptied into 70% alcohol in the field and taken back to the laboratory for sorting and counting under a stereo microscope. Data were averaged to obtain mean counts per metre for each site.

SWEET CORN IPM FIELD TRIALS

Two trials assessing different heliothis management options were undertaken at the Queensland Department of Primary Industries (QDPI) Gatton Research Station (S 27° 33' E 152° 20'). Four bays of sweet corn (H5) 12 rows wide were separated by maize (Hycorn 83) buffers 16 rows wide. The rows were 180 m long and 70 cm apart. Four rows of maize buffer were grown on both edges of the study site. There were 5.8 ± 0.4 corn plants/m. A randomised complete block design of treatments was used in the sweet corn bays for both trials.

Corn Trial 4

In Corn Trial 4 the plots were 30 m long x 12 rows, separated by buffers 15 m long. A 5 m buffer was provided at each end of the site. Clear walkways two metres wide were chipped at each end of the buffers to allow access to plots and to reduce the likelihood of insect virus being transmitted throughout the site. The trial was sown on 10 September 1996. The first silks appeared 73 days after sowing (DAS). Four different heliothis management strategies were evaluated, with each replicated four times. The treatments were:

a) Chemical: The plots were sprayed with the synthetic pyrethroid deltamethrin (Decis®) at a rate of 0.5 L/ha in a spray volume of 50 L/ha using a Stihl SR400 mistblower. Four chemical sprays were applied on 78, 80, 86 and 90 DAS.

b) NPV + *Trichogramma*: The plots were sprayed with NPV and *Trichogramma* nr. *brassicae* were released inundatively. The NPV was applied at a rate of 741 mL/ha in a spray volume of 52 L/ha using a Hardi knapsack sprayer. *T.* nr. *brassicae* were released as pupae in parasitised *Sitotroga cerealella* eggs. The eggs were suspended in a starch thickened water solution and sprayed onto leaves every metre in every second row using a hand held atomiser. The release rate was equivalent to 175,000 wasps/ha. The Instant Gel-It® thickener was prepared at 40 g product per litre of water, and the *Trichogramma* (parasitised *Sitotroga* eggs) were added just before they were released so that they were submerged in solution for less than 0.5 hours. The NPV was applied 86 and 90 DAS, and one *Trichogramma* release was carried out on 80 DAS.

c) Control: No action was taken in these plots.

d) *Bacillus thuringiensis* (*B.t.*): The plots were sprayed with *B.t.* (Dipel Forte®) at a rate of 425 g/ha in a spray volume of 52 L/ha using a Hardi knapsack sprayer on 86 and 90 DAS.

Heliothis eggs and larvae were assessed twice weekly by counting the numbers of eggs (white, brown or black parasitised) and larvae (very small < 3 mm; small 3-7 mm; medium 7-20 mm; large > 20 mm) on each of five consecutive plants at four randomly selected locations per plot. These counts were averaged to obtain a mean number per plant for each plot.

Suction samples were taken every week to assess predator and egg parasitoid numbers. A Stihl BG72 suction machine was used to collect the samples. A fine muslin bag was attached to the inlet tube of the machine using a velcro strap. One 10 m row of plants were sampled into the muslin bag per plot. The collection was immediately emptied into 70% alcohol in the field and taken back to the laboratory for sorting and counting under a stereo microscope. Data were averaged to obtain mean counts per treatment.

In the later part of the trial, when predators were most abundant, visual counts of predators were also recorded to evaluate the accuracy of suction samples. The numbers of predators observed on each of three consecutive plants at three randomly selected sites per plot were recorded. These data were averaged to obtain the mean number of predators per plant.

The levels of heliothis egg parasitism were monitored weekly by stapling egg cards onto the upper surfaces of corn leaves. The egg cards were stapled to leaves every seven metres in every second row, i.e. 25 cards /plot. They were collected after 48 hours and held in a constant temperature room until the eggs had either hatched or turned black (indicating that they were parasitised). The proportion of cards containing parasitised eggs was used to indicate *Trichogramma* activity in the crop.

A damage assessment of ripe cobs was completed on 98 DAS by inspecting 50 cobs randomly selected from each plot in the field. Each cob was checked for feeding damage by heliothis (including silk damage) and for the presence of larvae.

Corn Trial 5

In Corn Trial 5 the plots were 25 m long x 12 rows, separated by buffers 15 m long. A 5 m buffer was provided at each end of the site. Clear walkways two metres wide were chipped at each end of the buffers to allow access to plots and to reduce the likelihood of insect virus being transmitted throughout the site. The trial was sown on 24 February 1997. The first silks appeared 54 days after sowing (DAS). Five different heliothis management strategies were evaluated, with each replicated four times. The treatments were:

a) Chemical: The plots were sprayed with the synthetic pyrethroid deltamethrin (Decis®) at a rate of 0.5 L/ha in a spray volume of 50 L/ha using a Stihl SR400 mistblower. Four chemical sprays were applied on 56, 60, 63 and 67 DAS.

b) NPV + *Trichogramma*: The plots were sprayed with NPV and *T.* nr. *brassicae* were released inundatively. The NPV was applied at a rate of 741 mL/ha in a spray volume of 52 L/ha using a Hardi knapsack sprayer. *T.* nr. *brassicae* were released as pupae in parasitised *Sitotroga cerealella* eggs by one of two methods: 1) suspended in a thickened water solution and sprayed onto leaves every metre in every second row using a hand held atomiser, or 2) stuck onto paper cards and placed on corn plants at silk height every six metres in every second row (i.e. twenty cards/plot). In Corn Trial 5 AquaKeep® was used as the thickener and prepared at 2 g product per litre of water. The *Trichogramma* (parasitised *Sitotroga* eggs) were added just before they were

released so that they were submerged in solution for less than 0.5 hours. The NPV was applied 58 and 64 DAS, and *Trichogramma* were released 48, 50 and 57 DAS. *Trichogramma* releases one and two (48 and 50 DAS) were in the AquaKeep® solution, and the third release (57 DAS) was made using egg cards. The release rates were equivalent to 406,000; 762,000; and 414,000/ha respectively (males + females).

c) Control: No action was taken in these plots.

d) *Bacillus thuringiensis* (*B.t.*): Plots were sprayed with *B.t.* (Dipel Forte®) at a rate of 425 g/ha in a spray volume of 52L/ha using a Hardi knapsack sprayer on 58 and 64 DAS.

e) *Trichogramma*: *T.* nr. *brassicae* were released in these plots and no other management tactics were used. The releases were carried out in the same manner and on the same days as outlined in (b) above. The emergence of released *Trichogramma* was assessed by placing 50 parasitised *Sitotroga* eggs (taken from the material to be released in the field) individually into the wells of a microtitre tray. The tray was covered with sticky tape to contain the emerged wasps. Two trays were set up for each release: one was tied to a plant (at silk height) in the field, and the other was held in the laboratory in a constant temperature room. These trays were checked daily and the wells containing emerged wasps were noted.

Heliothis eggs and larvae were assessed twice weekly by counting the numbers of eggs (white, brown or black parasitised) and larvae (very small < 3 mm; small 3-7 mm; medium 7-20 mm; large >20 mm) on each of three consecutive plants at four randomly selected locations per plot. Visual counts of predators on the same plants were also recorded. These data were averaged to obtain the mean numbers per plant.

The levels of heliothis egg parasitism were assessed by 'tagging' eggs during the vegetative stage of the crop (one week before the start of silking) or tagging silks (one week after the start of silking).

Eggs were tagged by marking plants with coloured plastic tape and using a felt-tipped marker pen to circle and number freshly laid heliothis eggs. These eggs were then examined daily for six days and their fate recorded as either predated, parasitised or hatched. Eggs that disappeared 1-3 days after tagging were assumed to be eaten by chewing predators (egg remains were sometimes noticed). Eggs that were eaten by sucking predators were discoloured and collapsed. Eggs that disappeared 4-5 days after tagging were assumed to have hatched under the field temperatures experienced in the trial. Parasitised eggs turned black 4-5 days after tagging.

Sweet corn silks were tagged in a similar manner by marking plants with coloured plastic tape and silks by using a felt-tipped pen. Approximately 40 silks that had recently emerged and were free of heliothis eggs were tagged in each plot. Ten silks were collected from each plot 2, 4 and 9 days after tagging by removing them with secateurs and individually placing them into labelled brown paper bags. The bags were taken back to the laboratory and the numbers of heliothis eggs and larvae per silk were counted. The eggs were individually placed into the wells of microtitre trays and covered with clear plastic film. The trays were held in a constant temperature room until the eggs hatched or showed signs of parasitism.

Parasitised eggs were held until wasps emerged. The wasps were then counted, sexed and identified.

A damage assessment of ripe cobs was completed on 85 DAS by inspecting 100 cobs randomly selected from each plot in the field. Each cob was checked for feeding damage by heliothis (including silk damage) and for the presence of larvae.

RESULTS

LABORATORY INVESTIGATIONS

Lab Study 1: Species comparisons

Six strains of egg parasitoids parasitised more than ten heliothis eggs during four hours, with strain T1 parasitising the greatest number - 45.8 (Figure 1). These strains were either *Trichogramma* nr. *brassicae*, *Trichogramma funiculatum* Carver, or *Trichogramma pretiosum*.

Unfortunately not all of the strains that have displayed biocontrol potential are easily mass reared. *T. funiculatum* and some strains of *T.* nr. *brassicae* (e.g. strain T1) have been difficult to mass rear on eggs of the grain moth, *Sitotroga cerealella*. The commercial company that currently produces *Trichogramma* in Australia (Bugs for Bugs) uses *Sitotroga* eggs to mass produce egg parasitoids. Strains that are unsuitable for mass rearing on *Sitotroga* should therefore not be considered as biocontrol candidates.

Of the strains studied, *T. pretiosum* and some strains of *T.* nr. *brassicae* have displayed reasonable parasitism levels (more than 10 eggs in 4 hours) and are able to be mass reared on *Sitotroga* eggs. Bugs for Bugs currently mass rears *T.* nr. *brassicae*. The above findings suggest that this species is a suitable candidate for biocontrol of heliothis, so it was not necessary for the company to commence rearing a different species.

Lab Study 2: Short-term Storage of *Trichogramma* at Cool Temperatures

The total emergence of *Trichogramma* was greatly reduced after two weeks of cold storage (Figures 2-4). Peak emergence of wasps varied with the time they were introduced to cold storage, and between species (Figures 3, 4).

T. bactrae displayed two peaks of emergence when stored 6 days (Figure 3A) or 7 days after parasitisation (Figure 3B), occurring 3-4 or 2-3 days after removal from cold storage respectively. Those wasps stored at 8 days (Figure 3C) or 9 days (Figure 3D) after parasitisation displayed one peak of emergence, occurring two or one day after removal from cold storage respectively. Emergence peaked at 45-60% on any one day.

T. pretiosum generally displayed single peaks of emergence (Figure 4), to a maximum of 63% on a single day (1 week at 6 days (Figure 4A)).

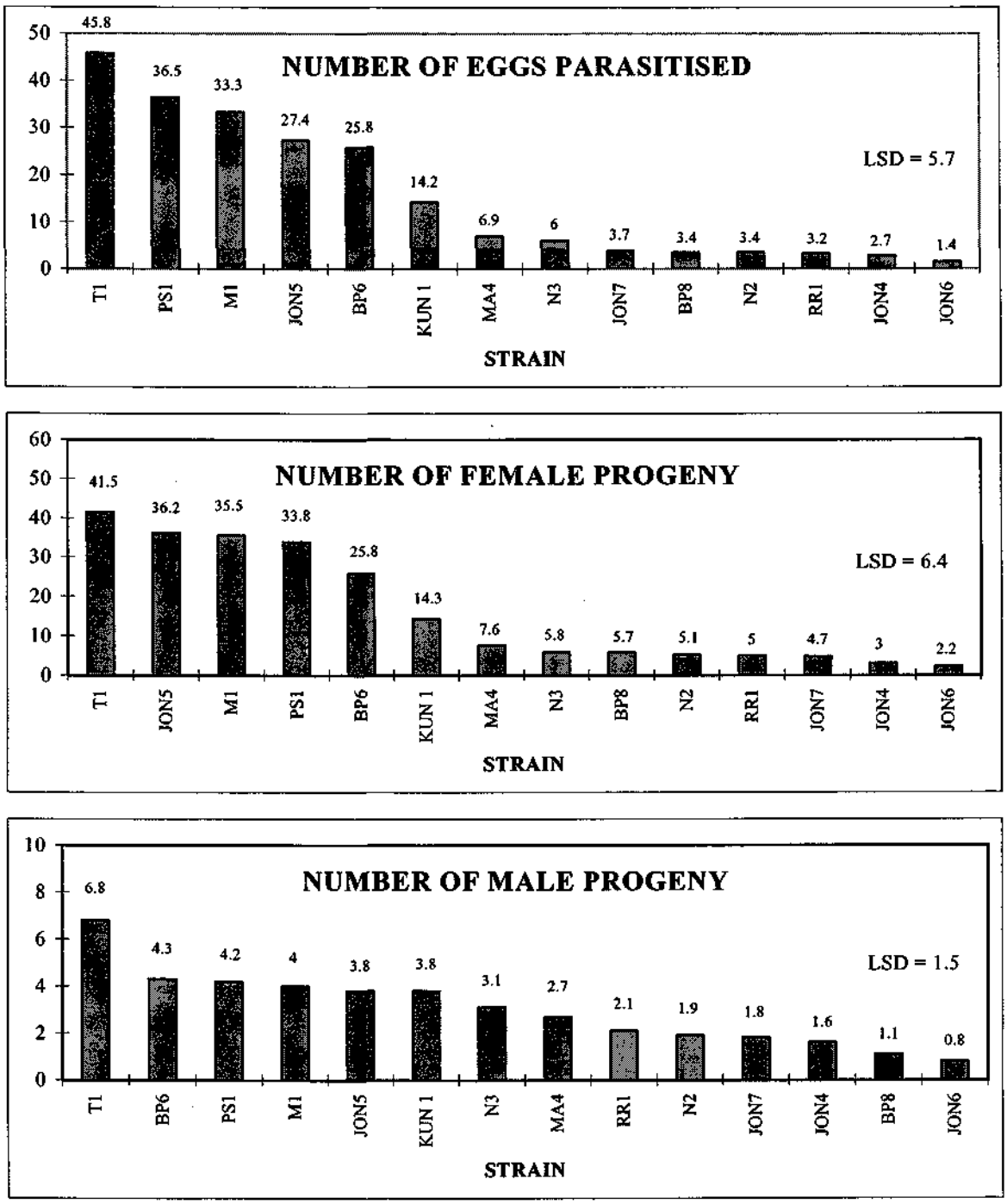


FIGURE 1: The variation in the number of heliothis (*H. armigera*) eggs parasitised, and number of progeny produced, by different strains of egg parasitoids. The wasps were exposed to the heliothis eggs for 4 hours. Values represent the mean ± standard error of 20 replicates. Data were analysed by analyse of variance, and the LSD values are provided (for $P<0.05$).

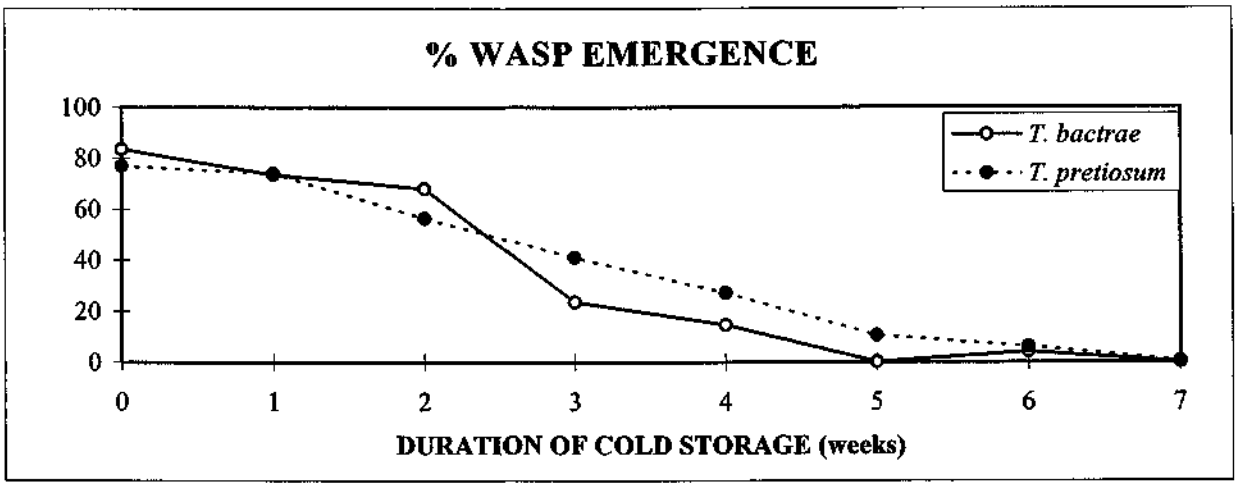


FIGURE 2: The variation in total emergence of two species of heliothis egg parasitoids reared on *Sitotroga cerealella* eggs. Parasitised eggs were placed in cold storage 8 days after parasitisation. The duration of cold storage was 0 (control), or 1-7 weeks. Storage Conditions: 8.7°C, 70% RH, 0:24 (L:D) photoperiod.

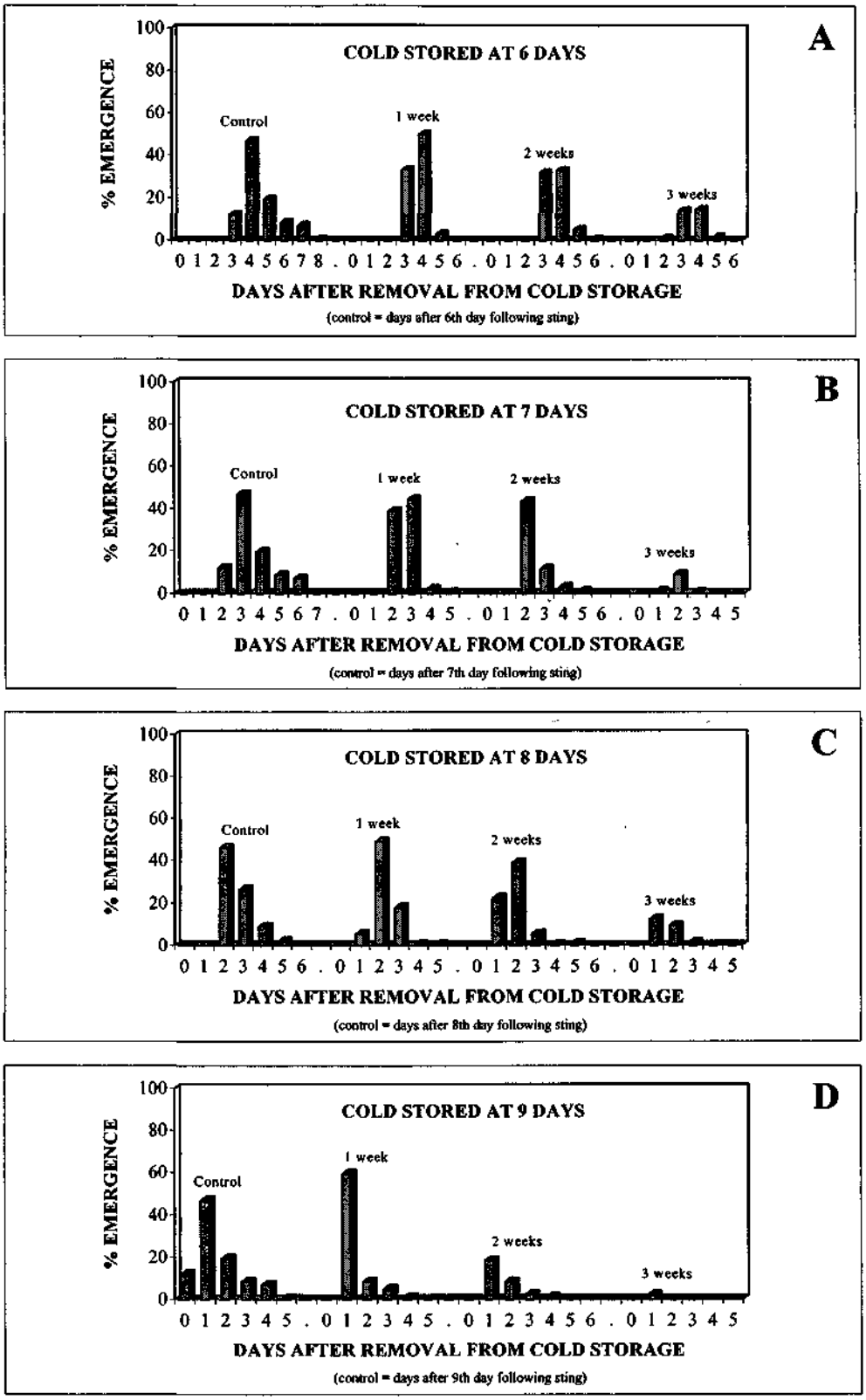


FIGURE 3: The variation in emergence of *Trichogrammatoidea bactrae* reared on *Sitotroga cerealella* eggs. Parasitised eggs were placed in cold storage on day 6, 7, 8 and 9 after parasitisation ('sting' date). The duration of cold storage was 0 (control), 1, 2 or 3 weeks. The emergence (%) was recorded for each day after removal from cold storage. Storage Conditions: 8.7°C, 70% RH, 0:24 (L:D) photoperiod.

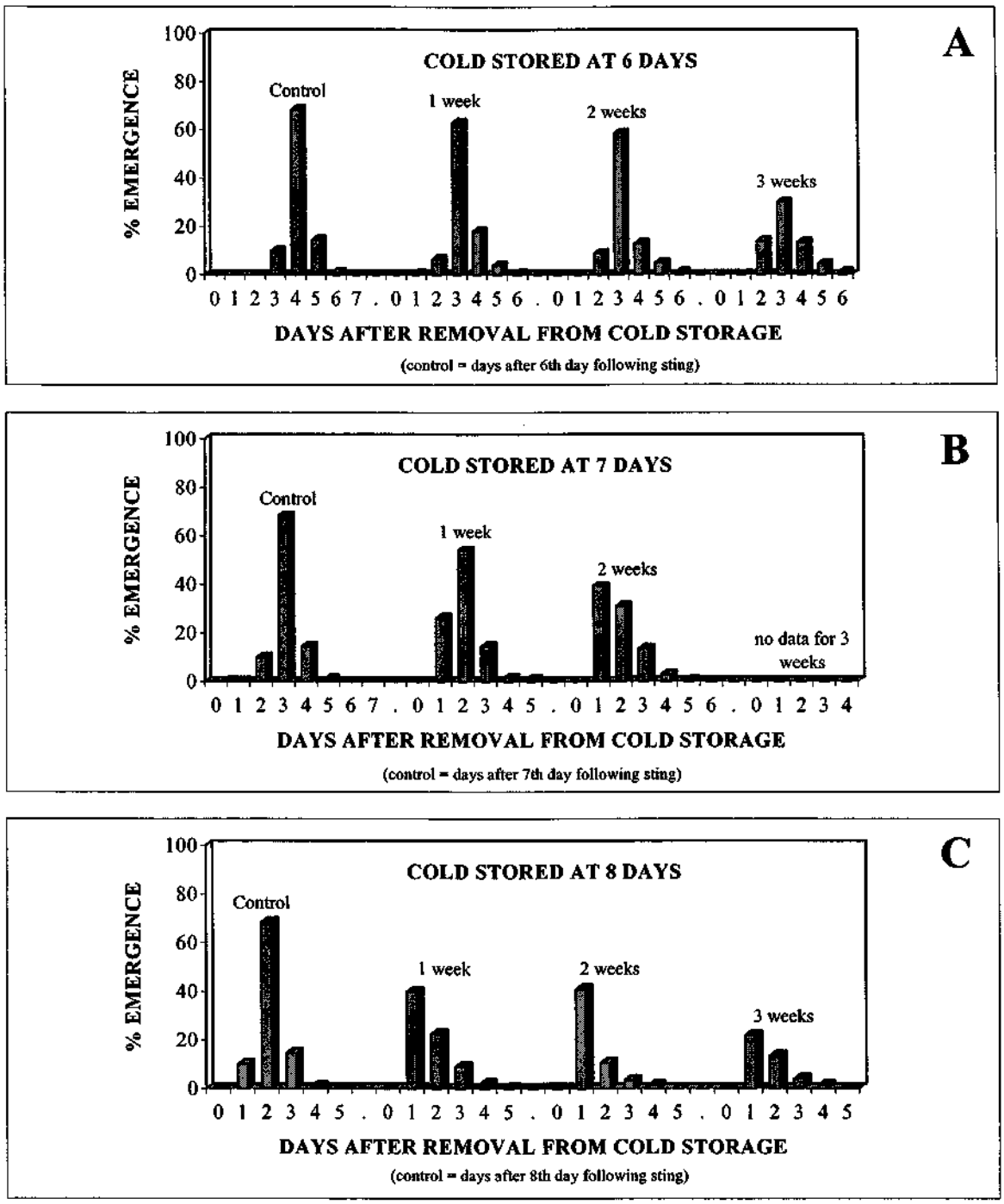


FIGURE 4: The variation in emergence of *Trichogramma pretiosum* reared on *Sitotroga cerealella* eggs. Parasitised eggs were placed in cold storage on day 6, 7, and 8 after parasitisation ('sting' date). The duration of cold storage was 0 (control), 1, 2 or 3 weeks. The emergence (%) was recorded for each day after removal from cold storage. Storage Conditions: 8.7°C, 70% RH, 0:24 (L:D) photoperiod.

Lab Study 3: Emergence of *Trichogramma* at High Temperatures

The emergence of egg parasitoids was greatly affected by exposure to high temperatures. Exposure to 51°C caused high mortality (90+%) for all species of egg parasitoids studied (Figures 5-7). At 44°C exposure for 1 and sometimes 2 hours could be tolerated, but exposures of 4 or 6 hours caused high mortality (90+%).

Soil temperatures in lettuce at DPI Gatton were monitored during field trials in January and March 1997, and exceeded 50°C for 2 hours and 44°C for 5 hours on some days. In contrast, soil temperatures in mature tropical hybrids of sweet corn rarely exceeded 30°C on normal summer days (Nov-Dec 1996 and Mar-Apr 1997). Closed canopy crops, where soil and presumably most leaf temperatures rarely exceed 30-35°C, are therefore more suitable for the successful emergence of *Trichogramma* wasps.

Lab Study 4: Egg Predation

Red and blue beetles and striped ladybirds eat more heliothis eggs and parasitised *Sitotroga* eggs than the other predators studied (Figure 8), however two-spotted ladybirds and pirate bugs are much smaller insects. No predation of parasitised *Sitotroga* eggs was recorded for pirate bugs.

The results indicate that all five species of predators studied will consume heliothis eggs, and all of the predatory beetles will consume parasitised *Sitotroga* eggs. These predators may therefore have an impact on heliothis egg numbers in the field, and may reduce the numbers of inundatively released *Trichogramma* (by eating wasp pupae inside parasitised *Sitotroga* eggs).

Lab Study 5: *Trichogramma* Release Techniques

The use of semolina/parasitised *Sitotroga* dry mixture dispensed from a hopper via a seed drill does not affect the survival of *Trichogramma* (Table 2). This technique has been used in lettuce and tomatoes.

The use of liquid suspensions of parasitised *Sitotroga* eggs to release *Trichogramma* is also viable. Emergence from the Instant Gel-It® is affected by the application technique, with reduced emergence occurring when applied in a liquid stream (44.7%) as opposed to a fine mist (82.9%) (Table 2). Liquid Instant Gel-It® dries to form a hard gelatinous layer over parasitised *Sitotroga* eggs. This layer is thicker when sprayed as a stream and the emerging *Trichogramma* wasps have difficulty in chewing through this layer. The use of AquaKeep® overcomes this problem - emergence was high (82.9%) when applied in a stream. AquaKeep® does not form a thick layer over the eggs.

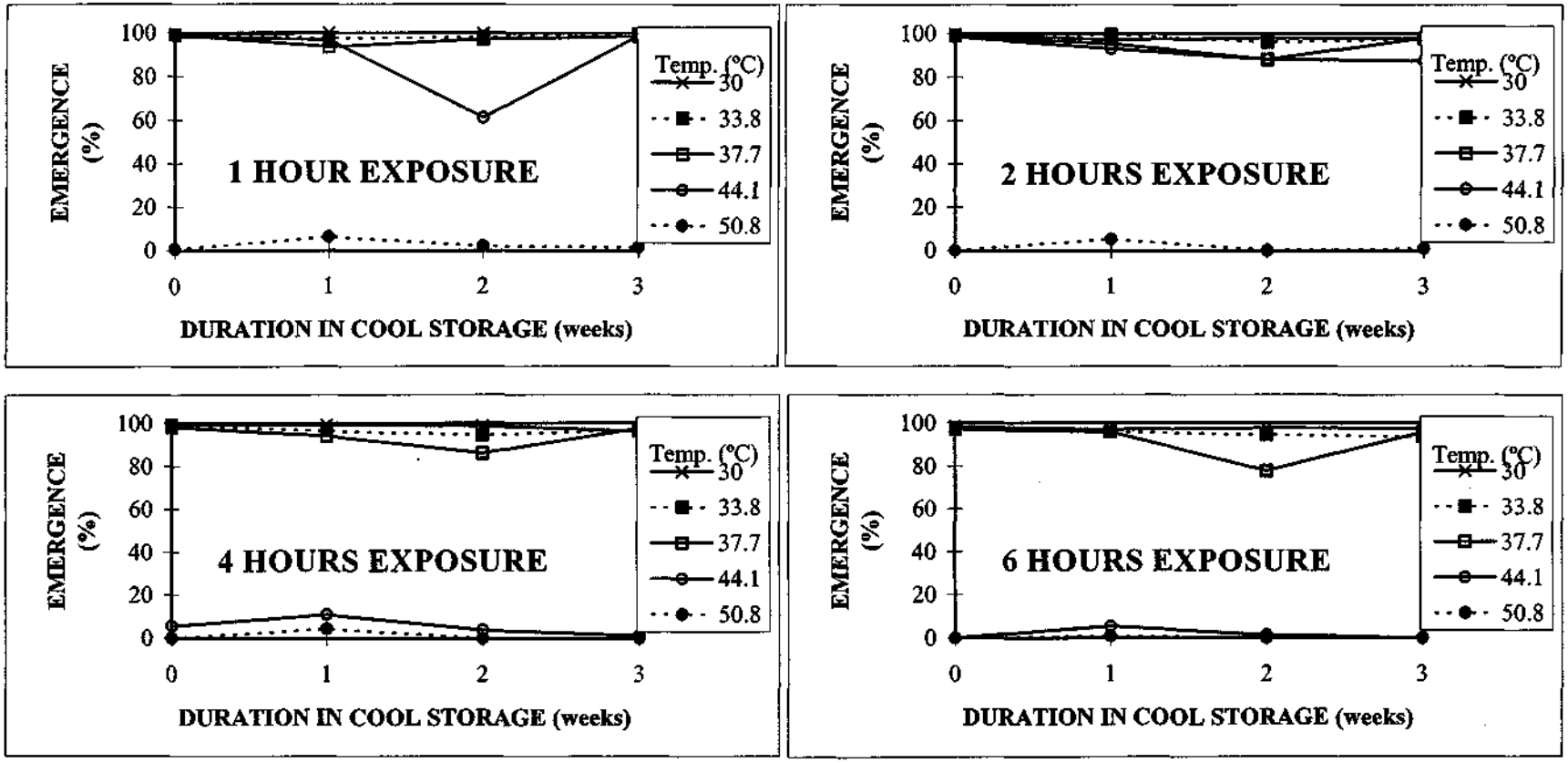


FIGURE 5: The variation in emergence of *Trichogramma* nr. *brassicae* when exposed to different constant temperatures for 1, 2, 4 or 6 hours (70% R.H. and constant light). The wasps were reared on eggs of the Angoumois grain moth, *Sitotroga cerealella*, for 7 days at approximately 25°C and 70% R.H. before exposure to these temperatures.

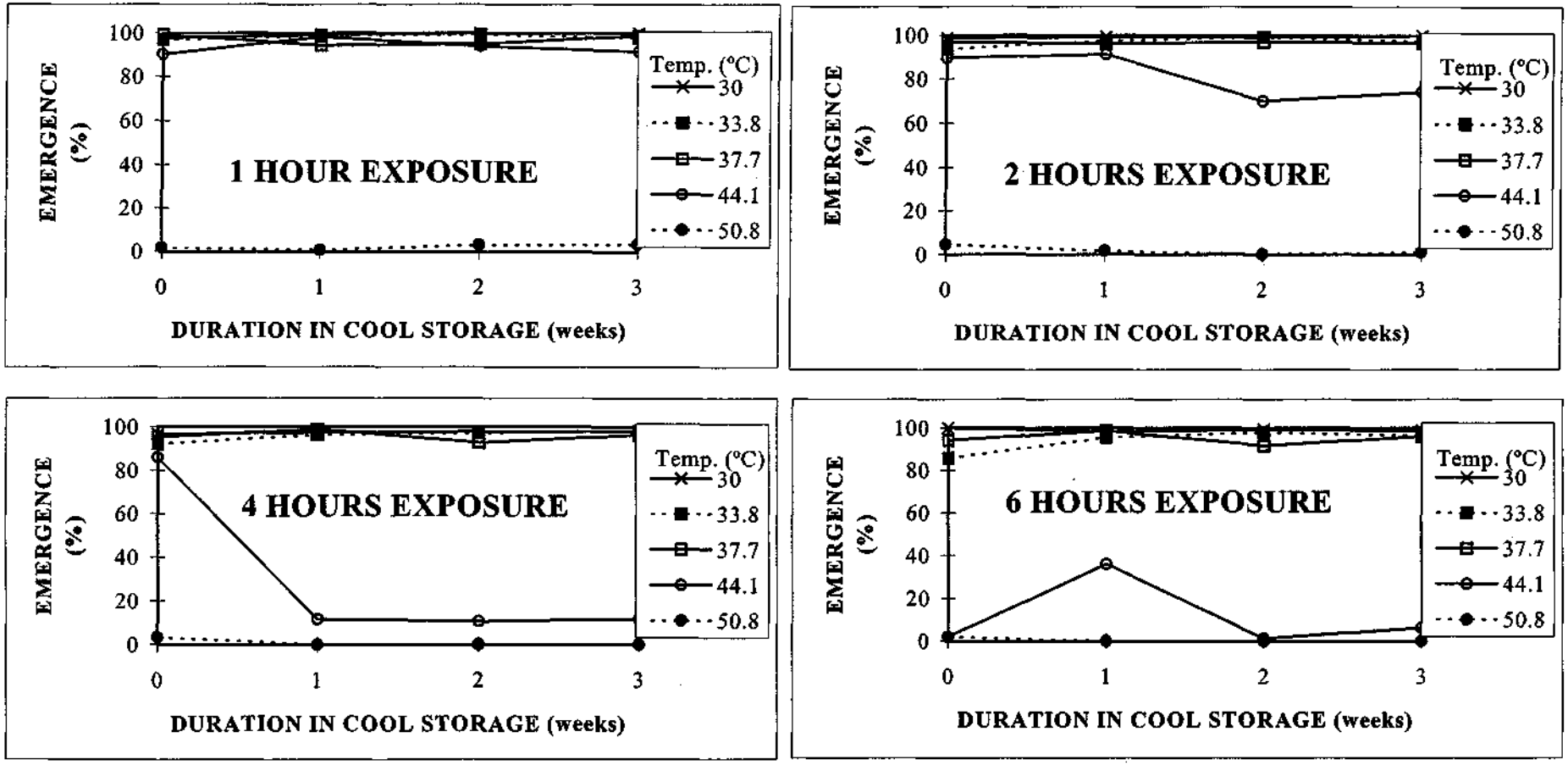


FIGURE 6: The variation in emergence of *Trichogramma pretiosum* when exposed to different constant temperatures for 1, 2, 4 or 6 hours (70% R.H. and constant light). The wasps were reared on eggs of the Angoumois grain moth, *Sitotroga cerealella*, for 7 days at approximately 25°C and 70% R.H. before exposure to these temperatures.

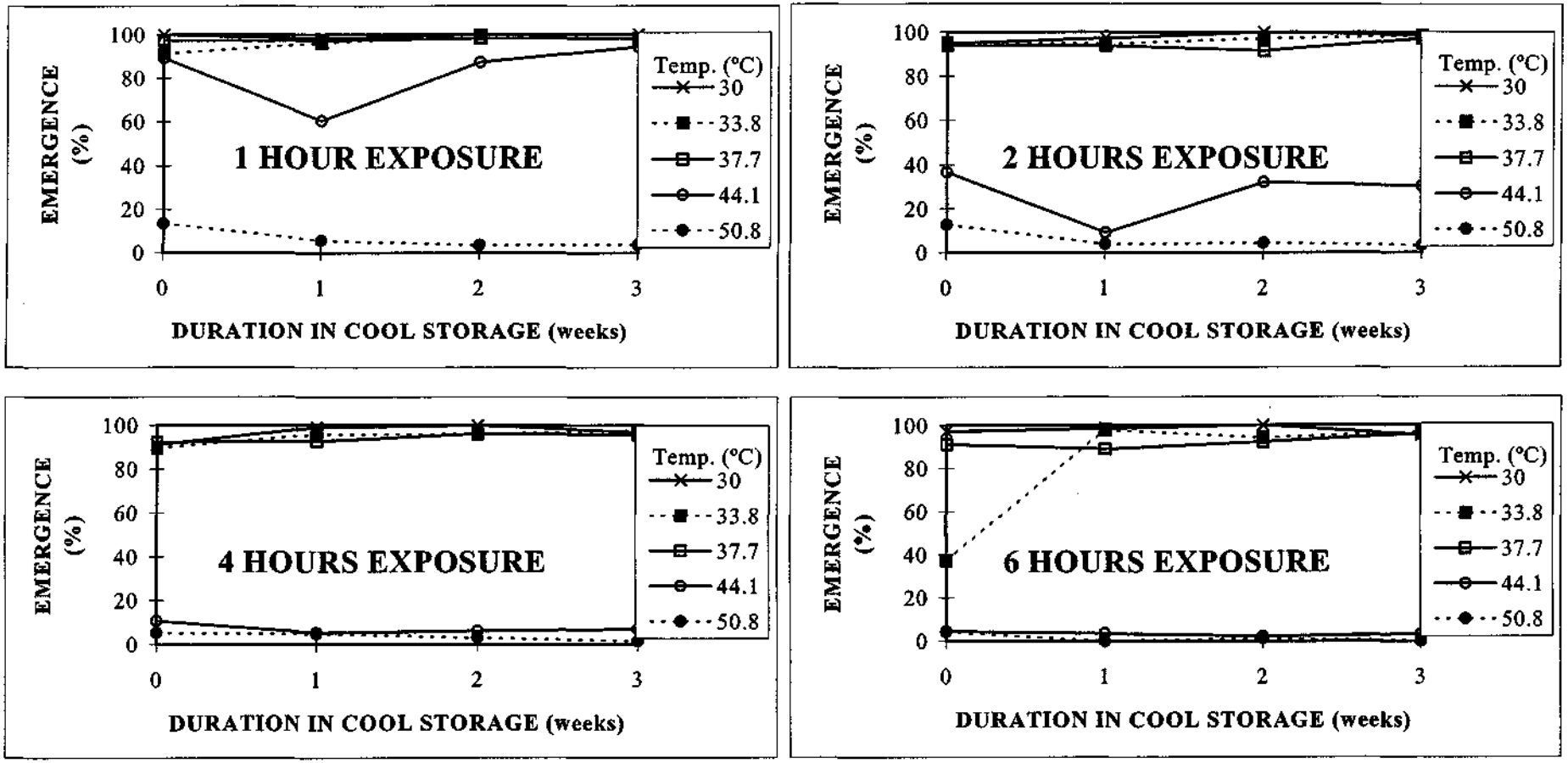


FIGURE 7: The variation in emergence of *Trichogrammatoidea bactrae* when exposed to different constant temperatures for 1, 2, 4 or 6 hours (70% R.H. and constant light). The wasps were reared on eggs of the Angoumois grain moth, *Sitotroga cerealella*, for 7 days at approximately 25°C and 70% R.H. before exposure to these temperatures.

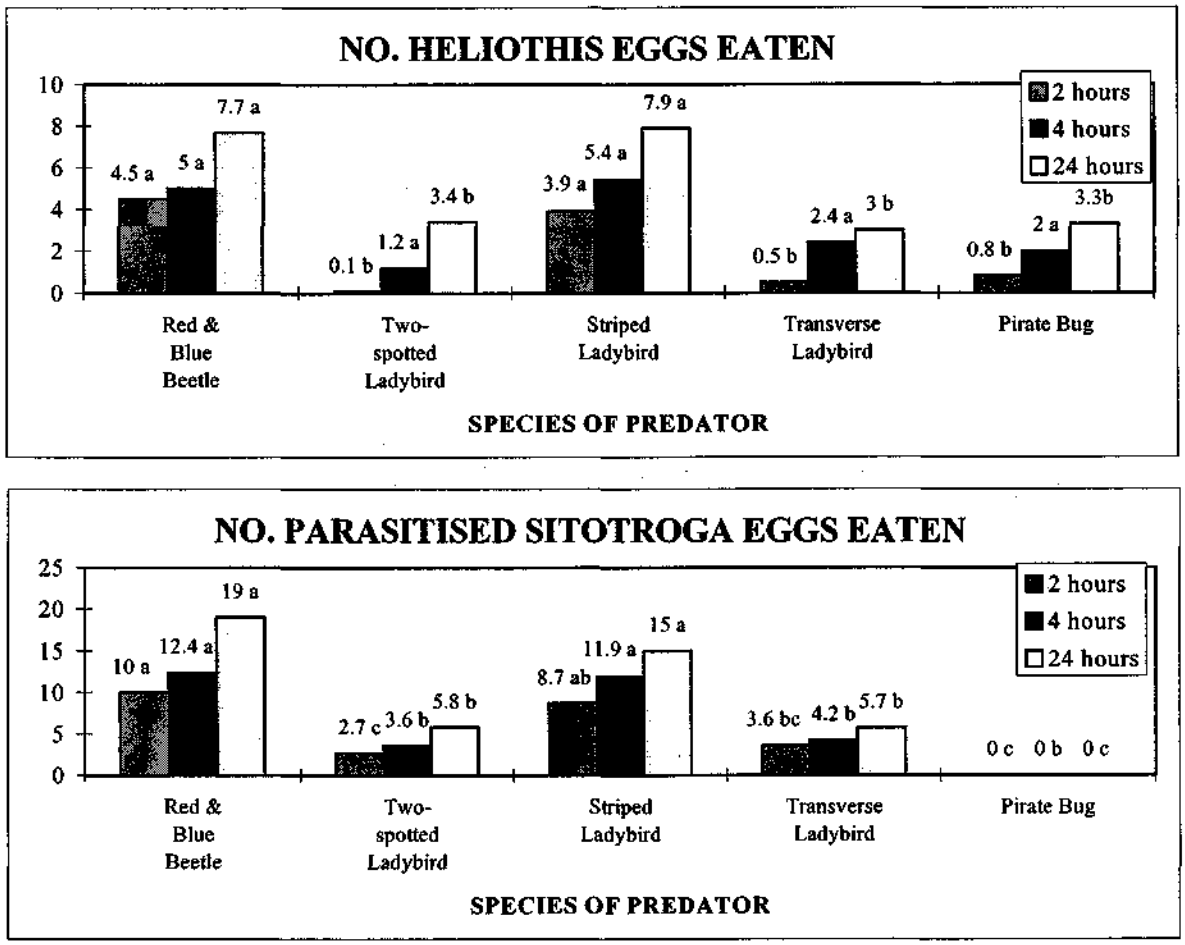


FIGURE 8: Predation of heliothis eggs and parasitised *Sitotroga* eggs in the laboratory at 25°C, 70% R.H., and 14:10 (L:D) photoperiod. Values represent the mean number of eggs eaten at various intervals after exposure (mean of ten replicates). Data were analysed by analysis of variance and the means (for a given exposure) were compared by Fisher's Least Significant Difference technique. Means for a given exposure followed by the same letter are not significantly different for $P<0.05$.

TABLE 2

The percentage emergence of adult *Trichogramma* nr. *brassicae* following different release techniques.

Control	Dry Mix [1]	Suspension A [2]	Suspension B [3]	Suspension C [4]
94.5	95.4	44.7	78.3	82.9

1 = Semolina/parasitised *Sitotroga* dry mix dispensed via a hopper/seed drill.
2 = Instant Gel-It® suspension applied with a pressurised 1 L hand-held sprayer.
3 = Instant Gel-It® suspension applied with a Stihl SR400 mistblower.
4 = AquaKeep® suspension applied with a pressurised 1 L hand-held sprayer.

LETTUCE TRIALS

Lettuce Trail 1

The heliothis infestation was low to moderate, and the seven releases of *Trichogramma* failed to have an impact on egg mortality (Figure 9A). There was no egg parasitism whatsoever recorded. Suction samples from the trial failed to detect any significant numbers of adult *Trichogramma* (Figure 9B). It was therefore concluded that the wasps were not remaining in the crop after emergence (assuming that they successfully emerged).

Very few parasitised *Sitotroga* eggs were recovered from the routine suction samples. On one occasion (21 October 1994) suction samples were taken immediately after field release of parasitised *Sitotroga* eggs by walking behind the tractor and sampling. Only 13.3 ± 6.7 *Sitotroga* eggs were recovered per 50 lettuce plants, despite being released at a rate of approximately 714 eggs / 50 plants. The trial site was exposed and conditions were almost always windy. It was concluded that many of the parasitised *Sitotroga* eggs were blown away from the lettuce plants as they were released from the hopper.

The emergence of wasps peaked approximately 24 hours after they were taken from cold storage, and extended over a 4-5 day period (Figure 10A). Given that many of the eggs were apparently blown away from the plants as they were released, few eggs probably remained in the crop after 24 hours. Consequently, the incidence of wasp emergence in the crop was most likely very low.

The total emergence of wasps varied according to the length of time in cold storage (Figure 10B). Storage times of more than one week reduced total emergence, suggesting that *Trichogramma* should be purchased as they are required and released as soon as possible. Cold storage of wasp pupae (in parasitised *Sitotroga* eggs) as an 'emergency supply' in case they are needed should be avoided.

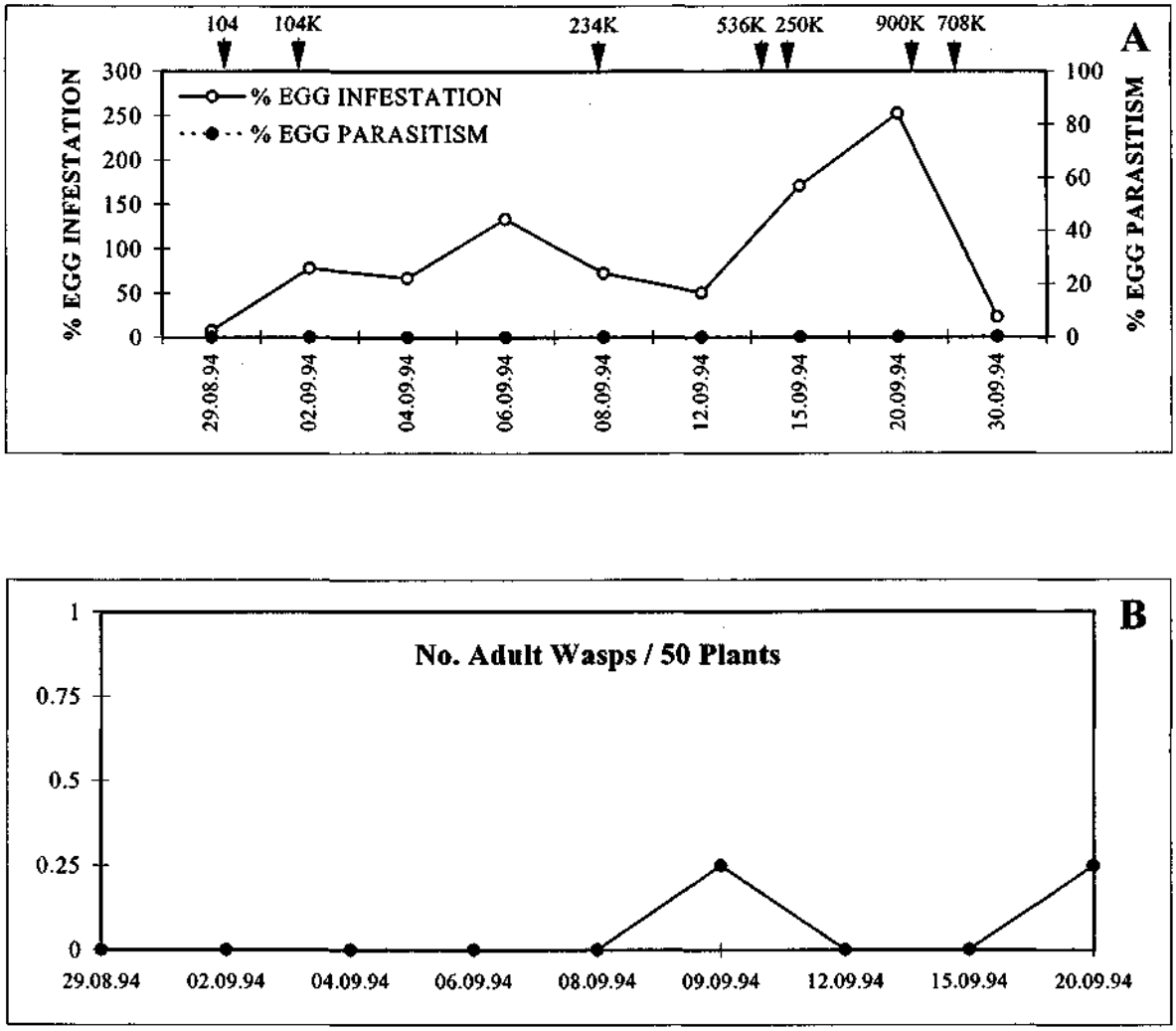


FIGURE 9: Heliothis egg infestation and egg parasitoid activity during Lettuce Trial 1 at Cambooya on the Darling Downs. **(A)** Heliothis egg infestation and parasitism following inundative releases of *Trichogramma*. The arrows indicate when *Trichogramma* nr. *brassicae* were released (1,000's/ha of parasitised *Sitotroga* eggs). **(B)** The numbers of *T*. nr. *brassicae* captured in suction samples from lettuce at Cambooya. Suction samples were taken from 50 plants. Values represent the mean of four samples.

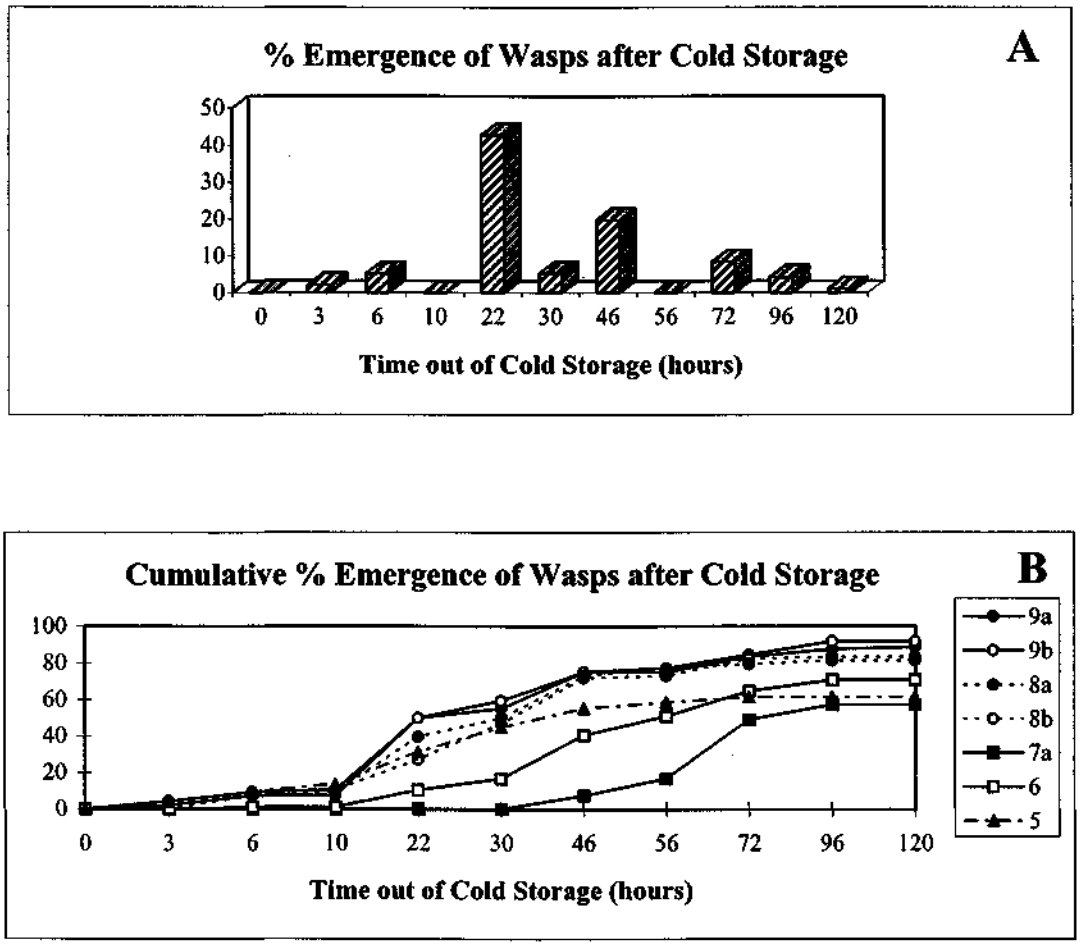


FIGURE 10: Emergence of *T.* nr. *brassicae* after being taken out of cold storage (at approx. 4°C) and held at approximately 25°C and 70% R.H.. **(A)** The emergence of adult wasps after cold storage of less than one week. **(B)** Cumulative emergence of wasps cold stored for different lengths of time. Cold storage times: Batch 9 = less than one week; Batch 8 = approx. one week; Batch 7 = 1-2 weeks; Batch 6 = 2-3 weeks; Batch 5 = 3-4 weeks.

Lettuce Trail 2

There were some significant differences between the levels of heliothis egg card parasitism attributed to different strains of egg parasitoids (Table 3). All *Trichogramma* species performed significantly better than *Trichogrammatoidea bactrae* overall (see pooled data Table 3). In general there was no significant difference between *T. pretiosum* and both strains of *T.* nr. *brassicae*, however there were 25% fewer *T. pretiosum* released per plot.

Emergence of most wasps peaked two days after release (DAR) (Table 4). The levels of parasitism recorded may have been underestimated because the egg cards were collected 2 and 3 DAR, i.e. egg cards may have only been exposed to peak wasp emergence for 24 hours or less. This may help explain the low levels of parasitism recorded (less than 25%) (Table 3).

Lettuce Trail 3

The levels of heliothis egg card parasitism were significantly higher in the plots were parasitoids were released than in the control, with no significant difference between *T. pretiosum* and *T.* nr. *brassicae.* (Table 5). The levels of parasitism (65% and 70% for *T. pretiosum* and *T.* nr. *brassicae* respectively) were higher than those recorded on smaller plants (maximum of 23%, Table 3). The emergence of wasps in this trial peaked 1 DAR for *T.* nr. *brassicae*, and 2 DAR for *T. pretiosum* (Table 6).

Lettuce Trial 4

The application of NPV was effective against heliothis larvae on lettuce that were resistant to commonly used chemical insecticides (Table 7). The combination of virus mortality and mortality due to natural causes (especially the egg-larval parasitoid *Chelonus* sp.) was high - only 5% of larvae collected after the first virus application, and 4% of those collected after the second virus application, pupated.

Lettuce Trial 5

The application of NPV or NPV + Coax® killed 83-93% of heliothis larvae (Table 8). The addition of Coax® increased mortality by 5% 3 DAS. These results are very encouraging. Almost all larvae were collected from the centre of lettuce plants, but apparently consumed sufficient virus to cause death at some stage. Additional research may be required to record the feeding behaviour of larvae. Those larvae that are sheltered in the centre of the lettuce plant would not be exposed to or consume virus unless they moved to the outer (sprayed) leaves to feed during certain times of the day.

TABLE 3

A comparison of the levels of heliothis egg parasitism recorded following small plot releases of different strains of egg parasitoids at Cambooya during Lettuce Trial 2.

Treatment	Release rate (females/plot)	2 DAR *	3 DAR *	pooled data
Control	0	2.2 ± 1.5 b	2.6 ± 1.4 c	2.3 ± 1.2 b
T. pretiosum	1210	11.2 ± 1.9 ab	23.0 ± 2.0 a	16.8 ± 2.1 a
T. nr. *brassicae* (strain BP)	1620	21.9 ± 8.0 a	10.9 ± 0.9 b	17.6 ± 5.4 a
T. nr. *brassicae* (strain QDPI)	1654	23.4 ± 8.4 a	17.3 ± 3.4 ab	20.4 ± 4.6 a
T. bactrae	1435	5.1 ± 2.5 ab	6.1 ± 2.0 bc	5.5 ± 1.7 b

* DAR = Days After Release. The proportion of egg cards parasitised (%) data are the mean ± standard error of four replicates. Egg cards were left in the field for 48 hours, and collected either 2 or 3 DAR. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Column means followed by the same letter are not significantly different for $P<0.05$.

TABLE 4

The daily emergence (%) of egg parasitoids for each day after release (DAR) at Cambooya during Lettuce Trial 2. Day of release = DAR 0.

DAR	*T. pretiosum*	*T.* nr. *brassicae* (strain BP)	*T.* nr. *brassicae* (strain QDPI)	*T. bactrae*
0	0	0	0	0
1	12	8	32	9
2	27	27	29	41
3	20	3	3	9
4	3	2	1	2
5+	2	1	6	1
no emergence	36	59	29	38

TABLE 5

A comparison of the levels of heliothis egg parasitism (% egg card parasitism) recorded following small plot releases of two different strains of egg parasitoids at Cambooya during Lettuce Trial 3.

Treatment	Release rate (females/plot)	2 DAR *
Control	0	7.1 ± 1.8 b
T. pretiosum	3695	64.6 ± 2.2 a
T. nr. *brassicae* (strain BP)	3324	70.2 ± 4.5 a

* DAR = Days After Release. The proportion of egg cards parasitised (%) data are the mean ± standard error of four replicates. Egg cards were left in the field for 48 hours. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Column means followed by the same letter are not significantly different for $P<0.05$.

TABLE 6

The daily emergence (%) of egg parasitoids for each day after release (DAR) at Cambooya during Lettuce Trial 3. Day of release = DAR 0.

DAR	*T. pretiosum*	*T.* nr. *brassicae* (strain BP)
0	0	1
1	11	46
2+	66	37
no emergence	23	16

TABLE 7

The fate of heliothis larvae collected from commercially sown lettuce in Toowoomba before and after application of NPV (GemStar®) - LettuceTrial 4. Values represent the proportion (%) of larvae that were healthy or died due to various causes.

DATE	NOTES *	NPV	*Microplitis* [1]	*Chelonus* [2]	Ascovirus	Healthy
26.03.95	Pre-spraying	0.0	2.5	13.7	0.0	83.8
27.03.95	1 day after spray 1	71.3	0.0	19.1	4.8	4.8
31.03.97	3 days after spray 2	60.7	7.1	25.0	3.6	3.6

* NPV was applied on March 26 and 28.

1 = % dying due the larval parasitoid *Microplitis demolitor*.

2 = % dying due to the egg-larval parasitoid *Chelonus* sp.

TABLE 8

The fate of heliothis larvae collected from a field planting of lettuce at QDPI Gatton Research Station after application of NPV (GemStar®) with and without Coax® - Lettuce Trial 5. Values represent the proportion (%) of larvae that were healthy or died due to NPV.

DATE	DAS *	Treatment	NPV	Healthy
04.03.97	1	Control	1.1 b	98.9 a
	1	NPV	85.9 a	14.1 b
	1	NPV + Coax	82.8 a	17.2 b
06.03.97	3	Control	2.5 b	97.5 a
	3	NPV	88 a	12 b
	3	NPV + Coax	92.8 a	7.2 b

* DAS = Days after Spraying. Values are the mean of four replicates. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Column means for a given date followed by the same letter are not significantly different for $P < 0.05$.

TABLE 9

The percentage NPV mortality of larvae exposed to lettuce leaf discs sprayed with NPV (GemStar®) or NPV + Coax®. Lettuce plants at QDPI Gatton Research Station were sprayed, and leaf discs were removed daily for five days after application - Lettuce Trial 6.

DAS *	GemStar	GemStar + Coax	Control
1	100	100	0
2	100	98.0	4.0
3	58.7	92.0	0
4	53.1	54.0	10.0
5	22.4	28.6	0

* DAS = Days after Spraying

TABLE 10

A comparison of the levels of heliothis egg parasitism recorded following small plot releases of two species of *Trichogramma* in sweet corn at Lowood - Corn Trial 1.

Treatment	% Egg Cards Parasitised	No. eggs/card Parasitised
Control	6.6 ± 0.9 b	11.5
T. pretiosum	83.3 ± 4.3 a	13.9
T. nr. *brassicae*	79.1 ± 1.1 a	8.2

The proportion of egg cards parasitised (%) data are the mean ± standard error of four replicates. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Means followed by the same letter are not significantly different for P<0.05.

TABLE 11

The daily and cumulative emergence (%) of adult *Trichogramma* for each day after release (DAR) at Lowood during Corn Trial 1. Day of Release = DAR 0.

	DAILY EMERGENCE		CUMULATIVE EMERGENCE	
DAR	*T. pretiosum*	*T.* nr. *brassicae*	*T. pretiosum*	*T.* nr. *brassicae*
0	53.6	45.5	53.6	45.5
1	22.7	34.3	76.3	79.8
2+	10.3	13.1	86.6	92.9
no emergence	13.4	7.1		

TABLE 12

A comparison of the levels of heliothis egg parasitism recorded following small plot releases of *T. pretiosum* in sweet corn - Corn Trial 2.

Treatment	% Egg Cards Parasitised	% Egg Cards Completely Eaten
Control	3.8 ± 1.3 b	39.3 ± 5.7 a
Release	38.5 ± 4.9 a	27.4 ± 2.8 a

Data are the mean ± standard error of six replicates. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Column means followed by the same letter are not significantly different for P<0.05.

Lettuce Trial 6

The bioassay of virus activity on sprayed lettuce plants found approximately 100% larval mortality for the first 2 days after spraying (DAS) (Table 9). NPV mortality 3 DAS was higher in the plot where Coax® was used (92%) than that where NPV alone was applied (59%). This suggests that the addition of Coax® may provide some UV protection and extend the potency of NPV from two to three days. This is worthy of additional investigation.

Larval mortality 4 and 5 DAS was similar in both virus treatments, i.e around 50% 4 DAS and 25% 5 DAS (Table 9).

SWEET CORN TRIALS

Corn Trial 1

High levels of heliothis egg card parasitism were found following the release of *Trichogramma* in the Lowood sweet corn trial - approx. 80% (Table 10). There was no significant difference in the levels of parasitism recorded due to either species of *Trichogramma*. Both species displayed potential at finding heliothis egg cards under field conditions. There were, however, twice as many *T.* nr. *brassicae* released *as T. pretiosum*.

There was rapid emergence of wasps when they were released in the field. Approximately 50% of wasps emerged on the day of release (Table 11), and this probably contributed to the high levels of egg card parasitism recorded.

Corn Trial 2

The level of heliothis egg parasitism was ten times higher in plots where *T. pretiosum* were released (Table 12), indicating that wasp releases can contribute to increased heliothis mortality. There was also evidence of predation of heliothis eggs, with 27% of egg cards in the release plots completely eaten (Table 12). Adult transverse ladybirds (*Coccinella transversalis*) and striped ladybirds (*Micraspis frenata*) were common throughout the trial site - 39% of plants contained ladybirds. The combined action of increased egg parasitism (38% in the release plots) and predation prevented widespread heliothis damage, and the Pacific Seeds staff were able to avoid spraying with chemicals.

Corn Trial 3

There was one peak of heliothis egg laying in the Pacific Seeds Trial that commenced just prior to the start of silking (Figure 11A, B). The egg cards used to assess the levels of heliothis egg parasitism also provided evidence of the action of predators in both sites. The proportion of cards completely eaten by predators reached 70% in the

release site and 49% in the control site (Figure 11C). A number of insect predators were noticed in both sites, including pirate bugs (*Orius* spp.), three-banded ladybirds (*Harmonia octomaculata* (Fabricius)), striped ladybirds (*Micraspis frenata*), and adult lacewings.

The egg parasitism reached high levels early season in both sites (up to 78% and 52% in the release and control sites respectively), but dropped to low levels at the most crucial stage of the crop, i.e. silking (Figure 12A). The high proportion of egg cards parasitised early season may have been because the cards were highly attractive to the wasps in the absence of naturally laid heliothis eggs (Figure 11A, B). Fewer egg cards were predated early season than mid-season (Figure 11C). The high level of parasitism in both (release and control) sites early season suggests that natural populations of egg parasitoids contributed to the parasitism levels. No attempt was made to identify the parasitoids recovered in this trial.

Low numbers of heliothis egg parasitoids were captured in suction samples, peaking at 0.43 and 0.23 wasps/m in the release and control sites respectively (Figure 12B). Two species of egg parasitoids were recovered in suction samples, *Trichogrammatoidea bactrae* Nagaraja and a species of *Trichogramma*.

SWEET CORN IPM FIELD TRIALS

Corn Trial 4

There were two peaks in heliothis egg laying during Corn Trial 4 (Figure 13). The first peak commenced 17 days before the start of silking, and the second peak coincided with the beginning of silking. No significant larval populations resulted from the first peak of egg laying activity (Figure 13). The predators in the crop apparently managed larvae at the vegetative stage. Brown lacewings (*Micromus tasmaniae* (Walker)), ladybirds (primarily the two-spotted ladybird, *Diomus notescens*, and the transverse ladybird, *Coccinella transversalis*) and pirate bugs (*Orius* spp.) were numerous throughout Corn Trial 4 (Figures 14, 15). There was predation of heliothis egg cards placed in the crop to assess egg parasitism - approximately 30% of cards showed signs of complete or partial predation during the first peak of egg laying (Table 13). The numbers of predators in the chemical treatment fell to low levels once spraying with deltamethrin commenced.

There was no significant correlation between suction samples and visual counts of key predators when the plants had finished vegetative growth (Figure 16). This suggested that suction samples were not an accurate indication of predator density, so they were not used in Corn Trial 5.

The second peak of egg laying (at silking) lead to a marked increase in larval infestation in all plots, with the greatest increase (from 5% to 84%) occurring in the chemical plots (Figure 13). Chemical applications commenced 5 days after silking started, and NPV/*B.t.* applications commenced 13 days after silking started.

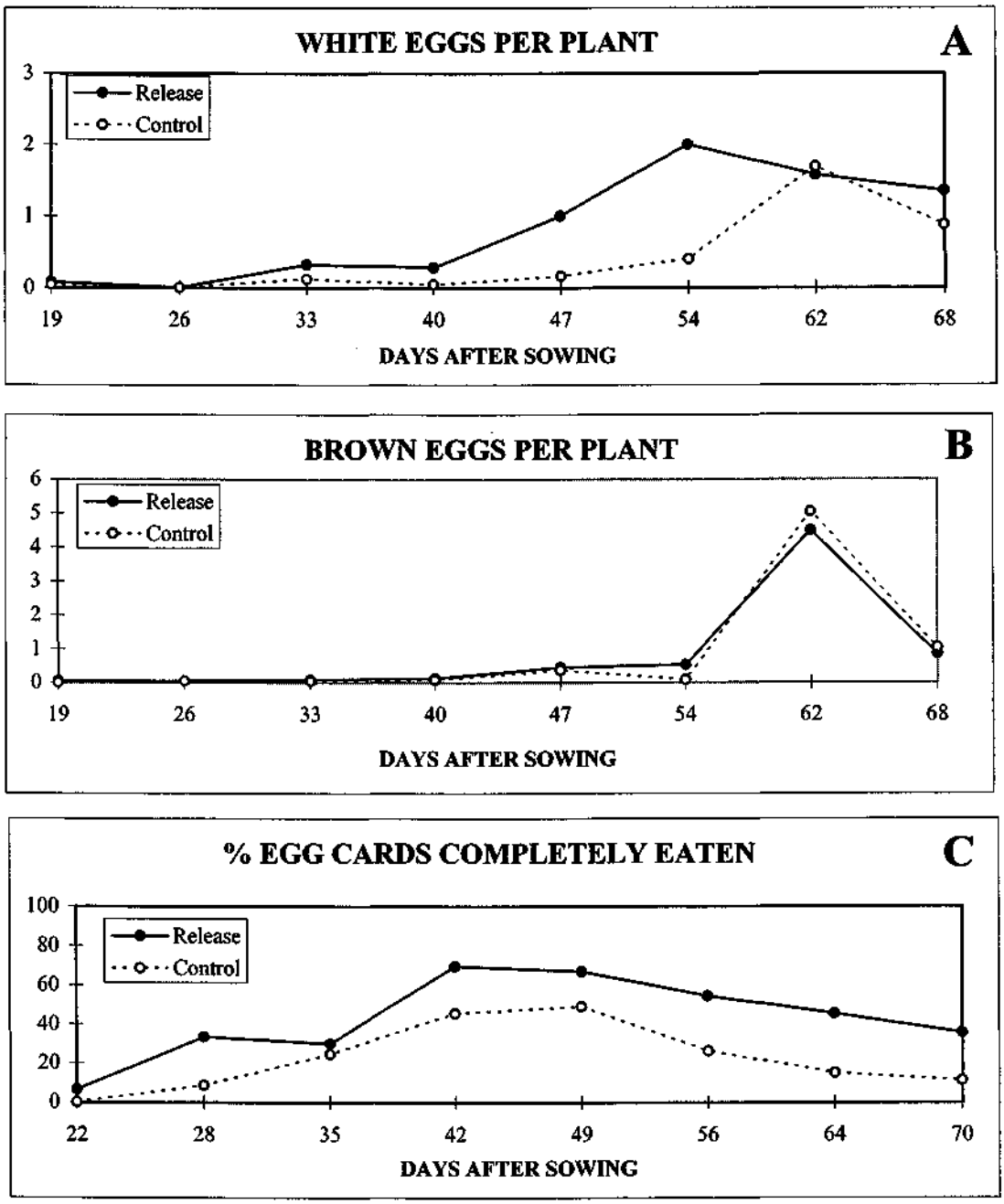


FIGURE 11: The number of heliothis *(H. armigera)* eggs on sweet corn during Corn Trial 3. *Trichogramma* nr. *brassicae* were inundatively released in the release site weekly (200,000 wasps/ha/release). **(A)** The number of white eggs per plant. Silking commenced approximately 58 DAS and 64 DAS in the release and control sites respectively. **(B)** The number of brown eggs per plant. **(C)** The proportion of heliothis egg cards in each site that were completely eaten by predators.

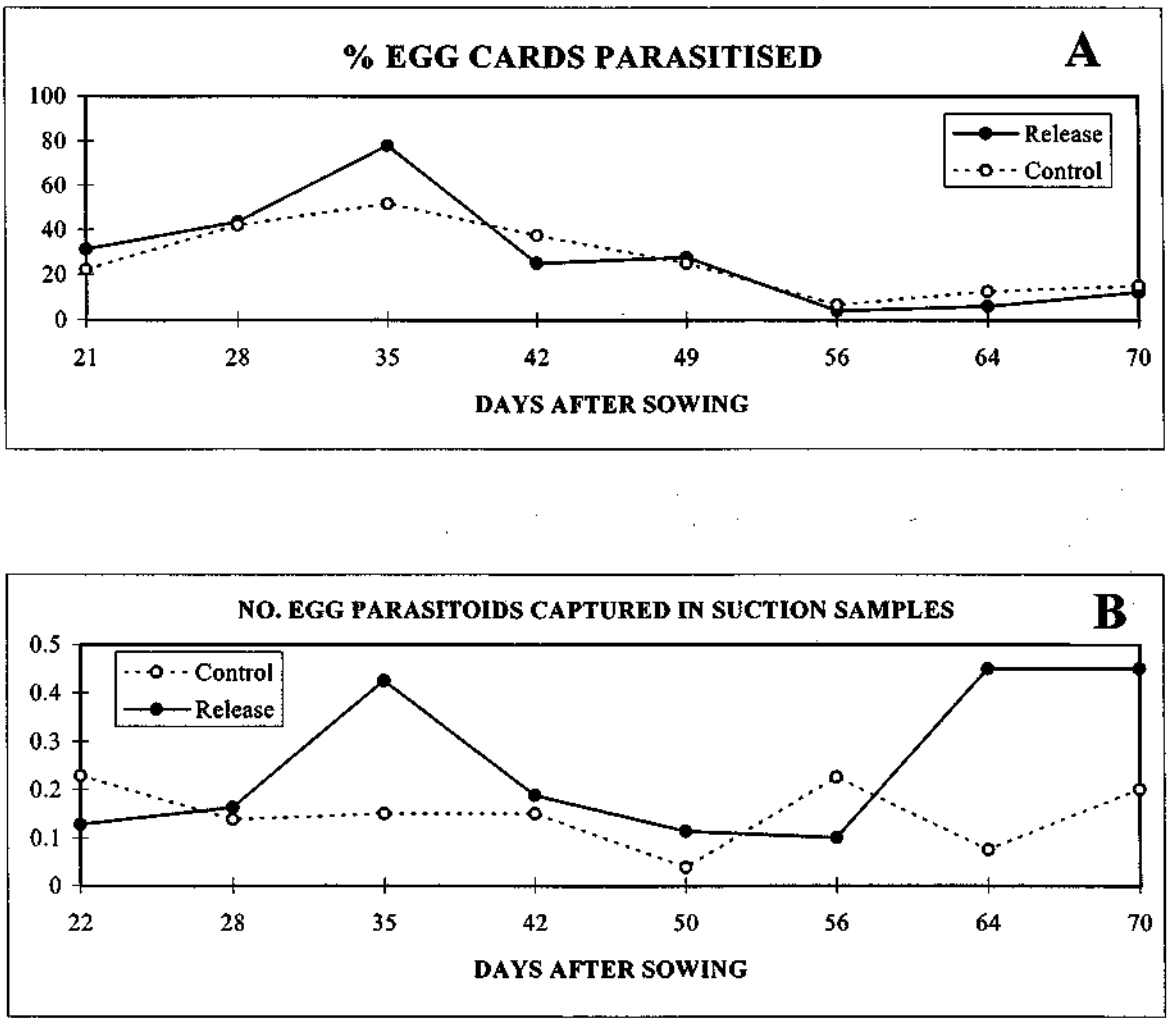


FIGURE 12: Indications of heliothis egg parasitoid activity on sweet corn during Corn Trial 3. *Trichogramma* nr. *brassicae* were inundatively released in the release site weekly (200,000 wasps/ha/release). **(A)** The proportion of egg cards containing parasitised eggs. **(B)** The numbers of heliothis egg parasitoids captured in suction samples (no. / m). Values represent the mean of four suction samples per site.

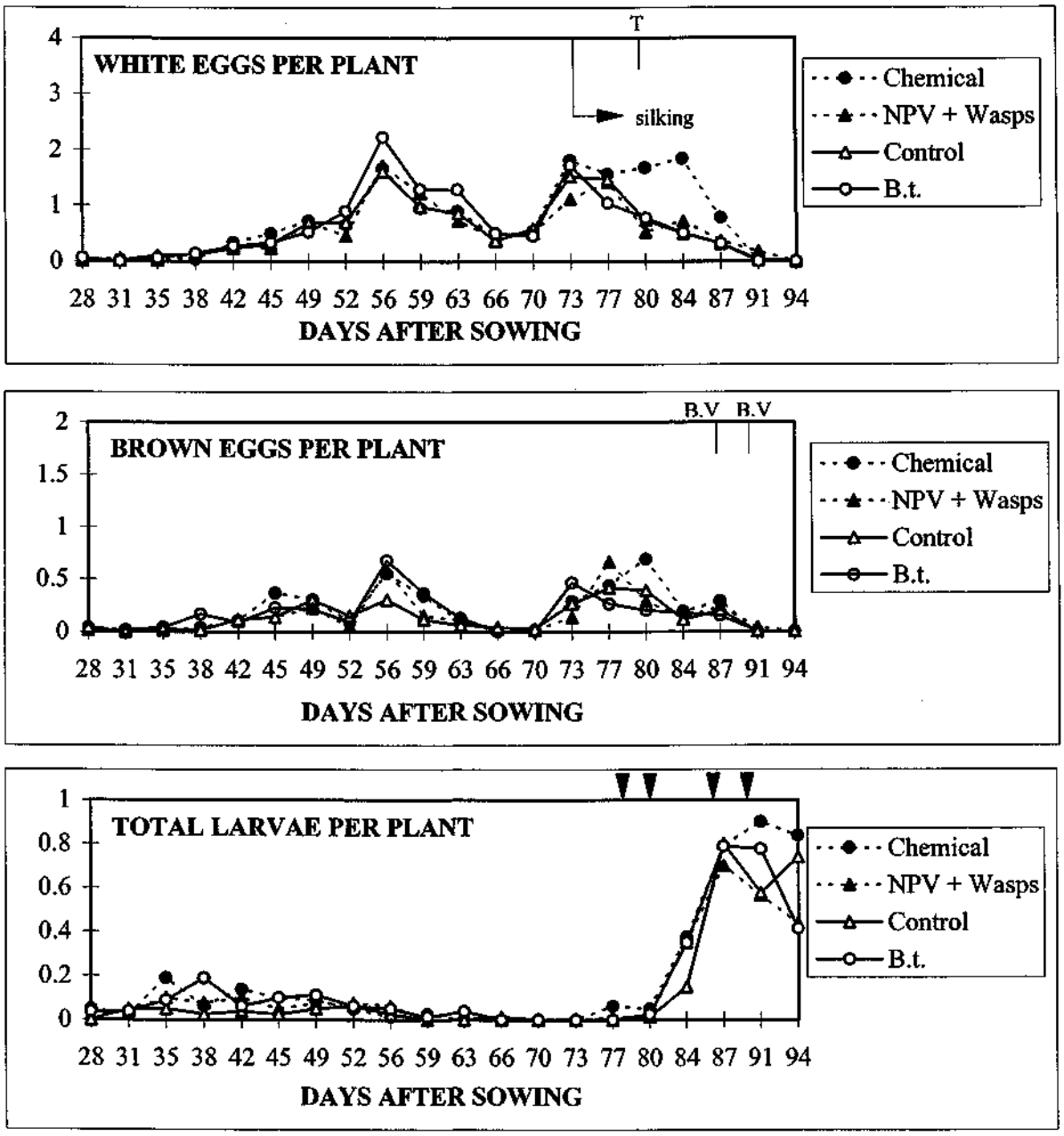


FIGURE 13: The number of heliothis (*H. armigera*) eggs and larvae on sweet corn at QDPI Gatton Research Station during Corn Trial 4. The arrows and lines indicate when management tactics were applied: Black arrows = chemicals, T = *Trichogramma*, V = NPV, B = *B.t.*.

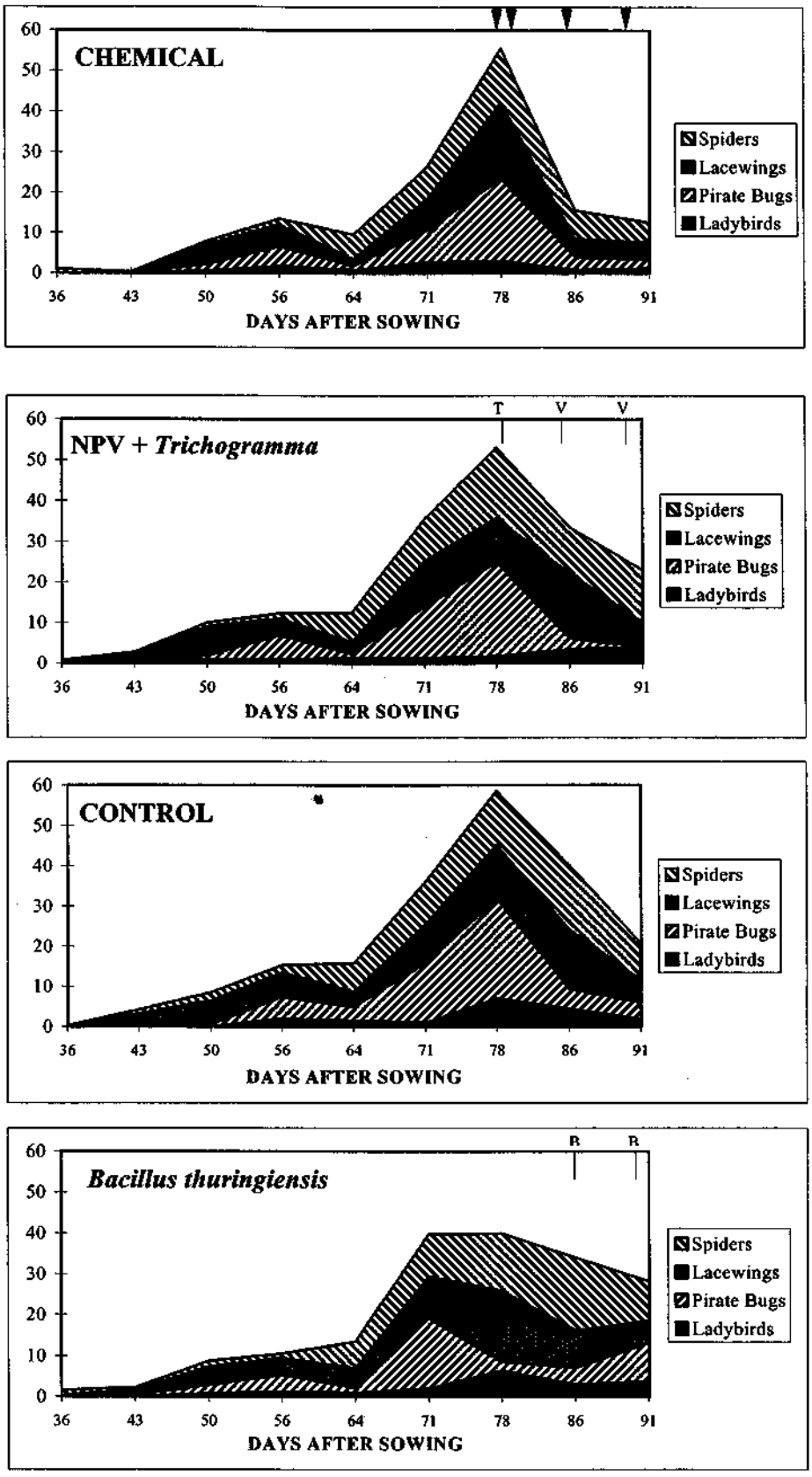


FIGURE 14: The number of predators collected from sweet corn at QDPI Gatton Research Station during Corn Trial 4. The values represent the numbers of predators (mean of four plots) collected with a suction machine over 10 m of crop row. The arrows and lines indicate when management tactics were applied: Black arrows = chemicals, T = *Trichogramma*, V = NPV, B = *B.t.*. Silking commenced 73 DAS.

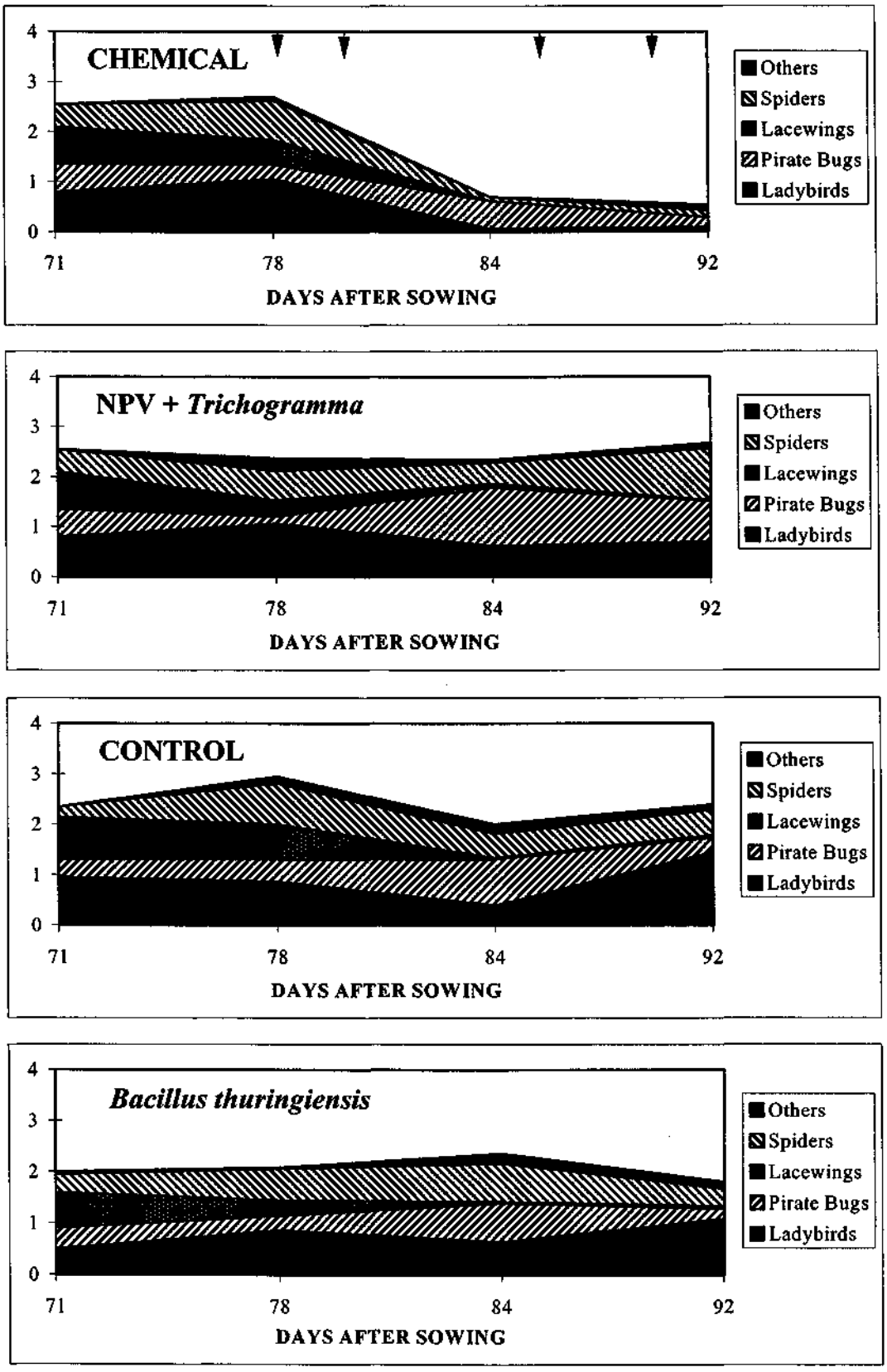


FIGURE 15: The number of predators on sweet corn at QDPI Gatton Research Station during Corn Trial 4. The values represent the numbers of predators observed per plant (mean of four plots). The black arrows indicate when chemicals were applied to the chemical plots. Silking commenced 73 DAS.

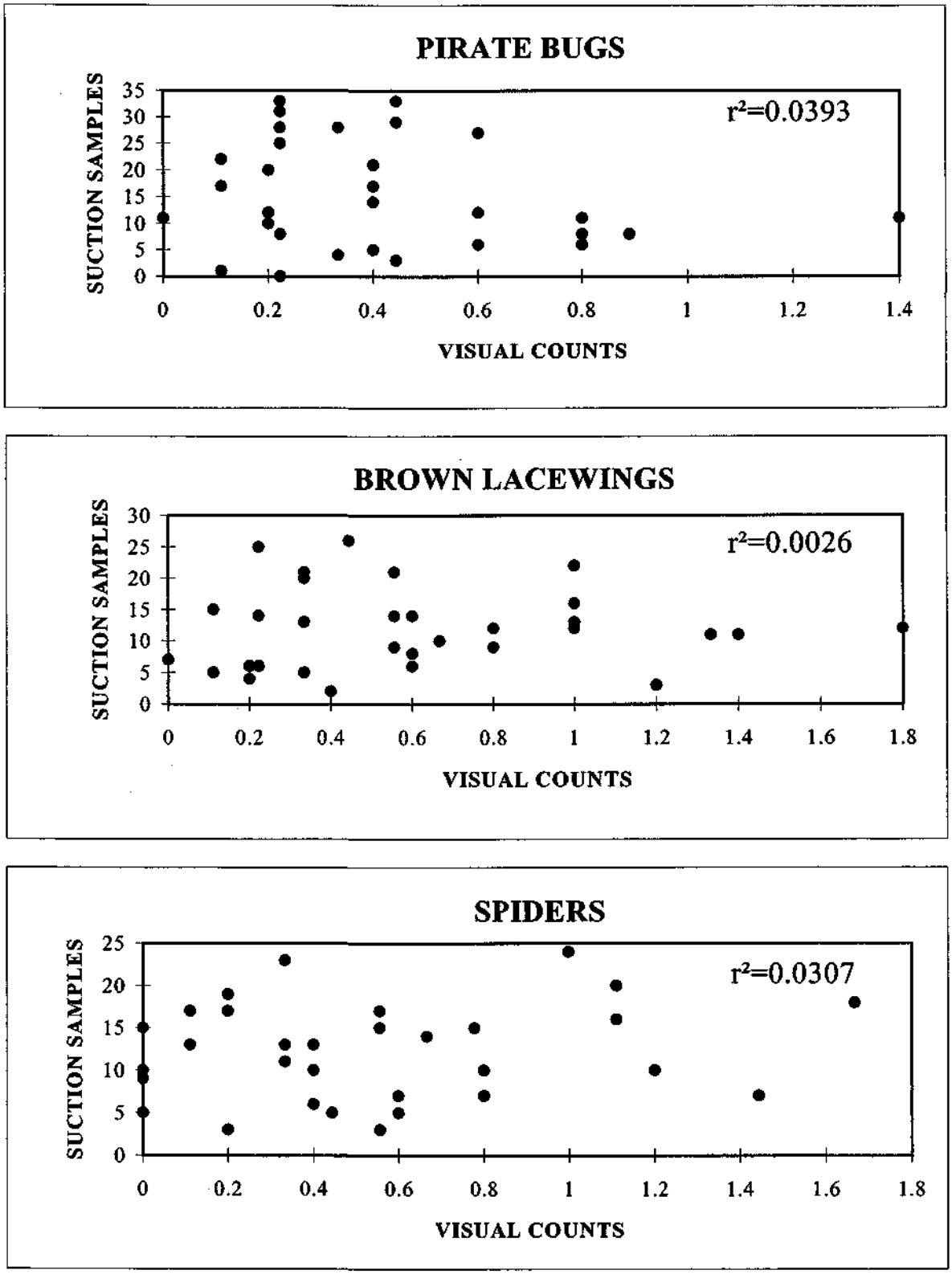


FIGURE 16: A comparison of visual predator counts (no./plant) with suction sample predator counts (no./10 m row) taken from sweet corn plots at QDPI Gatton Research Station during Corn Trial 4.

TABLE 13

Fate of heliothis egg cards collected from Sweet Corn at QDPI Gatton Research Station during Corn Trial 4. Cards containing at least ten eggs were stapled to the upper surface of corn leaves every 5 m in every second row (total of 25 egg cards per plot). The shaded columns indicate those data collected after chemical applications had commenced in the chemical plots (78 DAS).

DATE	18-Oct-96	25-Oct-96	1-Nov-96	8-Nov-96	15-Nov-96	22-Nov-96	29-Nov-96	6-Dec-96
DAS	38	45	52	59	66	73	80	87
% Hatched								
Chemical	78.8	79.2	73.0	70.4	60.3	47.6	61.1	57.5
NPV + wasps	80.9	81.2	69.5	74.6	53.3	60.2	64.2	46.8
Control	76.1	74.0	70.0	73.2	51.1	66.6	62.0	28.6
B.t.	78.7	81.5	67.1	58.0	57.2	64.6	57.6	29.7
% Parasitised								
Chemical	0.0	0.0	2.9	0.0	0.0	0.0	0.0	4.8
NPV + wasps	0.0	4.9	0.0	0.0	0.0	0.0	0.0	1.0
Control	0.0	1.1	0.0	0.0	2.1	1.1	0.0	2.1
B.t.	0.0	0.0	6.6	0.0	1.0	0.0	0.0	1.6
% Completely Eaten (a)								
Chemical	14.1	9.3	15.6	21.5	37.4	45.0	25.2	35.4
NPV + wasps	5.6	8.5	23.7	21.3	36.9	24.6	22.8	45.5
Control	9.2	15.5	21.2	20.9	39.6	25.0	23.0	67.7
B.t.	7.8	7.6	22.1	30.0	33.5	34.4	22.2	56.9
% Partly Eaten (b)								
Chemical	7.1	11.5	8.6	8.1	2.4	7.4	13.7	2.3
NPV + wasps	13.6	5.4	6.9	4.1	9.8	15.3	13.0	6.6
Control	14.7	9.5	8.9	5.8	7.3	7.3	15.0	1.7
B.t.	13.5	10.9	4.3	12.1	8.3	1.0	20.3	11.9
% Eaten (a+b)								
Chemical	21.2	20.8	24.2	29.6	39.8	52.4	38.9	37.6
NPV + wasps	19.2	13.9	30.5	25.4	46.7	39.8	35.8	52.2
Control	23.9	25.0	30.0	26.8	46.9	32.3	38.0	69.4
B.t.	21.3	18.5	26.3	42.0	41.8	35.5	42.4	68.7

The chemical applications (four sprays of deltamethrin) did not manage larvae and lead to 84% larval infestation just prior to harvest assessment - higher than any other treatment. The NPV + *Trichogramma* and *B.t.* treatments had the lowest larval infestations at this time - 44% and 41% respectively. There was no evidence to suggest that the single *Trichogramma* release had any impact on heliothis egg mortality (Figure 17).

The proportion of cobs with no damage was highest in the NPV + *Trichogramma* treatment (32%) and lowest in the chemical treatment (9%). Similarly, larval infestation of cobs was greatest in the chemical treatment and lowest in the NPV + *Trichogramma* treatment (Table 14, Figure 18).

Larval mortality due to NPV was highest in the treatment sprayed with NPV. There was, however, NPV mortality in all treatments suggesting that a natural epizootic of NPV had occurred throughout the site (Table 15).

Corn Trial 5

In Corn Trial 5 there was one peak in heliothis egg laying commencing approximately one week before silking started (Figure 19). Chemical applications commenced 2 days after silking started, and NPV/*B.t.* applications commenced 4 days after silking started. The chemical applications (four sprays of deltamethrin) again failed to manage larvae and lead to a larval infestation of 54% at the end of sampling. The NPV + *Trichogramma* treatment had the lowest larval infestations at this time (2%) (Figure 19).

Larval mortality due to NPV was high in the treatment sprayed with NPV (83-100%), and negligible in the other treatments (Table 16). The proportion of cobs with no damage was highest in the NPV + *Trichogramma* treatment (94%) and lowest in the chemical treatment (47%). Similarly, larval infestation of cobs was greatest in the chemical treatment and lowest in the NPV + *Trichogramma* treatment (Table 17, Figure 20).

Pirate bugs (*Orius* spp.) were the most abundant predators present (Figure 21). The number of predators fell to almost zero once deltamethrin was applied in the chemical treatment.

In contrast to Corn Trial 4, there was abundant egg parasitoid activity during Corn Trial 5 (Figure 22). A high level of egg parasitism (47%) was recorded at the vegetative stage of the crop (approximately one week before silking commenced (Figure 22B). This population of parasitoids apparently acted as a foundation for subsequent generations of *Trichogramma* that caused very high levels of egg parasitism when the crop was silking (Figure 22C). These egg parasitoids were identified *as Trichogramma pretiosum*, i.e. different to the commercially available species that was released in some treatments (*T.* nr. *brassicae*). *T. pretiosum* was the most abundant species collected throughout the study site (Table 18).

Naturally occurring *Trichogrammatoidea bactrae* were also recovered. It was therefore concluded that the three inundative releases of *T.* nr. *brassicae* had little impact on heliothis egg mortality.

The primary factor responsible for the high egg mortality found was an abundant natural population of *T. pretiosum*. These wasps may have originated from an adjacent unsprayed 2 ha planting of sorghum that flowered just prior to silking in the corn trial. The egg parasitism reached high levels in all treatments, including the chemical treatment (Figure 22A). *Trichogramma* seemed to rapidly re-invade the chemically sprayed plots, probably because these plots represented only 6% of the total study site. Despite this, the chemical applications still caused enough disruption to permit heliothis larval numbers to increase (Figure 19) and cause significant damage (Figure 20).

The emergence of inundatively released *T.* nr. *brassicae* was generally poor, with no emergence recorded from an attempted fourth release (Table 19). In addition to the poor overall emergence, emergence generally did not peak until at least 3 days after release (Table 20). Heliothis eggs laid just prior to, or on the day of release, would therefore have hatched or been unsuitable for parasitisation. These problems need to be addressed by the commercial suppliers of *Trichogramma* if they are going to be of value for use as inundatively released biological control agents of heliothis.

There were also some data suggesting that *Trichogramma* pupae (in parasitised *Sitotroga* eggs) were eaten by predators, and that the longer pupae spent in the field the greater the level of predation (Table 21). This is further evidence suggesting that wasps should emerge as soon as possible after release. Interestingly, there was no predation of egg cards in the treatments sprayed with chemicals, presumably because the predators were killed by the chemical.

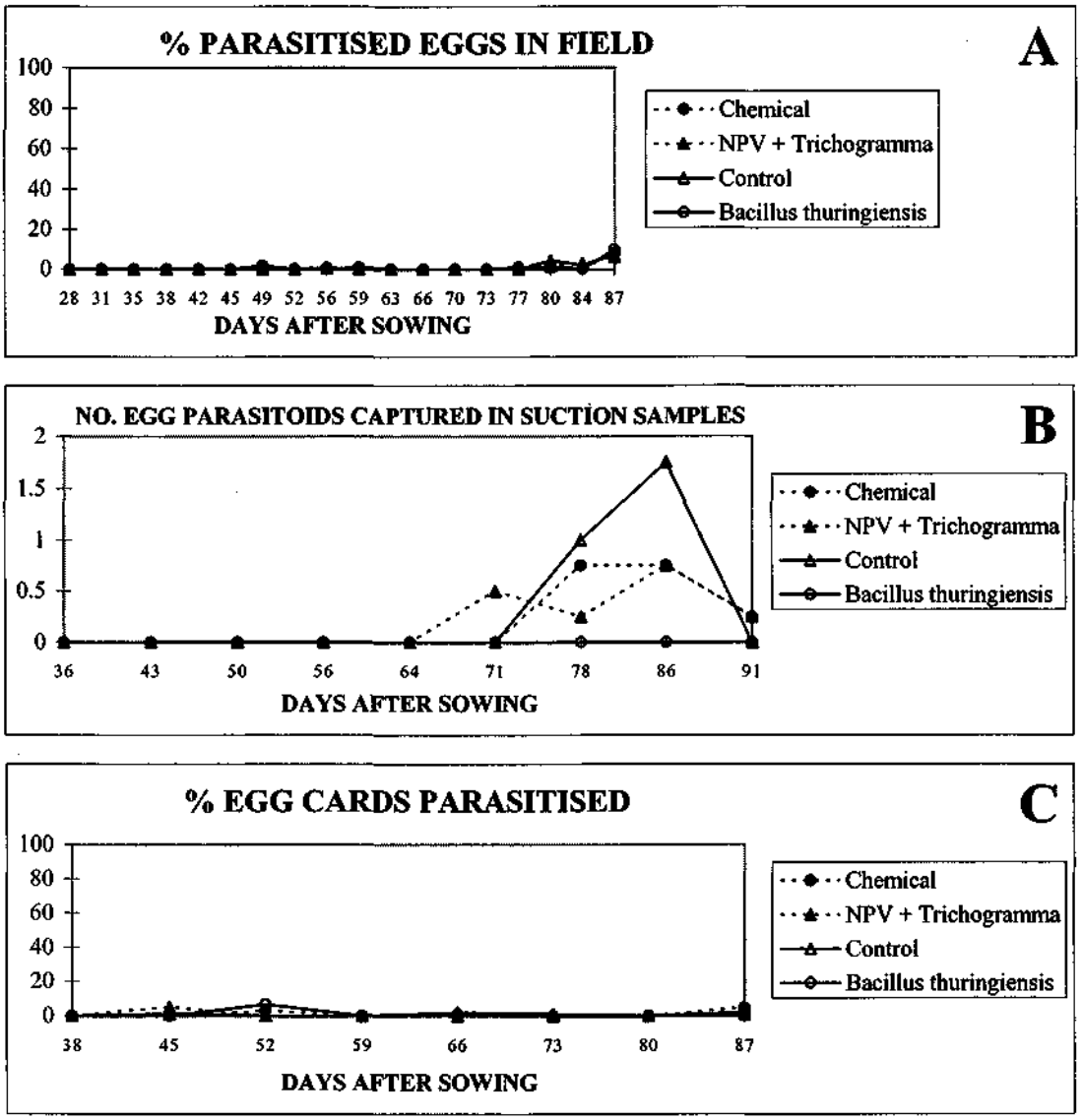


FIGURE 17: Indications of heliothis egg parasitoid activity in sweet corn at QDPI Gatton Research Station during CornTrial 4. **(A)** The percentage of parasitised (black) eggs recorded in the field. **(B)** The numbers of egg parasitoids collected in suction samples. Values represent the mean of four samples collected over a 10 m row length of crop. **(C)** The levels of *H. armigera* egg card parasitism. Values represent the mean of four plots (25 egg cards per plot).

TABLE 14

Damage assessment of sweet corn cobs produced during Corn Trial 4. Fifty cobs were randomly collected from each plot and inspected for heliothis damage and/or larval presence.

TREATMENTS	Nil Damage	Silk Damage (No Larvae)	Silk Damage (with Larvae)	Tip Damage	Cob Damage	Cobs with Larvae
Chemical	9.0 b	20.5 b	9.5 a	19.0 a	42.0 a	33.5 a
NPV + *Trichogramma*	32.0 a	31.0 ab	9.0 a	12.0 a	16.0 c	21.5 a
Control	18.0 ab	37.0 a	10.0 a	13.0 a	22.0 bc	24.0 a
Bacillus thuringiensis	13.5 a	34.0 ab	6.0 a	13.5 a	33.0 ab	22.0 a

Data are the mean percentages of four plots inspected 98 DAS. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Column means followed by the same letter are not significantly different for P<0.05.

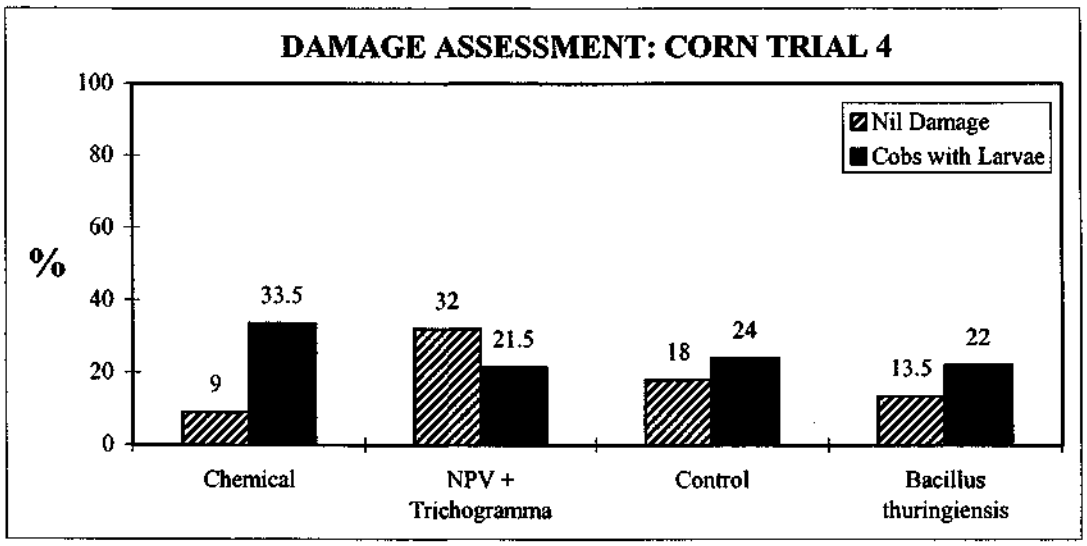


FIGURE 18: The variation of cob damage and larval infestation between treatments for sweet corn grown at QDPI Gatton Research Station during Corn Trial 4.

TABLE 15

Mortality of heliothis larvae during Corn Trial 4. Data are presented as x/y, where x = no. larvae dying due to NPV, and y = total no. larvae collected. NPV was applied on December 5 and December 9, 1996.

Sample	Date	Treatment	VS	S	M	L	Total NPV Mortality
Pre-Spray I	5 December 1996	Control	0/2	0/4	1/11	0/7	1/24 (4.2%)
		NPV+wasps	0/1	2/4	0/13	0/6	2/24 (8.3%)
		Bt	0/0	0/1	1/9	1/14	2/24 (8.3%)
		Chemical	0/1	1/2	0/9	0/9	1/24 (4.2%)
4-d Post Spray I and Pre-Spray II	9 December 1996	Control	0/0	2/4	3/9	1/11	6/24 (25.0%)
		NPV+wasps	1/1	1/1	7/12	4/10	13/24 (54.2%)
		Bt	0/0	1/ 2	2/7	1/15	4/24 (16.7%)
		Chemical	0/1	2/3	1/8	5/12	8/24 (33.3%)
4-d Post-Spray II	13 December 1996	Control	0/0	2/3	3/7	4/14	9/24(37.5%)
		NPV+wasps	0/0	4/4	8/12	6/8	18/24 (75.0%)
		Bt	0/0	1/ 2	3/9	4/13	8/24 (33.3%)
		Chemical	0/0	1/1	6/8	5/15	12/24 (50.0%)

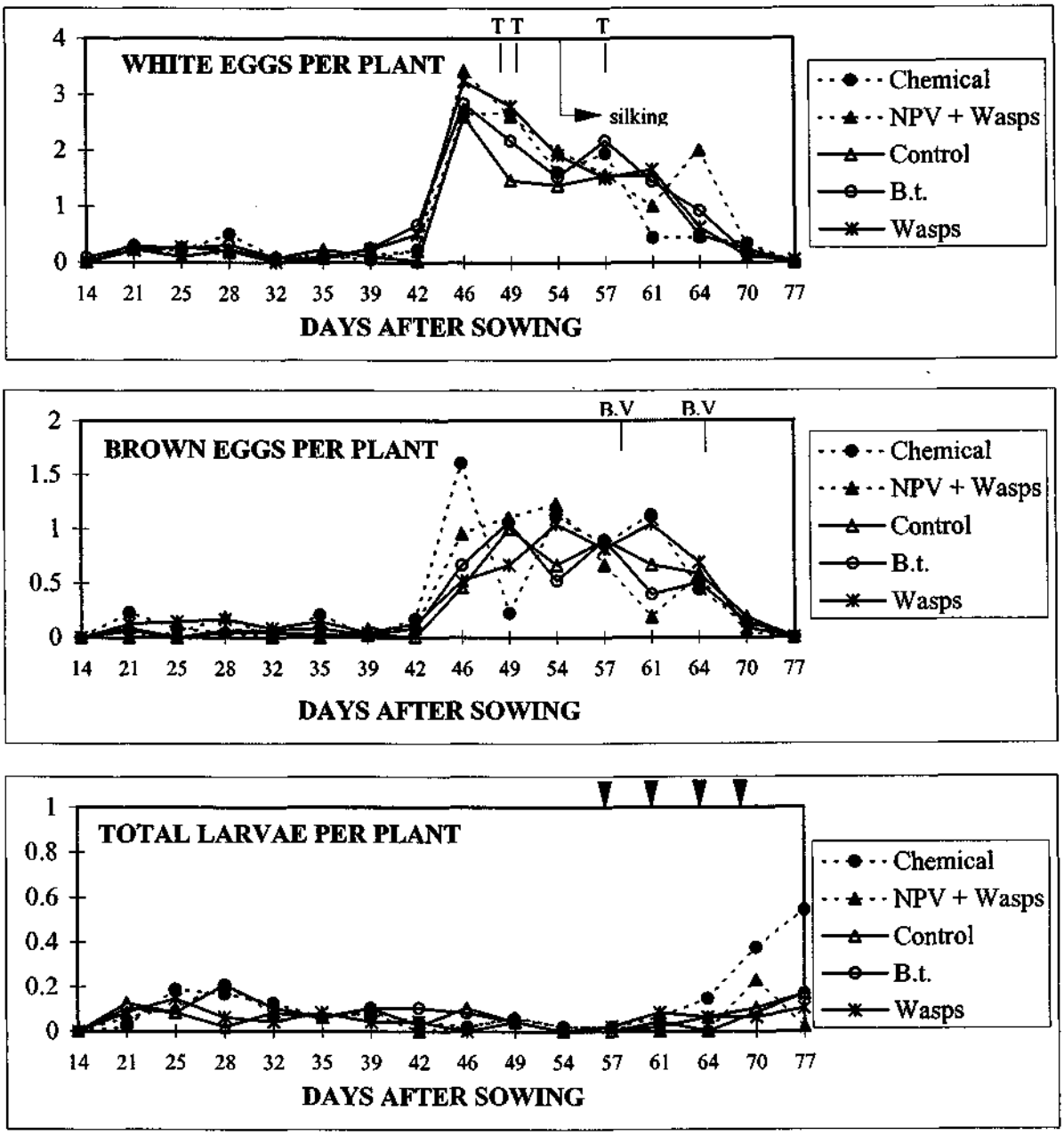


FIGURE 19: The number of heliothis (*H. armigera*) eggs and larvae on sweet corn at QDPI Gatton Research Station during Corn Trial 5. The arrows and lines indicate when management tactics were applied: Black arrows = chemicals, T = *Trichogramma,* V = NPV, B = *B.t.*.

TABLE 16

Mortality of larvae during Corn Trial 5. Data are presented as x/y, where x = no. larvae dying due to NPV, and y = total no. larvae collected. NPV was applied on April 5 and April 11, 1997.

Sample	Date	Treatment	VS	S	M	L	Total NPV Mortality
Pre-Spray I	5 April 1997	Control	0/10	0/2			0/12
		NPV+wasps	0/9	0/3			0/12
		Bt	0/4	0/6	0/2		0/12
		Chemical	0/4	0/7	0/1		0/12
3-d Post Spray I	8 April 1997	Control	0/10	0/2			0/12
		NPV+wasps	9/10	1/1	0/1		10/12 (83.3%)
		Bt	0/2	1/8	0/2		1/12 (8.3%)
		Chemical		0/4	0/8		0/12
Pre-Spray II	11 April 1997	Control	0/1	0/6	0/4		0/11
		NPV+wasps	2/2	4/4			6/6 (100%)
		Bt	0/3	1/4	0/1		1/8 (12.5%)
		Chemical	0/3	0/4	0/1	0/4	0/12
4-d Post-Spray II	15 April 1997	Control	0/1	0/1	1/5		1/7 (14.3%)
		NPV+wasps	4/4	2/2	2/3		8/9 (88.9%)
		Bt	0/3	0/2	0/3		0/8
		Chemical	0/2	0/5	0/3	0/2	0/12

TABLE 17

Damage assessment of sweet corn cobs produced during Corn Trial 5. One hundred cobs were randomly collected from each plot and inspected for heliothis damage and/or larval presence.

TREATMENT	Nil Damage	Silk Damage (No Larvae)	Silk Damage (with Larvae)	Tip Damage	Cob Damage	Cobs with Larvae
Chemical	46.5 d	11.0 a	13.5 a	13.8 a	15.3 a	33.8 a
NPV + *Trichogramma*	94.0 a	1.8 c	1.3 b	2.8 c	0.3 c	1.8 d
Control	76.8 c	6.3 ab	2.5 b	7.8 ab	6.8 ab	11.3 b
Bacillus thuringiensis	88.0 ab	3.5 bc	1.0 b	5.0 bc	2.5 bc	4.0 cd
Trichogramma	79.8 bc	4.0 bc	2.8 b	8.0 ab	5.5 b	7.8 bc

Data are the mean percentages of four plots inspected 85 DAS. Data were arcsine transformed and analysed by analysis of variance. The means were compared by Fisher's Least Significant Difference technique. Column means followed by the same letter are not significantly different for P<0.05.

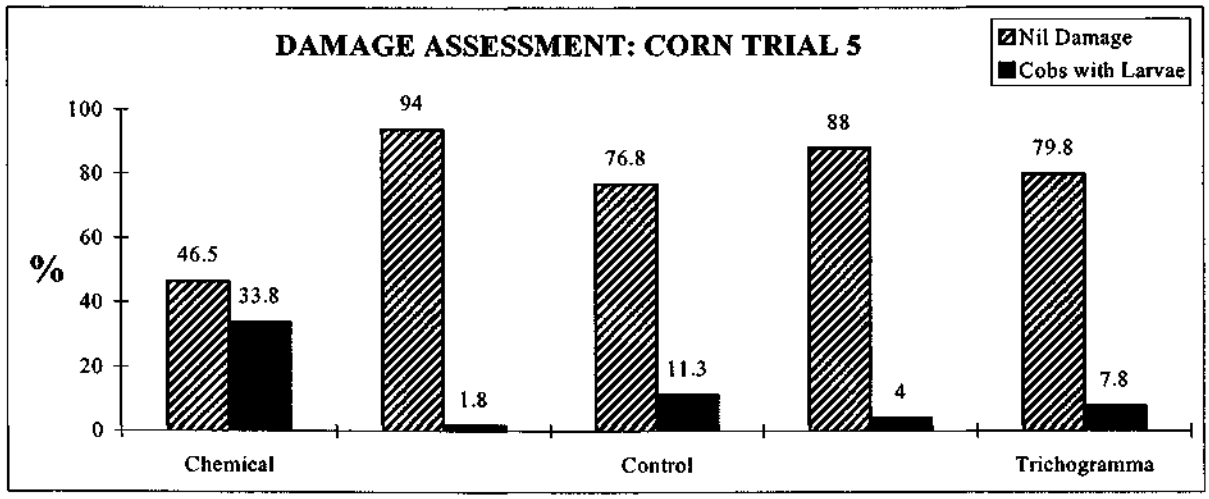


FIGURE 20: The variation of cob damage and larval infestation between treatments for sweet corn grown at QDPI Gatton Research Station during Corn Trial 5.

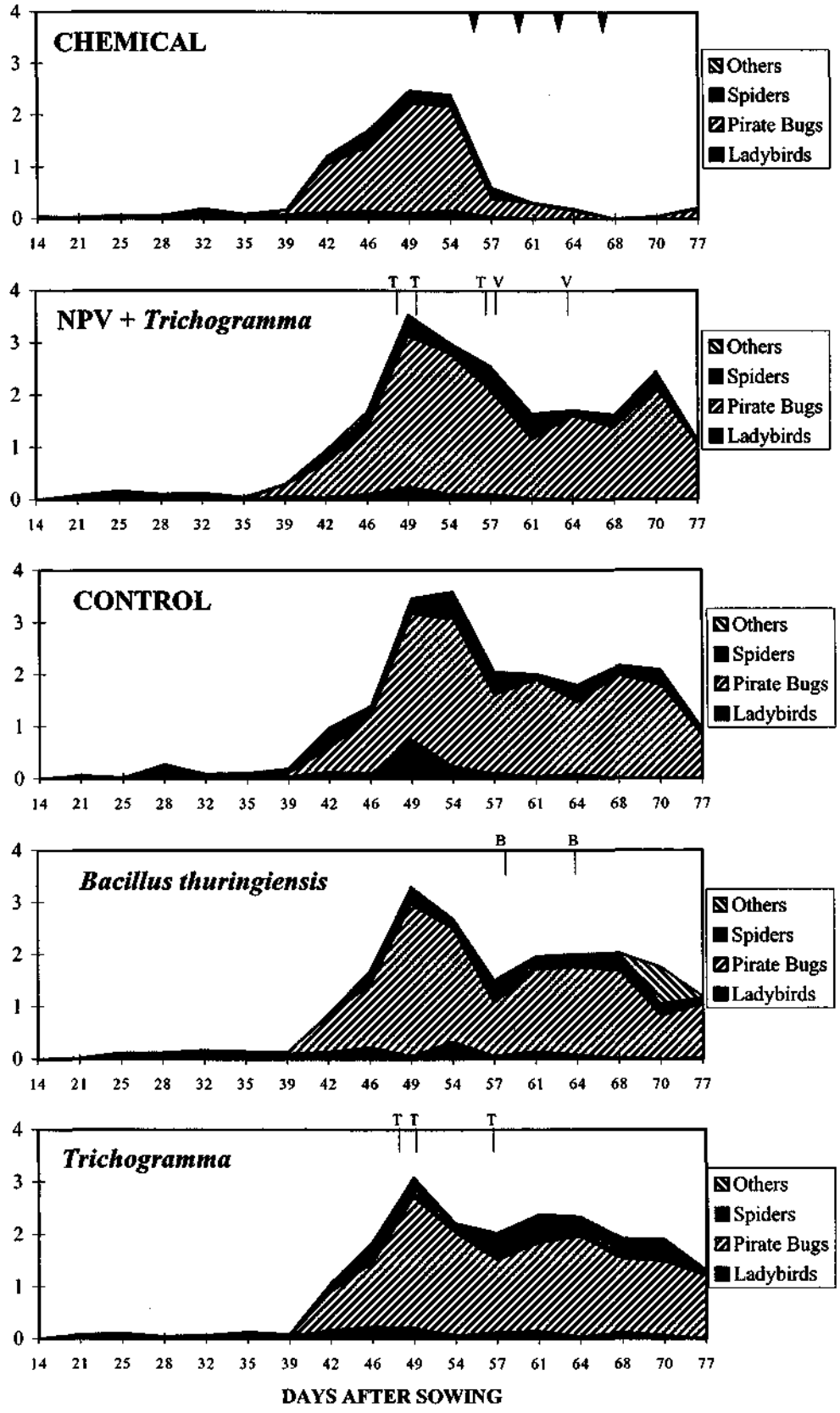


FIGURE 21: The number of predators on sweet corn at QDPI Gatton Research Station during Corn Trial 5. The values represent the numbers of predators observed per plant (mean of four plots). The arrows and lines indicate when management tactics were applied: Black arrows = chemicals, T = *Trichogramma*, V = NPV, B = *B.t.*. Silking commenced 54 DAS.

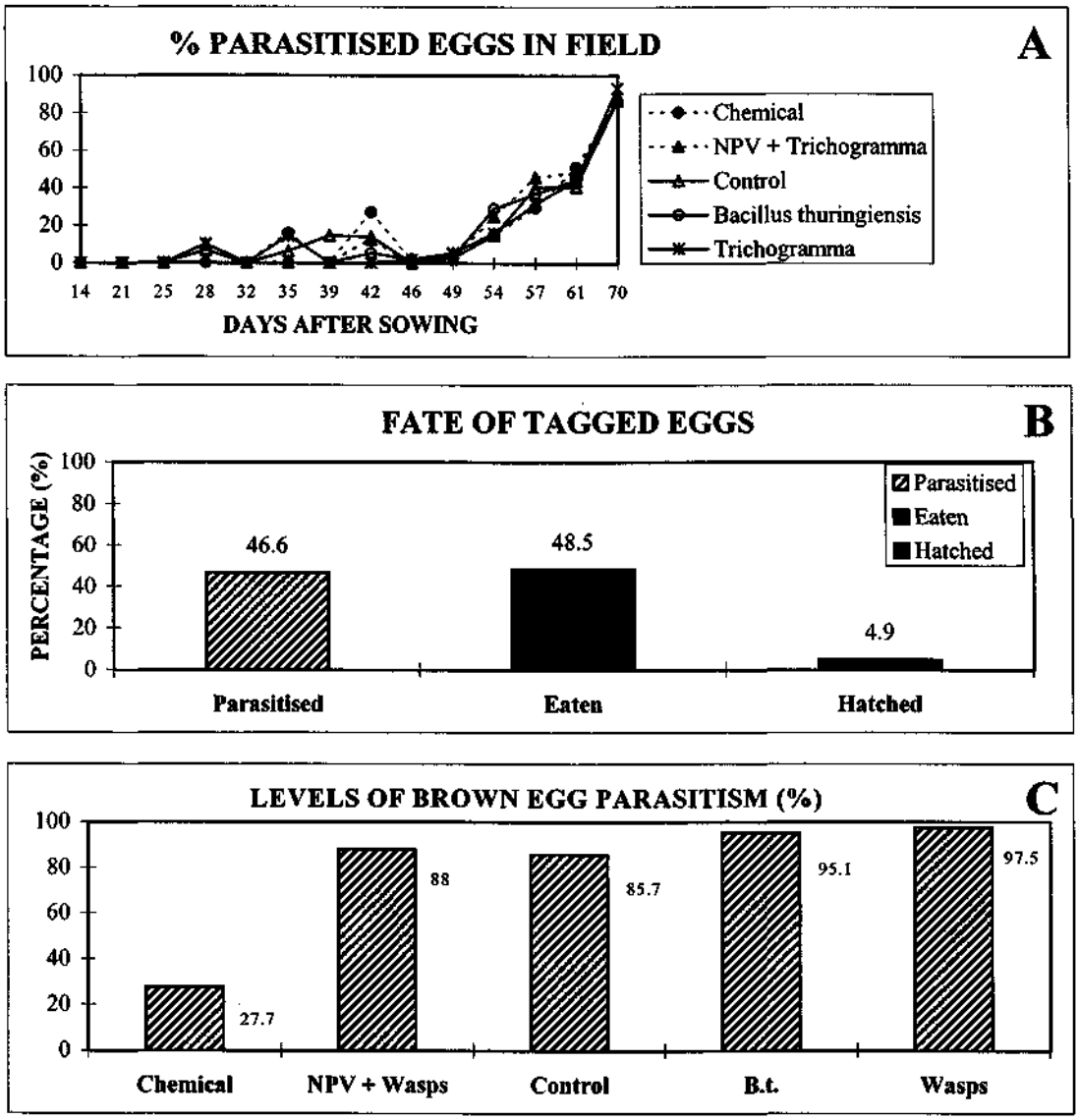


FIGURE 22: Indications of heliothis egg parasitoid activity during Corn Trial 5. **(A)** The percentage of parasitised (black) eggs recorded in the field. **(B)** The fate of white eggs tagged in the field on 25 March 1997 (approximately one week before silking commenced), in control plots. There were 5.2 eggs tagged /plant. **(C)** The levels of brown egg parasitism for eggs collected from brown silks on 14 April 1997 (approximately 2 weeks after silking commenced). There were 0.2, 1.1 and 7.3 white, brown and black (parasitised) eggs/silk respectively.

TABLE 18

The species of egg parasitoid recovered from all treatments throughout Corn Trial 5. Data are the number of parasitised eggs that produced a given parasitoid species.

Date	DAS	*T. pretiosum*	*T. brassicae*	*T. bactrae*	*Telenomus*
25.03.97	47	138	13	15	1
02.04.97	55	124	2	10	0
09.04.97	62	128	1	3	0
11.04.97	64	327	4	15	0
14.04.97	67	1332	27	102	0
16.04.97	69	233	4	21	0
23.04.97	76	6	0	1	0
Total No.		2288	51	167	1
Total %		**91.3**	**2.0**	**6.7**	**0.0**

TABLE 19
Emergence and release rate data for the inundative releases of *T.* nr. *brassicae* during Corn Trial 5.

Release No.	Date of Release	Sex Ratio (% female)	Total % Field Emergence	Release Rate / ha (males + females)
1	26.03.97	76	62	406,000
2	28.03.97	77	32	762,000
3	04.04.97	57	58	414,000
4 *	11.04.97	0	0	0

* No wasps emerged from the fourth release.

TABLE 20
The daily emergence (%) of *T.* nr *brassicae* for each day after release (DAR) during Corn Trial 5. Day of release = DAR 0.

DAR	Release #1		Release #2		Release #3	
	% Lab Emergence	% Field Emergence	% Lab Emergence	% Field Emergence	% Lab Emergence	% Field Emergence
0	0	0	0	0	0	0
1	0	0	0	6	0	0
2	38	6	10	0	0	0
3	50	32	16	8	0	2
4	4	18	38	4	2	22
5+	0	6	2	14	60	34
no emergence	8	38	34	68	38	42

TABLE 21
The percentage of egg cards showing signs of predation during Corn Trial 5.

Date Cards Put in Field	Treatment	DAR #	% Cards Predated *	No. Eggs Eaten / Card
26.03.97	*Trichogramma*	1	40	11.8 ± 6.2
	Trichogramma	2	50	18.4 ± 4.3
	Trichogramma	3	75	11.3 ± 2.9
04.04.97	*Trichogramma*	3	50	17.7 ± 5.4
	Chemical	3	0	0
10.04.97	*Trichogramma*	2	31	34.4 ± 8.9
	Chemical	2	0	0

Days After Release, i.e. the number of days that the egg cards were exposed to predators in the field.
* The percentage of cards containing parasitised *Sitotroga* eggs that showed signs of predation.

DISCUSSION

Two new pest management tactics were evaluated against heliothis in this project - *Trichogramma* wasps and NPV (GemStar®) - the potential of these 'products' are discussed below.

The Usefulness of *Trichogramma* Wasps and Predators

King and Coleman (1989) state that 'there is limited experimental evidence to support the commercial use of *Trichogramma* for *Heliothis* (*Helicoverpa*) control'. This statement was in reference to the use of inundative releases of *Trichogramma* against heliothis in cotton. Many researchers have demonstrated that *Trichogramma* can sometimes increase heliothis egg mortality, however none have demonstrated that wasp releases can significantly manage heliothis season long. The inconsistent effectiveness of *Trichogramma* in field trials makes it very difficult to successfully promote them as worthwhile biocontrol agents for heliothis management.

The variable effectiveness of *Trichogramma* was again obvious in this project. In lettuce, for example, egg parasitism in some small plot studies was high (up to 70% - Table 5), and in others it was low (up to 20% - Table 3). The commercial evaluation of *Trichogramma* against heliothis on lettuce (Lettuce Trial 1) found no evidence of egg parasitism at all, despite seven wasp releases at rates ranging from 100 to 900 thousand per hectare (Figure 9). These data indicate that *Trichogramma* have little potential against heliothis in lettuce. Why is this so?

Lettuce plants may be attacked by heliothis at any stage after the seedlings are transplanted. Initially the plants are small with very few leaves, and are surrounded by a lot of bare ground. This environment is not *Trichogramma* friendly. It is very difficult to successfully release *Trichogramma* pupae onto small plants using a hopper mounted on a tractor. The progress of the tractor over the terrain causes the delivery hoses to move about and much of the material that is released does not land on a plant. High soil temperatures will kill *Trichogramma* pupae and greatly reduce adult emergence (see Figures 5-7). Temperatures of 45°C or more are potentially deadly to the wasps, and are likely to be encountered during summer. Delayed wasp emergence compounds these problems because pupae are exposed to the environment for longer periods of time, thus increasing the likelihood of exposure to high temperatures and other environmental factors that may ultimately reduce the numbers of wasps emerging in the field.

Variable levels of egg parasitism following inundative wasp releases were also found in the sweet corn trials. High levels of egg parasitism were found in Corn Trial 1 (approx 80% - Table 10), but low levels were found in Trial 2 (approx. 40% - Table 12), and Trial 3 (Figure 12). There was also no evidence that inundative releases had any significant impact during the sweet corn IPM trials (Corn Trials 4 and 5) (e.g. Figure 17A). Although there were high levels of egg parasitism in Corn Trial 5 (Figure 22), very few of the released wasp (*T.* nr. *brassicae*) were recovered (Table

18). The high levels of egg parasitism recorded were due to a natural population of *T. pretiosum* (Table 18).

During corn trial 1, there was rapid emergence of *Trichogramma* in the field - approx. 50% of wasps emerged on the day of release (Table 11). In contrast to this, there was delayed emergence of wasps during Corn Trial 5, with most wasps emerging 3-5 days after release (Table 20). There was also no emergence from one attempted release (Table 19).

It is likely that rapid wasp emergence is crucial for successful egg parasitism. As mentioned above, delayed emergence may expose wasp pupae to adverse environmental conditions. Delayed emergence also increases the likelihood that wasp pupae may be eaten by predators (Table 21). Heliothis eggs can hatch in 2-3 days during summer. Delayed emergence of 3 or more days would therefore be useless against initial egg lays because the eggs would hatch before the wasps emerge.

It would be desirable for commercial producers of *Trichogramma* to predict when most of their wasps will emerge so that they are not released too early and exposed to adverse conditions (e.g. high temperatures or predation). The commercial production of *Trichogramma* is in its infancy in Australia and quality control measures to ensure rapid (and high) wasp emergence are being developed. One possibility is to include an 'indicator' vial of wasp pupae that is 24 hours older than the material to be released. The bulk material can be held until the indicator vial has emerged, and then released with the knowledge that it will emerge in the next 24 hours.

Trichogramma release techniques for broad acre crops are also still being developed. Initial work involved releasing a dry mixture of wasp pupae (in parasitised *Sitotroga* eggs) with a bulking agent (e.g. semolina). However, recent work has evaluated releasing *Trichogramma* in a liquid suspension that adheres pupae to plants. Some liquid suspensions have shown promise in small scale trials (Table 2), but additional research is necessary to develop systems for using suspensions in commercial cropping situations.

There are, as highlighted above, a number of practical problems involved with using *Trichogramma* as inundative biocontrol agents. These include availability, storage/emergence and release techniques. Until these issues are resolved it is unrealistic to expect primary producers to have confidence in using these wasps. *Trichogramma* can be further evaluated against heliothis once the production and release techniques are properly developed.

This scenario does not help the development of commercial *Trichogramma* production facilities. It is costly to develop and operate a commercial facility, and there is an element of risk involved in investing in a form of pest management that is (to date) not widely used. This is, to some extent, a 'Catch 22' situation. A commercial facility is needed to produce wasps for farm scale testing to convince primary producers that the concept has some merit. But why invest in something that has unknown market potential?

The DPI has provided *Trichogramma* production advice to five companies. Only one of these is still producing wasps (Bugs for Bugs). Fortunately there are currently

demands for *Trichogramma* to manage other insect pests. While this demand continues, commercial production will continue, and the opportunity for *Trichogramma* to be further evaluated against heliothis occurs.

Although there have been variable levels of egg mortality recorded following inundative releases of *Trichogramma*, egg parasitoids may still have an important role in heliothis IPM strategies. High natural populations of *Trichogramma* contributed to the low damage found in most treatments during Corn Trial 5 (Figure 22, Table 18). The key to successfully utilising these natural populations is: 1) recognising that they are present and active in a crop, and 2) adopting pest management practices that do not disrupt their action, i.e. by avoiding the use of broad spectrum chemical insecticides. High natural populations of egg parasitoids are regularly encountered in late sown summer crops. Wasp numbers presumably increase as the season goes on and more hosts are available.

A technique for rapidly assessing the levels of egg parasitism is necessary if natural wasp populations are to be utilised. As mentioned earlier (Plate 1), parasitised eggs change colour, becoming black. However this takes approximately four days, and healthy (unparasitised) heliothis eggs can hatch in 2-3 days.

Brown heliothis eggs are usually around 2 days old. If the levels of parasitism in these eggs could be rapidly assessed a management decision could be made regarding the value of applying a larvicide. A larvicide may be necessary if egg parasitism (hence mortality) was low, or unnecessary if egg parasitism was high. DNA based diagnostic techniques have been developed by the Centre for Tropical Pest Management (Bilston and Simpson, 1997) that have potential for assessing egg parasitism six hours after wasps have laid their eggs. Such tests would be a valuable tool in an IPM program, and need to be developed to a stage where they are available for commercial use.

It would also useful to have some indication of egg predation prior to making a spray decision. Predation, however, is more difficult to assess than parasitism. From a crop consultants perspective, decisions on whether or not predation is likely to be significant will have to be based on predator counts. During Corn Trial 5, key predator counts averaged 3 or more per plant prior to silking (Figure 21) and caused almost 50% egg mortality (Figure 22B). This, along with high levels of egg parasitism, killed 95% of all eggs at the vegetative stage (Figure 22B). Although there were over 5 eggs per plant at this stage (approx. 30 eggs per metre), they did not produce a significant larval population (Figure 19). A farmer finding 30 eggs per metre in a corn crop just prior to silking would probably consider a chemical application. However, as illustrated in this trial, it may not be necessary because natural enemies can reduce egg hatch enormously. Primary producers and crop scouts/consultants need to able to assess beneficials and feel secure in the knowledge that they will kill heliothis eggs. Further research is necessary to determine the numbers (and species) of predators necessary to manage various heliothis infestations.

Predator numbers of 2-2.5 per plant just prior to silking (Figure 15) did not prevent severe larval infestation during Corn Trial 4 (Figure 14). There was, however, almost no natural egg parasitism recorded during this trial (Figure 17). Predators should not be assessed in isolation. Parasitism must also be taken into account.

The usefulness of NPV (GemStar®)

The results indicate that NPV has potential for use against heliothis in both lettuce and sweet corn. The obvious advantage to using NPV is the additive benefits to be obtained by conserving natural enemies. In lettuce these benefits included the preservation of larval parasitoids (Table 7). The application of NPV was also effective against heliothis larvae on lettuce (Tables 7-9), killing 60-70% of chemically resistant larvae during Lettuce Trial 4 (Table 7), and 80-90% of larvae during Lettuce Trial 5 (Table 8). The 60-70% NPV mortality in Trial 4 was complemented by 25-35% mortality due to natural enemies and pathogens, resulting in a high (95+%) total mortality. NPV should not be thought of as a replacement or substitute for chemical insecticides, but as an alternative. The main differences between NPV and conventional chemical insecticides are: 1) NPV is a natural pathogen, 2) NPV usually takes 5-7 days to kill larvae, and 3) NPV does not kill anything except heliothis. NPV is not the new panacea for heliothis management. It does, however, have great potential in a true IPM program where it can supplement naturally occurring mortality.

NPV would be a valuable addition to heliothis management in lettuce. There are currently no selective insecticides (including *B.t.*) registered against heliothis in lettuce in Queensland. Additional data needs to be generated to assist the registration of these products.

NPV also has potential in sweet corn. The plots sprayed with NPV during Corn Trials 4 and 5 produced more undamaged cobs than the other treatments (Figures 18, 20). This was achieved in Corn Trial 4 by a combination of NPV mortality (Table 15) and conservation of predators (Figure 15).

During Corn Trial 5 the chemically treated plots suffered much more cob damage than all of the other treatments (Figure 20). This was presumably because the natural populations of egg parasitoids and predators were disrupted by the application of deltamethrin (Figures 18, 21). Almost 77% of cobs in the control plots were undamaged (Figure 20), illustrating the importance that natural egg parasitoids and predators had on managing heliothis. The application of NPV or *B.t.* significantly increased the proportion of undamaged cobs to 94 and 88% respectively (Table 17). The use of NPV, therefore, enhanced the level of control achieved from natural enemies alone. Only two applications of NPV/*B.t.* were made during Corn Trial 5.

The timing of the first NPV application may be important. In Corn Trial 4 the first NPV spray occurred 13 days after the first silks were noticed (Figure 13), while in Corn Trial 5 NPV was first applied 4 days after silking commenced (Figure 19). Trial 4 was severely infested and suffered a lot of cob damage (Figure 18), and Trial 5 was lightly infested and suffered very little cob damage (Figure 20). The timing of NPV application was probably more critical in Corn Trial 4 because there was very little egg mortality due to *Trichogramma* (Figure 17).

Data from this study suggest that the first NPV spray should be applied when silking commences. However, additional research is necessary to determine the best timing and number of NPV sprays required to effectively manage heliothis larvae.

CONCLUSIONS AND RECOMMENDATIONS

- Heliothis management must be directed at the egg and small larval stages. Large larvae typically feed in sheltered sites and are difficult to contact with insecticides. *H. armigera* is also resistant to most of the chemicals used against it. Research into new heliothis management techniques should be ongoing, and emphasis should be placed on those practices that are more sustainable than those based on chemicals alone.

- Inundative releases of *Trichogramma* wasps against heliothis eggs are ineffective in lettuce. Environmental conditions in lettuce crops are not *Trichogramma* friendly. Lettuce plants (especially young plants) are surrounded by a lot of bare ground. Released wasp pupae landing on the soil are likely to be exposed to lethal temperatures.

- Augmentative releases of *Trichogramma* wasps against heliothis eggs in sweet corn need further investigation. Research to date has been hampered by practical problems associated with the mass production and release of wasps in commercial situations. However, the commercial production of *Trichogramma* is in its infancy in Australia, and improved rearing, storage and release methods are currently being developed.

- Naturally occurring populations of egg parasitoids (*Trichogramma* and relatives) can cause significant heliothis mortality in sweet corn. High natural populations of these wasps are commonly encountered in summer sown corn in the Lockyer Valley. Pest management practices that recognise and conserve these populations need to be further developed and adopted.

- A technique for rapidly assessing the levels of egg parasitism is required to utilise *Trichogramma* in pest management programs. DNA based diagnostic techniques have been devised, but need to developed to a stage where they are available for commercial use. This may be as a field test kit, or as a service provided by specialists.

- Predators can also greatly reduce heliothis eggs numbers. Further research is necessary to determine the numbers (and species) of predators necessary to manage various heliothis infestations. Primary producers and crop scouts need to be able to assess parasitism and predation to make heliothis management decisions. These natural control tactics are an integral part of true Integrated Pest Management.

- NPV is effective against heliothis on lettuce and sweet corn, but should not be thought of as a substitute or replacement for chemicals that no longer work. NPV has great potential in IPM because it only kills heliothis, and is safe on parasitoids and predators. Additional research is required to determine the best way to use NPV, i.e. timing and number of applications. Additional data may be required to register NPV against heliothis in lettuce and sweet corn.

APPENDIX 1

INSECT REARING

Three species of insects were reared extensively throughout the duration of this project, viz. heliothis (*Helicoverpa armigera* (Hübner)), wasp egg parasitoids (*Trichogramma* species and *Trichogrammatoidea* species), and the Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier).

Heliothis (*Helicoverpa armigera*)

Heliothis were reared on a navy bean based diet which was modified from that described by Teakle and Jensen (1985).

Larval Diet Composition

a.	navy bean		258 g
	tap water		1410 ml
b.	agar		37.5 g
	tap water		909 ml
c. dry mix:	wheat germ		180 g
	torula yeast		150 g
	l-ascorbic acid		9 g
	nipagin		9 g
	sorbic acid		3 g
d.	formaldehyde (40%)		3 ml
e.	mould inhibitor		7.8 ml
	mould inhibitor preparation:		
	propionic acid	42 ml	
	phosphoric acid	4 ml	
	water	54 ml	

Moth Diet Composition

water	1000 ml
sugar	100 g
l-ascorbic acid	6 g
terramycin soluble powder	2 g

The navy bean/water (a) and agar/water (b) mixtures were steamed separately in covered beakers for two hours. Both mixtures were then cooled for 5-10 minutes to approximately 70°C. The beans, dry mix (c) and formaldehyde (d) were mixed in a waring blender. The agar and mould inhibitor (e) were then added and remixed.

The diet was poured into stainless steel larval rearing trays measuring 300x300x20 mm. Each tray was lined with plastic film before the diet was poured. This aided cleaning at the completion of rearing. The diet was allowed to cool at room temperature. An aluminium grid was then pressed into the diet to produce 196 square cells (20x20 mm) that were 20 mm deep. Separate rearing cells are required when rearing heliothis because the larvae are cannibalistic (Twine, 1971).

Loose heliothis eggs were dispensed onto the diet by inverting a plastic vial with a stainless steel gauze lid (8 strands/cm; 28 SWG; 0.9 mm aperture) over the tray and sprinkling the eggs onto the diet. Each tray was then covered with perforated aluminium sheeting (0.81 mm holes; 0.6 mm thick; 31% open area) to contain larvae to cells and prevent cannibalism or escape. Two large rubber bands held each perforated sheet in position. The covered trays were wrapped in brown paper and held in a constant temperature room (approximately 25°C and 70% R.H.) for 24 days until pupation.

The pupae were removed from the diet trays with feather forceps and washed in a sodium hypochlorite solution (0.167% a.i.) for three minutes and then rinsed in water. They were then placed in an aluminium tray with vermiculite, and the tray was placed in a mating cage (300x300x300 mm) covered with fine nylon cloth. A plastic container of sucrose solution (moth diet) was provided for emerging moths. Two cotton dental wicks were passed through the lid of each moth diet container and acted as feeding stations for the moths.

Moths emerged approximately 7 days after pupation and were allowed to mate in the mating cage for two days. The moths were then transferred to aluminium oviposition cages (after Teakle and Jensen, 1985). Moth diet was placed in each oviposition cage. The moths laid eggs on the paper towelling walls of the cages. The paper walls (containing the eggs) were removed daily. The eggs could be stored at 8±1°C for 4-5 days and maintain reasonably high levels of hatchability, or could be stored for longer periods (up to two weeks) if they were used for rearing egg parasitoids.

The eggs were removed from the paper towelling by gently washing them in a 0.167% a.i. sodium hypochlorite solution for three minutes and rinsing them in water. The eggs were poured into a plastic squeeze bottle and dispensed onto pieces of cotton cloth that were placed on flyscreen frames. These frames were stacked in a rack and the eggs were allowed to dry at room temperature (approximately three hours). The eggs were removed from the cotton cloth by brushing them in short, gentle strokes with a soft paint brush. They were then ready for dispensing onto larval diet trays.

Sitotroga cerealella (AGM)

An experimental facility for rearing egg parasitoids on AGM eggs was developed at QDPI Toowoomba. AGM larvae were reared on wheat using techniques adopted from those described by others (Morrison, 1985; Laing and Eden, 1990).

Chemically free wheat was used to rear AGM larvae. All wheat was heated in an incubator at 65°C for 24 hours before use to kill any insects or mites that may have been in the grain. The sterilised grain was treated with a miticide (dicofol - 0.113% a.i.) at a rate of 600 ml per 5 kg of wheat to prevent infestation by mite pests. The treated grain was then placed in a sealed plastic container and held in a refrigerator (approx. 4°C) for three days to restore the grain to a favourable moisture content of approximately 15% (Morrison, 1985).

Rectangular AGM larval rearing cribs (1200x600x20 mm) were constructed of 19x19 mm timber framing on two sides and a base. Three timber dividers (580 mm long) divided each crib into four compartments 275 mm wide. Raised aluminium mesh (Expamet 601A) was stapled either side of the timber frame and 2.5 kg of treated wheat was poured into each compartment. Two timber spacers (30 mm long) were stapled in each compartment to maintain the wheat at a uniform thickness of approximately 20 mm throughout the crib.

The cribs were held in units to collect emerging moths. Each moth unit consisted of a base funnel, a central section for holding the cribs, and a tapered top section that was connected to an external airflow suction system. Plastic castors were attached to each leg of a unit so that it could be moved for maintenance. The base was made from galvanised sheet metal (24 gauge) and consisted of a rectangular opening (1320x710 mm) tapering down to a cylinder 70 mm high and 186 mm in diameter. Two metal lugs protruded from the cylinder that permitted a 4 L plastic container to be attached to the bottom of the funnel. Two holes (120x60 mm) were cut into the plastic containers and covered with fine stainless steel gauze (24 strands/cm; 37.5 SWG; 0.25 mm aperture) to provide ventilation.

The base was riveted to a steel rectangular frame (19x19 mm steel) measuring 1320x710x800 mm. Galvanised sheet metal was permanently riveted to the two long sides of the central section. A hole (1000x80 mm) was cut in the bottom of each galvanised sheet and covered with fine stainless steel gauze (24 strands/cm; 37.5 SWG; 0.25 mm aperture). This gauze covered hole prevented moth escape and parasitoid invasion, and allowed air to be drawn over the cribs. The two short sides were enclosed by removable covers (710x635 mm), attached with magnetic tape. One was made from galvanised sheet metal, and the other was made from 3 mm thick clear perspex (this permitted internal viewing to check for moth emergence). The removable ends were necessary for cleaning the units.

The tapered top of the unit was also made of galvanised sheet metal and measured 1320x710x400 mm. The top tapered down to a cylinder 70 mm high and 160 mm in diameter. The top was connected to PVC ducting (160 mm diameter) with flexible plastic tubing. The ducting lead to a filter system driven by an in-line variable speed fan. This system drew air over the cribs, filtered out moth scales, and returned the air to the rearing room to maintain humidity.

The central section that held the cribs contained ten 'U' shaped galvanised sheet metal troughs (1320x40x25 mm) riveted to the bottom of the steel frame. These troughs acted as guides for the cribs which were slid into the hangers and held at approximately 80° by 'L' shaped galvanised sheet metal support brackets at the top of the steel frame. The guides were 50 mm apart.

The treated grain was infested with enough AGM eggs to produce two eggs per kernel of wheat. This required approximately 1.2 g of AGM eggs per kilogram of wheat (there were 28,300 kernels of wheat/kg, and 50,400 AGM eggs/g), or 12 g AGM eggs per 10 kg crib (3 g eggs per compartment). Cribs containing newly treated wheat were held horizontally in steel storage racks for infesting with AGM eggs. The wheat was misted with water from a hand held sprayer, and the eggs were sprinkled onto the wheat by shaking them from a small plastic bottle with a stainless steel gauze lid (8

strands/cm; 28 SWG; 0.9 m aperture). AGM eggs could be stored at 5±1°C (50±5% R.H.) for up to five days and still maintain high levels of egg hatch (> 75%). After this, egg hatchability declined rapidly.

Following infestation the cribs were placed 100 mm apart on steel horizontal storage racks to allow the AGM eggs to hatch, and the larvae to penetrate the grain and develop. After three weeks the cribs were transferred to the moth collection units and stored vertically to collect AGM moths as they emerged from the grain.

Temperature was maintained at approximately 25°C and relative humidity at 70% in the AGM rearing room. Lights were used when the room was occupied by working staff (usually 6 hours maximum per day).

Adult AGM commenced emerging approximately 21 days after infestation. The moths were blown into the plastic collection bottles at the bottom of each moth unit with a vacuum cleaner and were collected daily for five weeks. Used wheat was dumped and the cribs were washed and dried in the sun.

The moths were placed into stainless steel wire mesh (8 strands/cm, 26 SWG, 0.8 mm aperture) cylinders (240 mm long, 290 mm Ø) with plastic ends. A central, threaded stainless steel rod (6 mm Ø) held the plastic ends against the wire mesh. A hole (45 mm Ø) was drilled in one end of each cylinder to allow collection bottles to be emptied via a plastic funnel into the cylinder daily, and was sealed with a cork stopper.

The oviposition cylinders were placed in an enclosed unit (1040x730x640 mm). Two 6 mm thick clear acrylic bi-fold doors (1040x600 mm) at the top of the unit allowed a maximum of six cylinders to be placed in two rows on stainless steel rods (19 mm Ø) that were connected to a geared motor that rotated the cylinders four times per minute. A moth scale extraction fan was connected to a stainless steel tube (100 mm Ø) containing inlets located 3 mm from the top of cylinders placed in the unit. The fan removed moth scales from the top of the cylinders without drawing moths or eggs (at the bottom of the cylinders) from the unit. The fan and motor were connected to a timer with a manual over ride switch, and operated automatically for two minutes every hour. The manual over ride was used for five minutes when moths were first added to a cylinder to remove excess scales.

The tumbling of the moths dislodged the eggs, which fell through the wire mesh and onto stainless steel collection trays (570x200 mm) at the bottom of the unit. A second vacuum system continually drew a slight airstream over the collection trays to withdraw any minute moth scales falling out of the cylinders. AGM eggs were collected daily and sieved through a series of stainless steel mesh screens (9 strands/cm, 13 strands/cm, and 33 strands/cm). The finest screen separated mite (*Blattisocius keegani* Fox) eggs from AGM eggs. AGM egg production fluctuated throughout the project.

***Trichogramma* and relatives**

After collection, AGM eggs were held at 8±1°C to retard development. AGM eggs held for up to 28 days at this temperature could still be used to rear parasitoids. When needed, AGM eggs were weighed and sprinkled uniformly through a stainless steel wire screen (16 strands/cm) onto a clear perspex sheet (400x300x3 mm) misted with distilled water. A natural adhesive on the eggs stuck them to the perspex when they contacted moisture. After drying, eggs could be easily removed with a soft paint brush held diagonally to the perspex and moved downwards in short, gentle strokes.

Parasitoids were reared in a simplified 'window box' unit (Laing and Eden, 1990). The unit consisted of a commercially available plastic box (600x400x100 mm), with three 25 mm diameter gauze covered ventilation holes in each side. The ventilation holes were covered with fine stainless steel mesh (47 strands/cm, 44 SWG, 0.125 mm aperture) to prevent parasitoid escape while allowing ventilation. Two clear sheets (400x300x3 mm) sat neatly on the top rim of the box. Black tape (30 mm wide) was stuck around the perimeter of each perspex sheet. *Trichogramma* are positively phototactic (attracted to light), and the black tape served to keep the wasps away from the edges of the perspex. One perspex sheet was covered with the black cardboard (400x310 mm), and AGM eggs were stuck on the lower surface of the other sheet. The unit therefore consisted of a dark end and a light end. A spare perspex sheet could be covered with eggs and placed in the box after 24 hours.

During operation, parasitoid pupae ('sting stock') were placed in the box to provide approximately one parasitoid per 5 AGM eggs. Emerging parasitoids were positively phototactic and moved to the light end of the unit where they encountered unparasitised AGM eggs. New (unparasitised) AGM eggs on a separate perspex sheet could be introduced to the box after 24 hours by replacing the sheet under the black cardboard with a new sheet containing unparasitised eggs, and transferring the black cardboard to the sheet containing the parasitised eggs. In this way the parasitoids could be moved from one half of the unit to the other by alternating the dark ends. Parasitoids moved from the dark end to the light end and encountered unparasitised eggs. This process could be repeated for a number of days if required, provided sufficient parasitoids were alive, or could be introduced to the unit.

The unit was operated in a constant temperature room (approximately 25°C, and 70% R.H.) with lighting supplied for 14 hours per day by standard overhead fluorescent lighting. Parasitised AGM eggs turn black after four days (unparasitised eggs are white or red, depending on their stage of development) and adult wasps emerge after another six days at 25°C. Parasitised hosts were removed from the egg sheets with a soft paint brush and weighed, and could be used in field release programs or as sting stock to increase parasitoid numbers. Parasitised host eggs weigh slightly less than unparasitised hosts (54,900 and 50,400 eggs per gram respectively). Weights can be used to determine parasitoid numbers as one parasitoid emerges per parasitised AGM egg. Samples of parasitised AGM eggs should be regularly taken and the emerging parasitoids sexed and counted to determine sex ratios.

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