VG131

Development of a large scale pilot production facility for the Lepidopteran egg parasitoid Trichogramma spp.

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Know-how for Horticulture™

VG131

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PROJECT No. VG 131

FINAL REPORT

PROJECT TITLE:

Development of a large scale pilot production facility for the Lepidopteran egg parasitioid *Trichogramma spp.*

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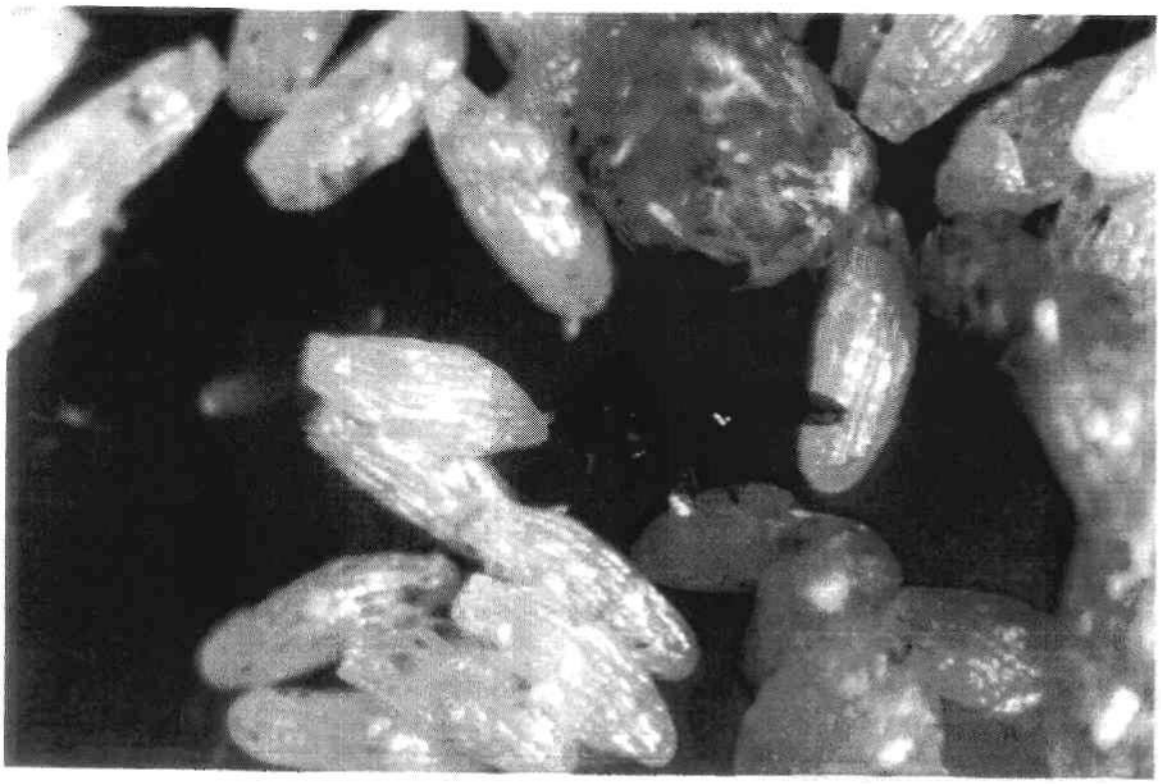

Trichogramma nr brassicae ovipositing in an egg of the Angoumius Grain Moth.

Final Report, Year 3 , Project No. VG 131:

Project Title: Development of a large scale pilot production facility for the Lepidopteran egg parasitoid *Trichogramma* spp.

1. Summary

(a) Industry summary:

Bio-Protection Pty Ltd. of Warwick, Qld, has established a pilot production insectary to mass rear *Trichogramma* spp. wasps for use in horticultural crops. The wasps are about .25 mm long and kill Lepidopteran pests by parasitizing their eggs. Two species, *T. nr. brassicae* and *T. funiculatum* are now being produced. A mass release trial of *T. nr. brassicae* in field tomatoes at Bundaberg, conducted by Croptech Research, achieved 34.3% parasitization with low level of Heliothis infestation. Croptech concluded that *Trichogramma* releases will be a "useful adjunct to a *Bacillus thuringiensis* control strategy." In a range finding trial conducted by Peter Bailey of the Department of Primary Industries South Australia, *T. funiculatum* showed commercial potential against Light Brown Apple Moth in a vineyard at Coonawarra.

(b) Technical summary:

A system was developed to produce large numbers (10 million per week) of *Trichogramma* spp. on host eggs of the Angoumius grain moth *Sitotroga cerealella* Oliver. Problems overcome in mass producing *S. cerealella* eggs have included low grain infestation of wheat by moth larvae, and infestation of the moth colony by the predatory mite, *Pyemotes vetricosis* Newport. The highest yield of *S. cerealella* eggs returned in production was 4.9 g per g of eggs used to set the grain. *T. nr. brassicae* production efficiency (parasites available for field release from host eggs exposed) over the period of February to June 1994 was 64.5%. A range finding trial with *T. nr. brassicae* against Heliothis in field tomatoes at Bundaberg gave parasitization of 62% in the release rows. A mass release trial of *T. nr. brassicae* in field tomatoes at Bundaberg achieved 34.3% parasitization with low level of Heliothis infestation. A range finding trial using *T. funiculatum* against Light Brown Apple Moth (on laboratory reared egg masses placed in the field) in grapes at Coonawarra resulted in parasitism rates of 80% or more in the release row.

2. Recommendations

(a) Extension/adoption by industry:

Bio-Protection Pty Ltd is a private company committed to the commercialization of biological control agents. As it has contributed to funding the research and development of the project all findings have been rapidly adopted to benefit the horticultural industry.

Increasing interest in *Trichogramma* has provided a positive impetus for commercialization. Ongoing trial work with the Queensland Department of Primary Industries, Institute for Horticultural Development of Victoria, Department of Primary Industries of South Australia and a number of crop consultants will provide valuable assistance in encouraging the use of *Trichogramma* by growers.

(b) Directions for continuing/future research:

The following issues require continuing research effort.

- The selection of the best strains of *Trichogramma* spp. to control various pest species.

- For selected strains, determine if continual rearing on factitious host eggs effects their performance in the field and if so select the most appropriate strategies for preventing loss of quality. Strategies could include. (a) Genetic manipulation of strains to prevent loss of fitness. (b) Revitalizing strains continually reared on factitious host eggs by rearing through the targeted host species at a predetermined frequency. (c) The establishment of a government agency with the responsiblility of maintaining the fitness of small colonies of selected strains of *Trichogramma* for use by commercial insectaries. This method ensures the availability of a quality product for the horticultural industry and has been used successfully in Germany. At the Institut fur Biolgischen Planzenschutz, Darmstadt, the fitness of small colonies of *Trichogramma* spp. are maintained by rearing them on target host eggs under the extremes of environmental conditons found in the field. Commercial insectaries obtain *Trichogramma* every year from the Institute's colonies to replace old colonies which have been continuously reared on factitious host eggs (Hassan, 1994).

- For the selected strains of *Trichogramma* spp. determine the effects on their quality of long term and short term cold storage.

• Development of IPM programs for various crops. This would include improving knowledge of timing and release rates for *Trichogramma* applications and determining the compatability of various pesticides for use with *Trichogramma* releases.

• Development and evaluation of efficient application methods

(c) Financial/commercial benefits:

Some of the *Trichogramma* spp. occurring in Australia should have the potential to become important biological agents for controlling *Heliocoverpa armigera*, and *H. puntigera* (Heliothis), *Epiphyas postivittana* (Light Brown Apple Moth) and other Lepidopteran pests. Commercial use of *Trichogramma* in the horticultural industry will lower the use of toxic chemical insecticides resulting in fewer pest chemical resistance problems, reduced residue problems in foods, and enhanced grower safety.

Trichogramma releases should slot easily into IPM strategies because of compatibility with naturally occurring beneficial species, biological control agents released by government and commercial insectaries, pheromone mating disruption systems, selective insect growth regulators, and *Bacillus thuringiensis* products.

3. Technical report

(a) Introduction:

Lepidoptera are major insect pests of many fruit and vegetable crops grown in Australia. The damage they cause often results in large financial losses to growers. Current control measures using widespread applications of broad spectrum insecticides have led to chemical resistance problems, secondary pest problems, application health risks, public concern about residues on food and insecticide legislative restrictions. To help circumvent these problems, *Trichogramma* spp. are mass reared in many countries as bio-insecticides and released in IPM programs against a range of Lepidopteran pests (countries include:- Russia, France, Switzerland, Germany, China, India, Canada, Mexico, and the United States). There are a number of *Trichogramma* spp. found in Australia and it is probable that some of these may prove very useful as biocontol agents in IPM programs here.

Limited research on *Trichogramma* spp. has been done in Australia. In Western Australia, imported *T. pretiosum* Riley was mass reared and released against Heliothis in Ord River cotton (Michael and Woods, 1980). High levels of Heliothis egg parasitism were recorded after releases but native species active in the control site were also effective bio-control agents. *T. nr. ivelae* Pang and Chen 1981 (renamed *T. nr. brassicae* Bezenko , by Dr. Mary Carver 1993) was released in tomatoes against Heliothis in a field trial at Burnley Victoria (McLaren and Rye, 1981). Damage to fruit by Heliothis was assessed at ca. 2.5% in the release plot and 13% in the control. Since 1985 the Queensland Department of Primary Industries has been evaluating native species of Trichogramma for use against Heliothis in cotton. To date its field trials with *T. caverae* and *Trichogrammatoidea bactrae* have been inconclusive because insufficient numbers of *Trichogramma* were available at times of large Heliothis egg laying (Scholz,1993). In 1990, *Trichogramma* spp. were not commercially available to Australian horticulturalists.

To produce *Trichogramma* spp. for field trial evaluation, and to make rapidly available the commercialization of this work for the benefit of horticultural producers; the techniques for large scale rearing of *Trichogramma* spp. are developed at a commercial pilot production facility established at Bio-Protection Pty Ltd. Because large numbers of *Trichogramma* are required in releases a cheap, dependable and efficient production process is required. Morrison (1985a) developed an effective method for rearing eggs of *Sitotroga cerealella* Olivier as production hosts for *Trichogramma* spp. This method has been modified at Bio-Protection after encountering problems with production. Furthermore, emphasis has been placed on providing a healthy work environment within the moth rearing facility. This has been accomplished by the installation of an air filtration plant to remove allergenic moth scale which is produced in large quantities when moths are reared en masse.

Morrison (1985b) devised a very efficient parasitization chamber for mass production of *T. pretiosum* in the eggs of *S. cerealella*. However, attempts to use this device were unsuccessful, because the species of *Trichogramma* reared were highly attracted to areas of high light intensity resulting in poor parisitization of eggs. To overcome this problem a parisitization chamber was constructed with a light source behind the host eggs.

A manual application system was used to distribute *Trichogramma* spp. in field trials. However, an efficient mechanized delivery system will be required if *Trichogramma* is to be

widely used in the horticultural industry. Bouse and Morrison (1985) developed an aerial release technique for Trichogramma which could be used in large scale agricultural applications. Gross et al (1981) devised an efficient *Trichogramma* spreader which was mounted on a tractor. This type of application device should be suitable for horticultural use. In fields of cotton and soybean it evenly distributed parasitized *H. zea* eggs throughout the crop by passing them at a set rate into an air draft from a centrifigal fan. Damage levels to parasitized eggs by the delivery process were considered slight. Work on the refinement of a similar style *Trichogramma* spreader has started. This equipment will have the advantage of low cost and ready availability of replacement parts.

With the aid of collaborating researchers the following field trials were conducted. *T nr. brassicae* Bezdenko was released against Heliothis in tomatoes, and *T. funiculatum* Carver against Light Brown Apple Moth in grapes.

(b) Materials and Methods.

Mass rearing Trichogramma,

Rearing of host eggs

Organic hard wheat with a protein content >12% was used as the rearing medium for *S. cerealella* larvae. Cribs (see Plate 1) holding ca. 6.5 kg of wheat were made from wooden frames (size 100 cm by 50 cm by 2 cm) and covered with expanded metal mesh screen (Expamet Galvabond No.213). The 2 cm thickness of the cribs should not be exceeded as this helps regulate the temperature for larval development (Morrison, 1985a). To prevent cribs from expanding when loaded a wooden divider was included in crib manufacture. All wheat and production equipment was heat sterilized to kill insect pests. Strict hygiene is maintained in the insectary to prevent the establishment of *Pyemotes ventricosis* which is a pest mite capable of rapidly destroying a *S. cerealella* colony. This includes daily vacuum cleaning of the insectary floors and the capture and destruction of any escaped moths. Regular checks are carried out for the early detection of *P. ventricosis*.

Grain moisture content is a major factor influencing infestation by *S. cerealella* larvae and should be ca. 15% (Morrison, 1985a; Laing and Eden 1990). Initially grain was sterilized within the cribs at ca. 66°C for 24 h and then thoroughly soaked by hose to return moisture (Morrison, 1985a). This method of returning moisture to the grain proved inadequate and resulted in poor infestation of 30-40%. Soaking the cribs in a water bath for periods up to 1.5

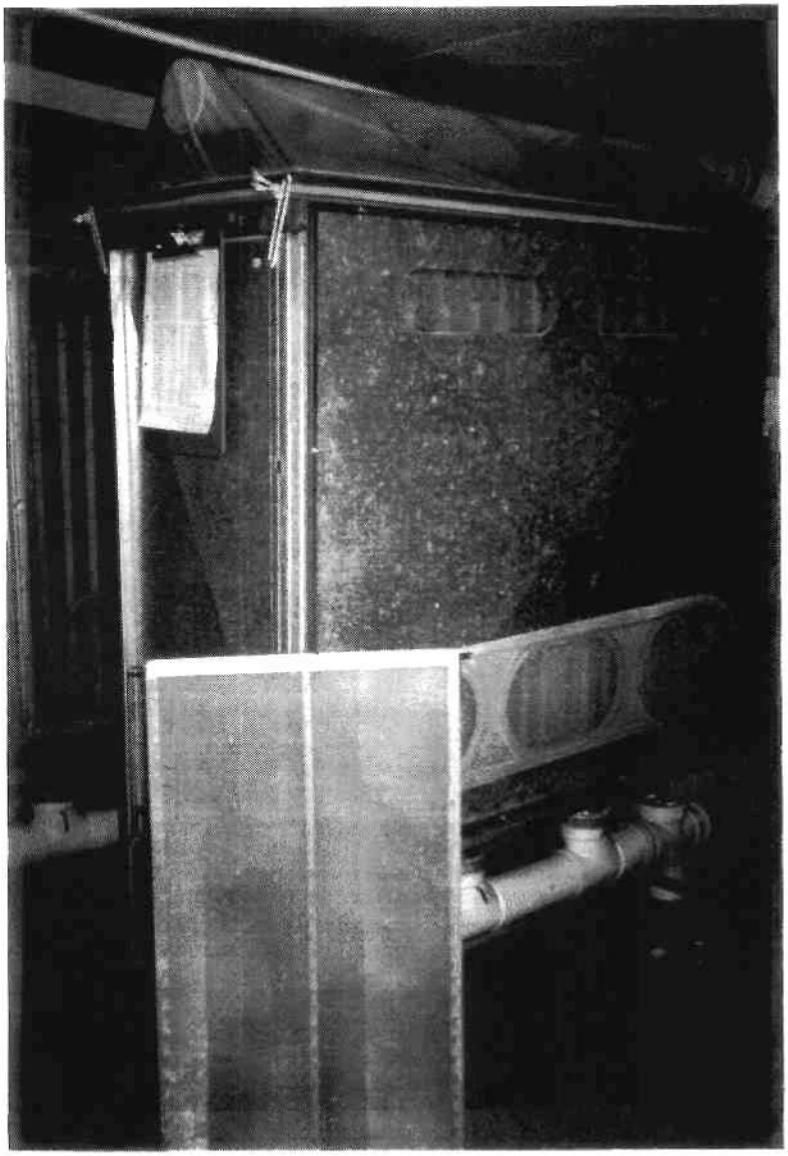

Plate 1. Shows a wheat holding crib (foreground) and moth collection cage with cribs inside. Two lugs visible at the top of the crib hang on parallel rails fixed inside of the top of the trolley.

h was tested but did not greatly improve infestation and resulted in severe fungal growth on the wheat. Grain infestation has been improved to 60-70% using the following grain treatment. Eighteen lots of ca. 6.5kg of wheat are placed in separate 20 litre drums and each is thoroughly mixed with 600 ml of water. The drums are sealed with air tight tops and allowed to stand for 24 h. Twice during this time the lids of the drums are briefly removed and the wheat is stirred. The drums are then placed in a sterilizing oven at 70°C for 18 h. After sterilizing, the moisture content of the wheat is ca. 15%, and it is loaded directly into the cribs taking care to stop grains from tightly packing down.

Next, each grain-filled crib is infested with 1 g of *S. cerealla* eggs (ca. 50, 000 eggs) per 1 kg of wheat (ca.28 000 kernels) as follows. Eggs for crib setting are stored at 10-15°C for up to 3 days after collection. Eggs are allowed to mature for 48 hours before they are used to set the cribs. This ensures the maximum emergence of larvae whilst the grain has high moisture content. The *S. cerealella* eggs are treated in a 10% formalin bath for 5 minutes to destroy any *P. ventricosus* that may be present (Morrison,1985a). The decontaminated eggs are rinsed for 10 minutes in running water to remove the formalin. Each gram of eggs is diluted with 50 ml of water and placed in a large measuring cylinder. The eggs are kept uniformly dispersed in the solution by means of a small electric stirrer. The bottom of the cylinder has a tap which is connected via a plastic tube of 12mm diameter to a 'T' wand made of electrical conduit. The bar of the wand is slightly narrower than a crib and has holes 1.5 mm diameter every 1 cm along its length. By gravity feed, 325 ml of egg solution per crib is quickly dispensed from the meauring cylinder through the wand giving a very uniform coverage of the wheat.

The infested cribs are stored horizontally in racks with 4 cm intervals between the cribs. The temperature and humidity in the rearing areas is maintained between 23-27°C and 70-80% respectively. The racks are isolated from the floor by oil baths which act as a barrier to mites. At day 15 the cribs are cooled by strong fans to ensure the temperature does not rise above 32°C. This is to remove excessive metabolic heat which can cause aestivation and sterility of moths (Morrison, 1985a). The crib temperatures are monitored by an electronic thermometer inserted in the middle of one crib of wheat.

At day 20 the cribs are enclosed in collecting cages (see Plate 1) which are isolated from pest insects by oil baths. The collecting cages hold 18 cribs hanging vertically with 3-5 cm spacings

between cribs. The top of the collecting cage has a mesh top and cribs are cooled by a flow of air drawn out through a canopy at the top of the unit. The canopy is connected by a duct to a large axial fan. This air is then passed through a filtration plant capable of removing sub micron size particles of moth scale before it is passed back into the rearing room. Initially the cribs were cooled by a ducted flow of air drawn from the bottom of the moth collection cages, but this was found to be inefficient because it opposed the principle of convection.

Moths start emerging around day 22 and are collected daily until day 40 when the trolly is heat sterilized for 24 h, cleaned and made ready for the next moth batch. Moths fall from the cribs into funnel collectors which are connected to the air filtration unit by a 100 mm duct. During collection the moths are blown along the duct where they collect in a metal receptacle.

After collection the moths are weighed and transferred to oviposition cages which are inserted into an egg collecting machine (see Plate 2). The oviposition cages were made from stainless steel wire mesh (9strands/cm, 28SWG, 0.9 mmm aperature) rolled into the shape of a cylinder (50 cm long 40 cm diam) and fastened by staples. The ends of the cylinders were made of building form ply 12 mm thickness. The mesh was slotted into a groove routed around the perimiter of the ends and was bolted to the cylinder by 4 threaded rods running through the cages. About 450 g of moths are inserted through a 4 cm hole provided at one end of the cylinder which is sealed by a rubber plug. A 14 cm square hole in the other end of the cylinder is used to empty out dead moths and is closed by a metal plate fastened by wing nuts. Each oviposition cylinder fits onto two parallel steel shafts one of which is attached to a drive motor the second shaft being a slave. The motor rotates the cylinders at ca. 4 revolutions/minute. The moths lay their eggs through the wire mesh and under each others wings and these are shaken loose when the cylinders are tumbled. A metal tray is fitted 10 cm below each oviposition cage to collect the moth eggs. The oviposition drums are rotated for a period of 9 min every 3 hours. Moth scale dust accumulates and clogs the surface of the drums and is removed by a vacuum system which is switched on when the drums are rotating. The vacuum apparatus consists of a brush and a narrow slotted cleaning head which rides at the top of and along the length of each cylinder. Further scale is removed by a second vacuum system which provides a constant draft across the egg collection trays. The moth egg collection unit is enclosed in a cabinet to isolate moth scale from working areas. Eggs are collected daily from the moths for a period of 3 days. After collection moth eggs are seived through two screens one 9 strands/cm and another 33 strands/cm. The coarse seive removes large bits of moth debris and the fine

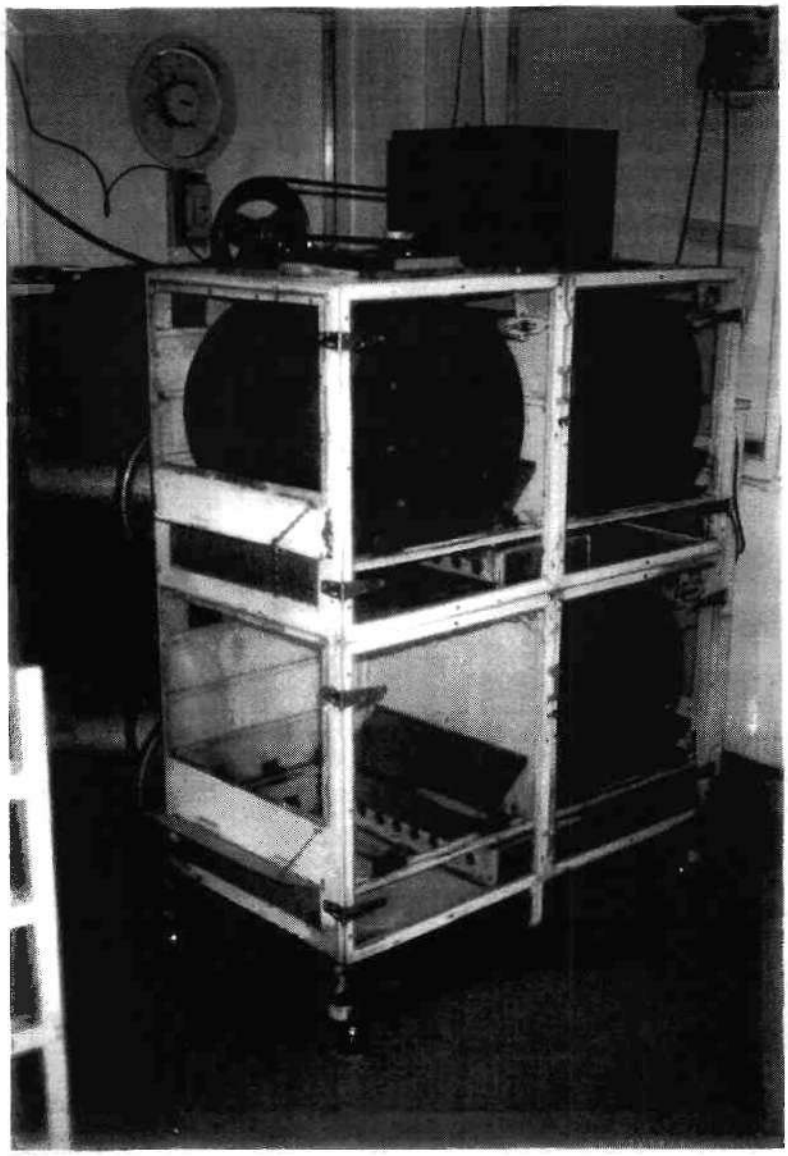

Plate 2. Egg collection machine showing essential construction details. Note the egg collection drums, the vacuum cleaning heads at the top of the drums and the egg collection trays.

screen contains the moth eggs and separates out *Blattisocius keegani* Fox eggs (Morrison, *pers. comm.*, 1991). *B. keegani* is a minor pest mite of *S. cerealella*. Fine moth dust adhering to the eggs after seiving is removed by pouring them through a low velocity air draft. The eggs are then used for rearing *Trichogramma* or for starting new batches of moths.

Rearing Trichogramma

All parasitoid rearing is done in controlled environment rooms 23-27°C and 60-80% RH. Field collected *Trichogramma* spp. were reared in glass vented bottles until there were sufficient numbers to supply the sting stock for a parasitization unit. Fresh *S. cereallella* eggs were supplied to newly emerged *Trichogramma* wasps in the jars by gluing them onto strips of stiff paper with pesticide free glue. The strips of eggs were labeled with the parasitization date so that the stage of parasitoid development could later be determined. Parasitized *S. cereallela* eggs turn from white to red in 24 hrs and black after 4 days. The parasitoids reared under the constant conditions described start to emerge ca. 9 days after parasitization.

Initial trials using a Morrison (1985b) type chamber gave poor parasitization percentages and seemed to be related to the parasitoids failure to parasitize eggs in lower light intensity areas of the chamber. A parasitization unit (see Plate 3) was then constructed (32.5cm wide, 94 cm long and 13 cm deep) with a glass floor in one half of the base, opaque in the other. Diffused light of intensity of ca. 900 lux at the glass floor of the chamber is supplied by two 20 watt flourescent tubes placed under the chamber. Two hinged doors with rubber strips make an insect proof seal at the ends of the unit for easy access. A small shelf in the dark end of the parasitization chamber holds sting stock.

Twenty grams of freshly collected *S. cerealella* eggs are attached to a clear sheet of acrylic plastic (30cm wide by 45 cm long) by pouring them over its top surface previously misted with a fine water spray. The eggs on the acrylic sheet are then slid into the light end of the chamber. Newly emerged sting stock attracted by the light fly to the light end of the chamber and parasitize the eggs for a period of 24 h. Fresh eggs are added daily to the light end of the chamber and at the same time the acrylic sheet with the previous days parasitized eggs is pushed to the dark end of the unit. After a couple of hours the positively phototrophic parasitoids are attracted back to the light end of the chamber and the acrylic sheet with the previous days parasitized eggs can be removed without losing many wasps. The parasitized eggs are gently brushed off the acrylic sheet and placed in a plastic container marked with the parasitizaton date.

Plate 3. Trichogramma parasitization chamber. The door at the dark end of the chamber is open revealing the shelf holding sting stock and the acrylic sheet upon which *S. cerealella* eggs are attached for parasitization.

One fith of the eggs is removed volumetrically and glued onto dated paper sheets and reserved as sting stock. Sting stock is placed in the chamber one day before it is ready to emerge; it remains in the chamber for a period of five days after insertion to ensure that all parasitoids have hatched. A ratio of 1 parasitoid to 5 host eggs is maintained in the chamber by the daily addition of sting stock to the unit at the time the previous days eggs are removed.

Production quality control has involved daily monitoring of % parasitism, and weekly monitoring of adult emergence, sex ratio of emerged adults, and % runting. All tests are carried out at 25°C and 75% R.H. The percentage parasitization of eggs is checked on the sixth day after the eggs are parasitized by counting the number of black eggs in a random sample of 150 eggs. Percentage emergence, sex ratio and percent runting is done weekly on a sample of 500 parasitized eggs

Field trials

Delivery system

A silk screen was used to print 1 cm^2 squares of insecticide free glue onto sheets of paper. *Trichogramma* parasitized *S. cerealella* eggs were rolled over the sheets of paper while the glue was wet. About 500 parasitized eggs stuck to each glued square of the paper sheets and these were guillotined into small cards. Distribution of *Trichogramma* in field trials was done manually by lodging or stapling the cards of parasitized eggs in the foliage of the crop.

T. nr. brassicae against Heliothois in field tomatoes at Bundaberg

A range finding trial was used to determine the parasitization of Heliothis and dispersal distances of *T. nr brassicae* Bezendko in tomatoes. John Hall of Croptech Research supervised the field work. *T. nr brasicae* were released along two rows of tomatoes in a block of 9 row bays 180 m long. A thousand parasitoids were released every 4 m of row. Parasitism samples were taken from rows at 4, 8, and 16 m from the release rows. Sampling was conducted at days 2, 8, 10, and16 after release. At each sampling 20 Heliothis eggs were collected from 4 points randomly selected in each row. Each egg was placed in a separate unit stored at 25°C and 60% RH and examined for wasp or grub emergence. Unviable eggs which produced neither grubs nor wasps were excluded from the analysis.

Croptech Research also did a mass release trial of *T. nr. brassicae* against Heliothis in tomatoes to determine parasitizaton percentages for two release rates. A large block of tomatoes with 2 m

row spacing was divided into 12 plots size 11 rows by 65 m long. Each of the following three treatments were replicated in 4 plots.

1. Release rate 375 000/ha. A release card of 500 parasitiods was placed at every stake, in every second row forming a 4 m by 4 m grid.
2. Release rate 250 000/ha. A release card of 500 parasitiods was placed at every stake, in every third row forming a 4 m by 6 m grid.
3. Control no parasitoids released.

Wasps were released on the 8th and 14th of October. Parasitism samples were collected on the 12th and 19th of October. A minimum of 20 Heliothis eggs per plot were collected labled and incubated to assess parasitism.

T. funiculatum against Epiphyas postvittana in vineyards

A range finding trial was used to determine parasitization of Light Brown Apple moth (Epiphyas postivittana) and dispersal distances of *T. funiculatum* in a vineyard at Coonawarra. Peter Bailey of the S.A. Dept. of Primary Industries supervised the field work. The *T. funiculatum* used in the trial were field collected at Coonawarra (identified by Dr M. Carver, CSIRO) and reared at Bio-Protection for 23 generations. The vineyard used in the trial had 78 rows of shiraz vines aligned roughly north south and at right angles to the prevailing westerly wind. Releases were made along the length (ca. 500m) of rows 30 and 60. Because Light brown Apple Moth (LBAM) numbers were unusually low strips of laboratory reared moth eggs were used to measure parasitization in the field. Strips of freshly-laid LBAM eggs were stapled to pine trellis posts in every third row along a single transect at right angles to the line of rows.

Parasitoids were released on the 15, 20, 21 October to correspond with the spring period of LBAM egg laying in the field and also on the 21 December to correspond with the summer generation. The release rate for the October releases was 25 000 parasitoids per 100m of row and December 8400 parasitoids per 100m of row.

(c) Results and discussion

Mass rearing Trichogramma

Rearing of host eggs

Effective mass rearing of *Trichogramma* spp. hinges upon the reliable production of large numbers of suitable host eggs. This has been largely achieved. Current egg production is ca.

720 g a week from 36 cribs set a week. However, there have been a number of difficulties and rearing techniques are still being refined. In March 1993, the predatory mite *P. ventricosis* was detected in the moth colony. The presence of this mite drastically reduced host egg production for several months. The mite probably infested the colony from a batch of grain improperly heat sterilised. After the mite was discovered a centrifugal ceiling fan was installed in the heat sterilising unit. This modification ensures a more uniform temperature throughout the sterilising chamber. For a period of several months after mites were detected, all cribs of wheat were treated with a miticide (Dicofol 15ml/L) at day 12 post egg infestation and every 4 days afterwards until the cribs were enclosed for moth collection. A 40 day cycle from crib set to termination is now used to minimize the risk of an explosion in *P. ventricosis* numbers.

Marked drops in egg production have occurred when the optimum rearing conditions for the moths were not maintained due to problems with environmental equipment. If humidity drops too low first instar larvae may be unable to penetrate the grain and older instar larvae can be moisture stressed resulting in increases in larval development time. If the temperature gets too high larvae aestivate resulting in erratic moth emergence and reduced fecundity. If humidity drops below 70% moth egg laying is reduced (Morrison, 1985a). All these factors have at various times had a negative impact on production but improvements in environmental equipment and controllers have now largely overcome these problems.

However, at best 4.9 g of *S. cerealella* eggs have been produced for every g used to infest grain. Although this result is better than 3.0g returned/g set reported by Scholz (1992), it is poor compared to a return of 10.9g reported by Morrison (1985) and 6.5 g by Laing and Eden (1990). Although improvements have been made in the percentage of larval infestation of grain it is still short of 80% achieved by Morrison (1985). A trial with soft rather than hard wheat gave no improvement in infestation of grain as did a trial supplying supplementary amino acids to larvae. Further trials are planned to see if improvements may be made by different grain preparation techniques.

Poor moth fecundity could be a contributing factor in the low number of grams of eggs returned per gram set. Low moth fecundity could result from hereditary or environmental causes. As humidity and temperature in the rearing facility are now close to the optimal conditions reported in the literature some investigations have been made to determine if egg yields could be increased by reducing stress on the moths during the collection process of moth eggs. Peak

egg laying was found to be moth density dependent with maximum output in the tumblers occurring with ca. 450 g of moths per drum.

Rearing of Trichogramma

The insectary has been producing 10 million *Trichogramma* per week and as future market trends become clearer scaling up to a large scale commercial production unit can take place.

Rearing *Trichogramma* on factitious host eggs for many generations can result in reduction in the parasitoids ability to search for and parasitize target host eggs. Furthermore, losses in fitness may eventually occur through inbreeding and by adverse selection pressures resulting from parasitization chamber design. In Switzerland the loss of fitness of *T. brassicae* , caused by rearing on factitious host eggs, is overcome by releasing and recapturing parasitoids in a greenhouse. Target host eggs are attached to corn plants to simulate field conditions (Bigler,1984).

The *T. nr brassicae* Bezendko (identified by Dr M. Carver, CSIRO) mass reared at Bio-Protection was field captured in sorghum near Bundaberg in April, 1993. After being reared for 23 generations on *S. cerealella* eggs the colony was regenerated from recaptured field release material. The recaptured parasitoids were identified as the same species by Dr. M. Carver. Although this field release and recapture was accomplished successfully it is unlikely that this could be done on a regular basis because of the high possibility of contamination of the original culture with native species. Better strategies for maintaining fitness need to be developed . Possible alternatives are (a) Genetic manipulation of strains to prevent loss of fitness. (b) Revitalizing strains continually reared on factitious host eggs by rearing through the targeted host species at a predetermined frequency. (c) The establishment of a government agency with the responsibility of maintaining the fitness of small colonies of selected strains of *Trichogramma* for use by commercial insectaries. This method ensures the availability of a quality product for the horticultural industry and has been used successfully in Germany.

For *T. nr. brassicae* over the period of February to June 1994 the means of daily percentage parasitization samples, weekly percecentage emergence, sex ratio and runting samples from production quality control records were respectivley, 80.6%, 92%, 3.4 females to 1 male, and 3.7%.

Field trials

Delivery system.

Because the locomotion range of the parasitoids is about 10 m, even distribution of *Trichogramma* in the field proved very laborious using the card system. Furthermore, eggs on the cards were prone to predation by ants and other insects because they were unprotected and highly concentrated. Nevertheless, in Switzerland and Germany *T. brassicae* are released commercially against the European Corn Borer using a similar system. There the cards are hooked manually on to plants using a pre-punched hole in the card. A much better French system, although still labour intensive, uses a small paper wax coated capsule. The capsules are tossed onto the ground or placed in foliage. *Trichogramma* are inserted into the capsules by a patented technique. The capsules have very small exit holes for the parasitoids and have the advantage of providing shelter from rain and predators. However, *Trichogramma* die in the capsules if they should fall on unshaded ground in hot weather, which would be a major problem in Australia's hot climate.

At Bio-Protection a modified seeder is being developed for application of *Trichogramma* into the field (see Plate 4). A small centrifugal fan at the bottom of the seeder is used to blow parasitized eggs onto pre-wetted foliage. *S. cerealella* eggs have a natural adhesive which is activated by water. Because the eggs are distributed randomly over the wetted folialage it is likely that predation of eggs will be low compared to the card system. Furthermore, by adding small quantities of sugar or molasses to the water spray it may be possible to provide the emerged parasitoids with a food source. Longevity and fecundity of *Trichogramma* in the field can be enhanced by providing a suitable food source (Hohmann *et al*, 1988). Initial trials of placement and delivery rates of *Trichogramma* using this equipment have been very promising, and trials in field tomatoes at Bundaberg are in progress.

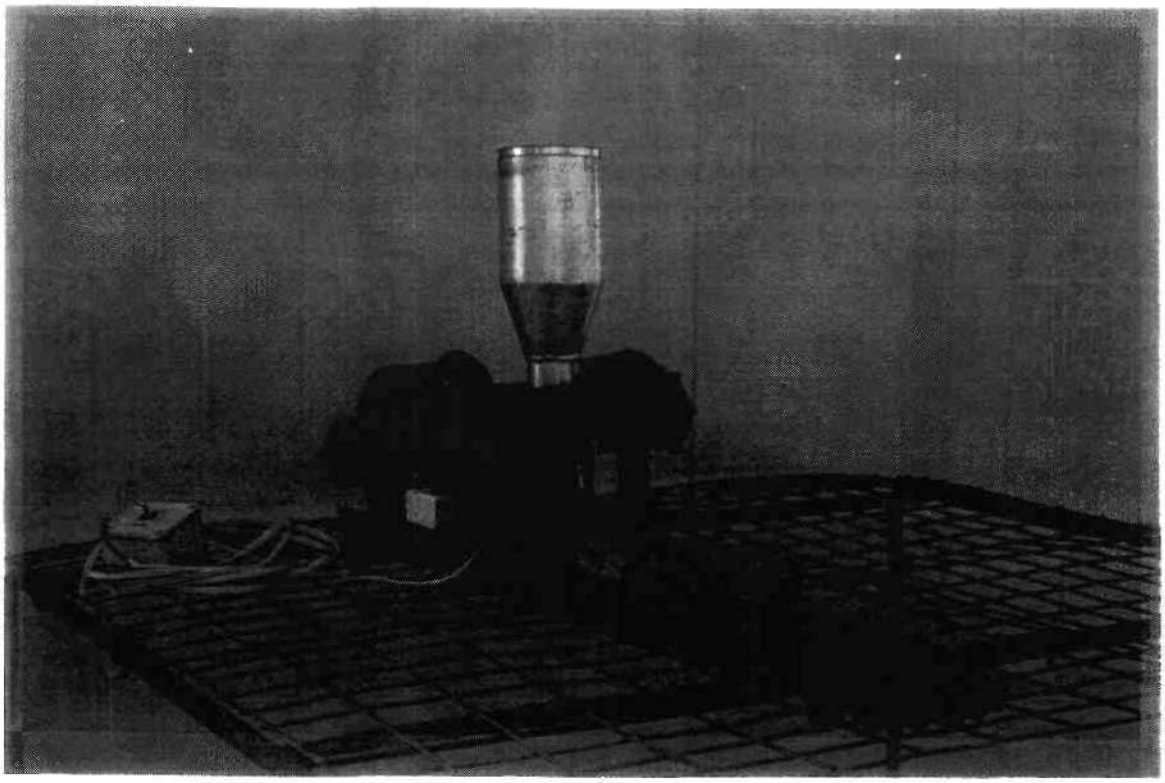

Plate 4. Prototype of *Trichogramma* field delivery machine. The essential parts of the unit are visible. At the top is a hopper which holds parasitized *S. cerealella* eggs. Beneath the hopper is a small seed drill. At the back of the unit is a centrifugal electric fan which is attached to the front manifold. Six plastic hoses attach to the manifold. Parasitized eggs are blown along the hoses and are ejected in close proximity to pre-wetted foliage.

T. nr. brassicae in field tomatoes at Bundaberg

The range finding trial (see Fig. 1) shows that percentage parasitism falls rapidly with distance from the release rows. Unfortunately, there are some gaps in the data due time constraints on field personnel. Because there was only slight % of parasitism at rows 16 m from the release rows background parasitism from native *Trichogramma* is slight or non existant. The large drop in parasitism at rows 4m from the release rows indicates that mass releases on a 4 m grid should provide the necessary overlap to give high parasitism percentages. The recorded increase in parasitism from day 10 to 16 in the release row is most probably due to the emergence of the first generation from the released parasitoids.

Fig. 2. shows the % parasitism for the mass release trial. This % for both release rates is lower than the release rows in the range finding trial and may be partly explained by low levels of Heiothis egg laying at the time of the trial. Predation of eggs on the release cards may have also contributed to the low parasitization recorded although this was not checked. In addition, some parasitized Heliothis eggs may have been taken by predators because sampling for parasitized eggs was done at days 4 and 5 after the release. The small size of the samples taken for parasitization checks would have magnified this problem. Note the parasitism rate is climbing as parasitoids from the first generation in the field emerge and supplement the second release. From the trial results John Hall concluded that *Trichogramma* should be a useful complement to *Bacillus thuringiensis* contol strategy·

T. funiculatum against Epiphyas postvittana in vineyards

In the spring release period parasitism rates approaching 90% were recorded in some rows (see Fig. 3). From the two release rows No. 30 and 60 the wasps have dispersed downwind over most rows and there appeared to be little upwind dispersal. In the summer release parasitoid activity was mainly confined to the vicinity of the two release rows (see Fig. 4); the high level of parasitism in row 61 and none in row 60 is probably a labelling error. The greater dispersal of wasps in the spring release compared to the summer release is probably related to the higher release rate and perhaps windier conditions in spring.

During the trial period LBAM egg strips reared in the laboratory were placed in a non release vineyard at Coonawarra. No parasitism was recorded for the period 29/10 to 2/11 in 45 strips placed throughout the vinyard, while for the period 21/12 to 23/12 parasitized eggs were found on 1 of 45 egg strips.

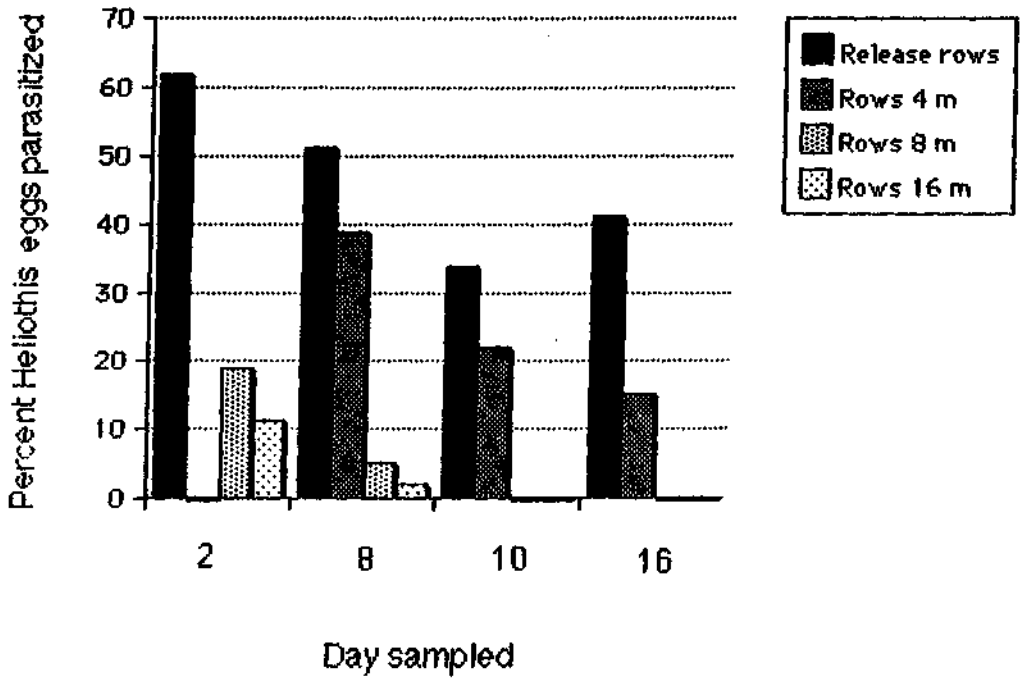

Figure 1. Range finding trial . Parasitism of Heliothis eggs by *T. nr. brassicae* in field tomatoes at Bundaberg

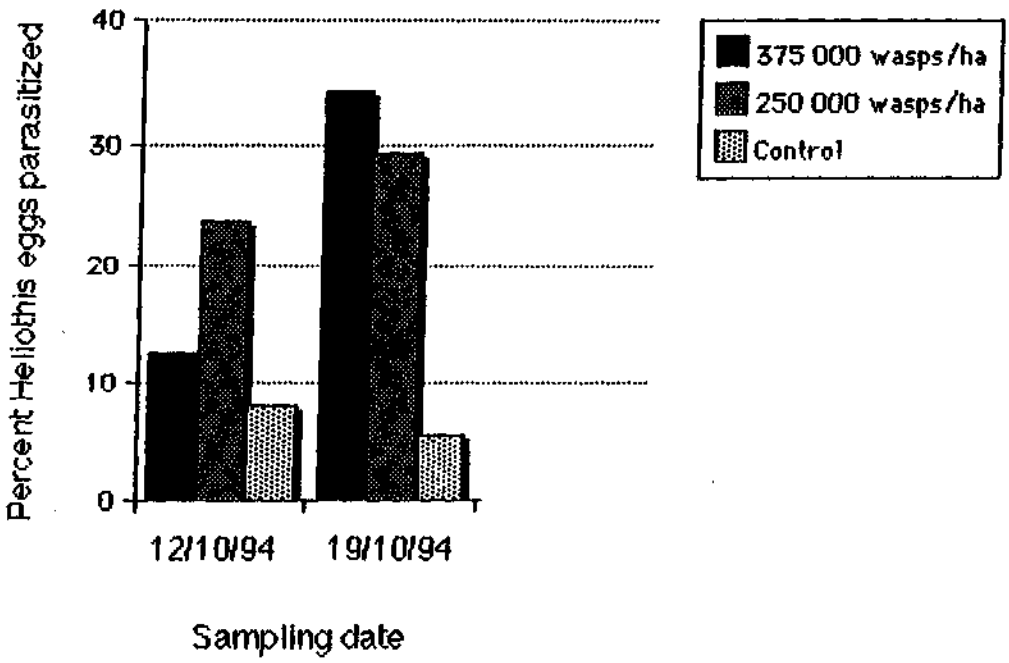

Figure 2. Mass release trial. Parasitism of Heliothis eggs by *T. nr. brassicae* in field tomatoes at Bundaberg.

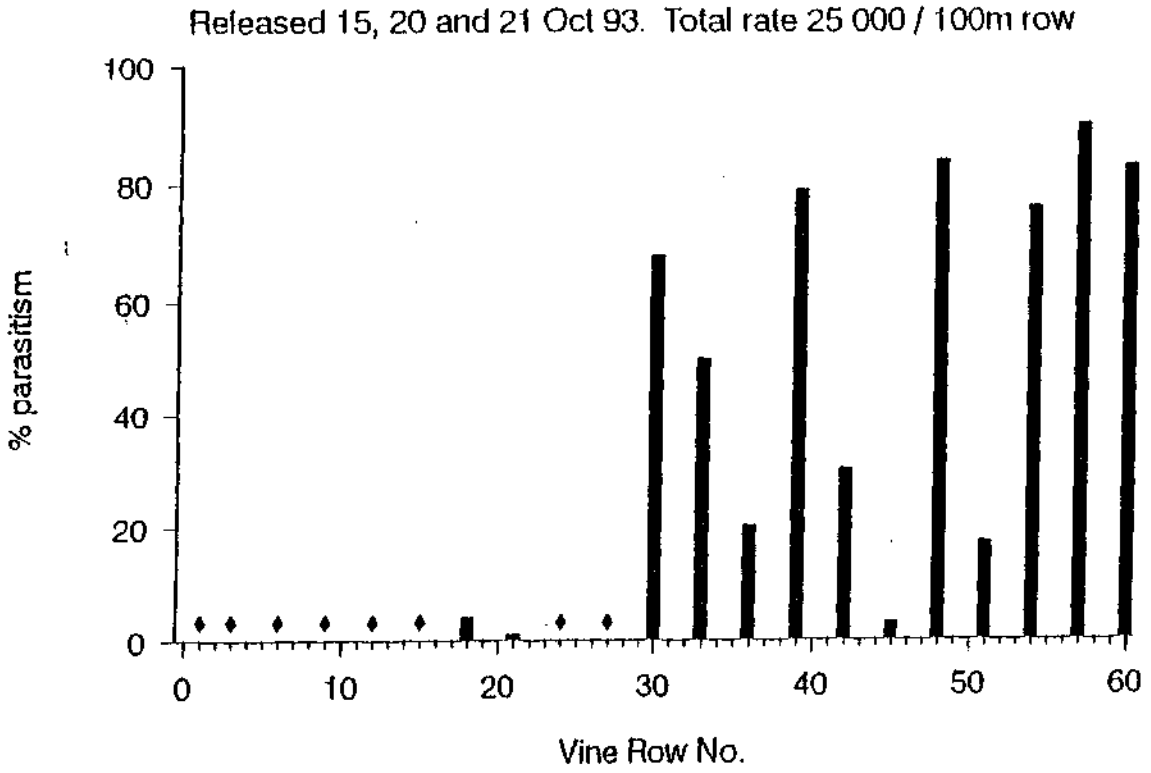

Figure 3. Range finding trial with *T. funiculatum* in grapes at Coonawarra. Parasitism of test LBAM eggs placed every third row in the period 1-4 November 1993. Diamond symbols indicate rows where no parasitism was detected. Wind blows from direction of row 1.

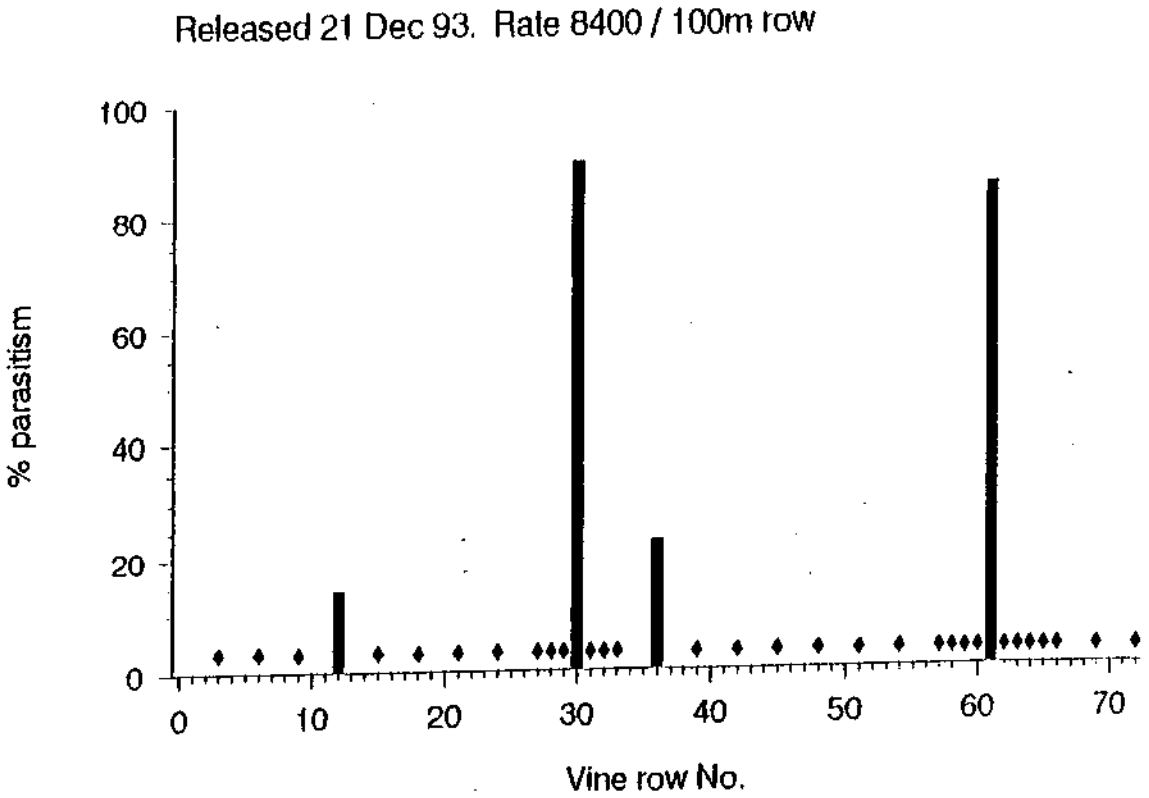

Figure 4. Range finding trial with *T. funiculatum* in grapes at Coonawarra. Parasitism of test LBAM eggs placed every third row in the period 23 December 1993 - 4 January 1994. Diamond symbols indicate rows where no parasitism was detected.

It is noteworthy, in another trial involving 3 separate releases of *T. funiculatum* against LBAM at Coonawarra ants removed all the eggs from the release cards before parasitoid emergence.

Peter Bailey concluded from this preliminary work that *T. funiculatum* showed commercial potential, although release systems which excluded predation need to be developed and reduction of LBAM numbers resulting from egg parasitism must be demonstrated.

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