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The role of insect cell lines in an artificial diet for the parasitoid wasp, *Trichogramma pretiosum*

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Abstract

Trichogramma species are mass-produced for biological control using host eggs. Artificial diets have been developed to reduce production costs, however, most include insect haemolymph as a major component, which still results in a significant expense. Medium conditioned with insect cell lines has produced some success as a haemolymph replacement in artificial diets for several parasitoid wasp species. *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae) was the first species to develop successfully to the adult stage on diets containing concentrated *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) cells. *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) was subsequently grown to the adult stage on a similar cell line diet. This success encouraged a systematic investigation into the use of insect cell lines in *Trichogramma* artificial diets. We compared the effect of diets containing insect cells with diets containing conditioned cell line media. Diets containing insect cells produced significantly more pupae than diets containing conditioned medium and, although not significant, produced a higher number of adults. Second, we compared the effect of diets containing cell lines established from ovary-associated tissue of *H. zea* and embryo tissue of *Aedes albopictus* (Skuse) (Diptera: Culicidae) on *T. pretiosum* development. *Trichogramma pretiosum* development was not significantly different on diets containing cells from the two origins and tissue types. Third, the effect of cell storage on *T. pretiosum* development was observed. *Heliothis zea* cells in medium were stored at 4 °C and room temperature (22 °C) for one, two, four and seven days before addition to artificial diets. Cell viability was calculated for these storage treatments. *Heliothis zea* cells could be stored at 4 °C for up to seven days with no detrimental effect on *T. pretiosum* development. *Trichogramma pretiosum* development did not depend on cell viability. The use of insect cell lines as a haemolymph replacement has the potential to significantly reduce production costs and simplify *Trichogramma* artificial diets with the eventual aim of replacing host production in mass rearing facilities.

Keywords: Artificial diet; Hymenoptera; Insect cell line; In vitro production; Parasitoid; *Trichogramma pretiosum*; Trichogrammatidae

1. Introduction

Due to its excellent growth-promoting factors, insect haemolymph is often a major ingredient in *Trichogramma* artificial diets. The remaining components are usually crude products such as milk, egg yolk, yeast, and salt solution (Consoli and Parra, 1996a,b; Grenier, 1994; Notarte and Merritt, 2001; Strand and Vinson, 1985). However, the production cost of insect haemolymph can be prohibitive (Grenier et al., 1995). Although the importance of specific growth-promoting factors in vertebrate cell lines has been known for some time, it was not until recently that invertebrate cell line culture became of interest (Ferkovich and Oberlander, 1991). Insect cell lines and conditioned cell line media have been investigated as replacements for haemolymph in insect artificial diets (Ferkovich et al., 1991, 1994, 1999; Notarte and Merritt, 2001).

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The perceived importance of particulate solids in diets for trichogrammatid parasitoids (Jarjees et al., 1998; Wu et al., 2000) led Notarte and Merritt (2001) to investigate the efficacy of cells from perpetual insect cell lines as a haemolymph substitute in an artificial diet for the egg parasitoid, *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae). Insect cells established from ovary-associated tissue of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) were used as the main component of a diet for *T. australicum* along with milk, yeast, and Grace's insect medium (Notarte and Merritt, 2001). The experiments produced growth to adulthood, making *T. australicum* the first parasitoid species to be successfully grown on medium containing cultured insect cells as the major ingredient. In that study, no systematic attempt was made to assess the relative importance of the cell lines and other ingredients in the diet. To do so, a series of experiments were carried out by Heslin et al. (submitted) to show that the more common parasitoid, *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) could successfully develop on Notarte and Merritt's (2001) insect cell line diet and in a bid to further refine the diet, individual ingredients were systematically removed, showing that insect cells and chicken egg yolk were essential for development while Grace's insect medium had a detrimental effect.

Media conditioned with cell line have shown limited success for rearing other parasitoid species. Several parasitoids have been grown to the pupal stage on cell line media conditioned with various insect cells. The insect cells were grown in media for several days to allow growth factors to be produced and the conditioned supernatant then used in artificial diets, allowing the beneficial growth factors to become available to the parasitoids (Ferkovich and Oberlander, 1991). Conditioned cell line media have been used to grow the egg parasitoid *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) (Hu et al., 1999), the larval parasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) (Ferkovich et al., 1991, 1994), and the ectoparasitoid, *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) (Ferkovich et al., 1999) to the pupal stage, but no adults were produced.

Insect cell lines can be established from cells originating from many species and tissue types (Ferkovich and Oberlander, 1991). Hu et al. (1999) investigated the effects of medium conditioned with cell lines from different origins on growth of *E. puttleri*. Tests of cell lines derived from 11 insect species and three tissue types revealed significantly different responses to the various conditioned media that were incorporated into artificial diets. They surmised that growth factors produced by embryonic cell lines are similar to growth factors supplied by the natural host egg, providing the most effective growth for *E. puttleri*.

As the use of insect cell lines in *Trichogramma* artificial diets is very recent, little is known about the effects of cell origin on *Trichogramma* development. Consequently, the current study investigates the development of *T. pretiosum* in artificial diets containing cells established from two sources; ovaries of *H. zea* and embryo cells from the mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae).

Few experiments have been conducted to determine whether the presence of insect cells in the diet results in more wasps developing than in conditioned media diets. Experiments carried out by Hu et al. (1999), produced variable results. Some cell line conditioned media performed better when cells were present, while in others, performance decreased. Consequently, we have investigated whether cells per se were necessary for *Trichogramma* development by growing *T. pretiosum* on diets containing either a high density of *H. zea* cells or conditioned cell line medium without insect cells.

Correct storage of insect cell lines prior to use is also important. Hu et al. (1999) found storage of diets containing cell line conditioned media at 4 °C for seven and 14 days resulted in reduced growth of *E. puttleri*. Diets containing conditioned media alone gave better growth than media containing cells, leading to the proposal that the insect cells may produce toxins or that the dead cells were toxic to the parasitoid. To determine the effect of cell line storage on the development of *T. pretiosum*, *H. zea* cells stored at different temperatures for one, two, four, and seven days were assessed as diet ingredients.

2. Materials and methods

2.1. Insects

Trichogramma pretiosum were obtained from the commercial facility, Bugs for Bugs (Mundubbera, Qld, Australia) where they were maintained on *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs. In the laboratory, stock cultures of *T. pretiosum* were reared on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) eggs at 25 °C ± 1 °C, 50% ± 10% relative humidity (RH), and 12L:12D. Newly emerged *T. pretiosum* adults were used in all tests. *Helicoverpa armigera* pupae were supplied weekly by the Department of Primary Industries and Fisheries, Toowoomba and Indooroopilly (Queensland, Australia).

Trichogramma pretiosum eggs used in the experiment were obtained from superparasitised *H. armigera* eggs. *Helicoverpa armigera* eggs less than 24 h old were collected on nappy (diaper) liners (Johnson and Johnson). Eggs were placed in a vial with adult wasps for 5–6 h under laboratory conditions (25 ± 1 °C, 50 ± 10% RH, and 12L:12D). After parasitisation, *H. armigera* eggs were surface sterilised with 2% sodium hypochlorite