In vitro rearing of *Edovum puttleri*, an egg parasitoid of the Colorado potato beetle – development from egg through the pupal stage

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Abstract. A variety of semi-defined artificial diets were developed and tested for their ability to support the *in vitro* development of *Edovum puttleri*. In the most effective diet, 2.6% of *E. puttleri* pupated. This diet contained high levels of hen egg yolk combined with *Manduca sexta* larval hemolymph, or with a mixture of *M. sexta* egg homogenate and larval hemolymph. Egg homogenate alone (without the addition of hemolymph) was not capable of supporting the parasitoid’s development. Thus, hemolymph appears to contain unidentified factor(s) important for inducing pupation of the wasp. Addition of *M. sexta* pupal fat body tissue extract (in place of hemolymph) also promoted pupation of *E. puttleri*. Gypsy moth (*Lymantria dispar*) larval hemolymph could not replace *M. sexta* larval hemolymph. Fractionation irreversibly reduced the growth-promoting effects of *M. sexta* larval hemolymph. However, the most effective fraction contained components whose molecular weights were ≥1000 kDa. In diets that were devoid of insect materials, the best results were achieved when hen egg yolk, FreAmine, yeast extract, lactalbumin, trehalose, fetal bovine serum and bovine milk were included. This is the first report of an artificial diet for *in vitro* rearing an eulophid parasitoid from the egg through the pupal stage.

Key words: artificial diet, Coleoptera, Chrysomelidae, Eulophidae, Hymenoptera, metamorphosis, molting

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say (Col.: Chrysomelidae), is an economically important pest of potato, tomato, and eggplant crops in North America and Europe (Schalk and Stoner, 1979; Hare, 1980; Puttler and Long, 1983). Control of CPB costs growers hundreds of millions of dollars every year (Schroder and Athanas, 1989). In addition, use of conventional chemical pesticides for controlling CPB has had a negative impact on the ecosystem. Population density of minor insect pests has
increased due to suppression of natural enemies. Spraying with chemicals has induced resistance to pesticides, caused contamination of ground water, and had a deleterious effect on animal life (Schroder R.F., Insect Biocontrol Lab, ARS, USDA, Beltsville, MD, USA, 1997, personal communication). Increased use of biological control agents, as part of an Integrated Pest Management (IPM) program, would alleviate some of the problems associated with a pesticide-based insect control program (Greany et al., 1984).

_E. puttleri_ Grissel (Hym., Eulophidae) has been used as a biological agent for controlling CPB on eggplant (Williams, 1987). The parasitoid completes its life cycle in one half the time required by the CPB, and the ratio of females to male parasitoids is 2:1. Therefore, _E. puttleri_ can produce two generations for every generation of CPB. These characteristics tend to ensure that the parasitoid will establish itself relatively quickly in the field during the growing season (Schröder, personal communication). After mass release of _E. puttleri_ in New Jersey eggplant fields, the population of CPB decreased below the economic damage threshold (46.8% of the beetle eggs in the field were parasitized and 73.9% of the beetle eggs in egg masses were destroyed (Williams, 1987)), and no additional applications of chemical pesticides were required (Palmer D., New Jersey Department of Agriculture, West Trenton, NJ, USA, 1996, personal communication). However, the cost of mass rearing this parasitoid on CPB eggs is high (approximately USD 900/acre, Palmer, personal communication). Development of an _in vitro_ rearing system for _E. puttleri_ could provide a new cost-effective technology to produce large numbers of parasitoids for mass release.

To date, _in vitro_ rearing of egg parasitoids has been achieved for 15 trichogrammatid species, one tetrastichid, one eupelmid, one scelionid, and one encyrtid species (Grenier et al., 1994). In contrast, there have been no reports concerning the _in vitro_ rearing of an eulophid parasitoid. Here we report for the first time the development of an artificial diet capable of supporting the growth of _E. puttleri_ from the egg through the pupal stage.

**Materials and methods**

_Insect culture_

Both _E. puttleri_ and _L. decemlineata_ were originally provided by the New Jersey Department of Agriculture (NJDA). CPB were reared on potato plants in a growth chamber at 24 ± 1 °C, r.h. 70%, L:D regimen of 16:8, light intensity 600 lux. The method of rearing _E. puttleri_ was derived from Palmer (1996). The parasitoid was reared on its natural host, CPB eggs, and maintained in 4-liter jars at 25 ± 1 °C, r.h. 55%, L:D regimen of 14:10, light intensity 1750