IN VITRO REARING OF *TOXONEURON NIGRICEPS*, A LARVAL ENDOPARASITOID OF *HELIOTHIS VIRESCENS* – DEVELOPMENT FROM EARLY SECOND INSTAR TO THIRD INSTAR

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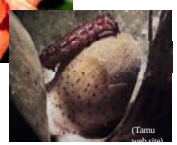
ABSTRACT

The early second instar (8 days after parasitization) larvae of *Toxoneuron nigriceps* were incubated in the artificial rearing media supplemented with unparasitiized and parasitized hemolymph of the host, *Heliothis virescens*. The growth (increase in length and width), development (molting), and survival of the incubated larvae were observed. The incubated larvae demonstrated growth in all the nine types of media tested including the control medium devoid of host hemolymph. However, the semisolid form of the rearing media supplemented with the hemolymph from the late fifth instar parasitized host larvae (day 5, 7, and 9) promoted better growth and development than the liquid form. Some of the in vitro reared third instar *T. nigriceps* larvae demonstrated behavioral changes that could be expressed as the preparation for cocoon formation or pupation. However, neither cocoon nor pupation occurred.

The Host – *Heliothis virescens*



Economically important pest; lays 300-500 eggs per female

5-7 larval instars, 18 – 31 days of developmental time

WHY THIS STUDY?

- Potential biological control agent for tobacco budworm (80% parasitism-Lewis et al., 1972)
- A good model system to study:
  - – the immature development and feeding behavior of the parasitoid
  - –the physiological interaction between the host and the endoparasitoid

OBJECTIVES

- To evaluate the effects of host hemolymph (host factors or parasitoid directed host factors) in an artificial rearing medium (ARM).
  - –To develop a suitable artificial basic medium (ABM)
  - –To evaluate the growth of *T. nigriceps* larvae in the ARM with and without host hemolymph
  - –To determine the suitability of a semisolid and a liquid rearing medium

The Parasitoid – *Toxoneuron nigriceps*

Stings all stages of the host larvae; however, prefers third and fourth instars.
Egg hatches between 32-48 hours.

Larva feeds on host hemolymph; remains as first instar until the host larva enters the fifth instar.
Three larval instars; third instar egresses out from the host.

More like a predator at the final stage

METHODOLOGY

•Parasitization

4th instar at the head capsule slippage stage (Webb & Dahlman 1985)

•Hemolymph Extraction (UP1,P1,UP3,P3,UP5, P5, P7 and P9)

Day 1, 3, and 5 of the unparasitized (UP1, UP3 and UP5) and day 1, 3, 5, 7 and 9 of the parasitized (P1, P3, P5, P7 and P9) fifth instar *H. virescens* larvae.

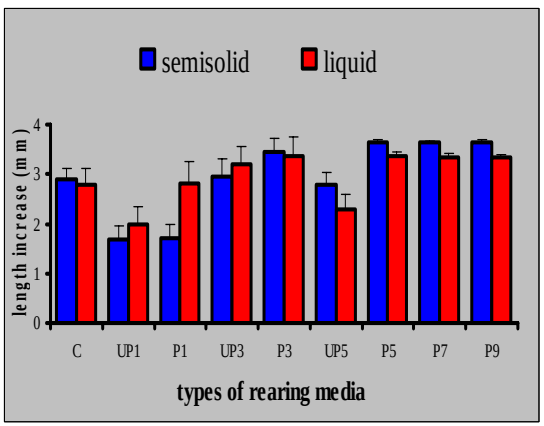
•Preparation of the Artificial Basic Medium (ABM) (Modification of Hu & Vinson,1997)

- Prepared the liquid (L) and semisolid (SS) artificial rearing media (ARM) with the addition of host hemolymph and agar; control (C) was ARM without the hemolymph.
- Incubated the early second instar (8 days after parasitization) *T. nigriceps* larvae in the artificial rearing media.
- Observed the increase in size (length X width), molting (Lewis & Vinson 1968), and survival of the incubated larvae for 10 days.

RESULTS

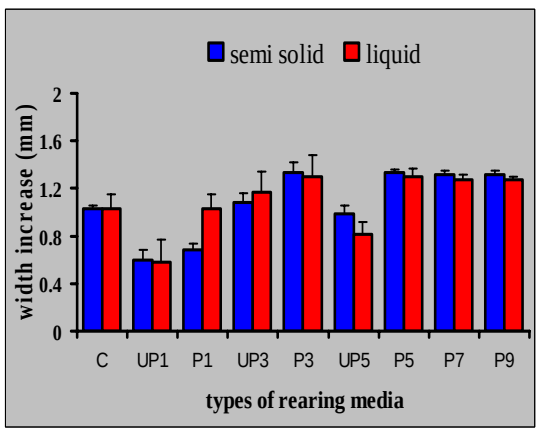
Length increase

Length before-3.5 mm

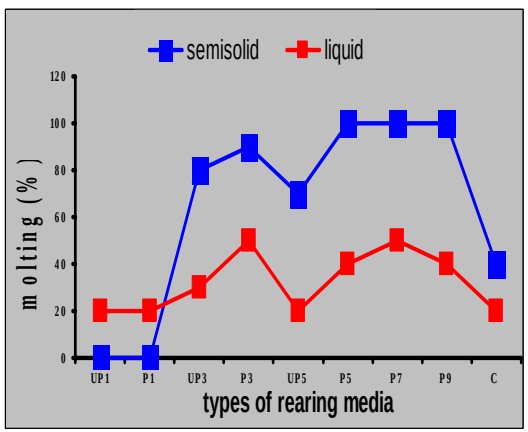


Width increase

Width before – 0.86mm



Molting



Larval Size

The average size of the *in vitro* reared third instar larvae was smaller (7mm long and 2.2 mm wide) than their counterparts *in vivo* (9 mm long and 2.6 mm wide). In addition, the color of the third instar larvae was transparent and fragile, whereas in vivo, they were opaque and sturdy.

Survival

None of the incubated larvae survived the observation period (10d). None of the newly molted third instar larvae were alive for more than three days after molting.

CONCLUSIONS

The early second instar *T. nigriceps* larvae demonstrated growth in all media including the control medium devoid of host hemolymph**.** However, molting was much higher in the semisolid media, especially those with the hemolymph from the late fifth instar (day 5, 7, and 9) parasitized host. Pouring of the media to one side of the well in the shape of a crescent moon and keeping the culture plate slanting upwards improved the growth of the larvae by giving them the freedom to move in and out of the media and thereby reducing the chance of drowning. Some of the in vitro reared third instars showed signs of cocoon formation (evidenced by an oral secretion) and expressed behavioral changes such as larvae rolling over, and often shrinking, and expanding the body that suggested the initiation of pupation. However, neither cocoon formation nor pupation occurred.

DISCUSSION

Consumption of agar – beneficial or detrimental?

Molting was higher in the semisolid than in the liquid form of the rearing media. It is not clear how digestible agar is or its nutritional value. However, if undigested, it would be of no nutritional value and it could influence molting if molting is due to volume increase in the gut. In either case the agar could have become a detriment and an evaluation of other thickening agents may be fruitful.

REMAINING CHALLENGES

Rearing early eggs through first instars

Developing a diet for the tissue predator phase