MORPHOLOGY AND FUNCTIONS OF CALYX FLUID FILAMENTS
IN THE REPRODUCTIVE TRACTS OF ENDOPARASITOID,
MICROPLITIS MEDIATOR [HYM. : BRACONIDAE]

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Very long virus-like filaments were found in calyx region of the reproductive
tracts of the braconid parasitoid, Microplitis mediator Haliday, which attacks the
Noctuid, Pseudaletia (= Leucania) separata Walker. These filaments are nuclear in
origin; Feulgen and methyl-green pyronin reactions revealed cytochemically the
presence of DNA. Filaments are attached to the surface of the chorion of an egg until
hatching and function to suppress the encapsulation reaction of the host. Further-
more, it was observed that serosal cells, 18-h and 24-h from oviposition, released
substances possibly related to the inhibition of encapsulation.

KEY-WORDS: Microplitis mediator; calyx fluid; endoparasitoid; common army-
worm; Pseudaletia separata.

In recent years, particles from calyx region of the reproductive tract have been observed
in endoparasitoids and found to be virus-like particle (reviewed by Stoltz & Vinson, 1979).
The morphology of the virus-like particles in the calyx fluid of Braconidae and Ichneumoni-
dae has been carefully examined and found to be essentially particle-like or rod-like shape.
So far, long virus-like particles have been found only in the part of calyx fluid in the 3 spe-
cies, Microplitis croceipes (Cresson), Apanteles congregatus (Say) (Stoltz et al., 1976), and
Apanteles hyphantriae Riley (Stoltz & Vinson, 1979). Interestingly, the long particles of
Apanteles congregatus are 1 constituent of the calyx fluid and are not present in the calyx of
newly eclosed females (Stoltz & Vinson, 1979). None of these virus-like particles except
those of Venturia canescens Gravenhorst (Rotheram, 1973) is attached to the surface of the
egg; they are capable of inhibiting encapsulation of parasitoid eggs (Norton & Vinson,
1977; Vinson, 1977; Stoltz & Vinson, 1979; Edson et al., 1980). The oviposited egg in the
host hemocoel is not coated by virus-like particles. Microplitis mediator, however, was
found to have pure calyx fluid containing long virus-like filaments. The present investigation
was undertaken to determine whether this long filaments possessed the ability to inhibit the
defense reaction of the host. In addition, the possible influence of serosal cells (teratocytes)
associated with the developing parasitoid in protecting the parasitoid larvae from encapsula-
tion were investigated.
MATERIALS AND METHODS

Larvae of the Noctuid host, *Pseudaletia (= Leucania) separata* Walker, were reared in plastic cups on corn leaves at 25°C and under a 16-h photoperiod. Adult, *Microplitis mediator* Haliday, were kept in a glass tube containing drops of honey solution. One 3rd instar host on a damaged leaf was exposed to 1 ♀ wasp. At the time of oviposition, the parasitized larvae were quickly removed to avoid superparasitization. The injection of eggs or larvae was carried out using a glass micropipette inserted into the proleg of the 6th abdominal segment. Prior to this, the host larvae were narcotized with CO₂ to prevent loss of hemolymph. The time of encapsulation was determined by examining host larvae dissected in physiological saline (PS: 9.993 g NaCl, 0.3 g KCl, 0.2 g MgSO₄ · 7H₂O, 0.2 g CaCl₂ · 2H₂O, 0.003 M phosphate buffer pH 7.0 in 1 liter H₂O), observed with a binocular or inverted microscope. Even when hemocytes layer around the injected substance was thin, encapsulation reaction were recorded as positive.

HISTOLOGICAL STUDIES

The presence of DNA was determined by the Feulgen reaction and methyl green-pyronin staining on 5 μm sections of ovaries fixed in Carnoy’s solution (alcohol : chloroform : acetic acid = 6 : 3 : 1) and embedded in paraffin. The methyl-green pyronin dyes were purified by chloroform and used to stain some slides in combination with the digestion by RNase.

For electron microscopy of female reproductive tracts, the 18 h eggs and 24 h eggs following oviposition were dissected into a primary fixative consisting of 2.5 % glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 2 h. They were then postfixed in cacodylate-buffered 1 % osmium tetroxide for 2 h at 4°C. The fixed materials were dehydrated in a graded series of ethanols, and embedded in Spurr’s medium for observation by transmission electron microscopy or dried with a critical point dryer and coated with gold for scanning electron microscopy. Measurements of virus-like filaments were made from enlargements of electron micrographs.

EFFECT OF CALYX FLUID ON HOST-DEFENSE REACTION

After being removed from the female wasps, the ovaries were rinsed in PS, and placed in a concavity slideglass. Firstly, the venom glands and reservoir were removed with forceps to avoid contamination of the calyx fluid. The other parts of ovary were cut out with 2 tungsten needles and then the calyx fluid was collected with micropipette. Care was taken to insure that no tissues or cells would be taken up with fluid. The fluid was then cooled for preservation. The eggs in the lateral oviducts were collected with a micropipette and washed a number of times with PS. A egg or the 1st instar of a parasitoid from a parasitized host was injected, with and without calyx fluid, into the 4th instar of an unparasitized host to examine the function of this fluid.

RESULTS

DETERMINATION OF ENCAPSULATION-TIME FOR LIVING MATERIALS

Living material has been found to require a long time for encapsulation than lifeless material (Lynn & Vinson, 1977). At 27°C, cactus thorn-encapsulation occurred completely within 8 h, but that of *Cardiochiles nigriceps* eggs (living material) in *Heliothis zea* showed