PROGRESS TOWARDS DEVELOPMENT OF AN ARTIFICIAL DIET AND AN *IN VITRO* REARING SYSTEM FOR *MICROPLITIS CROCEIPES*[1]

P. D. Greany, S. M. Ferkovich, and W. R. Clark

Insect Attractants, Behavior, & Basic Biology Research Laboratory, ARS, USDA, Gainesville, Florida 32604

ABSTRACT

Current knowledge of the artificial culture of *Microplitis croceipes* (Cresson) is summarized; unique aspects of the physiological requirements of endoparasites relevant to *in vitro* culture are identified; remaining needs are stated, and anticipated potential benefits from this technology are outlined.

INTRODUCTION

One of the most promising candidates for biological control of *Heliothis* spp. is the larval endoparasite *Microplitis croceipes* (Cresson) (Hopper and King 1984, Powell and King 1984, Stadelbacher et al. 1984, King et al. 1986). In addition to effecting high levels of parasitism, it has shown great tolerance to several of the pesticides commonly employed for management of *Heliothis* spp. in cotton (King et al. 1986).

The greatest constraint to increased use of *M. croceipes* for biological control of *Heliothis* spp. probably is the expense of mass production caused by the high cost of rearing *Heliothis* spp. larvae. Knipling and Stadelbacher (1983) optimistically estimated that it might be possible to produce *M. croceipes* for $25.00 per 1,000. Even with improved rearing methods (Powell and Hartley 1987), a more realistic figure might be $250.00 per 1,000 (J. Powell, personal communication).

It is possible that the cost of production might be significantly reduced if an artificial rearing system can be developed. Moreover, we believe that many other worthwhile findings on the biology and physiology of this important species will be compiled in the course of determining this parasite's rearing requirements. Some of these findings should enable ancillary technological advances in the use of this and related species of parasites.

With these incentives, we have been conducting research to discover the requirements of *M. croceipes* eggs and larvae *in vitro*. To date, no hymenopterous larval endoparasite has been successfully cultured from egg to adult *in vitro*, although numerous egg and pupal parasites have been successfully reared artificially (Greany 1986, Greany et al. 1984, Thompson 1986, Strand et al. 1988). Thompson (1983, 1986) suggested that larval endoparasites probably will be more difficult to culture *in vitro* than parasites with other habits because of their intimate integration with the physiology of their hosts. This reflects the fact that larval endoparasites must allow their hosts to continue to feed and develop, in contrast to egg and pupal parasites, whose hosts usually do not undergo transitions such as molting during parasite development, and which may support parasite development even when dead.

1/Hymenoptera: Braconidae

We are considering these complexities in our efforts to rear M. croceipes in vitro. Fortunately, the host relationships of M. croceipes are being studied by several other investigators as well (many of whom also are contributors to this supplement), and this should facilitate our progress. We are taking an eclectic approach by considering the specialized requirements of larval endoparasites, along with findings on the requirements of other parasites. Larval endoparasites have many unique attributes that must be considered in developing an in vitro culture system. Several of these host-parasite interactions and adaptations have recently been shown to involve viruses that are injected by the female wasps upon oviposition. The roles of these viruses, termed "polydnaviruses", were reviewed by Stoltz (1986). In addition to abrogation of host cellular immunity, the viruses have been shown to affect host hemolymph protein profiles, both quantitatively and qualitatively, and ecdysteroid and trehalose titers. These adaptations are discussed below.

A key issue in the host-parasite relationship is that of endocrine interactions (Mellini 1983, Beckage 1985, Lawrence 1988). Some species, termed "conformers" (Lawrence 1986a), synchronize their molts with hormonal changes induced by their host's hormonal patterns. Lawrence (1986a) suggests that this may be nutritionally beneficial for regulator species. Perhaps the best studied example of a regulator is Cotesia congregata (Mason), which elevates the juvenile hormone (JH) titer of Manduca sexta (L.) larvae to its own presumed advantage (Beckage and Templeton 1986 and references therein). Unfortunately, nothing is known about the effects of M. croceipes on the JH titer of their hosts.

Heliothis spp. larvae parasitized by M. croceipes are developmentally arrested after making the initial transition to the wandering stage and exhibit lowered ecdysteroid levels (Webb and Dahlman 1985, 1986), but the significance of this finding has not yet been established in terms of the nutritional physiology of the M. croceipes larvae. It has recently been found that polydnaviruses injected by Campoletis sonorensis (Cameron) infect cells of the host's prothoracic glands, with a concomitant reduction in the host's ecdysteroid titers (Dover et al. 1987). A similar mechanism might account for the observed reductions in the ecdysteroid titers of Heliothis spp. larvae parasitized by M. croceipes (Webb and Dahlman 1986).

Another issue to consider in simulating the host environment is the induction of unique host hemolymph proteins by polydnaviruses. Beckage and Templeton (1986) showed that M. sexta larvae parasitized by C. congregata display unique hemolymph proteins, at least one of which is induced by viruses injected by the parasite females. As yet, no one has elucidated the physiological significance of parasite-induced proteins; one possibility is that they may play a nutritional role but this has not yet been shown. Insofar as M. croceipes is concerned, however, Ferkovich and Dillard (1986) did not find any newly-induced proteins in Heliothis zea larvae parasitized by M. croceipes. This was based upon use of one-dimensional SDS polyacrylamide gels; higher resolution 2-D gels might reveal new polypeptides.

Another finding of interest is that Heliothis spp. larvae parasitized by M. croceipes exhibit an elevated trehalose level (Dahlman and Vinson 1975), which again was ascribed to viruses injected during oviposition (Dahlman and Vinson 1977). This hyperglycemic condition should be considered when mimicking the natural nutritional milieu.

An additional aspect of the host-parasite relationship that merits further investigation is the role of teratocytes in the nutrition of M. croceipes larvae. These specialized cells proved of importance to the successful in vitro growth of the egg parasite Telenomus heliothidis Ashmead (Strand et al. 1987). Earlier, Strand et al. (1985, 1986) found that these cells produce proteolytic enzymes important in breaking down host tissues. The presence of teratocytes of M. croceipes was important for growth of 1st

instar larvae in vitro (Greany, unpublished), and this role clearly deserves additional study toward optimizing the culture medium.

Much additional basic research likely will be needed before M. croceipes can be successfully reared in vitro. Presently, we are characterizing the critical nutritional and physiological requirements of M. croceipes eggs and larvae, and we have made significant progress toward this goal, as described below. This work has revealed many gaps in our knowledge of the physiology and physiological interactions of M. croceipes and Heliothis spp. that should be investigated in vivo as well as in vitro. Simultaneous efforts are being made to develop a surrogate host that is acceptable for oviposition by M. croceipes females. The status of our work and future prospects are outlined below.

PROGRESS TO DATE

Prior work on in vitro culture of M. croceipes was described in detail by Greany (1986). It was then possible to rear this species from germband stage eggs to fully-grown 1st instar larvae in completely artificial (but undefined) media. Growth rates were slow compared to those for larvae reared in vivo, and while many larvae attained fully-grown 1st instar size and form, none molted spontaneously. Up to 75% of pregermband M. croceipes eggs developed and hatched when cocultured with fat body from H. zea larvae. Heat-treated hemolymph plasma from a non-permissive host, Manduca sexta (L.), also fostered development of newly-laid M. croceipes eggs.

Our current efforts are being directed toward isolating and biochemically characterizing the egg development stimulating polypeptide (EDSP) from M. sexta and H. zea hemolymph. This agent promotes the development of pregermband M. croceipes eggs beyond the critical point of germband formation. We plan to investigate whether it is the protein itself or a factor that is transported by the protein which ultimately accounts for pregermband egg stimulation. The latter is suggested because Ferkovich and Dillard (1986) saw no evidence for uptake of radiolabeled host proteins by M. croceipes eggs cultured in vitro or in vivo, although they did observe rapid uptake of radiolabeled free amino acids. By way of example, JH normally is transported in hemolymph by a specific carrier protein which helps sequester it from degradation by general esterases (Goodman and Chang 1984). If the active agent is indeed a hormone, it could be provided in vitro without the need to use the natural carrier protein. In any case, it will be essential to provide the growth factor in some form to stimulate early egg development, without which the eggs are unable to develop from the pre- to postgermband stage.

As mentioned above, we also found that the presence of teratocytes was important for normal growth of the 1st instar M. croceipes larvae in vitro (Greany, unpublished). In the absence of these specialized extraembryonic cells, which enlarge dramatically in vitro just as in vivo, parasite larval growth occurs slowly and the larvae are deformed. By investigating the biochemical alterations in teratocyte-conditioned medium, it may be possible to improve upon the basal medium employed by initially formulating the medium so as to duplicate conditioned medium. Because Strand et al. (1987) documented protease production by teratocytes, special consideration will be given to the production by M. croceipes teratocytes of proteases that may degrade tissues and/or hemolymph proteins.

It also will be important to stimulate molting by fully-grown 1st instar M. croceipes larvae, which remain motile but developmentally-arrested for at least two months. Lawrence (1986b) found that 1st instar larvae of the endoparasite Biosteres longicaudatus Ashmead molted in vitro only after providing a pulse of 20-OH ecdysone. In our preliminary tests, a few M. croceipes larvae were induced to molt to the second instar through administration of a 24 hr pulse of 10^{-8} M 20-OH ecdysone (Greany,

unpublished). However, providing the hormone continuously did not elicit molting. Not only was the 1st instar head capsule shed, but the larvae then resumed growth. As mentioned above, H. virescens larvae parasitized by M. croceipes display reduced ecdysteroid titers and fail to pupate normally after wandering (Webb and Dahlman 1986). Whether host hormones influence M. croceipes development still is unknown, but in vitro results suggest that there may be some hormonal interactions or specific requirements for molting of the parasites.

Development of a surrogate host is progressing well. Tilden and Ferkovich (1987) discovered that M. croceipes females will readily oviposit into agar substrates impregnated with a low molecular weight factor present in host hemolymph. Additional studies to identify the active material are underway (Heath et al. 1988). This is a great asset since we can potentially use this agent to induce oviposition into surrogate hosts that also provide the nutrients and other factors required for parasite egg and larval development. Fortuituously, host movement is not required to release oviposition behavior since the M. croceipes females readily oviposited into nonmotile surrogate hosts.

FUTURE DIRECTIONS

Much additional work is needed to characterize the optimal physical aspects of a surrogate host. One requirement will be to facilitate parasite respiration without allowing excessive water loss, but the medium still must be protected from bacteria and fungi. Antibiotics that are selectively active against pathogenic microorganisms likely will be needed as well.

Once the problems cited above are overcome, much work will be required to increase the rearing system to allow for mass production. It will then be desirable to perform rigorous quality control tests on host-reared vs. artificially-reared parasites to ensure efficacy in the field, and to make genetic improvements as required to maintain competitive abilities of the parasites in the natural environment.

Development of in vitro culture techniques will foster many new and exciting possibilities. For example, in vitro technology might be coupled with gene transfer techniques to allow for the introduction of genes for increased pesticide resistance into M. croceipes eggs. For this purpose, preblastoderm eggs could be injected with a plasmid of a suitable DNA construct. Once it is possible to rear the parasites entirely in vitro, they could be left for development in the artificial culture medium; meanwhile, injected eggs could be reintroduced into immunosuppressed hosts for continued development. Transformants then could be used as founders of a pesticide-resistant strain. Genetic transformation through DNA injection already is being used routinely for Drosophila melanogaster (Meigan) eggs (Rubin and Spradling 1982, Spradling and Rubin 1982, Scavarda and Hartl 1984, Brennan et al. 1984) as well as for plant protoplasts and animal cells (Crossway et al. 1986).

Another potential application of this technology is in vitro fertilization of parasite eggs, which could permit production of only female progeny. This might also allow otherwise reproductively isolated species each possessing desirable traits to be "mated" artificially (Greany et al. 1984).

Finally, by using in vitro techniques, which permit control of the composition of the culture medium, it also may be possible to settle the long-standing question of the influence of preimaginal conditioning of the subsequent oviposition behavior of parasite females (Thorpe and Jones 1937, Thorpe 1939). This has been the subject of controversy for many years, but it has not been possible to determine whether female parasites prefer ovipositing into hosts of the same species in which they themselves developed. The availability of an artificial medium should allow direct

tests of the influence of the preimaginal environment on the behavior of the resulting female wasp.

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