REARING *MICROPLITIS DEMOLITOR* WILKINSON (HYMENOPTERA: BRACONIDAE) IN THE LABORATORY FOR USE IN STUDIES OF SEMIOCHEMICAL MEDIATED SEARCHING BEHAVIOR

F. Hérard[1], M. A. Keller[2], and W. J. Lewis[2]
(Accepted for publication 8 September 1987)

ABSTRACT

Microplitis demolitor Wilkinson were reared in *Heliothis zea* (Boddie) larvae fed artificial diet and cowpea seedlings. The effect of temperature on development time, food and humidity on adult longevity, and cage design on mating and subsequent sex ratio of the progeny of *M. demolitor* were tested and are discussed. It was determined that individual pairs of males and females should be held in mating chambers for at least 24 hours in order to obtain a high percentage of inseminated parent females. Females used for parasitization should be at least 24 hours old in order to obtain the maximum number of female progeny. A 25-day longevity of female parasitoids was obtained when they were fed unadulterated honey and a separate water source, and confined in 90-95% relative humidity. A parasitoid survival of 74% was obtained in the hosts when they were reared individually on artificial diet at 27° C. Our rearing techniques were shown to produce parasitoids with a consistent high host searching response as shown by tests involving use of volatile semiochemicals in a wind tunnel.

Key Words: *Microplitis demolitor*, rearing, growth and development, environmental holding conditions.

J. Entomol. Sci. 23(2): 105-111 (April 1988)

INTRODUCTION

When more than 35% of parasitism of *Heliothis* spp. by *Microplitis demolitor* Wilkinson was observed in several field crops, especially soybeans, in Queensland, Australia, it was imported to the U.S.A. from Australia in 1981 through the U.S.D.A. Research Quarantine Facility in Stoneville, Mississippi as a potential biocontrol agent (Shepard et al., 1983). These authors tested 10 possible lepidopterous hosts for the parasitoid which successfully completed development only in the 4 following species: Cotton bollworm, *Heliothis zea* (Boddie), Tobacco budworm, *Heliothis virescens* (F.), Soybean looper, *Pseudoplusia includens* (Walker) and cabbage looper, *Trichoplusia ni* (Hübner).

The biology of *M. demolitor* was first described by Hafez (1951) and later by Shepard et al. (1983) in which these latter workers considered this knowledge to be helpful in understanding the possible use of *M. demolitor* in pest management programs. With this objective in mind, some aspects of *M. demolitor* foraging behavior, namely flight responses of experienced and inexperienced females to volatile semiochemicals emitted by a plant-host complex in a wind tunnel were conducted (Hérard et al., 1987a, 1987b).

[1] European Parasite Laboratory, USDA, ARS, 17 rue de la Masse, Béhoust 78910 Orgerus, France.
[2] Insect Attractants, Behavior, and Basic Biology Research Laboratory, USDA, ARS, Gainesville, FL. 32604. Duty station: USDA, ARS, P. O. Box 748, Tifton, GA 31793.

Good data for behavioral responses require rearing procedures that produce organisms of a consistent high quality. We report herein methods of maintaining laboratory cultures of *M. demolitor* on hosts fed artificial or natural diet along with some techniques for extending longevity of the adult parasitoids and maintaining a more desirable sex-ratio (1:1) of the progeny.

MATERIALS AND METHODS

Culture of M. demolitor on hosts fed artificial diet.

Initial rearing procedure. — *M. demolitor* is a larval parasite which preferably attacks 1st through 3rd instar host larvae (Shepard et al., 1983). The hosts used in our study were *H. zea* larvae produced in the insect rearing facility at the Insect Biology and Population Management Research Laboratory, A.R.S., U.S.D.A., in Tifton, GA., on an artificial diet and with the technique described by Burton (1969). Initial stocks of *M. demolitor* were obtained from a laboratory culture at the Southern Field Crop Insect Management Laboratory, A.R.S., U.S.D.A., in Stoneville, Mississippi.

Two hundred to 300 M. demolitor cocoons were placed at 27 ± 1°C in a 13.5-liter acrylic emergence cage equipped with 20 cm^2 ventilation holes covered by nylon mesh. Two cotton pads in 30 ml cups were impregnated with either honey-water or free water as food and water.

Thirty females were taken from this cage and used for parasitization of other hosts. Groups of 70 2nd instar *H. zea* larvae were exposed to 2 female parasitoids in 480 ml waxed cardboard cups. A layer of artificial diet was first poured in the bottom of the cups prior to the introduction of the 10 host larvae.

Modified rearing procedure. — We modified our procedures for handling the emerging adults. Individual *M. demolitor* cocoons were isolated in 30 ml plastic cups with a droplet of unadulterated honey as food for the emerging adults. Upon emergence, individual female and male adults were paired for a few hours in a 480 ml waxed cardboard cup at 27 ± 1° C. A few droplets of the honey were deposited under the transparent lids of the cups. Unadulterated honey was preferred over a honey-water mixture which oxidized rapidly unless changed often, and, in turn, decreased adult survival. Water was supplied by a moistened cotton pad contained in an open 30 ml plastic cup located on the floor of the mating chamber. In this manner, we also maintained a high relative humidity of ca. 90% which was apparently beneficial to the parasitoids, because increased longevity was observed. Females were held with the males in individual mating chambers until the behavioral tests which was generally 7 to 10 days after emergence (Hérard et al., 1987a, 1987b).

The mated females selected for subsequent colonization were held for at least 24 hours in the mating chamber, since most of the eggs laid the first day following mating produced males. After this, ten second instar *H. zea* larvae were exposed for 4 hours to each mated female in 480 ml waxed cardboard cups. Ten additional larvae were exposed to the same females during the next 4 hours, so that each female *M. demolitor* parasitized 20 hosts over a period of 8 hours. We did not expose hosts to the parasitoids in the presence of the artifical diet of the host since Shepard et al. (1983) reported that any contact with the diet by the parasitoid resulted in extensive cleaning efforts. Thus, oviposition was greatly reduced and some females died before parasitizing all the hosts. Parasitized larvae

were placed individually in 30 ml plastic cups with artificial diet until parasitoid larvae emerged from their hosts and spun a cocoon.

It appeared important to hold the cups of parasitized hosts at a minimum temperature of 27° C. At this temperature, or higher, when the parasitoid larvae were fully developed, the host would move to the edge or top of the container and become motionless. When parasitoid larvae emerged from the host, the cocoon was spun beside the host and out of contact with the oxidized host food. Under these conditions, we obtained a survival of 74% of the parasitoid pupae. At lower temperatures 22 - 23° C, about 50% of the parasitized hosts remained on the diet and parasitoid cocoons in turn made contact with the diet. Microbial contamination of the parasitoid by the diet may have occurred since 93% of the pupae died. Before emergence of adult parasitoids, the cocoons were isolated from the rearing containers and placed individually in emergence cups as described above. The average percentage of females obtained in each generation during 11 months using this procedure was a mean of 62% with a range of 51 - 77%.

Sex ratio of progeny. — We noted that *M. demolitor* emerge from cocoons with mature eggs which can be oviposited the first day. Newly emerged parasitoid males and females were paired and maintained with food for 12 hours before being provided with hosts for parasitization. Each female was allowed to lay only 1 egg in each 2nd instar host larva. Females of one group laid 5 eggs a day while those of another group were allowed to lay 3 eggs a day for 7 days. The parasitized hosts were then reared individually on artificial diet until emergence of adult parasitoids.

Culture of M. demolitor in hosts fed plants.

Following the method of Hérard et al. (1987b), we also reared *M. demolitor* from *H. zea* fed on pink eye, purple hull, cowpea seedlings that had been grown in the greenhouse. Cowpea seeds were germinated in perforated aluminum pans filled with a mixture of sand and compost. About 10 *H. zea* eggs were placed on artificial diet in each capped 30 ml plastic cup for hatching and subsequent feeding for two days. Then, ten to fifteen 2-day old 1st instar host larvae were transferred using a small artist brush, to 480 ml cups. The larvae were then exposed to a 2 day-old mated female parasitoid for 4 hours in these capped cups. Each female was allowed to oviposit in 20 to 30 host larvae. Oviposition occurred immediately upon detection of the host by the female. Three to 4 hours were required to get 100% parasitism in each group of 10 - 15 hosts exposed. Two hundred parasitized *H. zea* larvae were placed on two pans of 10 cm-high cowpea seedlings, held in a 70-liter cage at 24 ± 1° C and 50 ± 10% relative humidity. The perforated aluminum pans containing the germinated cowpeas were placed in slightly taller pans of the same diameter filled with water. The parasitized host larvae were transferred to new pans of cowpea seedlings when leaves were 75% consumed. At the end of the parasitoid larval development, the parasitized hosts became motionless on a cowpea leaf, or sometimes on the cage surface. Parasitoid larvae emerged from the 4th instar of the host and then spun a cocoon attached to the substrate beside the host or the cage surface. The cocoons were removed from the plants or the cage surface and placed in capped 30 ml plastic cups with a droplet of unadulterated honey until adult emergence. Adult parasitoids were handled in the same way as those reared from hosts fed artificial diet.

RESULTS AND DISCUSSION

Development time. — Time of parasitoid development from egg to pupation in *H. zea* fed artificial diet was 7.0 days when held at 27 ± 1° C and 60 ± 10% relative humidity. Shepard et al. (1983) recorded 9.0 day development time at 26 ± 2° C in the same host. We observed development time from pupation to adult emergence to be 4 days for most of the males and 5 days for most of the females. Thus the total average developmental time for all immature stages was about 11 days for male and 12 days for female parasitoids in *H. zea.*

The average longevity of adult parasitoids placed in 13.5-liter cages did not exceed 7 days. We observed an average 20-day male longevity (maximum 26 days) and an average 25-day female longevity (maximum 35 days) in our modified rearing procedure with 27 ± 1° C and 90% relative humidity. The 8 days of longevity for adult male and female parasitoids reported by Shepard et al. (1983) at 26.7° C was similar to that we observed in the 13.5 liter aerated acrylic cage, at the same temperature and relative humidity of 50 - 60%. Consequently, it seems that longevity of adult *M. demolitor* is highly correlated with the degree of ambient humidity. We observed a 25-day longevity in females which laid 35 eggs or less in their life. Average female longevity was also reduced to 12 days by intense oviposition as when they were allowed to lay 140 eggs in groups of 20 hosts a day for 7 days.

Sex ratio of progeny. — Males and females were expected to mate immediately after emergence and female-male sex ratio of progeny in the initial rearing was approximately 1:1 which indicated mating occurred but later decreased to 1:10. Hafez (1951) and Shepard et al. (1983) reported the occurrence of arrhenotokous parthenogenecity in *M. demolitor* i. e. unmated females produce all male offspring; mated females produce both sexes. We felt that a high number of unmated females in our colony was the cause of low female-high male ratio of progeny. Hafez (1951) reported that a female-male ratio of progeny (4:100) was increased to 57:100 when male and female parasitoids were allowed to mate after being separated for 24 hours after emergence. We assumed that high numbers of parasitoid individuals in the 13.5-liter acrylic cage led to mating disruption by an excess of sex pheromone in the limited space. Normally, virgin *M. demolitor* females emit a sex pheromone which attracts males and facilitates mating. Males respond to an emitting female by spreading and fluttering of the wings while walking toward the pheromone source. In fact, we observed in the 13.5-liter cage that many males responded to female pheromone, but were often unable to locate the source efficiently. They had a tendency to attempt copulation with any encountered individual regardless of sex. This justified development of the modified rearing procedure as described above.

The sex ratio of individuals from parasitoid eggs laid daily from day 1 to 7 was calculated and are reported in Tables 1 and 2. Only the offspring of parent females producing both sexes was included in this calculation. Only 20% of parent females laid unfertilized eggs which produced only male progeny. Another 10% stung the presented hosts regularly without laying eggs. Tables 1 and 2 show that a higher number of male than female eggs was laid during the first 24 hours following emergence of the parents. This suggests that the 43% of the parent females which oviposited eggs during the 1st day following emergence were still non-inseminated since all progeny were males. On the second day of parasitization, only one parent

Table 1. Daily records of sex ratio of progeny from 6 *M. demolitor* females. Each female was allowed 1 sting each on 5 separate *H. zea*[*] larvae each day for 7 days[†, ‡].

	Hosts not producing parasitoids		Parasitoid progeny		
day no.	No. pupated	No. died	♀	♂	♀:♂ ratio
1	1	7	9	13	1:1.5
2	1	6	18	5	3.6:1
3	0	4	16	10	1.6:1
4	0	6	15	9	1.7:1
5	1	6	16	7	2.3:1
6	0	2	18	10	1.8:1
7	0	4	17	9	1.9:1
Total	3	35	109	63	1.7:1

[*] Host larvae fed artificial diet.
[†] Oviposition was in a total of 30 host larvae per day. Only progeny from inseminated parent females producing both sexes are reported.
[‡] Parent females were held in presence of a male for the 7 days.

Table 2. Daily records of sex ratio of progeny from 8 *M. demolitor* females. Each female was allowed 1 sting each on 3 separate *H. zea* larvae[*] each day for 7 days[†, ‡].

	Hosts not producing parasitoids		Parasitoid progeny		
day no.	No. pupated	No. died	♀	♂	♀:♂ ratio
1	3	5	7	9	1:1.3
2	0	1	18	5	3.6:1
3	0	5	12	7	1.7:1
4	0	4	16	4	4:1
5	0	3	15	6	2.5:1
6	1	3	10	10	1:1
7	1	4	11	8	1.4:1
Total	5	25	89	49	1.8:1

[*] Host larvae fed artificial diet.
[†] Oviposition was in a total of 24 host larvae per day. Only progeny from inseminated parent females producing both sexes are reported.
[‡] Parent females were held in presence of a male for the 7 days.

female appeared non-fertile. This female began to lay eggs producing females on the 5th day following mating. All other parent females producing progeny of both sexes were thus inseminated 24 hours after mating.

From the second day after mating, more female progeny were produced daily. After 7 days of oviposition, 1.7 to 1.8 times more female progeny were produced. It was important to obtain an optimum number of females of *M. demolitor* because only females were involved in our host searching behavior tests (Hérard et al., 1987a, 1987b). Moreover, the benefits of a higher number of females in each generation,

and the need for fewer numbers of hosts, diet and other expenses needed to perpetuate the parasitoid culture adequately were important. Our findings thus confirmed those of Hafez (1951) that *M. demolitor* parents should be held together for at least 24 hours before being used for parasitization of hosts.

Culture of M. demolitor in hosts fed plants. — When parasitized hosts were reared at the same temperature on either artificial diet or plants, parasitoid development time was the same. About 50% mortality occurred in parasitized *H. zea* larvae reared on plants before completion of parasitoid development due to the following: (1) a few 1st and 2nd instar host larvae fell off the cowpea seedlings and drowned in moistened crevices of soil; (2) the rather high density of parasitized hosts on plants led to some cannibalism; and (3) a few host larvae were infected with virus. Surviving parasitoid adults had the behavioral qualities (Hérard et al., 1987a, 1987b) we required.

Our *M. demolitor* rearing procedures were modified and improved to avoid a possible mating disruption caused by sex pheromone emitted by the parasitoid in a confined space. We obtained a high percentage of inseminated parent females, and thus an increased percentage of females in the progeny, by the following methods: (1) confining males and females as individual pairs in mating chambers; and (2) use of mated females for parasitization only after a minimum of 24 hours of pairing. An increased longevity of adult parasitoids was accomplished by feeding them unadulterated honey and a separate water source and keeping them in a constantly high relative humidity of 90%. We found that the survival of the ovipositing female parasitoids was improved when not exposed to the hosts in the presence of artificial diet. Also, a high temperature of 27° C was found to be important to the survival of host larvae and parasitoid pupae when hosts were fed artificial diet. The parasitoids were also reared satisfactorily from *H. zea* larvae fed cowpea seedlings.

These parasitoids were used to study the flight response to olfactory stimuli by groups of female *M. demolitor* (Hérard et al., 1987a, 1987b). Consistency of the female response was strongly dependent on their physiological homogeneity which was insured by the improved rearing procedures and reduced the number of individuals needed for the studies. The procedures described here were used successfully for rearing *M. demolitor* for about 11 months.

ACKNOWLEDGMENT

We are most grateful to the following: Lisa Hill and Joe Spurlin who maintained insect and plant cultures; Deryck Perkins and Margaret Wood who generously provided us with host larvae and artificial diet; and Jim Tumlinson, Ted Turlings, Fred Eller, Lucas Noldus, Yvonne Drost and Olivier Zanen for helpful discussions and suggestions. Raymond Moore, Walker Jones, David Perkins, Geneviève Prévost and Deryck Perkins gave valuable suggestions for manuscript revisions.

LITERATURE CITED

Burton, R. L. 1969. Mass rearing the corn earworm in the laboratory. USDA Tech. Bull., ARS series 33-134: 8 pp.

Hafez, M. 1951. Notes on the introduction and biology of *Microplitis demolitor* Wilk. (Hym.: Braconidae). Bull. Soc. Fouad ler Entom. 35: 107-21.

Hérard, F., M. A. Keller, W. J. Lewis and J. H. Tumlinson. 1987a. Beneficial arthropod behavior mediated by airborne semiochemicals. III. Influence of age and experience on flight chamber responses of *Microplitis demolitor* Wilkinson. J. Chem. Ecol. Submitted.

Hérard, F., M. A. Keller, W. J. Lewis and J. H. Tumlinson. 1987b. Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. J. Chem. Ecol. Submitted.

Shepard, M., J. E. Powell, and W. A. Jones. 1983. Biology of *Microplitis demolitor* (Hym.: Braconidae), an imported parasitoid of *Heliothis* (Lep.: Noctuidae) spp. and the soybean looper, *Pseudoplusia includens* (Lep.: Noctuidae). Environ. Entomol. 12: 641-45.