Laboratory Rearing of the Mexican Bean Beetle[1] and the Parasite, *Pediobius foveolatus*,[2] with Emphasis on Parasite Longevity and Host-Parasite Ratios[3]

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ABSTRACT

The parasitic wasp *Pediobius foveolatus* (Crawford), was successfully reared in the laboratory on *Epilachna varivestis* Mulsant. The feasibility of maintaining overwintering stocks of this parasite was demonstrated. The optimum parasite-host ratio used in terms of adult parasites produced/host larvae was 0.4:1. Reproductive capacity of adult ♀ diminished with age, and those held for longer than 90 days at 13°C failed to successfully reproduce. The optimum age for reproduction was less than 12 days.

The parasitic eulophid wasp *Pediobius foveolatus* (Crawford) has been effectively utilized in the suppression of the Mexican bean beetle, *Epilachna varivestis* Mulsant, on soybeans in Maryland (Stevens et al. 1975). The parasite is unable to overwinter and must be released annually. This necessitates maintenance of overwintering stocks and subsequent increase to sufficient numbers for inoculative releases every spring. This paper deals with laboratory rearing of the Mexican bean beetle and *Pediobius foveolatus*, emphasizing the effects of parasite age and parasite-host ratios on production of the parasite.

Materials and Methods

Rearing of Mexican Bean Beetle

Campbell and Brett (1966), Kogan (1972), and others have conducted host plant selection and nutritional studies with the Mexican bean beetle in the laboratory. Kogan (1971) reported rearing 3rd and 4th instars on artificial media, but continuous rearing of large numbers of Mexican bean beetles has not been reported. Since a satisfactory artificial diet is not available, large numbers of all stages of this insect were reared on lima beans, *Phaseolus lunatus* L., under controlled conditions in the laboratory.

Plant Material.—Soil medium, premixed in a cement mixer, consisted of the following materials: ½ bu (17.62 liters) each of sphagnum and Michigan peat moss, 1 bu (35.24 liters) vermiculite, 150 g ground limestone, and 150 g 5-10-5 granular fertilizer. 'Henderson' bush lima beans, 5 seeds/pot, were planted in 11-cm clay pots containing the soil medium. All plant material was grown in the greenhouse at 24–27°C under 15-h light.

Rearing and Storage.—Insect colonies were established with adult beetles, 2–3 wk old, collected from soybeans on the Eastern Shore of Maryland in late Sept. and early Oct. Adults, in groups of 200, were maintained in holding cages constructed with wooden frames 40×40×43 cm with 32-mesh saran screen on top and all sides. This type of construction provided good ventilation and permitted maximum lighting within the cage. Tops were constructed to lift free and a tight seal was formed by cementing a narrow strip of foam rubber on the underside of each top. Cages contained 9 pots of bean foliage which were changed 3 times weekly.

In addition to adult beetles used as an immediate source of eggs, several thousand field-collected adults, 200 to a group, were placed in cold storage at 16°C to be used as egg layers at a later date. These insects were placed in pt ice cream cartons, each with a 4-dram (60 ml) shell vial of 2% sugar water and a cotton wick. Cartons and vials were changed as needed, generally on a monthly basis.

Bean leaves containing egg masses were collected from the adult holding cages and cut into squares somewhat larger than the egg mass. These were dipped into a 0.1% hypochlorite solution for 10 min to maintain aseptic conditions. Groups of 5–10 egg masses were placed in 100-mm diam glass petri dishes containing a cotton wick moistened with distilled water. These dishes were then set out for incubation or placed in cold storage at 16°C, under constant darkness, for later use.

Immediately after hatching, small larvae, still in clusters on bean leaves, were transferred to 3–5-wk-old bean plants. Plants were placed in 70×105-cm galvanized metal pans and bottom-watered periodically to maintain plant material in good condition. To maintain an adequate supply of foliar material, larvae were periodically divided into smaller groups by cutting the old foliage and placing it on fresh plant material in additional pans. Larvae readily transferred from one to the other. After larvae had reached the late 3rd or 4th instars, they were harvested for use in rearing the parasite. Those larvae that pupated were placed in holding cages for adult emergence. New adults were fed until sexually mature and began mating and ovipositing. They were then used or placed in cold storage for later use.

All rearing and incubation of eggs was done in a

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walk-in rearing room maintained at 24–27°C, 70± 20% RH, and 15-h light at 700±50 ft-c.

Rearing of Pediobius foveolatus

Large 3rd instars, 4th instars, or prepupae of the Mexican bean beetle were placed in pt ice cream cartons and adult parasites were transferred to the same cartons with a dampened camel-hair brush. Only females were introduced into the cartons. Cartons had fine-mesh nylon covering a hole cut into each lid. One medium-sized lima bean leaf was added to each carton 2 or 3 times a day until all larvae had mummified or pupated. A drop of honey was placed on the mesh lid and the lids were sprayed daily with a light mist of water. The cartons were kept in an incubator at 22°C with a 16-h photoperiod. After 7 days, the parasites were removed with a camel-hair brush, mummified larvae were transferred individually to 1-oz plastic media cups and returned to storage in the 22°C incubator. If non-mummified larvae remained in the original carton, they were fed as before and harvested again after another 4 days. A 3rd harvest was sometimes necessary after another 4 days. Mexican bean beetle adults that emerged in these containers were discarded or transferred to bean beetle colonies.

New-generation adult parasites usually began emerging from harvested mummies after 5 days and normally most completed emergence within 19 days of the 1st harvest. Under these conditions, the complete expected cycle from oviposition to emergence of adults fell within the range of 12–26 days. Newly-emerged parasites were transferred into pt cartons and placed in storage at 13°C, fed honey, and sprayed daily with a mist of water. Few adult parasites died before being placed in storage if they received honey and water within 12-h of emergence. The newly-emerged parasites were kept under darkness at 13°C until they were needed for production of the next generation.

Developmental Rate.—We determined the developmental rate of the parasite by isolating host larvae immediately after they were once stung and holding them individually in 1-oz (29.5 ml) media cups as previously described at 22°C until the new-generation adults emerged. A sample of 250 mummies were so isolated over a period of several months. Records of the time elapsed from oviposition to emergence were kept. Under these conditions, the complete life cycle of the parasite is 10–23 days.

Fecundity of Parasite.—Individual ♀ parasites, not previously exposed to host larvae, were placed in pt ice cream cartons with 20 host larvae. After 2 days, the parasites were transferred to a new carton with 20 more larvae. This was repeated every 2 days until the parasite died. Host larvae so exposed to parasites were held until they completed normal development, died, or mummified. Mummies were isolated individually in 1-oz media cups as previously described and records of adult parasite emergence were maintained. Dead larvae and mummies from which no parasites emerged were dissected to determine the presence of parasite larvae, pupae, or adults. A total of 49 individual parasite ♀ were studied in this manner.

Sex Ratio

At various times throughout the period of these studies, groups of laboratory-reared and field-collected mummies were isolated and the sexes of the individual parasites emerging from them were recorded. A total of 4026 adult parasites was sexed in this manner.

Effect of Parasite Age on Fecundity

Variously-aged parasite adults that had been stored at 13°C were placed in pt cartons with larvae of the Mexican bean beetle. The age groups tested are listed in Table 1. From 8–12 ♀ parasites were used with from 20–40 larvae per carton, depending on the ratio of parasite to host desired. We used a ratio of 0.4 parasite ♀ to 1 host larva for the major portion of the study. However, ratios of from 0.2–0.6 parasites to 1 larva were included. The parasitized larvae were isolated as previously described, and records were kept of live adult parasite emergence from mummified larvae. For the purpose of laboratory production, only mummified larvae from which the parasites emerged were considered successfully parasitized.

Effect of Parasite-Host Ratio on Laboratory Production

From 4–25 ♀ parasites were introduced into each pt carton containing from 10–45 Mexican bean beetle larvae, depending on the desired parasite ratio. The ratios studied are listed in Table 2. Only adult female parasites of from 1–12 days of age were used. All parasites were taken directly from storage at 13°C immediately prior to introducing them into the pt cartons containing larvae. All mummified larvae were isolated as previously described and records of parasite emergence were kept. Only mummified larvae from which parasites emerged were considered as successfully parasitized.

Results and Discussion

Rearing of Mexican Bean Beetle

Plant Material.—Several references listed in Kogan (1972) state that many varieties of snap beans, *Phaseolus vulgaris* L., and lima beans serve as preferred host of the Mexican bean beetle. Since lima beans provide more foliage material and mature more slowly than snap beans, they were used as the food source in our rearing program. Less mature plants were preferred by the Mexican bean beetle. This is understandable because the beetle ingests the juices and softer tissues, rejecting the cellulose and other harder tissues of the leaf before ingestion (Kapur 1948).

Kogan (1972) and Elden et al. (1974) suggested that Mexican bean beetles attacking soybeans still prefer snap and lima beans when given a choice. This response indicated to us that beetles reared on lima beans could be used to screen for host plant resistance in soybeans and other host plants. Rearing Mexican bean beetles on soybeans took longer and was less productive than rearing on lima beans.

Soil medium used in this program offered several advantages over conventional soil mixes. Seeds planted in this medium had a higher germination rate, and resulting plants were large enough to use in 3 wk as compared to 7 wk when grown in soil. Leaves from 3–5-wk-old plants grown in this mixture were also more uniform in size and age.

Rearing and Storage.—Adults taken directly from the field or storage began laying eggs within 1–2 wk. A partial reproductive diapause appeared to exist in adults taken from the field late in the growing season as well as in those taken from cold storage. Cold storage appeared to have little or no effect on egg production after the diapause was broken by the 15-h photoperiod. Egg laying increased for 4–5 wk and then went into a sharp decline, with most of the colony dead by the 7th wk. Adults taken from the field showed little or no mortality for up to 5 mo in cold storage.

Eggs stored at 16°C began to hatch after 2 weeks. Within 3 wk, 75% of the eggs had hatched. For this reason, only unhatched eggs 2 wk or younger were incubated for use in the rearing program. Previous attempts to delay egg hatch by holding them under a lower temperature (4°C) proved unsuccessful. Eggs taken out of cold storage within the 2-wk period hatched in 2–5 days depending on time in storage. There was no apparent change in egg viability due to cold storage at 16°C. Egg masses averaged 40±5 eggs.

The developmental period for each of the 4 larval instars averaged 4 days. The prepupal period averaged 2 days and the pupal period 5 days. The avg period from egg to adult was 28 days. These figures were very similar to those obtained by Campbell and Brett (1966). Lower temperatures, reduced light intensity, and mature bean plants all appeared to have an adverse effect on the rate of bean beetle development.

A constant supply of eggs, larvae, and adults were maintained using this method. Successive generations of laboratory-reared adults were not used to propagate the insect colony until the stock of field-collected adults was depleted. Up to 1000 larvae were reared/wk with no evidence of decline in size and vigor of individuals.

Rearing of Pediobius foveolatus

Pediobius foveolatus is a gregarious parasite, and adults emerge from a hole in a parasitized host over a period of a few seconds. Although we assumed that mating takes place within the mummified host larva prior to emergence, we have observed mating within a few minutes to several hours after emergence. If host material is available, ♀ may immediately begin ovipositing after emergence. Unmated ♀ also oviposit, but their progeny are all ♂. Characteristically, the ♀ parasite works her way between the spines on the dorsal and lateral surfaces of the bean beetle larva, grasps the spines with her legs and inserts her ovipositor through the host cuticle while she is more or less vertically positioned with respect to the host surface. Within 5–6 days after oviposition, the host larva turns dark brown, assumes a cylindrical, cigar-shape, and remains fastened to the leaf at the caudal tip. The new adult parasites may begin emerging from the host mummy as early as 10 days after oviposition when held at 22°C. Third and 4th instar hosts are preferred by the parasite, although we have observed oviposition on 2nd instars when preferred stages are not available.

Developmental Rate

The avg time for development of *P. foveolatus* from oviposition to adult emergence was 16.2 (SD = 2.4) days at 22°C. Of the sample of 237 successfully parasitized host larvae, the developmental time ranged from 10–23 days. The median emergence time was 16 days. Lall (1961) reported an avg developmental time of 16 days for *P. foveolatus* in *Epilachna* spp. at 22°C, and 11 days at 25.6°C. Angalet et al. (1968) reported that the avg developmental time at 25°C was 30 days using *E. varivestis* as the host. Our observations are that at 25°C the developmental time is approximately equal to that reported by Lall (1961).

Fecundity of Parasite

The avg number of host larvae parasitized/♀ *P. foveolatus* was 20.3 (range, 1–81; SD = 17.9). Approximately 57% of the parasitized mummies did not produce live adult parasites. Dissection of these mummies showed they all contained dead larvae, pupae, or adults of *P. foveolatus*. The avg number of adults that emerged from each successfully parasitized mummy was 11.1 (range, 1–27; SD = 4.8). The avg production of live progeny (adults) was 88/♀. Lall (1961) reported from 10–50 progeny/♀. The higher mortality in this study than obtained with only 1–12-day-old parasites (Table 1) is because each parasite was allowed to parasitize host larvae until she died. Most of the mummies from older parasites produced no live progeny. In a sam-

Table 1.—The effect of age on reproduction of parasites stored at 13°C.

Parasite age (days)	No. MxBB[a] larvae exposed to parasite[b]	No. MxBB larvae from which parasites emerged	% successfully parasitized[c]
1–12	11.959	6,983	58.4
13–24	982	245	26.0
25–36	581	51	8.8
37–90	440	6	1.4
91–180	413	0	0

[a] MxBB = Mexican bean beetle.
[b] Parasite-host ratio varied from 0.3–0.6.
[c] Does not include parasitized larvae from which parasites did not emerge.

Table 2.—The effect of parasite-host ratio of *Pediobius foveolatus* to Mexican bean beetle larvae on the laboratory production of the parasite.

Ratio parasite to host[a]	No. host larvae exposed to parasite	No. host larvae successfully parasitized[b]	% host larvae successfully parasitized	No. parasites produced	Avg no. parasites produced per parasitized larva	Av no. parasites produced per exposed larva	Avg no. parasites produced per parasite used
2.0	21	11	52	204	18.5	9.7	4.9
.8	108	108	50	1,895	17.5	8.8	11.0
.7	305	131	43	2,111	16.1	6.9	9.9
.6	626	205	33	3,060	14.9	4.9	8.1
.5	731	385	65	6,391	16.6	8.7	17.5
.4	6,320	4,259	67	64,311	15.1	10.2	25.4
.3	2,705	1,438	53	20,420	14.2	7.5	25.2
.2	2,570	1,174	46	13,263	11.3	5.2	25.8
.1	900	346	38	3,627	10.5	4.0	40.3

[a] Parasites used in this study were 1–12 days old.
[b] Does not include parasitized larvae from which parasites did not emerge.

ple of 4686 field-collected mummies that were brought into the laboratory during the 1974 season, adult parasites emerged from 76% of the mummies. This indicates that most of the actively ovipositing females in the field are relatively young.

Sex Ratio

The ratio of ♀ : ♂ varied with each group of laboratory-reared parasites and also with samples derived from field-collected mummies. The sex ratios of various laboratory-reared groups ranged from 1 ♂ :2.5 ♀ to groups with 100% ♀. The overall sex ratio of 1114 laboratory-reared individuals was 1 ♂ :6.75 ♀, and of 2912 field-collected individuals was 1 ♂: 1.33 ♀. This apparent difference between laboratory and field indicates a possible rearing effect on sex ratio. Known unmated ♀ produced only ♂ progeny.

Effect of Parasite Age on Fecundity

The effect of the age of the parasite on its ability to reproduce in the laboratory is clearly evident in Table 1. From the standpoint of most efficient production of parasites, it is obviously detrimental to hold parasites longer than 12 days before utilizing them. If availability of host material is critical, stocks can be maintained by storing for up to 24 days, but storage beyond this point results in a dramatic drop in production. We have never been able to produce an additional generation from individuals held beyond 90 days, although we have successfully kept adult parasites alive for as long as 9 months. Adults older than 90 days will actively oviposit in host larvae, but we have never successfully reared out the next generation. Dissection of these "parasitized" larvae has revealed remains of all stages of development of the parasite up to the pupa.

Effect of Parasite-Host Ratio on Production

The most adequate ratio of parasite to host, and the one we have used most frequently in the laboratory is 0.4:1 (Table 2). We normally achieved this ratio by placing 8 ♀ parasites in a pt carton with 20 Mexican bean beetle larvae. This ratio yields the highest number of parasites/host larva exposed. This is important since the limiting factor during the overwintering period may be availability of host larvae. If availability of host larvae is not a restricting factor, a lower ratio (e.g., 0.1) will yield a higher return in terms of parasites produced per parasite used. This lower ratio is useful when trying to increase stocks as rapidly as possible for release in the spring and early summer.

Conclusions

The parasite, *Pediobius foveolatus*, can be effectively maintained in the laboratory during the winter and increased sufficiently for inoculative releases in the spring and early summer. Sufficient Mexican bean beetle larvae can be reared by the described method to sustain the parasite rearing program and allow for accomplishing desired objectives.

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