Physiological Attributes of Reproductively Active and Dormant Mexican Bean Beetles[1,2]

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ABSTRACT

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Measurements of several physiological parameters of adult Mexican bean beetles, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), throughout the season indicate a reproductive diapause. Both sexes of diapausing Mexican bean beetles had an increased dry weight, enlarged fat bodies, reduced inactive gonads, reduced respiration rates and lower water contents than reproductively active beetles. The type of host plant (i.e., lima bean vs soybean) influenced values of certain parameters, e.g., dry weight, fat body index and gonad length of male beetles were significantly higher for individuals reared on lima bean rather than soybean.

Reproductive diapause has been demonstrated in several families of Coleoptera including Chrysomelidae, Curculionidae (Beck 1963, Danilevskii 1965), Scolytidae (Norris 1964), Tenebrionidae (Lees 1956) and Carabidae (Krehan 1970). In addition, members of the Coccinellidae have been shown to have an adult reproductive diapause occurring as hibernation, aestivation or aestivo-hibernation (Hagen 1962, Hodek 1967). Auclair (1959) suggested but did not conclusively demonstrate that the Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant, undergoes an adult reproductive diapause. However, evidence for adult diapause has been presented for two related species, *Epilachna vigintioctopunctata* F. (Sakurai 1969) and *E. chrysomelina* (F.) (Hariri 1970).

Hodek (1967, 1973) has summarized the morphological and physiological changes which take place in diapausing coccinellid adults. These include empty digestive tracts and enlarged fat bodies in both sexes. The females usually possess inactive, reduced ovaries while in the males the activity of the follicular tissue of the testes apparently does not stop in diapausing individuals, with spermatogenesis ceasing only during the coldest parts of the dormancy period. Respiration decreases with the onset of diapause while water content is variable but generally low in diapausing coccinellids (Chapman 1969).

The following study was undertaken to measure selected physiological parameters of adult MBB over time and to determine seasonal changes in these parameters that might indicate the presence of a reproductive diapause. The same physiological parameters were used to compare differences in adult beetles due to host plant.

Materials and Methods

These studies were conducted during the growing seasons of 1976 and 1977 and the winter of 1976-1977 at the Central Crops Research Station (CCRS), Clayton, NC. The initial stock of beetles used during 1976 was collected from soybeans in Currituck Co., NC, soon after beetle emergence from overwintering sites. These beetles were then held in field cages on either soybeans (Ransom variety) or lima beans (Henderson bush variety). As the foliage was consumed, the cages were moved to fresh foliage. Soybeans were planted on June 2 and 26 and the lima beans were planted on June 30, August 1 and 29. A sample of adults from each host was taken at ca. 10-day intervals throughout the growing season.

Oviposition was examined by holding a sub-sample of the beetles collected from each host on either cut foliage of field-grown soybean in water or young (1-3 wk old) pot-grown lima bean. Three replicates of 3 male and 3 female beetles each were placed on each of the test hosts in screen cages in an open air insectary. At 4-day intervals, the egg masses and eggs were counted and removed, dead adults replaced with individuals field-collected at the same time and plant material was replaced as necessary. Observations were discontinued after 3 wk or 2-3 egg massess were oviposited, whichever came first. Data are expressed as preovipositional period (days from collection to first egg mass in each replicate), egg masses/female and eggs/female.

On each sampling date, the respiration rates of 11 males and 11 females from each field host were measured individually following the technique described by Englemann (1963). Respiration was measured within 24 h of collection at 30°C for 3 h at 30 min intervals and is expressed as μl O_2 consumed/mg dry weight/h.

Immediately after respirometry, the fresh weights of each beetle were determined using a Metler Model H34 balance and expressed in mg. Seven beetles of both sexes were then dried to a constant weight at 70°C. Water content was determined from the fresh and dried weights and expressed as % water of fresh weight.

The eight beetles (4 males and 4 females) remaining from respirometry were then dissected in 0.85% saline, and the fat content, size of gonads and ovarian development noted. Fat content was rated on a visual scale of 0-4 with 0 = no easily discernible fat body, 1 = only small amount of fat body, 2 = a moderate amount of fat body, 3 = considerable amount of fat body and 4 = fat body abundant, filling body cavity. The length and width of either a testis or ovary from each beetle was measured in mm. Degree of ovarian development was given a visual rating based on the following scale: 0 = ovaries empty and undeveloped, 1 = accumulation of yolk material but eggs not clearly shaped, 2 = a

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single small partially developed egg in the ovariole, 3 = a partially developed egg in the ovariole with an accumulation of yolk material behind, 4 = a nearly fully developed egg with a partially developed egg behind and, 5 = a fully developed egg near calyx with other partially developed eggs behind.

Beetles used during the winter of 1976-1977 were collected from senescing soybeans in Pasquotank Co., NC and held in screen wire cages in a mixed hardwood-pine stand at CCRS. Natural overwintering populations of Mexican bean beetles were also sampled in Currituck, Duplin, Jones, Pamlico and Pasquotank Co., approximately every 3 wk throughout the winter. These beetles presumably developed on soybeans that were defoliated by Mexican bean beetles in the vicinity of the overwintering sites (Sprenkel and Rabb, unpublished report).

The initial population of beetles used during the 1977 growing season was collected from a snap bean trap crop soon after emergence from overwintering sites at CCRS. As in 1976, the beetles were caged on either lima beans or soybeans throughout the growing season. Samples of adult beetles were taken at approximately 10-day intervals. With the exception of oviposition, all parameters measured on the overwintering populations and the reproductive populations of 1977 were the same as those measured on the 1976 populations.

Results

Female MBB collected from soybean during the summers of 1976 and 1977 (nondiapausing) and those soybean-fed beetles taken from artificial hibernacula in the fall of 1976 and winter of 1976-1977 (diapausing) had a respiration rate of 3.0 μl O_2/mg/h, a water content of 68.1% and a fat body index of 2.6. Male MBB from the same collections did not differ significantly (5% level) from the females in these three parameters. Male and female MBB differed significantly (5% level) in dry weight (11.0 and 12.5 mg, respectively).

In 1976 and 1977, field host did not have a significant effect over the entire season on any of the oviposition parameters measured. In 1977, test host did not have a significant effect on preovipositional period or number of egg masses per female. However, there were significantly more (5% level) eggs per female on the lima bean host (23.9 eggs/female) than on the soybean host (16.3 eggs/female) over the sampling period.

In 1977, the interaction of field host and test host was examined. There was no significant effect due to this interaction on egg masses per female (Table 1). However, there were significantly more eggs per female among those beetles taken from field soybeans and tested on lima beans in the insectary than any other interaction. The same combination resulted in a signficantly longer preovipositional period (5.7 days) than the two treatments where the field host and the ovipositional host remained the same.

Table 1.—A comparison of ovipositional behavior of the Mexican bean beetle as affected by field host and test host interactions.

Field Host[1]	Test Host[1]	Preovip. Period Days	Egg Masses Per ♀	Eggs Per ♀
L	S	5.3ab	0.42a	16.6b
L	L	4.4b	0.50a	20.6b
S	S	4.0b	0.54a	17.6b
S	L	5.7a	0.61a	28.4a

[1] L=Lima bean, S=Soybean.

Monthly means of selected physiological parameters of MBB collected at CCRS from July to December 1976 and from January to March and July to November 1977 using soybeans as a host are presented in Fig. 1. Respiration rates based on dry weights for both male and female beetles were higher in July than any other month. From August to October, rates ranged from 3.4 to 3.8 μl O_2/mg dry weight/h. Following a drop in respiration rate for both sexes between October and November the rates stabilized at ca. 1.5 μl O_2/mg dry weight/h. Fat body reserves showed a gradual increase from August to November after which the amount of fat body stabilized near maximum capacity. Changes in gonad size were evident for both sexes being more pronounced in the males. The greatest reduction in gonad size took place between September and October. Testes were small from November to January and could not be discerned from the fat body in samples taken in January. An increase in the size of the testes was noted in samples taken during February to April.

Mean dry weight of male beetles for the period July to October was 10.9 mg while for the period November to March the dry weight was 13.5 mg. For female beetles during the same periods, mean dry weights increased from 11.8 to 13.3 mg. For the same two periods % water content of the males decreased from 69.3 to 62.4% while the females decreased from 69.3 to 66.4%.

A summary of the physiological parameters measured on Mexican bean beetles collected from five counties in eastern North Carolina is presented in Fig. 2. The values

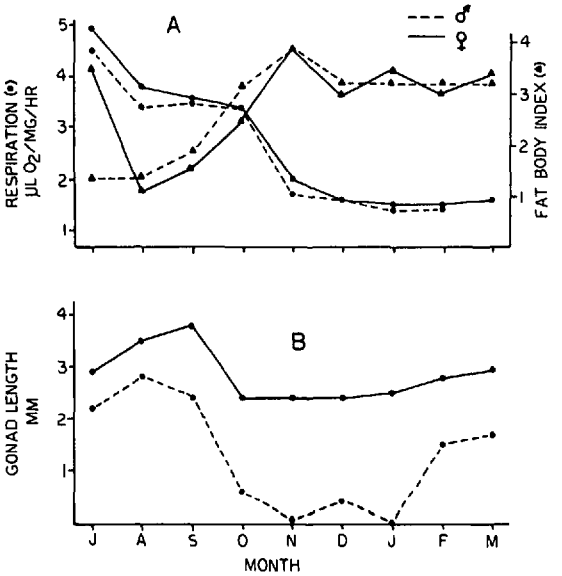

FIG. 1.—Summary of monthly changes in dry weight respiration and fat body index (A) and gonad length (B) of active Mexican bean beetles caged on soybean or dormant Mexican bean beetles which had fed on soybean in 1976-1977.

obtained from November through May were obtained from beetles collected from natural hibernacula while the June sample was made up of beetles collected from soybeans in the vicinity of the overwintering sites. Respiration rates based on dry weight for both sexes remained low ($\leq$ 2.1 μl O_2/mg dry weight/h) throughout the period the beetles were in the hibernacula. Respiration rates in June increased to 3.4 and 4.8 μl O_2/mg dry weight/h for females and males, respectively. Dry weights of both sexes were higher prior to February than they were after March.

Gonad length of the females remained relatively constant throughout the period the beetles were in the hibernacula (Fig. 2). Changes in the size of the ovaries were evident only in the June sample after the females left the overwintering sites. From November through January, the male gonads were small and could not be distinguished from the fat body, but from February through April showed an increase in size. Fat body of both sexes remained at capacity (fat body index = 4) through the February sampling period after which there was a gradual decline through May. The quantity of fat body dropped from an index of ca. 3 in May to less than 1 in June, after the beetles left the overwintering sites.

Percent water content for the period November through May ranged from 53.7 to 57.6% for the males and 54.1 to 60.1% for females. After spring emergence, there was an increase in % water content to 65.0 and 66.5% for the males and females, respectively.

A comparison of several physiological parameters between MBB reared on soybeans and lima beans is presented in Table 2. The data are means for samples collected July 26 through October 13, 1976 and July 11 through November 8, 1977. There was not a significant difference (5% level) between the two hosts for female beetles in respiration rate based on dry weight, % water content, fat body index or gonad length. For male beetles, there was no significant difference in respiration rate based on dry weight, but measurements for dry wieght, fat body index and gonad length were significantly higher for those beetles with a lima bean host. Percent water content was significantly higher in the male beetles from a soybean host.

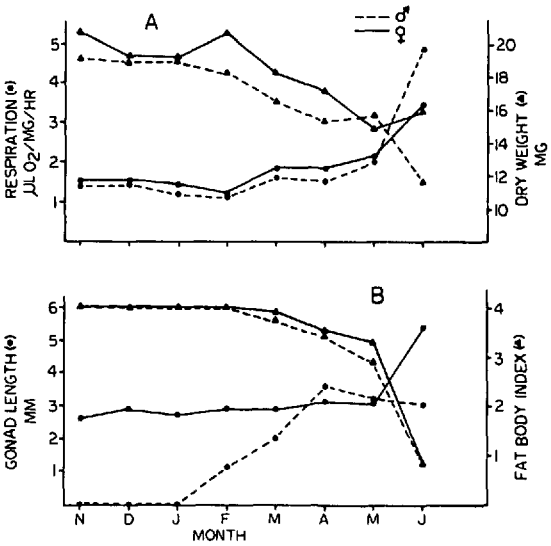

FIG. 2.—Summary of monthly changes in dry weight respiration and dry weight (A) and gonad length and fat body index (B) for Mexican bean beetles collected from five natural overwintering sites during November-May and soybean foliage in June in eastern North Carolina. Values given are means for the five locations.

Discussion

Dormancy in the MBB was found to be similar in many respects to that in other coccinellids where dormancy has been studied. Greatly enlarged fat bodies as found in dormant MBB are apparently widespread in the Coccinellidae (Hodek 1973). The apparent absorption of the ovaries on entering the overwintering sites has been noted previously in the coccinellid, *Stethorus punctillum* (Putman 1955). While dormant, the ovaries are generally greatly reduced (Hodek 1973). The MBB differs from *Adalia bipunctata* (Hariri 1966) and *Semiadalia undecimnotata* (Hodek and Landa 1971) where early development of the ovaries and oocyte formation begins while the beetles are still dormant and before food is taken. In female MBB, this development was not observed. Water content in hibernating MBB remained fairly constant as in *Hippodamia convergens* (Hagen 1962) and was lower than in active beetles, similar to observations made by Hodek (1973).

Relatively few studies have been reported which examined the respiration rates of dormant and active Coccinellidae. Those that exist generally show a decrease in respiration rates in dormant beetles. Stewart, et al. (1967) found a respiration rate in *H. convergens* of 12 μl O_2/beetle/h after arrival at the aestivation site. This rate decreased to between 5 and 9 μl O_2/beetle/h during aestivo-hibernation from July to February. Sakurai (1969) reported a respiration rate for *Epilachna vigintioctopunctata* in June just prior to emergence from the overwintering site of 0.5 μl O_2/mg fresh weight/h. This rate increased 2× to 1 μl O_2/mg fresh weight/h in Au-

Table 2.—A comparison of the effects of host plant on selected physiological attributes of male and female Mexican bean beetles collected from two host plants.

Sex	Host	Respiration μl O_2/mg/hr	Dry Wt. mg	Water Content %	Fat Body Index	Gonad Length mm
Female	Soybean	3.6	12.0	69.4	2.1	3.2
	Lima Bean	3.9	13.6	67.1	2.1	3.4
Male	Soybean	3.4	10.0*	68.6*	2.3*	1.8*
	Lima Bean	3.7	12.1	65.7	2.4	1.9

* Significant difference (5% level) between hosts.

gust when the beetles were feeding in the field. In the present study, respiration rates based on dry weights increased by 1.6 to 2.3× for female and male beetles, respectively.

One striking difference in the MBB from that reported previously for other coccinellids is in the changes in the testes during dormancy. Hodek (1973) indicated that testes in Coccinellidae unlike the ovaries undergo little or no change during the dormant period. However, the testes of the MBB undergo a considerable change and are greatly reduced as are the ovaries.

Based on observations in the present study, evidence is presented that the MBB undergoes an imaginal diapause as was suggested by Auclair (1959). This evidence includes enlarged fat bodies, reduced inactive ovaries and reduced respiration rate and water content in dormant beetles. All of these factors have been cited by Hodek (1973) as being typical in diapausing Coccinellidae.

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