# EFFECT OF LIGHT ON THE TEMPERATURE PREFERENDUM OF LARVAE OF THE MEXICAN BEAN BEETLE, EPILACHNA VARIVESTIS<sup>1</sup>

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## ABSTRACT

Second-instar larvae were allowed to find their preferred temperature on a circular thermal gradient disc, under conditions of 40-foot-candle and of 1-foot-candle illumination. Tests in dim light were made with 1,633 insects, in bright light with 1,052, and each individual was used once only. In dim light the preferred temperature was between  $70^{\circ}$  and  $75^{\circ}$  F.; in bright light the preference was less pronounced, and between  $90^{\circ}$  and 100° Differences in pretreatment or in the evaporation rate during tests (the only other experimental variables)

Deal (1941) defined the optimum temperature of an insect as the temperature at which the greatest number of insects are reproduced in a given period of time, and the temperature preferendum as the temperature to which an insect moves if given its choice in a temperature gradi-These definitions, of course, may be exent. tended to environmental factors other than temperature. Considerable evidence (Sweetman 1932, Marcovitch and Stanley 1930, Miller 1930, and others) has accumulated regarding the optimum conditions of the physical environment of the Mexican bean beetle in all its stages of development. Much less information is available regarding the preferenda of this insect for the various environmental factors, and the behavior resulting from possible interactions among them. There is a distinct possibility that for a given factor, such as temperature, the preferendum and the optimum may differ; and a further possibility that one preferendum may be changed with the variation of other factors. The data presented here may serve to illustrate such a behavior pattern.

### APPARATUS AND METHODS

The concentric thermal gradient disc and chamber originally described by Wellington (1948) was modified in the following particulars: (1)The peripheral conduit of the circular brass disc carried hot water rather than cold; (2) a second conduit under the center of the disc carried cold water; (3) a piece of white broadcloth was used to cover the surface of the disc and held in place by the pressure of an iron ring inside the wall of the peripheral water conduit. The whole gradient was enclosed in a plywood box with a circular plexiglass cover provided with ports which could be opened for the introduction of instruments or insects. Two separate circulating water systems

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appeared to have no bearing on the shift of temperature preference, and a statistical test indicated little likelihood of correctly attributing the observed result to a sampling error. Two possible conclusions are suggested: (1) the temperature preferendum for this insect in this stage may be altered by light conditions and therefore is not completely related to, or identical with, the temperature optimum, or (2) the temperature optimum may be changed with changing light.

were used. The hot-water temperature<sup>4</sup> was 120° at the inlet and 117° when it left the disc. The cold-water temperatures<sup>4</sup> were  $40.5^{\circ}$  and  $41^{\circ}$  at inlet and outlet, respectively. The temperature



FIG. 1.-Distribution of Mexican bean beetle larvae in a temperature gradient under bright- and dim-light conditions.

gradient thus produced on the disc ranged from  $60^{\circ}$  a few inches from the center to  $110^{\circ}$  a few inches inside the edge. The surface of the cloth was calibrated with a thermocouple on quadrant

4Unless otherwise stated, all temperatures are on the Fahrenheit scale.

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lines, and the roughly concentric isothermal lines drawn. During the course of the work the calibration of the surface was frequently rechecked, and the maximum deviation of any point was less than  $2.0^{\circ}$ . Inasmuch as bean beetle larvae respond to a range of at least 5°, this accuracy was considered adequate.

All treatments reported here were made in a controlled temperature-humidity room, whose temperature was maintained at  $75^{\circ}-77^{\circ}$  and whose relative humidity was held between 58% and 66%. Under these conditions, with the cover of the gradient chamber removed, the evaporation rate of water above the  $75^{\circ}$  isotherm was 0.27 cu. mm./min. in a capillary tube. This condition of evaporation rate is here termed a



FIG. 2.—Comparison of moist and dry gradients for effect on temperature preference. Tests in dim light; pretreatment 75% relative humidity. \*Above 100°.

"dry temperature gradient." With the exception of 19 runs, all tests were made in this dry gradient. "Moist temperature gradient," used in 15 dimlight and 4 bright-light runs, was obtained with the cover on the chamber, the room relative humidity at 90% to 95%, and the evaporation rate at the 75° isotherm 0.21 cu. mm./min.

Light was provided by either three 15-watt daylight fluorescent tubes 4 feet above the disc, or one 15-watt tube below the disc. The first produced a "bright" light of 40 foot-candles, the latter a "dim" light of 1 foot-candle, on the surface of the disc. These light intensities were checked with a commercial photometer.

Since previous experience had demonstrated that second-instar bean beetle larvae were more consistent in their behavior on the disc than other stages, the present work utilized only this instar. Insects were reared under greenhouse conditions, and before use were conditioned by a pretreatment of several hours in darkness at either 50%, 75%, or 100% relative humidity. Individuals were used only once. Larvae were liberated on the disc at random, in groups of approximately 50, and allowed to walk at will for 1 hour. Counts



FIG. 3.—Comparison of moist and dry gradients for effect on temperature preference. Test in bright light; pretreatment 75% relative humidity.

were made every 15 minutes of the number in each isothermal area. The four readings thus obtained for each isotherm were summed and divided by four to provide the raw data of the following tables and graphs. A total of 1,633 insects made up the dim-light curve; 1,052 the bright-light one.

# RESULTS AND DISCUSSION

The summation curves of the temperature preference for all insects handled in dim light vs. all those treated in bright light, regardless of the variables represented by differences in pretreatment or the evaporation rate, are presented in

Isotherm, "F.	Number of larvae							
	40 foot-candles				1 foot-candle			
	Pretreatment, RH				Pretreatment, RH			
	50% 6	75%	100%	Summation	50%	75%	100%	Summation
Below 60	9.25	5.00	19.75	34.00	1.25	17.25	6.50	25.00
60 - 65	12.25	18.25	34.25	64.75	6.50	109.00	16.25	131.75
65-70	11.75	23.25	48.00	83.00	17.50	182.00	22.00	221.50
70- 75	10.25	32.25	53.50	96.00	13.75	243.00	21.00	277.75
75 - 80	13.25	43.25	43.25	99.75	13.75	148.00	18.25	180.00
80- 85	11.00	82.25	32.00	125.25	8.00	150.25	13.25	171.50
85 90	13.75	45.75	43.50	103.00	7.25	141.75	12.25	161.25
90- 95	15.50	48.25	72.25	136.00	7.50	98.00	15.50	121.00
95-100	19.75	35.25	82.00	137.00	7.75	80.75	18.50	107.00
100 - 105	18.00	29.50	29.00	76.50	5.50	87.00	19.50	112.00
105 - 110	12.25	18.50	26.00	56.75	9.25	37.50	23.25	70.00
Above 110	3.25	22.25	14.50	40.00	2.25	42.25	9.75	54.25
Totals	150.25	403.75	498.00	1,052.00	100.25	1,336.75	196.00	1,633.00

Table 1. Effect of bright and dim light on Mexican bean beetle larvae in a temperature gradient.

figure 1 and the data on which these curves are based appear in table 1. The dim-light curve shows a pronounced mode in the  $70^{\circ}-75^{\circ}$  iso-therm. This agrees well with the  $77^{\circ}$  optimum reported by Marcovitch and Stanley (1930) and the  $22^{\circ}$  C. optimum found by Sweetman (1932). The slight rise at the  $100^{\circ}$   $-105^{\circ}$  isotherm is almost certainly attributable to the fact that larvae wandered into this hot area and could not escape. Similar data were presented by Marcovitch and Stanley (1930) who reported the death of "small" larvae in 7.5 minutes at 106°. The point is of some importance in considering the bright-light curve. From data recorded in the 15-minuteinterval readings it appears possible for a secondinstar bean beetle larva to leave any of the warmer isotherm areas after it has entered it and remained long enough to be counted. Though it is very probable that at least some of the beetles in the 90° 100° mode of the bright-light curve were killed by the temperature, it seems significant that there is no mode, even a spurious one, at these points in the dim-light curve. Under bright-light conditions the mode is much less pronounced and occurs between 90° and 100°. It appears that under the influence of more intense light the temperature preferendum of these larvae has shifted from  $70^{\circ}$   $75^{\circ}$  to some less well-defined region between  $90^{\circ}$  and  $100^{\circ}$ .

If this is indeed the case, it leads to either one of two conclusions: (1) the preferred temperature is independent of the optimum temperature for these insects, or (2) the optimum temperature may be changed by a change in lighting. By common consensus, the optimum is that temperature at which there is the highest survival and/or the greatest reproduction, and the inference has been made that in general the optimum temperature may vary somewhat under different humidity conditions but is otherwise rather constant. There is no reason to assume, a priori, that the temperature preferendum and the temperature optimum are the same, and some reason to expect that the physiological processes which lead to long life and high reproduction are independent of light. Experimental evidence on this point is lacking, but it appears that the first alternative above may be nearer the truth than the second.



FIG. 4.—Comparison of different pretreatment relative humidities for effect on temperature preference. Dim light.

# EFFECT OF VARIABLES

Evaporation Rate in Gradient Chamber.—Mention has been made of a series of tests in which a moist gradient was used. Two comparable curves are shown in figure 2. The moist-gradient tests were made with a pretreatment of 75%relative humidity and dim light in 15 runs involving 752 beetles. Available for comparison are 12 runs made in the dry gradient but otherwise under the same test and pretreatment conditions, using 585 beetles. The curves show some differences, but not with respect to the point in question here: the preferred temperature isotherm is the same in either a moist or dry gradient.



FIG. 5.-Comparison of different pretreatment relative humidities for effect on temperature preference. Bright light.

Another pair of curves illustrating this comparison are shown in figure 3. Compared here are two bright-light curves of four runs each, 75% R.H. pretreatment. The curves represent 202 beetles each. Under bright-light conditions the modes do not correspond, yet both are decidedly in a higher isotherm than those of figure 2. It is of interest to note that in both figure 2 and figure

3 the curve of the moist gradient shows a disproportionately sharper, higher peak.

Pretreatments.-The two groups of insects compared in figure 1 each received three different pretreatment processes. In the dim-light group, 100 specimens in two runs were pretreated at 50%R.H.: 1,337 specimens in 27 runs were subjected to 75% R.H. pretreatment; and 196 beetles in 4 runs were pretreated at 100% R.H. In the bright-light group, the 50% R.H. pretreatments was represented by 3 runs and 150 specimens; 75% R.H. by 8 runs totaling 404 specimens; and 10 runs of 498 specimens received a pretreatment at 100% R.H. The two sets of three curves each are shown in figures 4 and 5, respectively.

All three dim-light curves (fig. 4) show a principal mode in the 70°-75° isotherm or in a lower isotherm. Of the bright-light curves, only that for the 100% R.H. pretreatment shows a mode comparable to any of the dim-light curves, and the mode of this curve at the  $70^{\circ}$ -75° isotherm is considerably exceeded by the one at  $90^{\circ}-100^{\circ}$ . When the equivalent pretreatment curves are compared between figures 4 and 5, it seems clear (with the possible exception mentioned above) that the difference in temperature preferendum between bright and dim light can not be attributed to the differences in pretreatment.

Sampling.—The two experimental variables in this work have been discussed above and shown to have no bearing on the apparent shift of the preferendum as conditions of lighting changed. There remains the possibility that the two sets of runs under consideration were, after all, merely chance variants of the same population. A ttest for the difference between the bright-light and the dim-light means shows a P < 0.0001, thus indicating a very small probability that these two samples were randomly drawn from a single population which varied with respect to temperature preference.

### CONCLUSIONS

The above data suggest that either (1) the temperature preferendum of the second-instar Mexican bean beetle larva is not a physiological constant associated with the optimum, but is capable of being changed under different lighting conditions, or (2) the temperature optimum may be changed by a change in lighting. The first of these alternatives seems the more probable.

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# FURTHER STUDIES ON THE RELATIONSHIP BETWEEN PARENTAL AGE AND THE LIFE CYCLE OF THE MEALWORM, TENEBRIO MOLITOR

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### ABSTRACT

Eggs were collected at intervals of 2 weeks throughout the egg-laying period from beetles maintained at  $30^\circ$ ,  $25^\circ$  and  $20^\circ$  C. Eggs and larvae were maintained at the same temperature as was used for the parent beetles. Parental age had no effect on the duration of the egg stage or on the weights of the eggs or newly hatched larvae. At each temperature the percentage of eggs which hatched decreased with an increase in parental age, from approximately 90 for those laid during the first 2 months to about 50 for those laid 4 months after emergence. At each temperature, larvae from young parents grew at a slower rate than those from the same parents after they had aged 9 weeks. At  $30^\circ$  there

Ludwig (1956) noted that the duration of the larval stage of the mealworm, Tenebrio molitor Linn., was shorter in offspring produced by old than in those produced by young beetles. This than in those produced by young beetles. study was extended by Tracey (1958), who showed that larvae from old began a rapid increase in weight at an earlier age than those from young parents. She also found that at 25° C., the larval stage was shortened and the number of larval molts decreased with an increase in parental age. In her experiments, the durations of adult life at both 25° and 30° C. were also decreased with an increase in parental age. The present experiments were undertaken to determine at what parental age these effects become evident. In Tracey's experiments, the insects were kept in humidifiers at a relative humidity of 75 percent. In the present work, they were kept outside the humidifier and the effects of direct water feeding, during a part of or during the entire larval stage, on the life cycle were determined.

#### MATERIAL AND METHODS

Newly emerged beetles were kept in chickgrowing mash in specimen dishes at temperatures were no other effects of parental age, but at  $25^{\circ}$  and  $20^{\circ}$  larvae from young parents required a significantly longer time to complete development and had more molts than those from the same parents after they had aged 1 month or longer. At these temperatures, an increase in parental age resulted in a decrease in the duration of adult life. This effect was not evident until the parents had aged 9 weeks in Series A (water was added to the food after 6 weeks of larval life) and 6 weeks in Series B at  $25^{\circ}$  C. (water was added to the food throughout the larval period). In every case larvae from Series B grew at a faster rate and required less time for development than those of Series A.

of 30°, 25°, 20° and 15° C. Moisture was supplied by covering the cultures with damp cloths which were moistened several times a week. After several days, the beetles were transferred to other specimen dishes containing white flour and kept at the same temperatures as the cultures from which they were taken. A bottle of water, plugged with moist cotton, was placed in each Eggs were collected daily by sifting the dish. When a sufficient number of eggs had flour. been obtained, the beetles were returned to the chick-growing mash. After 2 weeks, at each temperature, the beetles were again placed in flour and eggs collected. This process was repeated at intervals of 2 weeks throughout the life of the culture. Hence, at each temperature, eggs were collected at intervals of 2 weeks from the same parents from the start until the end of the oviposition period. The age of the parent beetles was always calculated from the day of emergence.

Groups of eggs obtained at each temperature and from each parental age were weighed. Eggs were placed in humidifiers over a saturated solution of NaCl (relative humidity, 75 percent) and kept at the same temperature as was used for the parent beetles. In each group, determinations were made on the duration of the egg stage and on the percentage of eggs which hatched.

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