Reports

A Relationship between Photoperiod and Cold-Storage Treatment in the Spruce Budworm

Abstract. Exposure of diapausing second-instar larvae of *Choristoneura fumiferana* to continuous light at 21°C, after only 6 weeks' storage at 0°C, induces emergence of larvae that will not emerge in photoperiods of 15 or 18 hours. Following the normal storage period (20 weeks) there is no such effect. Complete dark or 12-hour photoperiods inhibit emergence after storage periods of all lengths.

The spruce budworm, *Choristoneura fumiferana* (Clem.), is a strictly univoltine species, most individuals entering diapause in the second instar without feeding, regardless of the photoperiod to which they have been exposed since the egg stage (*1*). However, as described below, these larvae are sensitive to length of photoperiod after having been stored at a low temperature while yet in their hibernacula. Also of interest is the finding that exposure to continuous light effects the termination of diapause and the emergence of larvae that have received inadequate cold treatment (*2*).

Families of the first laboratory generation of two wild populations were stored at 0°C, the larvae being in their hibernacula in petri dishes (*1, 3*). After 6, 10, 14, and 26 weeks, respectively, families were returned to the rearing room (temperature, 21°C, relative humidity, 70 percent), where each family was split into two equal portions by cutting the gauze-parafilm covering bearing the larvae in their hibernacula. Family fractions thus formed were distributed so that several families were represented in each light treatment. Photoperiods of 12, 15, 18, and 24 hours, respectively, were provided, controlled by a day-night clock switch in the circuit to the light tables (*1*); continuous dark was provided on an adjacent shelf. Emergence took place over freshly thawed balsam fir buds in petri dishes placed on the light tables, the pieces of gauze bearing the unemerged insects being moved to dishes of fresh food at regular intervals (*3*) until all the living larvae had emerged. Emergence was determined by checks of the gauze and by checks of the establishment of larvae on the food after 10 to 14 days.

Some of the important features of the results appear in Fig. 1; only data for 6 and 14 weeks' storage are included here, those for other storage periods being similar. In the figure, values for cumulative emergence, expressed as a percentage of the larvae alive at the end of the storage period, are plotted against time of emergence. A separate curve is shown for each photoperiod, and the points plotted represent the means of several family portions within each treatment.

As is shown in the figure, the initial emergences, after storage periods of both 6 and 14 weeks, were greater for photoperiods of 15 and 18 hours than for photoperiods of 0, 12, or 24 hours. This effect was sufficient to make the mean emergence rates (over the entire emergence period) of larvae subjected to 15- and 18-hour photoperiods significantly higher than the rate for larvae under other treatments (*t* tests on split families). After 26 weeks' storage there were no longer significant differences. The total percentage emergence also was higher for photoperiods of 15 and 18 hours than for photoperiods of 0 or 12 hours, even after 26 weeks' storage. This difference was least pronounced when light treatment followed the shortest storage period. These results definitely show that, after overwintering, the spruce budworm responds to length of photoperiod, both in rate of emergence and in percentage of individuals emerging. That light periods of 15 and 18 hours per day should be the most effective is not surprising, since there already is some evidence that the budworm is a "long-day" insect (*1*).

With an increase in length of storage from 6 to 14 weeks, both the rate of emergence and the percentage of individuals emerging, for 15- or 18-hour photoperiods, increased quite markedly, as was expected. However, longer storage had less effect on the emergence of larvae receiving 0 or 12 hours of light per day. Even after 20 weeks' storage, when there were only negligible differences between the effects of the other treatments (with emergence about 40 percent), emergence for 0 or 12 hours of light per day did not exceed 12 percent. This inhibition of emergence by short days is further evidence of a sensitivity of this insect to length of photoperiod at this stage.

Although emergence was initially slower, the numbers of insects that emerged under continuous-light treatment after short storage periods were considerably greater than the numbers that emerged for any other photoperiod. (The slight reduction in total emergence after 14 weeks' storage probably represents increased mortality as a result of storage; a further reduction was observed after 26 weeks.) It is probable that, after all four storage periods, continuous light induced the eventual emergence of most of the viable insects. Increase in length of storage time reduced the proportion of larvae which, having failed to emerge in 15- or 18-hour photoperiods, emerged under continuous light. After 26 weeks, when the cold-storage requirements of all insects had been met, continuous light was no more effective than photoperiods of 15 or 18 hours. It appears, therefore, that exposure to continuous light during the emergence period in some way overcomes the

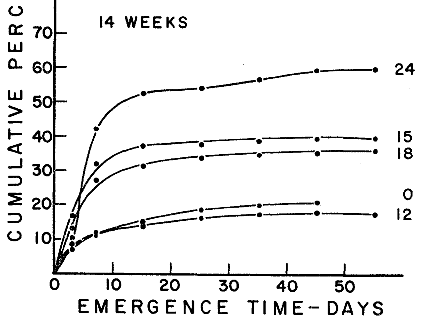

Fig. 1. Comparison of the effects of length of photoperiod on the emergence of spruce budworm after short overwintering times of 6 and 14 weeks.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

effect of insufficient cold treatment. After 6 weeks' storage, at least 10 days of continuous light were needed to effect emergence of larvae which would not emerge in more normal photoperiods. The minimum treatment required was less for families that had been stored an additional 8 weeks. There should be no question, however, about the nature of the diapause in this budworm, since even exposure to continuous light failed to produce appreciable emergence after storage for less than from 4 to 6 weeks.

It is interesting to recall at this point that in budworm stocks selected over several generations for nondiapause, the best response was achieved only when the larvae were exposed to continuous light (*1*). The nature of the effects of continuous light and the relation to the humoral control of diapause merits further investigation. The action of continuous light may normally be deleterious and may possibly be similar to the effects, in *Cecropia* silkworm, of surgical manipulation, during the last few weeks of the chilling period, in hastening the restoration of the brain's endocrine activity (*4, 5*).

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References and Notes

1. G. T. Harvey, *Can. J. Zool.* 35, 549 (1957).
2. This paper is contribution No. 481 of the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.
3. G. Stehr, *Can. Entomologist* 86, 423 (1954).
4. C. M. Williams, *Biol. Bull.* 110, 210 (1956).
5. A paper giving further details of the results and of the effects of various overwintering treatments on subsequent growth and development is in preparation.

16 June 1958

Respiratory Discharge of the Pons

Central respiratory discharges have so far been recorded mainly in the medulla oblongata (*1*), which was known to cause maximal inspiratory or expiratory response by electric stimulation. On the other hand, an involvement of the pons in the neurogenesis of respiration has been widely accepted. A region which inhibits the activity of the apneustic center and so produces normal respiratory rhythm has been called the pneumotaxic center and was located in the anterior pons (*2*).

To localize this area more precisely, electrolytic lesions (*3*) and electrical stimulations (*4*) were used, and it was concluded that the pneumotaxic center lies in the extreme dorsolateral portion of the anterior pontine tegmentum or in the dorsolateral reticular formation of the isthmus of the pons. Rhythmicity of respiration was attributed to two mechanisms which are mutually replaceable—the vagal reflex and the pneumotaxic center—although the mode of firing of the latter remains obscure. Electric sounding of the pons, therefore, may offer a possibility for a more precise localization and may clarify the function of the pneumotaxic center.

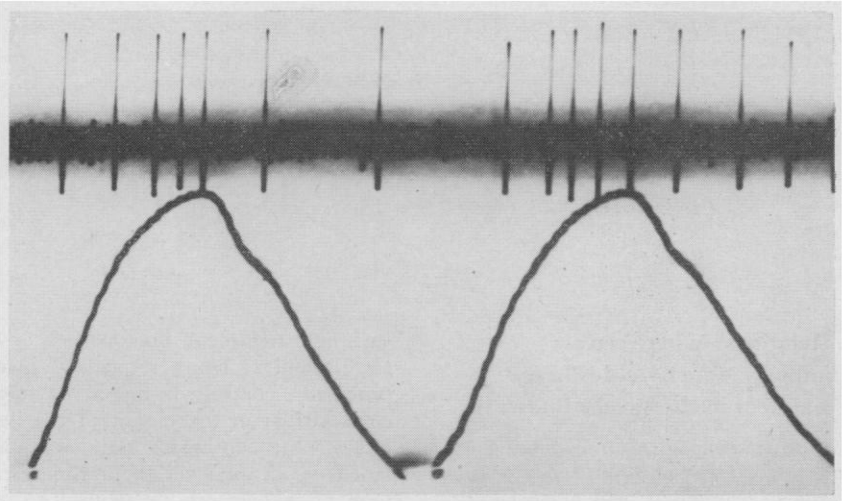

Fig. 1. (Top) Respiratory spike discharges obtained in the pons (9 mm rostral from the obex, 2.5 mm lateral from the mid-line, and at a depth of 2 mm from the surface), and (bottom) respiratory movement (peaks are inspiratory phase).

After anesthetization with urethane (1.2 g/kg), rabbits were tracheotomized, and both carotid arteries were ligatured. Occipital craniotomy was performed, but the cerebellum was left intact. Care was taken to minimize bleeding. The pons and medulla oblongata were explored by means of stereotaxic apparatus carrying a monopolar steel electrode (of about 10 μ diameter), insulated except at the tip. Changes of potential and the respiratory movements of the chest were simultaneously photographed on the screen of a two-beam cathode-ray oscillograph.

Inspiratory and expiratory discharges were obtained in the medulla; bursts or increase in frequency of continuous discharges occurred coincidentally with the inspiratory or expiratory movements. The respiratory discharges were relatively sparse and failed to separate distinctly the inspiratory and expiratory region. Periodic bursts of impulses corresponding with respiratory rhythm, however, were also obtained in the pons at a depth of from 1 to 3 mm from the dorsal surface. The active loci were restricted to a relatively small region—from 2 to 2.5 mm lateral from the mid-line and from 7 to 11 mm rostral from the obex—approximately corresponding to the nucleus reticularis pontis caudalis of Meessen and Olszewski. Pontine respiratory discharges, by contrast with respiratory discharges obtained in the medulla, did not correspond either to the inspiratory or to the expiratory phase but appeared as shown in Fig. 1 in the course of inspiration and disappeared in the course of expiration. The frequency of spikes increased with the progress of inspiration and declined with the onset of expiration. These figures resemble vagal afferent discharge, the frequency of which varied with the degree of lung inflation (*5*). The vagus-like discharges obtained from the pons were composed of positive spikes, while inspiratory and expiratory discharges of the medulla were composed of negative ones. The meaning of this, however, remains, for the present, a matter for speculation.

In the vagotomized rabbit, transection of the mid-pons at the upper end of the acoustic tubercles failed to affect the respiratory rhythm so long as the section was limited to the medial part. Apneustic breathing resulted, however, when the section was made to extend 3.5 mm lateral from the mid-line. The functional similarity of vagal afferent and pneumotaxic centers is well known; hence, the discharge here observed in the pons may be a representation of pneumotaxic activity and function in converting the inspiratory to the expiratory act (*6*).

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3. P. C. Tang, *Am. J. Physiol.* 172, 645 (1953).
4. S. H. Ngai, S. C. Wang, *ibid.* 190, 343 (1957).
5. E. D. Adrian, *J. Physiol. (London)* 79, 332 (1933).
6. This work was reported at the 35th general meeting of the Physiological Society of Japan, held from 3 to 5 May 1958.

5 June 1958