Reference: *Biol. Bull.*, **146**: 11–19. (February, 1974)

PHOTOPERIODIC CONTROL OF DEVELOPMENT IN *CHAOBORUS AMERICANUS* WITH SPECIAL REFERENCE TO PHOTOPERIODIC ACTION SPECTRA

WILLIAM E. BRADSHAW [1]

The University of Michigan and The Biological Laboratories, Harvard University

Despite the profusion of literature on photoperiodism of insects and related forms, there are relatively few reports which provide energy-compensated action spectra (Lees, 1953, 1966, 1971; Norris, Howell, Hayes, Adler, Sullivan, and Schechter, 1969; Bradshaw, 1972). Experiments concerned with the action spectra of photoperiodism in insects may be divided into three categories. The first involves the use of light sources and/or distribution of various wavelengths without regard to intensity (Kogure, 1933; Geyspitz, 1957; Williams, Adkisson, and Walcott, 1965). The second category of experiment establishes action spectra by equalizing energy or flux density of incident photons (de Wilde and Bonga, 1958; Müller, 1964; Harris, Lloyd, Lane, and Burt, 1969). If one is dealing with the appropriate energy or photon density level, this type of experiment is very useful. Often the energy value chosen is too high. Thus, Müller (1964) chose 160 ergs cm^{-2} sec^{-1} and found that the homopteran, *Euscelis,* responded to an entire series of wavelengths from violet to orange; presumably, many of these wavelengths would have been ineffective at lower intensities. The third category of experiments includes the action spectra where energy of flux density and wavelength are varied to give a standard response. It is this type of experiment which gives the most information. Indeed, once one knows the photon efficiency spectrum, then an equal flux density spectrum can be used effectively to monitor changes in sensitivity.

A suitable insect for action spectrum studies is the larva of the non-biting mosquito, *Chaoborus americanus.* Apart from their eyes and hydrostatic organs, the larvae are devoid of pigment and are available in the huge quantities necessary. The overwintering larvae rely upon photoperiodic and trophic signals for the maintenance or termination of diapause (Bradshaw, 1969). Ordinarily, food and long days must be present simultaneously and not sequentially to elicit development. The larvae are polymorphic and in some years, long days alone suffice to terminate diapause among the larger, yellower larvae (Bradshaw, 1973). In any year after prolonged chilling, the larger, yellower larvae will develop if fed on short-day photoperiod. Food contributes to post-diapause survivorship but for the termination of diapause *per se,* feeding constitutes an environmental cue independently of nutrition (Bradshaw, 1970). The present investigation considers responsiveness of diapausing larvae to photoperiod, the spectral sensitivity of this responsiveness, and the transition from photoperiod-dependent to photoperiod-independent development.

[1] Present Address: Biology Department, University of Oregon, Eugene, Oregon 97403.

Materials and Methods

Large numbers of larvae were collected from beneath the ice in George Pond, a small eutrophic kettle hole on the University of Michigan's E. S. George Reserve, near Pinckney, Michigan. The larvae were transferred on the day of capture to open jars and the latter stored in 5° C incubators programmed to provide 8 hours of light per day. No food was added. For experimentation, the larvae were warmed to room temperature and the largest yellow larvae sorted from the rest. The large yellow larvae are the fastest responding morph in the wild population (Bradshaw, 1973) and provided the most uniform results in experiments of the sort presented here. Ordinarily, larvae were exposed to an experimental regimen for only a few days and then placed under short-day conditions without food for an additional 5 to 10 days. The reason for using only a few days exposure to the test regimen lies in the need to conduct experiments during the annual period when the diapausing larvae are most responsive to photoperiod. Further prolongation of each experiment would not have permitted determining the response of the animals over the required range of intensities and wavelengths. Food in all cases consisted of mosquito larvae, *Culex pipiens,* provided in saturating amounts each day. For reasons given earlier (Bradshaw, 1969), the appearance of pupae was used to score the termination of diapause and resumption of development.

Experimental Results

Responsiveness of larvae to photoperiod

The following experiments are intended to assess the range of photophases affecting development. Sample populations of diapausing larvae were provided 12–17 hours of light per day in one hour increments at 23 ± 1½° C. Fifty larvae collected January 20, 1968, were exposed to each test daylength with food for

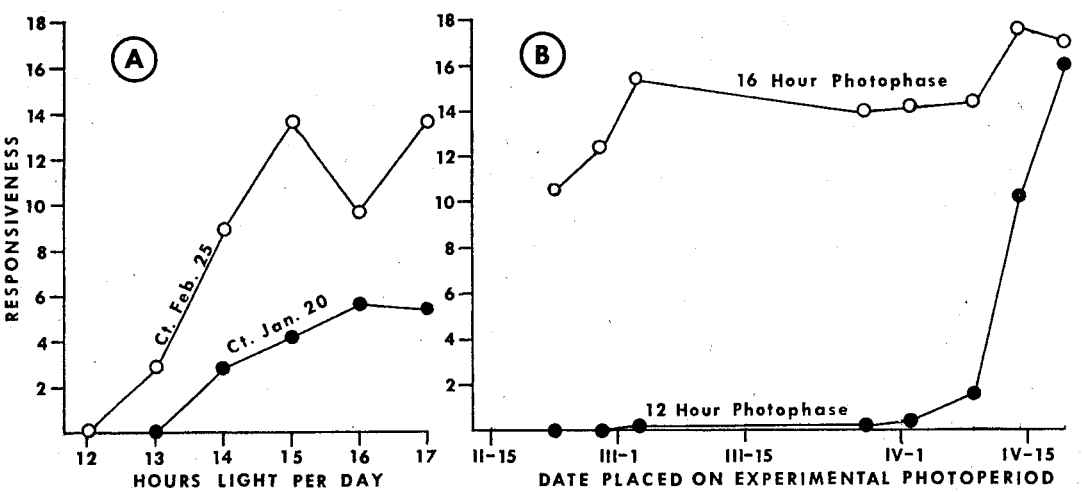

Figure 1. Responsiveness of diapausing larvae to photoperiod. (1A) Responsiveness (per cent pupation per day of exposure to experimental regimen) of larvae caught January 20 and February 25, 1968, and exposed to various photophases April 12 and April 22, 1968, respectively. (1B) Responsiveness of larvae caught February 13, 1969, stored under short-day conditions at 5°C, and exposed to long- or short-day photoperiods on various dates in 1969.

seven days, starting April 12, 1968. Fifty larvae collected February 25, 1968, were exposed to each test daylength with food for five days starting April 22, 1968. After seven and five days, respectively, the food was removed and the larvae placed on a 12L: 12D regimen at 23 ± 1½° C for a total experimental time of 15 days. Development was then scored and the experiment terminated. Responsiveness of larvae was calculated as per cent pupation divided by the number of days exposure to the test daylength.

Figure 1A shows that responsiveness generally increased with longer photophases. At any given daylength, however, the larvae collected earlier in the year were less responsive than those collected at a later date.

Changes in responsiveness during the year were further examined with respect to long- and short-day photoperiods, as defined by the above experiments. Diapausing larvae caught February 11, 1969, were periodically removed from the stock incubator from February 22, to April 17, 1969. One hundred larvae were exposed to either 12 or 16 hours of light per day with food for 5 days at 23 ± 1½° C. The food was then removed and both sets of larvae placed on a 12L: 12D regimen for 10 additional days at the same temperature. After a total experimental time of 15 days, development was scored and the experiment terminated. Responsiveness was calculated as above.

Figure 1B shows that responsiveness to both long and short days with food increased during the year. The change in responsiveness to long-day photoperiod was gradual; that to short-day photoperiod was abrupt. Responsiveness to short days increased tenfold over a 10 day period.

Action spectra

The following experiments test the effectiveness of various wavelengths in provoking the termination of diapause. Diapausing larvae were exposed to a short day of white light (12L: 12D) onto which was added four hours of monochromatic light (Bradshaw, 1972). If the larvae "saw" the colored light, the regimen was interpreted as long-day and pupation ensued; otherwise, the larvae would remain in diapause. For each experiment at each wavelength studied, 50 diapausing larvae were exposed to the test regimen for 5 or 7 days with food at 23 ± 1½° C. The food was then removed and the larvae placed under a short-day regimen at the same temperature. After 5 additional days, development was scored and the experiment terminated. Duplicate long-day (16L: 8D) and short-day (12L: 12D) controls were run in parallel with every experiment. For each wavelength studied, intensity of incident light was varied in equal energy logarithmic increments until the response to monochromatic light was no longer significantly different from the long-day, white-light control (normal variate not significant at the 5% level of confidence).

To allow for changes in responsiveness, error introduced in sorting the animals, and fluctuations in temperature, the raw percentage data were transformed to a standardized percentage value by the equation:

$$\text{Standardized \% Response} = \frac{(\text{raw experimental \%}) - (\text{\% response of short-day control})}{(\text{\% response of long-day control}) - (\text{\% response of short-day control})}$$

A dose-response curve was then calculated for each wavelength using these standardized percentage values. Action spectra were finally constructed for the threshold (arbitrarily, the 10% intercept), 50%, and saturation (arbitrarily, the 90% intercept) responses in the dawn and in the dusk.

Both at dawn and at dusk, diapausing larvae were most sensitive to light at 540 nm in the yellow-green region of the spectrum (Figure 2). For a saturating response, larvae were about two orders of magnitude more sensitive to light at 540 nm in the dawn than in the dusk. The shape of the known portions of the spectrum for these responses is similar to that of saturation, namely, around 540 nm. The action spectra demonstrate that overwintering larvae are extremely

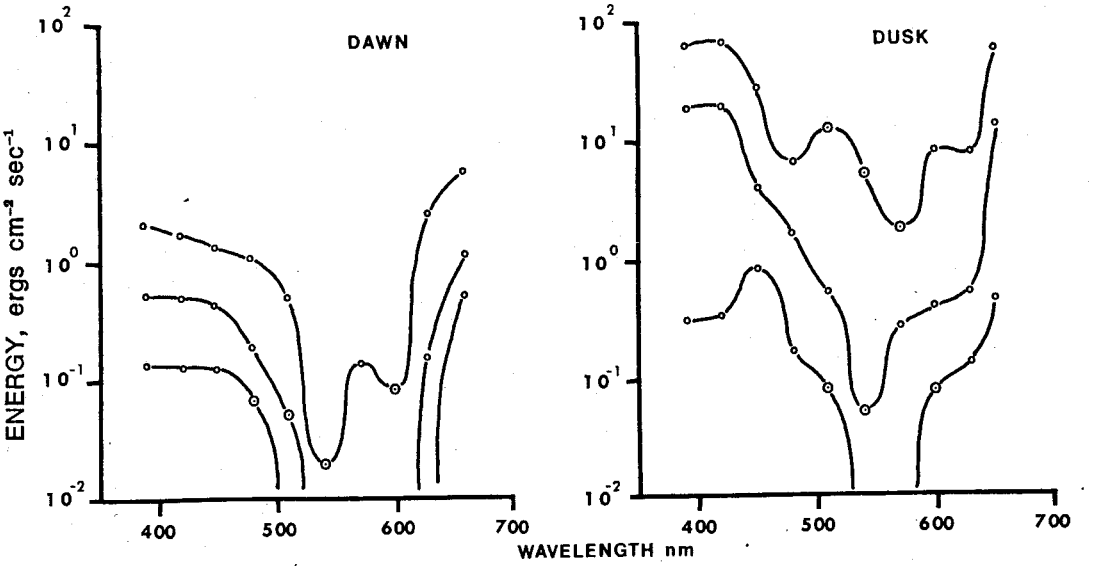

FIGURE 2. Action spectra for photoperiodic induction of development in response to dawn and dusk pulses of monochromatic light. Energy necessary to promote a threshold (bottom curve), 50% (middle curve), or saturation (top curve) response is plotted as a function of wavelength; open circles, interpolated point; dotted circles, extrapolated point.

sensitive to small quantities of light at 540 nm, probably less than 10^{-2} ergs cm^{-2} sec^{-1}.

Sensitivity to light during the dark period

Sensitivity to light during the dark period was assessed by providing asymmetric skeleton photoperiods. Samples of 50 larvae without food were exposed to eight hours of white light plus a 30 minute white-light pulse at various times during the dark period. Thus, successive samples would receive 8L: ½D: ½L: 15D, 8L: 1D: ½L: 14½D, . . . 8L: 15D: ½L: ½D. Two control experiments of 100 larvae each were run concurrently and consisted of a 17L: 17D regimen without food. Larvae experienced asymmetric skeleton or control photoperiods for 10 days at 23± 1½° C after which they were transferred to short-day photoperiod (12L: 12D) at the same temperature. Development was scored after 5 additional days and the experiment terminated.

As shown in Figure 3, 30 minute light breaks were most effective at two times during the dark period. Seventy-six and 71 per cent of the larvae were stimulated to pupate by light pulses ending 14½ and 16½ hours after dawn, respectively. A higher percentage of larvae were stimulated to develop by 17 hours of continuous illumination per day than by any asymmetric skeleton photoperiod.

Discussion

Previous data (Bradshaw, 1969) indicated only that the transition from photoperiod-dependent to photoperiod-independent development in *C. americanus* occurred between February and Late April. The results in Figure 1B show that the transition took place over a 10 day period in early April, 1969. Bradshaw (1969) proposed that the adaptive significance of the shift in photoperiod dependency relates to the shallow pond habitat of *C. americanus*. A thaw accompanied by a plankton bloom may take place in January or February. Refreezing of the pond can follow such a thaw through April. Major reliance upon photoperiodic cues during the winter thus insures against development while the pond is likely to refreeze; when an unfrozen pond is likely to persist, increased responsiveness to trophic cues ensures that *C. americanus* will be able to capitalize on the large spring plankton blooms.

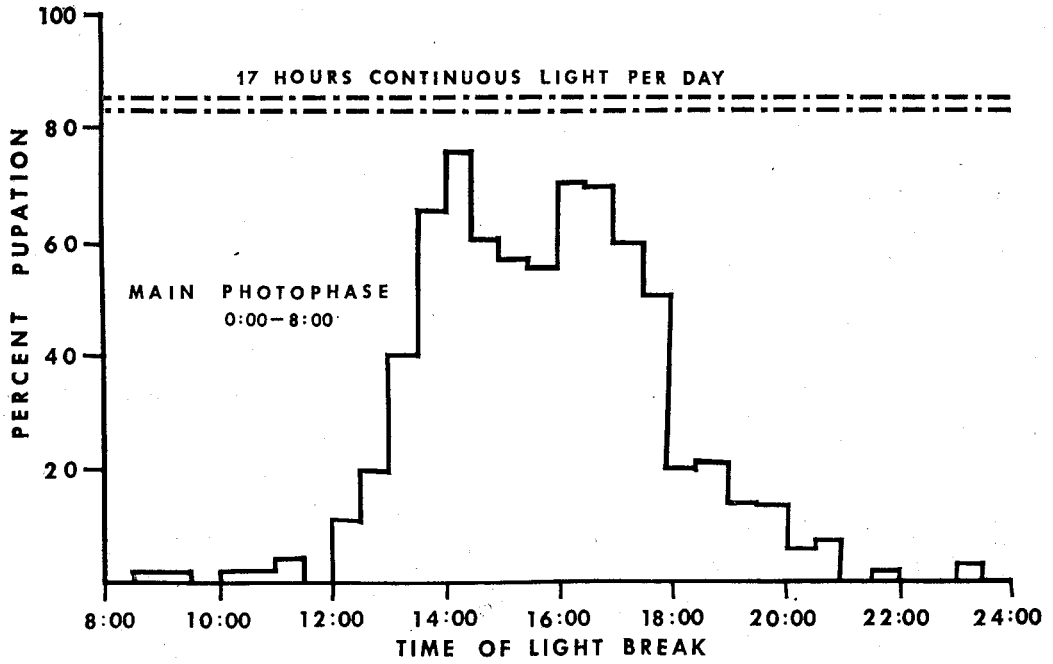

Figure 3. Development in response to half-hour light breaks during a 16 hour dark period of an otherwise short-day regimen (8L:16D). The broken lines at the top of the figure represent the response of two control populations exposed to continual long days (17L:7D).

Apart from affecting the qualitative shift in cues relied upon for development, chilling has a more quantitative effect on photoperiodism. As seen in Figure 1A, chilling enhances responsiveness to long and intermediate photophases. The im-

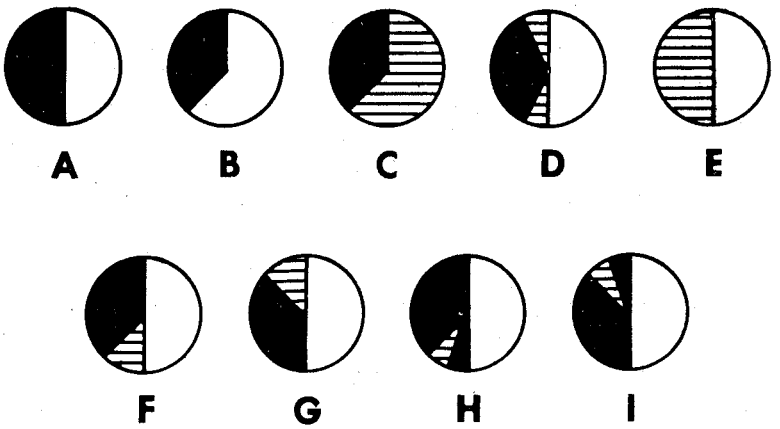

FIGURE 4. Experimental designs for determination of action spectra. Black indicates dark period; shaded, experimental monochromatic light; open, white light; A, hypothetical short day, no response; B, hypothetical long day, full response; C, action spectrum determined with a monochromatic long day in conjunction with a short night; D, action spectrum determined with both dawn and dusk extentions of a white-light short day; E, action spectrum determined with a monochromatic "night" and a white-light short day; F, G, action spectra determined with monochromatic extentions of a white-light short day into long day in the dusk and dawn, respectively; H, I, action spectra determined with monochromatic light breaks at the sensitive period early and late in the subjective night, respectively.

portant implication is that both the critical photoperiod and the number of days required for the termination of diapause are decreasing during the winter and early spring. At the same time, environmental daylengths are rapidly increasing. The net result is to endow the intrinsic accuracy of the photoperiodic clock with increased precision. Consequently, the transition from diapause-maintaining to diapause-terminating daylengths should take place more rapidly under natural conditions than is implied by response to static daylengths in the laboratory.

Given a photoperiodic system, there are many ways to set up an energy-compensated action spectrum (Fig. 4). Each of these methods has its own limitations. If one assumes that a short-day response is evoked by a regimen such as 4A and a long-day response by 4B, then an action spectrum could be determined by substituting monochromatic light for white light (4C), for dark (4E), or by adding short bursts of monochromatic light to both ends of a white-light, short-day regimen (4D). These three methods have the drawback that they only assay for wavelengths and intensities common to both dawn and dusk. Both peak and level of sensitivity could easily change during the day and would not be revealed by a spectrum derived by methods 4C–4E. Furthermore, 4C and 4E do not give a long-day, short-day alternative but rather constant dark *vs* long days (4C) or constant light *vs* short days (4E). Two separate spectra are necessary: one at either end of the white-light photophase. Such spectra would be obtained by regimens 4F and 4G. Alternatively, since light breaks during the dark period of an otherwise short-day regimen (asymmetric skeleton photoperiods) are capable of mimicking an entire photophase (Bünning and Joerrens, 1960; Adkisson, 1963; Pittendrigh and Minis, 1964), comparable spectra could conceivably be obtained by the regimens 4H and 4I. Barker, Cohen, and Mayer (1964) and Lees (1966, 1971) have used this concept. Unfortunately, action spectra defined by light

breaks as in 4H and 4I have several limitations. First, the periods of sensitivity must be well defined to make certain one is providing light during the sensitive period; secondly, a whole family of action spectra must be constructed to account for time-intensity reciprocity as observed by Lees (1966, 1971); and thirdly, the period of time between lights-off and the pulse of monochromatic light in the early subjective night may allow the receptor pigment system to dark-adapt. Consequently, the spectra in the present study were determined by the methods in Figure 4F and 4G.

The spectra in Figure 2 show that the photoperiodic clock in *C. americanus* is most responsive to 540 nm light both during the dawn and during the dusk. No other energy-compensated spectra have been determined for other insects using dawn extentions of monochromatic light. There are other energy-compensated spectra using dusk extentions which show that insects may generally have a peak sensitivity in the blue region of the spectrum (Norris, *et al.,* 1969; Harris, *et al.,* 1969). The reason why peak sensitivity in *C. americanus* does not lie further towards the blue probably relates to the larval habitat—characteristically small, shallow Nearctic ponds. In the spring, summer, and early fall, the smallest of these ponds may become covered by the broad-leaved canopy. Larger ponds are usually subject to heavy growths of the green algae, *Nitella* and *Chara,* as well as dense overgrowths of duckweed, *Lemna.* The peak sensitivity of *C. americanus* at 540 nm thus lies where the overlying chlorophylls, carotenes, and xanthophylls are absorbing at a minimum (Rabinowitch and Govindjee, 1969, pages 106–107).

Different action spectra for dawn and dusk would not distinguish between separate pigment systems and distinct photoisomers of the same system. In the case of *C. americanus,* the similarity of action spectra at dawn and at dusk argues that similar or identical photoisomers of a single pigment complex underlie both the dawn and dusk responses. The data presented here are, however, not sufficient to speculate on the type of pigment involved.

The increase in sensitivity at dawn (Fig. 2) could be the result of either an endogenous sensitivity rhythm in the receptor pigment system or light-adaptation during the day. The response of *C. americanus* to asymmetric skeleton photoperiods (Fig. 3) provides the basis for resolving these two possibilities. If the larvae were intrinsically more sensitive to light in the dawn, light breaks in the late subjective night would have elicited more development than those in the early subjective night. The results in Figure 3 show that sensitivity in the late subjective night is no different from or lower than that in the early subjective night. The peak in response at 16½ hours is lower than that at 14½ hours and there is less area under the curve between midnight and dawn than there is between dusk and midnight. The simplest explanation is that of a single pigment system in which the light-adapted pigment is less responsive to light than the dark-adapted one. In Figure 3, the 6 hours of light intervening between lights-off and the first peak in responsiveness permits the pigment to dark adapt. Responsiveness in the early subjective night is then equal to that in the late subjective night. Winfree (1972) has observed a slow dark-adaptation in the resetting of the ecdysis clock in *Drosophila* after transferring pupae from constant light to constant dark. In the case of *Drosophila,* response to phase shifting stimuli required about three days to dark-adapt by an order of magnitude. Though the response of *C. americanus*

suggests a faster and greater degree of dark-adaptation, the idea of dark-adaptation by a biochronometric system is not novel. One wonders if dark-adaptation plays a predominate role in determining the sensitivities of photoperiodic mechanisms under natural conditions. If so, action spectra of interrupted nights as performed by Lees (1966, 1971) may have major implications on the physiology of photoperiodism but the extended day technique used in the present study will be more valuable in gaining insight into the ecology of photoperiodism.

I thank Drs. Richard Cellarius, Hiroshi Ikuma, Peter Kaufman, Tahir Rizki, David Shappirio, and Conrad Yocum for their helpful counsel, Messrs. John Herkhof, James Mite, and Stephen O'Neill for their valuable technical assistance, and Drs. Christina Holzapfel, James Truman, and Carroll Williams for their consideration of the manuscript in its present form. Support from the National Institutes of Health in the form of Grants GM06101 to David Shappirio and 2 F02 GM41926 and 5 T01 GM00989 to the author are gratefully acknowledged.

Summary

1. Responsiveness of diapausing *Chaoborus americanus* larvae to photoperiod increases with chilling. Both the critical photoperiod and the number of long or intermediate days required to terminate diapause decrease at the time of year when environmental photophases are rapidly increasing. Consequently, the transition from diapause-maintaining to diapause-terminating daylengths probably takes place faster in nature than is indicated by responsiveness to static photoperiods in the laboratory.

2. After prolonged chilling, short days no longer maintain diapause and food alone suffices to evoke development. The switch from photoperiod-dependent to photoperiod-independent development takes place over only a ten day period.

3. Energies of monochromatic light from 390 to 660 nm required to elicit 10%, 50%, and 90% development were determined by extending an otherwise white-light, short-day regimen to long-day with monochromatic light at dawn or at dusk. Monochromatic light at 540 nm was most effective at evoking development both during dawn and dusk but larvae were about two orders of magnitude more sensitive during the dawn.

4. Response to half hour light breaks during an otherwise short-day regimen suggests that the relative insensitivity at dusk is due to light-adaptation of the receptor pigment system during the white-light day. Greater insight into the ecology of photoperiodism will therefore probably be derived from action spectra based on the extended day technique than from those based on light breaks during the dark period.

LITERATURE CITED

Adkisson, P. L., 1963. Time measurement in the photoperiodic induction of diapause in the pink bollworm. *Texas Agric. Exp. Stat. Progr. Rep.*, **2274**.

Barker, R. J., C. F. Cohen, and A. Mayer, 1964. Photoflashes: a potent new tool for control of insect populations. *Science*, **145**: 1145–1197.

BRADSHAW, W. E., 1969. Major environmental factors inducing the termination of larval diapause in *Chaoborus americanus* Johannsen (Diptera: Cluicidae). *Biol. Bull.*, **136**: 2–8.

BRADSHAW, W. E., 1970. Interaction of food and photoperiod in the termination of larval diapause in *Chaoborus americanus* (Diptera: Culicidae). *Biol. Bull.*, **139**: 476–484.

BRADSHAW, W. E., 1972. Action spectra for photoperiodic response in a diapausing mosquito. *Science,* **172**: 1361–1362.

BRADSHAW, W. E., 1973. Homeostasis and polymorphism in vernal development of *Chaoborus americanus. Ecology,* **54**: 1247–1259.

BÜNNING, E., AND G. JOERRENS, 1960. Tagesperiodische antagonistiche Schwankungen der Blau-violett- und Gelbrot-empfindlichkeit as Grundlage der photoperiodischen Diapause-Induktion bei *Pieris brassicae. Z. Naturforsch.*, **15**: 205–213.

GEYSPITZ, K. F., 1957. The mechanism of acceptance of light stimuli in the photoperiodic reaction of Lepidoptera larvae. *Zool. Zh.*, **36**: 548–560.

HARRIS, F. A., E. P. LLOYD, H. C. LANE, AND E. C. BURT, 1969. Influence of light on diapause in the boll weevil. II. Dependency of diapause response on narrow band of visible radiation and a broad band of infrared radiation used to extend the photoperiod. *J. Econ. Ent.*, **62**: 854–857.

KOGURE, M., 1933. The influence of light and temperature on certain characters of the silkworm, *Bombyx mori. J. Dept. Agric. Kyushu Univ.*, **4**: 1–93.

LEES, A. D., 1953. Environmental factors controlling the evocation and termination of diapause in the fruit tree red spider mite, *Metatetranychus ulmi* Koch (Acarina: Tetranychidae). *Ann. Appl. Biol.*, **40**: 449–486.

LEES, A. D., 1966. Photoperiodic timing mechanisms in insects. *Nature,* **210**: 986–989.

LEES, A. D., 1971. The relevance of action spectra in the study of insect photoperiodism. Pages 372–380 in M. Menaker, Ed., *Biochronometry.* National Academy of Sciences, Washington, D.C.

MÜLLER, H. J., 1964. Über die Wirkung verschiedener Spektralbereiche bei der photoperiodischen Induction der Saisonformen von *Euscelis plebejus* Fall. (Homoptera: Jassidae). *Zool. Zb.*, **70**: 411–426.

NORRIS, K. H., F. HOWELL, D. K. HAYES, V. E. ADLER, W. N. SULLIVAN, AND M. S. SCHECHTER, 1969. The action spectrum for breaking diapause in the codling moth, *Laspeyresia pomonella* (L.), and the oak silkworm, *Antheraea pernyi* Guer. *Proc. Nat. Acad. Sci. U. S.*, **63**: 1120–1127.

PITTENDRIGH, C. S., AND D. H. MINIS, 1964. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Amer. Natur.*, **98**: 261–294.

RABINOWITCH, E., AND GOVINDJEE, 1969. *Photosynthesis.* John Wiley, New York, 273 pp.

WILDE, J. E. DE, AND H. BONGA, 1958. Observations on threshold intensity to different wavelength of photoperiodic response in the Colorado beetle (*Leptinotarsa decemlineata* Say). *Entomol. Exp. Appl.*, **1**: 301–307.

WILLIAMS, C. M., P. L. ADKISSON, AND C. WALCOTT, 1965. Physiology of insect diapause: XV. The transmission of photoperiod signals to the brain of the oak silkworm, *Antheraea pernyi. Biol. Bull.*, **128**: 497–507.

WINFREE, A. T., 1972. Slow dark-adaptation in *Drosophila's* circadian clock. *J. Comp. Physiol.*, **77**: 418–434.