hemolymph and the solvent for NEM and for L-cysteine hydrochloride was 0.51*M* NaCl solution buffered at *p*H 7.8 with 0.01*M* tris(hydroxymethyl) aminomethane. The electrical conductivity of this diluent was approximately that of hemolymph from a horseshoe crab kept in artificial sea water (*7*). L-Cysteine HCl solutions were adjusted to *p*H 7.8 with 0.51*M* NaOH. The NEM concentrations of standard solutions were checked spectrophotometrically (*8*). All materials were maintained at 22°C. The diluted hemolymph was transferred immediately to a glass tube, agitated, and observed. The time required for macroscopic agglutination was recorded. Five minutes after collection an estimate of the relative number of agglutinated cells was obtained microscopically. Inhibition of agglutination was considered complete when the hemocytes, packed by centrifugation, could be suspended again as free cells 6 minutes after collection.

The experiments are summarized in Fig. 1. As previously reported (*2*), agglutination occurred spontaneously in 10 to 20 seconds in the absence of inhibitors. By the end of 5 minutes, more than 98 percent of the hemocytes were agglutinated. With concentrations of NEM greater than $0.9 \times 10^{-3}M$, agglutination time was prolonged and the number of agglutinated cells was decreased. Inhibition of agglutination was complete in three-fourths of the studies when either $9 \times 10^{-3}M$ or $11.1 \times 10^{-3}M$ NEM was used. In the other studies at these concentrations, less than 5 percent of the cells were agglutinated after centrifugation and suspension.

Several reagents for arresting *Limulus* hemocyte agglutination have been described. These include neodymium chloride, lanthanum nitrate, ammonium sulfate (*2*) and formaldehyde (*9*). Although effective, the use of these materials sheds little light on the mechanism of hemocyte agglutination. The inhibitory action of NEM on hemocyte agglutination indicates a possible role of sulfhydryl groups in the process. The specificity of NEM could be questioned because of the relatively high concentrations required. However, when hemolymph was collected in the test mixture which contained equimolar concentrations of $9 \times 10^{-3}M$ cysteine and NEM, hemocyte agglutination occurred. Cysteine and NEM react stoichiometrically (*8*). Cysteine alone had no effect on the hemocyte agglutination. Thus these experiments support the concept that NEM inhibits hemocyte agglutination by reacting with sulfhydryl groups.

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Daily Rhythm in the Susceptibility of an Insect to a Toxic Agent

Abstract. *Adult boll weevils exhibited a daily rhythm in their susceptibility to standardized doses of the insecticide, methyl parathion. The mortality produced by the insecticide was intimately related to the time of day at which the toxicant was applied. The rhythm appeared to be photoperiodically entrained and, regardless of the length of day or "clock time-of-day of treatment," a period of greatest resistance always occurred at dawn and recurred at 6-hour intervals throughout the 24-hour cycle. The greatest difference in response occurred in a photoperiod having 10 hours of light per 24-hour cycle. Here, the same dose of methyl parathion killed approximately 10 percent of the weevils treated at dawn but almost 90 percent of those treated only 3 hours later.*

A daily rhythm in the susceptibility of mice to certain toxic agents has been reported by Halberg and associates (*1*). In one series of experiments, these authors observed differences in mortalities ranging from 3 to 85 percent in standardized groups of mice injected with equivalent dosages of *Escherichia coli* endotoxin. The differences in mortality were related to the time of day during which the various groups of mice were injected.

The possibility that a similar phenomenon might occur in the reaction of insects to insecticides was a subject of discussion between us and C. S. Pittendrigh, of Princeton University, during his visit to our laboratory in 1963. In this conversation, as in his publications concerning circadian rhythms (*2*), Pittendrigh stressed the importance of the photoperiod in the entrainment of daily rhythms. Consequently, the following experiments were conducted.

The adult boll weevil, *Anthonomus grandis* Boh., was selected as the test animal, and methyl parathion (O, O-dimethyl-O-*p*-nitrophenylphosphorothioate) as the toxicant. Methyl parathion, a powerful cholinesterase inhibitor, is extremely toxic to the boll weevil even at low concentrations. The compound is an excellent contact insecticide, kills quickly, and has short residual properties. These qualities made this toxicant an appropriate choice for our study.

Adult weevils were reared from flower buds of infested cotton, growing from nearby fields. Buds containing the immature stages of the insect were collected and placed in special cages and held at room temperature under the day and night conditions of midsummer at College Station, Texas. On the day of emergence, the adult weevils were collected and transferred to incubators that automatically maintained particular photoperiods. Three photoperiods, having light phases of 10, 12, and 14 hours per 24-hour cycle, were used. Temperature in all cases was maintained at 27°C. Homogeneous groups of weevils were entrained to each of the three photoperiods for 10 days prior to insecticidal treatment.

Insecticide treatments were made by placing the weevils on glass surfaces coated with standardized amounts of methyl parathion. To accomplish this, the inner surfaces of glass vials (30-ml capacity) were uniformly coated with 0.85 ml of an acetone solution containing 0.0025 percent methyl parathion (technical grade, 80 percent purity). The vials were coated 30 minutes before use, and the solvent was evaporated. At 3-hour intervals beginning with "dawn," or lights on, and continuing throughout the day and night,

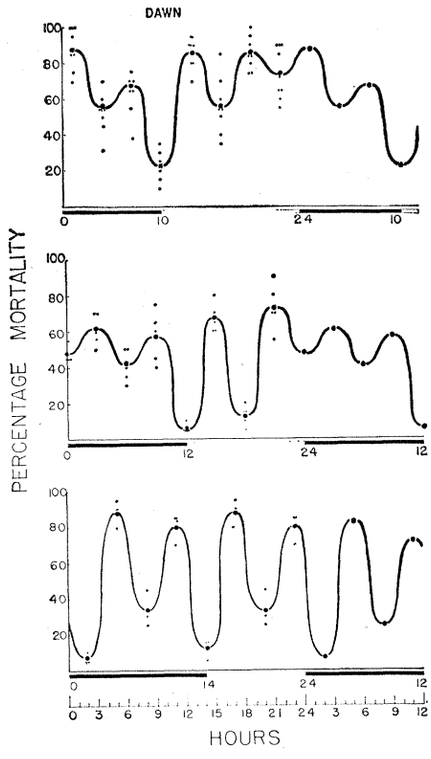

Fig. 1. Susceptibility of the adult boll weevil to methyl parathion as related to time of day of treatment. Curves connecting the large black dots are drawn through the mean percentage mortality produced by each series of treatments. The beginning of the dark period is designated as hour "0"; however, for clarity a second dark period has been plotted. "Clock time" for the dawn of each photoperiod was as follows: 14-hour photophase 6:00 a.m.; 12-hour, 7:00 a.m.; and 10-hour, 9:00 a.m.

groups of weevils were exposed to the same dose of methyl parathion for a 15-minute period. At the end of this period, the weevils were removed from the treated surfaces and returned to the original photoperiod. Treatments made at night were accomplished with the aid of a red photographic light. The insecticide treatments were repeated three to eight times on new groups of weevils over a period of several days. Mortalities were based on records made 24 hours after treatment and were adjusted, by Abbott's formula (*5*), for the small percentages of natural mortality occurring in the untreated control groups.

The weevils showed a definite daily rhythm in their susceptibility to methyl parathion (Fig. 1). Under each photoperiod, the peaks of resistance or susceptibility recurred approximately every 6 hours. Although the basic pattern of the response curve appeared to be the same under each of the photoperiods, the amplitudes of the curves apparently were influenced by the length of the photophase, the daily differences in degree of susceptibility becoming greater as the light periods were shortened. Thus the most dramatic difference occurred under the photoperiod with a 10-hour light cycle. Here the same dose of methyl parathion killed approximately 10 percent of the weevils treated at dawn but almost 90 percent of those treated only 3 hours later.

There also was evidence that the rhythm was entrained to the daily photoperiod. In all photoperiods, the weevils showed a great degree of resistance to methyl parathion at dawn (the beginning of the light period). This has more significance when one realizes that the experiment was conducted so that the clock time of dawn, or lights on, of each photoperiod was as follows: 14-hour photophase, 6:00 a.m.; 12-hour photophase, 7:00 a.m.; and 10-hour photophase, 9:00 a.m. Thus doses of methyl parathion applied at the same clock time, 6:00 a.m., killed slightly more than 20 percent of the weevils entrained to the 14-hour photophase, but nearly 80 percent of those entrained to the 10-hour light cycle. Under these photoperiods, the 6:00 a.m. treatment occurred at dawn of the 14-hour photophase but 3 hours before the dawn of the 10-hour light cycle. This seems to be sufficient proof that it was not the clock time of the day at which the insecticide was applied that was important in determining the susceptibility of the boll weevil to methyl parathion. Rather, it was the phase in the daily rhythm during which the insect was exposed to the toxicant that was important. And this, in turn, apparently was related to the time of occurrence of dawn in each of the photocycles tested.

The cause for this rhythm of susceptibility to the toxicant is not known. Because methyl parathion is a powerful inhibitor of cholinesterase, the results of our study may reflect a cycling of the levels of this enzyme in the boll weevil. However, the daily activity patterns of the insect, or differences in rates of penetration, or absorption, or translocation of the insecticide to the appropriate sites of action may also be important.

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Photoperiodic Reversibility of Diapause Induction in an Insect

Abstract. *The diapause of the pink bollworm is under photoperiodic control. Diapause is prevented when the dark phases of the daily photoperiod are 8 to 10 hours in duration. If the dark period is extended to 12 hours, diapause is induced. Intercalation of 8- or 10-hour nights may reverse the diapause induction caused by exposure to photocycles having 12-hour dark phases. The 10-hour night was much more effective in reversing induction than the 8-hour night. The intensity of diapause, in part, appeared to be dependent on the previous photoperiodic experiences of the test animals.*

The larval diapause of the pink bollworm, *Pectinophora gossypiella* (Saunders), is under photoperiodic control and occurs when nights are 11 hours or longer (*1*, *2*). Moreover, diapause caused by exposure of the early larval instars to long nights can be reversed for the most part by subsequent exposure of the late instars to short nights. Larvae so treated usually proceed to pupation without any delay in development. This clearly suggests that early inhibition of the endocrine processes controlling development may be released later by the appropriate stimulus. The present study was designed to help elucidate the action of photoperiod on the endocrine system.

Pink bollworms were reared on a cottonseed meal diet fortified with 1 percent Wesson Oil (by weight) (*1*). Newly hatched larvae were placed on the diet and exposed to particular photoperiods at 27°C. Each treatment was replicated three times, and 50 larvae were tested each time. The larvae were held under the test photoperiods for 16 days, long enough for development to proceed well into the last larval instar. At the end of this period, the larvae were transferred to