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MANIPULATION OF PHOTOPERIOD TO CONTROL INSECTS

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ABSTRACT

Studies are being carried out in our laboratories on the physical and biochemical mechanisms involved in diapause. The manipulation of photoperiods to prevent diapause in the fall or to break diapause prematurely in the spring could perhaps be developed as a supplementary method of control along with other non-insecticidal methods such as the use of the sterile male technique, sex pheromones, predators and parasites, etc. in integrated control of insects.

In action spectrum studies, we have determined that the blue region of the spectrum (400-500 nm) is most effective in the termination of diapause and probably in its prevention also. The light acts on the midportion of the brain, and a pigment or pigment-protein is almost surely involved as the initial stimulus even before brain hormone, juvenile hormone, or ecdysone are implicated. Our studies have shown that insect brains contain small amounts of pigments having absorption peaks mainly in the 400-500 nm region. Me have not yet been able to determine which among the many pigments (or pigment-proteins) present in Insects such as hemes, bile pigments, pterldlnes, carotenoids, oftmochromes, etc. is involved.

Preliminary outdoor experiments carried out to determine the feasibility of controlling diapause with light have given promising results. Experimental plots were illuminated in the fall with fluorescent lamps or mercury vapor lights containing a high percentage of blue light to prolong the apparent length of daylight, thus conditioning the insects to react as though exposed to summertime photoperiods. Diapause was prevented in 70-90% of European corn borer larvae (Ostrinia nubilalis H.) in corn plants and of codling moth larvae (Laspeyresia pomonella L.) in apples.

There are six main approaches to pest control which are outlined in Table 1 (15). Studies are being carried out in our own laboratory on basic aspects of photoperiodism and diapause, with the aim of developing still another method for the control of insects by manipulation of the photophase or by utilization of related biochemical phenomena.

Table 1.--Approaches to pest control.

1. Conventional pesticides.
2. Resistant crops.
3. Cultural and mechanical methods.
4. Biological (parasites, predators, pathogens).
5. Attractants (physical, chemical baits, pheromones).
6. Genetic methods (sterile male, chemical sterilant).

In temperate climates, many insects diapause (or hibernate) over the winter, while the term aestivation is more commonly applied to the state in which they can survive long dry periods in warmer climates (2, 23, 24). Some of the most important characteristics of diapause include (1) the absence of or a low level of feeding and movement, (2) lowering of metabolism, (3) decrease in the levels of oxidizing enzymes, (4) decrease in body water content, (5) increase in fatty reserves, and (6) resistance to low temperatures. These characteristics are protective mechanisms which enable the insects to survive low temperatures and the lack of food during the winter (2, 6a, 23, 24).

Our particular aim has been to find means of preventing diapause in the fall or of terminating it prematurely in the spring. In either case, the population of insects will be reduced because nondiapausing stages of insects will either die from the cold or starve to death. It is obvious, however, that even though such a method were economically feasible, it would almost certainly have to be used in conjunction with one or more of the other methods of insect control in an integrated attack.

Diapause can be induced by subjecting the insect in the susceptible stage of development to an appropriate regimen of light (photophase) and darkness (scotophase), usually about 13 hr or less of light and about 11 hr or more of darkness in a 24 hr day. Conditions in late summer and fall, as the length of daylight becomes shorter, are therefore conducive for the induction of diapause. Fig. 1 illustrates the effects of various photoperiodic regimens on the incidence of diapause in 4 pest species, the oriental fruit moth, Grapholitha molesta (Busck), the pink bollworm, Pectinophora gossypiella (Saunders), the cotton bollworm, Heliothis zea (Boddie), and the boll weevil, Anthonomus grandis (Boheman). Between 21°C and 27.5°C diapause is most effectively induced with 11-13 hr of daylight per day; shorter or longer periods of light than this are usually less effective or are ineffective in inducing diapause.

Although diapause can be induced by exposure of susceptible insects to light periods corresponding to the length of the daylight in the fall (short daylight periods), it has been found (1, 2, 4) that the use of light interruptions or light breaks interjected into the dark periods at an appropriate time can actually reverse this effect and prevent the insect from going into diapause. Similar effects on the growth and flowering of plants had been observed by Bünning and others (6). Light breaks can vary from a minute or two to an hour or two depending on the species of insect and the temperature. The classical work of

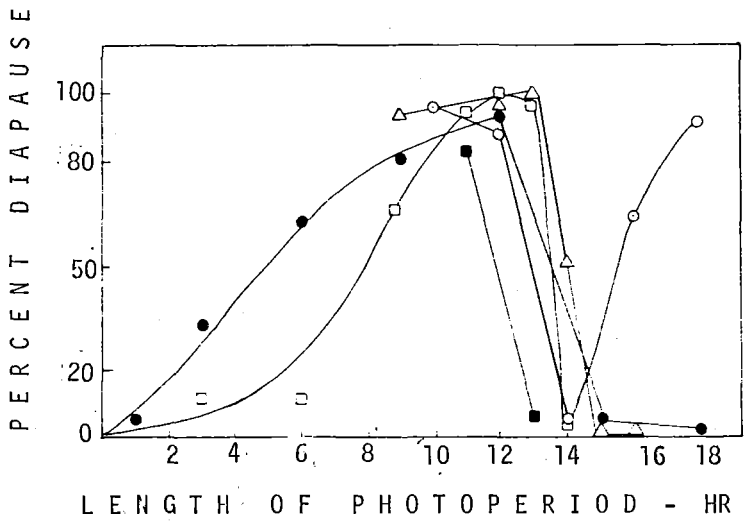

Fig. 1.--Diapause response of 4 species of insects to varied light regimens. Redrawn from original data as follows: □ Oriental fruit moth larvae - 24°C, Dickson (1949); ● Oriental fruit moth larvae - 26°C, Dickson (1949); ⊙ pink bollworm larvae - 27.5°C, Lukefahr et al. (1964); △ cotton bollworm larvae - 25°C, Goryshin (1958); ■ boll weevil, all stages, 21°C, Earle and Newsom (1964).

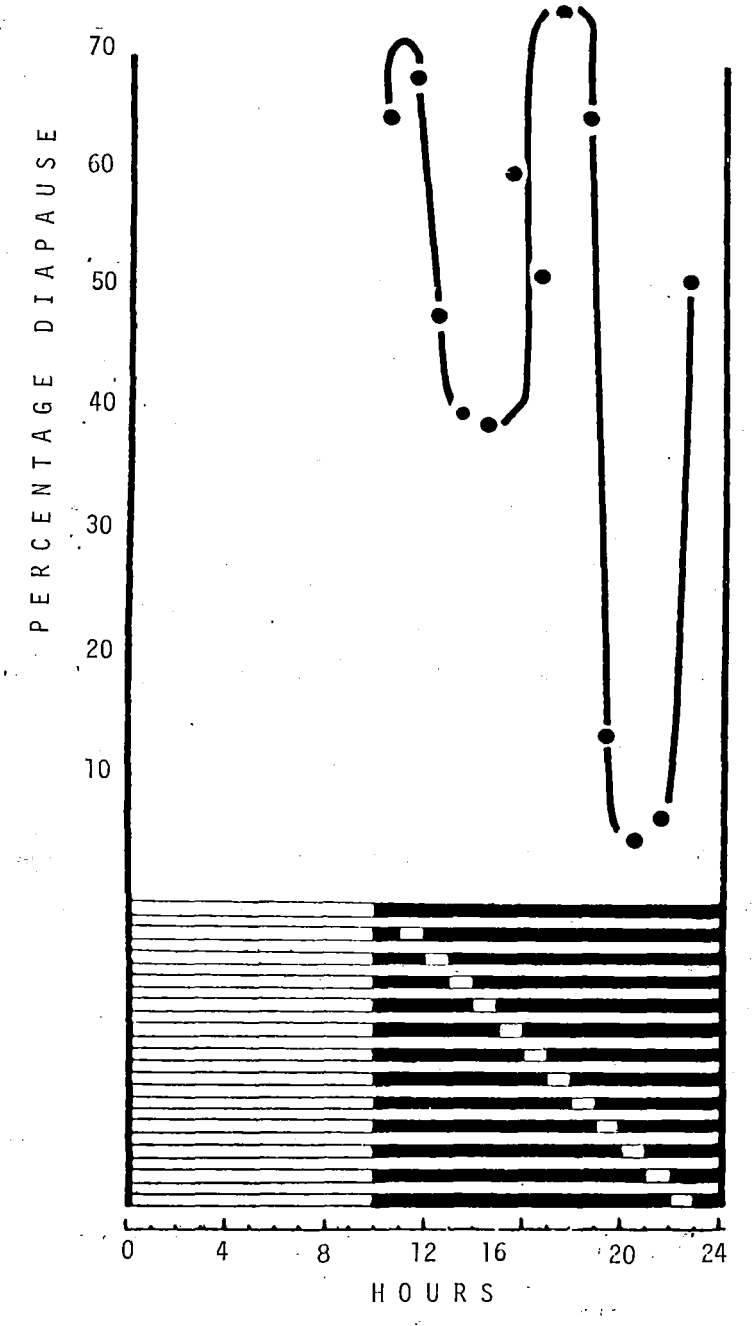

Fig. 2.--Effect of 1 hr light interruptions during a 14 hr dark period on the incidence of diapause in the pink bollworm. Note that maximum response occurred about 14 to 16 hrs after dawn (hr zero) and about 10 hrs after dusk (hr 12) (from Adkisson, 1963).

Adkisson (1, 2) (Figure 2) illustrates the effect of 1 hr light breaks at different times of the night on the incidence of diapause in the pink bollworm.

Diapause of many species is terminated in nature by the insects having been chilled to low temperatures during the winter and then being brought up to the warmer spring-time temperatures, with the increase in daylight playing little or no role in breaking diapause. In some instancesq the increase in humidity encountered in the spring also potentiates termination of diapause (Williams, 23).

There are, however, some species, such as the oak silkworm (Antheraea pernyi Guérin Ménéville) and the codling moth (Laspeyresia pomonella L.), in which diapause can be terminated by long daylight days without the insect having to be chilled (Williams 22, 24, 25; Norris et al., 19). Such species are excellent for laboratory studies of some aspects of diapause as will be shown later.

Temperature plays a role that is particularly important in the use of photophase manipulation outdoors in the fall when temperatures are below 20°C for a portion of the time. Under laboratory conditions at constant temperature, if the temperature is high enough, the appropriate photoperiodic regimen will fail to induce diapause. If the temperature is too low, even long-day photoperiods will not terminate diapause in a susceptible species. When studying diapause in Pieris brassicael Bünning (6) showed that 26°C was a temperature high enough to prevent photoperiodic control; no matter what the photophase, no diapause occurred.

The influence of temperature on critical daylength between 15° and 21°C was slight, with diapause being induced in a large percentage of the pupae in regimens of LD 14:10 (14 hr light, 10 hr darkness each day), LD 12:12, or LD 10:14. We observed a similar phenomenon when diapause of codling moth larvae was terminated by exposure to long day photoperiods (LD 16:8) for 56 days at different temperatures between 15 and 35°C. Ninety percent or more of the insects maintained above 21.4° broke diapause and pupated, while below this temperature the insects did not pupate. Under short day photoperiods (LD 8:16) the insects remained as larvae and did not pupate over the entire range.

The overall goals of the experimental work on diapause of our group are as follows:

(1) to determine wavelengths of light which are involved in the control of diapause;

(2) to determine the chromophore or pigment responsible for the absorption of the effective wavelengths of light;

(3) to determine the mechanism by which the reception of light either promotes or inhibits the development of insects to a sexually mature state; and finally

(4) to utilize this information for the control of insects by artificial manipulation of the diapause response.

Based on the work of Williams (22, 23), Williams and Adkisson (24), Williams et al. (25), Gilbert (9), and Schneiderman and Gilbert (20, 21) and others, as well as on our own, it seems likely that

after light of an appropriate photoperiod impinges on the median portion of the brain of the insect for the requisite amount of time, a trophic hormone, called the brain hormone, is released. In their studies on Bombyx mori, Ishizaki and Ichikawa (14) conclude that this hormone is a peptide. The intermediate steps between photoreception and release of the brain hormone are not clear but may involve both cyclic AMP and control of the levels of one or more biogenic amines such as serotonin and melatonin, possibly with changes in membrane permeability as well. The brain hormone (20, 21, 23) affects the prothoracic gland, thus controlling the level of ecdysone or molting hormone. On the other hand, the juvenile hormone, produced in the corpora allata, prevents molting; thus the balance between ecdysone and juvenile hormone is a controlling factor in insect growth and development.

Some biochemical differences between diapausing and nondiapausing insects which we have observed in our own laboratory are illustrated in Table 2. Nondiapausing larvae of the European corn borer (Ostrinia nubilalis Hübner) on LD 10:14 at 24°C were 18-25 days old and insects in diapause on LD 10:14 at 24°C were over 40 days old from the hatch date. It should be noted that in diapausing insects apparent RNA, DNA and protein synthesis are all lowered as well as the activities of a number of enzymes.

To find the chromophore which activates the first step in breaking diapause, a logical approach is the determination of the action spectrum for this process. An action spectrum is a plot of the intensity of light needed to cause a biological response, usually at the 50% level,

Table 2.--Metabolic activity in diapausing and nondiapausing European corn borer larvae - percent of activity found in insects reared in LD 16:8 at 24°C

	Nondiapausing		
	Fifth instar		Diapausing
	LD 16:8	LD 10:14	LD 10:14
Process or Enzyme	%	%	%
	In vivo		
DNA synthesis (thymidine[a/] incorp.)	100	70	29
RNA synthesis (uridine[a/] incorp.)	100	100	50
Protein synthesis (leucine[a/] incorp.)	100	104	41
	In vitro		
Phosphodiesterase [bis(p-nitrophenyl) phosphate]	100	119	21
Monoamine oxidase [tryptamine]	100	96	13
Microsomal epoxidation of aldrin to dieldrin	100	--	100

a/ Radioactively labeled.

against wavelength. A precedent for the determination of such spectra and for the participation of photoreceptor pigments in photoperiodic processes is found in the work of Hendricks (12), Hendricks and Borthwick (13), and Borthwick and Hendricks (5) in connection with germination of seeds and flowering of plants. The bile pigment-protein, phytochrome, has been implicated in a number of photoperiodic responses of plants. The red form of phytochrome, P_{660}, is present in plants grown in the dark, such as pea seedlings. When phytochrome is irradiated with red light (P_{660}), it is converted to the active form P_{730}. P_{730} activates seed germination and either promotes or inhibits growth and flowering, depending upon whether one is dealing with a short or a long day plant. It should be noted that the phytochrome reaction is photoreversible.

We are searching for an analogous pigment in insect brains and have started by studying the breaking of diapause in unchilled, diapausing oak silkworm pupae and codling moth larvae. The determination of the action spectrum for breaking diapause in these 2 insects has been described in detail elsewhere (19). We exposed the diapausing insects in holders to various portions of the visible spectrum using a spectrograph designed by Norris (18). Since a regimen of LD 10:14 at 24°C keeps insects in diapause, while LD 16:8 at 24°C results in termination of diapause we maintained the insects on a schedule of 10 hr of white fluorescent light, which was then shut off and followed by 6 hr of spectral light. These experiments were performed at 24°C, as described by Norris et al (19).

The data in Figures 3 and 4 from Norris et al. (19) show that the most effective wavelengths for termination of diapause in both species of insects lie in the blue region of the spectrum.

Since the photoperiodic control of diapause has been shown to be due to the action of light directly on the midportion of the brain (23, 24, 25), we have been examining the spectrophotometric absorption characteristics of insect tissues, especially brains, from various species of insects, using a special spectrophotometer designed by Norris and Butler (18) and following their methodology (18) in which absorption spectra such as are shown in Figures 5 and 6 can be obtained on as few as 25 whole oak silkworm brains or on 70 codling moth brains. Oak silkworm brains contain compounds which absorb between 400-500 nm. Possibilities for pigments absorbing in this region include hemes, bile pigments and carotenoids. We believe that codling moth brains contain hemes, since absorption is enhanced at points 1, 2 and 3 in Figure 6, when the absorption spectrum of brain tissue reduced with dithionite solution is obtained at liquid nitrogen temperatures as described by Norris and Butler (18).

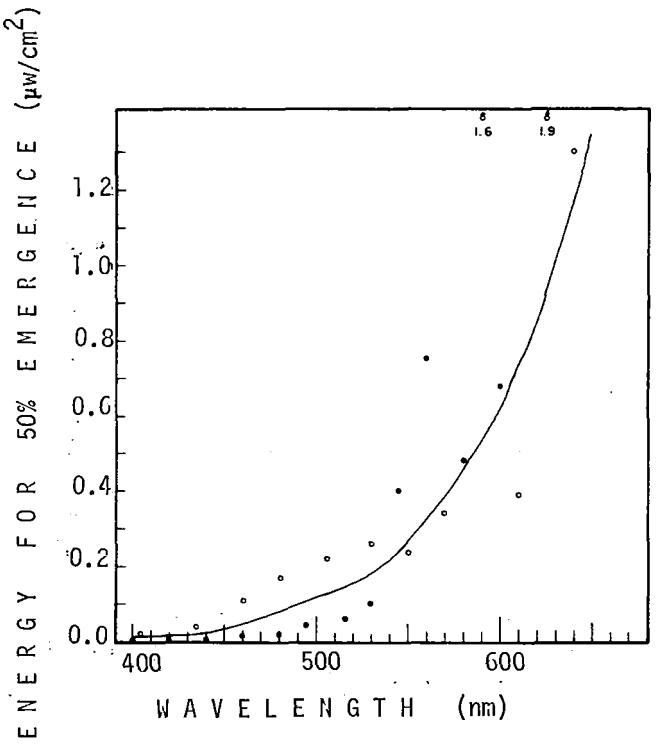

Fig. 3.--Action spectrum for breaking diapause in oak silkworm pupae. Solid circles are data from Expt 1 with a single filter spectrograph (20-25 nm bandpass). Open circles are data from Expt 2 with a double filter spectrograph (10-13 nm bandpass). The curve represents the best estimate of the spectrum from the combined experiments. (From Norris et al., 1969).

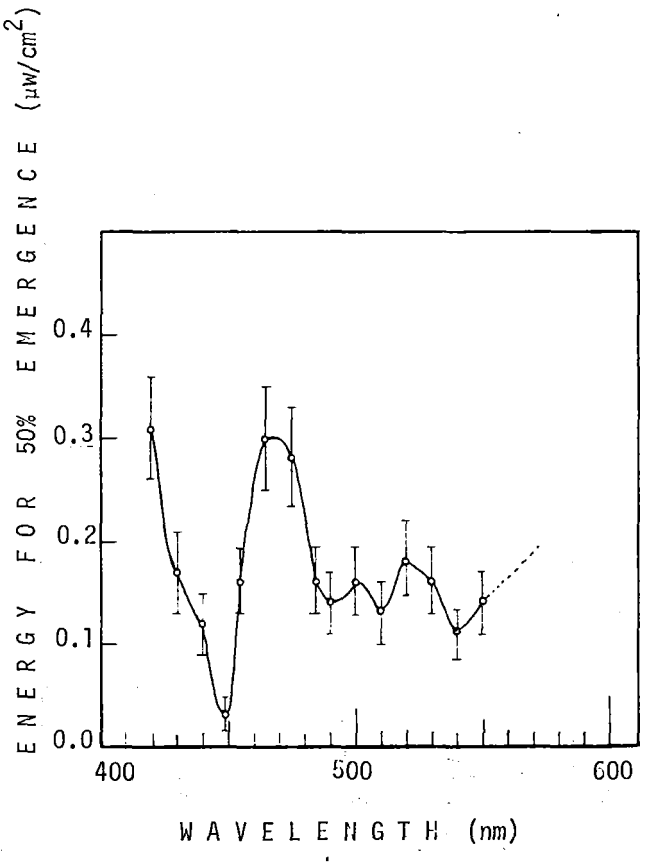

Fig. 4.--Action spectrum for breaking diapause in codling moth larvae. The error bars show the estimated uncertainty at each point. (From Norris et al., 1969).

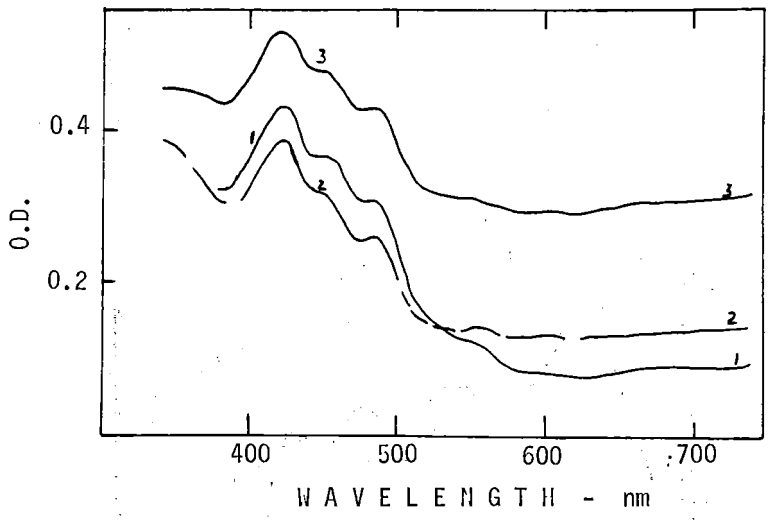

Fig. 5.--Absorption spectrum obtained from 26 intact oak silkworm brains. 1. As isolated from insects, after storage at -178°C for 48 hr. 2. Brains 5 min. after treatment with sodium dithonite solution. 3. Curve 10 min after treatment with sodium dithionite solution. O.D. = optical density.

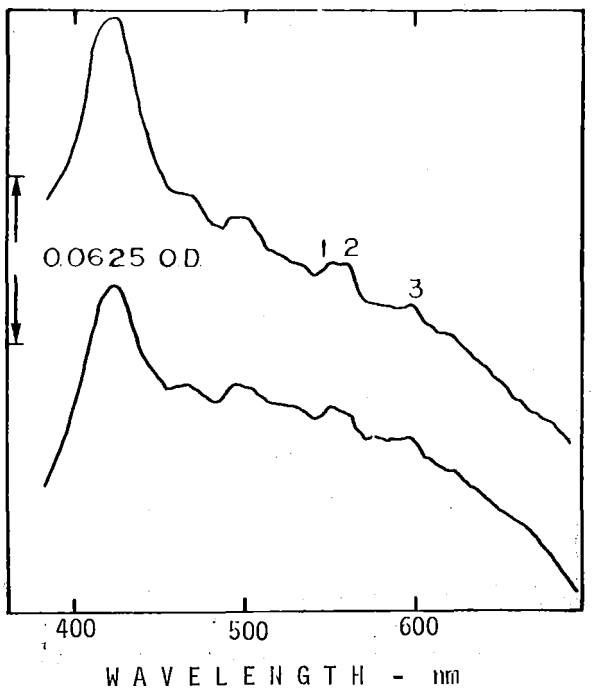

Fig. 6.--Absorption spectrum of 70 codling moth brains after reduction with sodium dithionite and cooling to liquid N_2 temperature. The 2 curves represent different orientations of the same sample in the light path. O.D. = optical density.

Our major efforts in field tests during the last 3 summers have been devoted to experiments on the prevention of diapause in insects under outdoor conditions. In spite of the large amount of laboratory work done on photoperiodism and diapause, it is surprising that so little work has been done outdoors. The only report of which we are aware is that by Ankersmit (3) in the Netherlands. He showed that 2-minute light breaks given 16-1/2 hrs after sunrise were effective in preventing diapause in about 80% of the population of the leaf-eating insect Adoxophyes reticulana Hb on apple trees. This work was done independently of ours about 3 years ago.

In our outdoor experiments (11) during the first 2 weeks of June, we planted a number of field plots, each with two 3.7m rows of Seneca Chief sweet corn. The plots were enclosed July 4 with Saran screen cages, 3.7 X 3.7 X 1.9m. In four plots, daylight was extended to 17 hr by turning lights on before sunset. Fixtures containing a total of eight 4-ft fluorescent tubes which were combinations of blue and daylight types were hung in these four plots. A second group of plots without any lights acted as controls. To make sure there were enough insects, the corn plants were infested with European corn borer egg masses, 1 egg mass per stalk of corn. Also, groups of young apples artificially infested with first instar codling moth larvae were exposed in plastic boxes held on racks in both lighted and control plots.

Ordinarily, adult European corn borer moths do not emerge in Beltsville, Md. in September; the insect diapauses as a mature larva. However, in the cages where the photophase had been lengthened to 17 hr, 76% adults emerged whereas none was observed in the unlighted control. The emerged corn borer adults in the cages were collected, identified and tabulated. Cornstalks were gathered after a killing frost in December and the corn borer larvae and pupae were counted after the stalks were slit open. We also observed numerous codling moth pupae and adults in the containers of apples held exposed in the 17 hr day plots. After a killing frost the apples were cut open and additional larvae were counted. Results of this experiment, conducted in 1969, are shown in Table 3. These data show that when the daylight was extended artificially to 17 hr diapause was prevented in 70-76% of the insects.

In 1970, in addition to repetition of the experiments in cages, we used 2 1000-watt mercury arc lamps, with each on a pole on either side of a plot 12m X 12m planted with Seneca Chief corn. The intensities of light ranged from about 140 to 360 lux at ground level in the Saran screened cages and from about 20 to 130 lux in the plots irradiated with mercury arc lamps. Adult European corn borer moths from the open plot could not be counted and therefore we determined pupal cases and larvae remaining in a sample of the corn stalks. Table 4 shows the results obtained in the field in 1970. Diapause was prevented in 70-96% of the insects when the photoperiod was extended to 17 hr. The reproducibility of our results when the photoperiod was extended to 17 hr

Table 3.--The effect of manipulated photoperiods on the incidence of diapause in larvae in the field from August to December 1969. (Total number found by collection and dissection).

	European corn borers		Codling moths	
Light regimen	Total No.	% Not in diapause[1/]	Total No.	% Not in diapause[1/]
A - 17 hr light	570	76[a]	23	70[c]
B - Light breaks[2/]	819	1	35	6[d]
C - Natural light-dark	304	0	51	8[d]

1/ In each group the confidence limits at the 99% level for the percentages with different superscripts do not overlap.

2/ Two spans of light each 22 minutes in length interjected in the dark period at 3 hr after sunset and at 2 hr before dawn.

Table 4.--Effect of manipulated photoperiods on the incidence of diapause in the European corn borer and the codling moth, 1970

Light regimen	European corn borers		Codling moths	
	Total No.	% Not in diapause	Total No.	% Not in diapause
Saran screened cages--12' X 12' X 7'--with 8 40-watt fluorescent lamps				
17 hr light	458	95	35	90
Light pulses 1 min light-3 min dark during a 24 hr period				
15 to 17 hr after sunrise	447	3	30	0
Natural light-dark	533	1	42	0
Open plot -- 40' X 40' -- with 2 1000-watt mercury lamps				
17 hr light	175	70	not run	
Natural light-dark	103	9	not run	

in both 1969 and 1970 is very encouraging. We are hopeful that this method of reduction of the overwintering pest population in combination with other techniques will find a place in the activities of entomologists of the future.

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PERSISTENCE AND FATE OF PESTICIDES IN SOILS, WATER AND CROPS: SIGNIFICANCE TO HUMANS

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1. The persistence and fate of pesticidal chemicals is dependent on the nature of the chemical itself and a multitude of environmental factors, such as soil types, temperature, light, moisture, microorganisms, etc.

2. It is for these reasons that no absolute "Half-Life" can be attributed to any of these chemicals.

3. The movement of pesticidal chemicals in and through soils is primarily a function of the water solubility of the chemical and of the adsorptive capacities of the soil type.

4. Insecticides like parathion and aldrin adhere to loam soil particles to such an extent that only traces of them could be removed from soils with water. Their presence in water percolated through agricultural loam soils was either nil or far below their water solubility.

5. It appears unlikely that commonly used insecticides are moved within water through soils. They could, however, be transported with washed off soil particles.

6. Insecticides of very low water solubility, such as aldrin, precipitate in mud-water systems and are to some extent being degraded by microbio-processes within the lake or river bottom mud. Soil microorganisms in general, play an important part in the degradation of pesticidal chemicals.

7. Detergents, such as ABS and LAS, prolong the persistence of parathion and diazinon in soil and also have a synergistic effect on both insecticides in soils.

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* *Full paper published in Fate of Pesticides in Environment. Ed. A. S. Tahori Gordon & Breach, London, p. 1-21.*

8. Polychlorinated biphenyl plasticizers (P.C.B.) are biologically active substances that apparently prevent detoxification of certain insecticides, thus synergizing their activity. The P.C.B.'s can also interfere with the analyses of certain chlorinated insecticides.

9. The absorption of insecticidal residues from soils into crops is smallest in those soils that contain high percentages of organic matter.

10. The penetration of some insecticides into roots of many plants is large. However, the translocation of these chemicals into the green plant parts is relatively very small.

11. Disruptive effects on the synthesis of DNA, RNA and protein in human cells (HeLa) in tissue culture could be demonstrated with aldrin and DDT at 125 p.p.m., but also with aspirin and sodium chloride. At concentrations of 10 p.p.m., however, these disruptions had mostly disappeared, thus illustrating the problem of dose-response relationship.

12. Concentrated research efforts are needed today that would be directed towards problems related to:

    a) The pharmacological significance of pesticidal residues and other environmental pollutants in human or other biological systems.

    b) The potential interaction of synthetic chemicals on subcellular levels.