Photoperiod Manipulation of Insect Diapause: A Method of Pest Control?

Abstract. *Extension of day length by artificial light in selected field plots in the fall prevented 76 percent of European corn borer* [Ostrinia nubilalis *(Hübner)*] *larvae and 70 percent of codling moth* [Laspeyresia pomonella *(L.)*] *larvae from entering diapause. Nondiapausing insects cannot survive rigorous winter conditions.*

In many insect species of the temperate zone the induction of diapause (a dormant overwintering state) or its prevention can be controlled in the laboratory by manipulation of the photoperiod. Exposure of most species to 1 to 3 weeks of "short days" with 13 or less hours of light per 24 hours induces, whereas exposure to "long days" prevents, diapause. Diapause can also be prevented by interruption of the scotophases (dark periods) of otherwise inductive (short day) photoperiods (*1*).

Prevention of diapause in pest insects by artificial manipulation of the photoperiod might be an alternative to chemical pesticides as a means of control in nature. Nondiapausing European corn borer [*Ostrinia nubilalis* (Hübner)] larvae placed in the field at Beltsville over the fall and winter months did not survive. European corn borer and codling moth [*Laspeyresia pomonella* (L.)] adults and pupae do not diapause, and insects in these developmental stages also succumb to outdoor winter conditions (*2*). The only demonstration of the effectiveness of manipulations of photoperiod under field conditions, where temperature, relative humidity, and light intensity vary, was made by Ankersmit (*3*) independently of our own work. He found that a 2-minute light break each night about 16.5 hours after sunrise prevented diapause in 80 percent of larval *Adoxophyes orano* on small apple trees in mid-August. We now report prevention of larval diapause in field populations of the European corn borer and the codling moth by manipulation of the photoperiod.

During the first 2 weeks of June 1969, 12 field plots, each 3.7 m^2, were planted with two 3.7-m rows of Seneca Chief sweet corn; the plots were enclosed after 4 July with Saran screen cages, 3.7 by 3.7 by 1.9 m (*4*). Four of these plots were in an isolated area (A) of the Agricultural Research Center at Beltsville, Maryland. In these plots daylight was extended to 17 hours by turning on lights before sunset in two fixtures containing a total of eight 4-foot (1.22-m) fluorescent tubes (combinations of 40-watt blue F 40B and 38.5-watt daylight F 48T12/D types) hung from the metal frame of each cage 20.3 cm from the top.

The second group in area B contained eight similar caged plots and served as the control, because no artificial light was provided. The length of the natural day from sunrise to sunset varied from 13 hours 55 minutes on 9 August to 9 hours 35 minutes on 5 December (*5*).

On 9 August, the corn which was in the early silk stage in areas A and B was infested by pinning a wax paper disk 10 mm in diameter containing one European corn-borer egg mass (10 to 30 eggs) in the blackhead stage (furnished by the Agricultural Research Service Laboratory at Ankeny, Iowa) on a leaf three nodes up from the lowest ear on each stalk. Also, on 20 August, groups of 670 young apples (2.5 to 4 cm in diameter) artificially infested with first-instar codling moth larvae (obtained from the Agricultural Research Service Laboratory at Yakima, Washington) in individual open 2-ounce (1 ounce is 29.6 ml) plastic cups were arranged in clear plastic sweater boxes (28 by 41 by 5 cm) with covers. Cotton was packed between the cover and the rim of each box so that larvae could not escape. A count could thus be made of any larvae which crawled out of the apples or pupated inside the plastic boxes or on the cotton packing. These boxes were placed on metal shelves in one plot in each of the two areas.

Table 1. The effect of manipulated photoperiods on the incidence of diapause in larvae of the European corn borer and the codling moth in the field from August to December. (Total number is total population of larvae, pupae, and adults found by collection and dissection. However, only one-fourth of the corn plants were examined, so the number of larvae found by this method was multiplied by 4.)

Light regimen	European corn borers		Codling moths	
	Total No.	Not in diapause* (%)	Total No.	Not in diapause* (%)
17 hour light	570	76[a]	23	70[c]
Natural light-dark	304	0[b]	51	8[a]

* In each group the confidence limits at the 99 percent level for the percentages with different superscripts do not overlap.

Ordinarily, adult European corn borer moths do not emerge in this area in September; the insects diapause as mature larvae. It was noticed that adult European corn borer moths began to emerge on 18 September in the plots in area A (Table 1) where the photophase had been lengthened to 17 hours. No adults were observed in the unlighted control area (B). As many of the adults as possible were collected and counted (Table 1). The identity of the moths was verified by the Systematic Entomology Laboratory. Exact confidence limits for the percentages of the population which emerged as adults were determined by means of binomial distribution tables (*6*). Also, on 5 December, every fourth hill of corn in all plots was harvested, and the ears, foliage, and stalks were dissected and examined for diapausing larvae (Table 1).

The boxes containing the apples were examined periodically for codling moth pupae and adults. The presence of either of these two stages indicated that diapause had not occurred and that the insects had continued their growth and development instead of diapausing as mature larvae. Then, on 1 December, the boxes were removed from the cages, and the final count of living larvae was made by dissecting the apples (Table 1). Seventy percent of the codling moths in area A had not gone into diapause and had continued their growth and development; 8 percent in area B were not in diapause.

Between 18 October and 1 December, we found 29 adult celery leaf tier moths *Udea rubigalis* (Guenée) in the cages in area A and one in area B. These insects had infested the kale and collards which had also been planted in these plots. Since nothing was known about the initial population of this species in any of the areas or of the effects of photoperiod on their diapause, we can only speculate that emergence of these adult moths may also have been caused by the manipulation of the photoperiod.

Although we also carried out an experiment with 20-minute light breaks, it was not successful, probably because of inadequate intensity, duration, or

timing of the breaks for the insects tested.

We have demonstrated that the diapause of a large percentage of European core borer and codling moth larvae can be prevented in the field by extending the day length artificially to 17 hours. Normally these nondiapausing insects die because they are unable to survive the winter conditions and the lack of food (*2*). In addition, there could be mortality of overwintering diapausing insects so that the combined effects of prevention of diapause by light manipulation and the natural mortality over the winter could cause a great reduction in the population emerging in the spring.

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References and Notes

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2. Unpublished observations in our laboratory.
3. G. W. Ankersmit, *Entomol. Exp. Appl.* **11**, 231 (1968).
4. The Saran screen cages were from the Chicopee Manufacturing Co., Cornelia, Georgia.
5. *Sunrise and Sunset at Washington, District of Columbia, Table No. 1061* (Nautical Almanac Office, U.S. Observatory, Washington, D.C., 1969).
6. K. Diem, *Statistical Tables* (Geigy Chemical Corporation, Ardsley, New York, 1962), pp. 85–103.
7. We thank T. Brindley, B. Butt, and F. R. Lawson (Entomology Research Division) for their help, and K. H. Norris (Market Quality Research Division) for his assistance in selecting the light sources.

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Molting in Land Crabs: Stimulation by Leg Removal

Abstract. *If the Bermuda land crab,* Gecarcinus lateralis, *loses numerous walking legs or both chelipeds, it undergoes almost immediate preparations for molting with attendant limb regeneration. Injections of the arthropod-molting hormone, ecdysterone, have no effect in either intact animals or those missing legs.*

The ability to experimentally control the molt cycle of Crustacea is important for at least three reasons: (i) it provides information on the physiology of molting; (ii) it facilitates studies of growth and regeneration; and (iii) it has potential economic importance. Molting in Crustacea is thought to be controlled by the interplay of a so-called molting hormone, the steroid ecdysterone (see *1*) and the molt inhibitory hormone (MIH) (*2*). Although MIH has not been isolated from X organs, it is thought to be produced there and stored in the sinus glands (*2*). Both X organs and sinus glands are located in the eyestalks, and one means of causing precocious molts is removal of both eyestalks (*3*). Although extirpation of eyestalks is effective as a trigger in the land crab *Gecarcinus lateralis* (*4, 5*), after surgery the animals become lethargic and do not generally survive ecdysis. At best, under certain conditions (when a shallow dish of dilute seawater at a salinity of 15 parts per thousand is available as the only source of water) some specimens live for a week after ecdysis (*6*). The behavior of animals without eyestalks in the period before ecdysis and their greatly decreased viability after ecdysis suggest that they are not entirely normal. Moreover, since the eyestalks are not regenerated, even if the animals survive in the laboratory, they could not live long if they were returned to nature.

We and others have observed that land crabs missing many legs (walking legs, or chelipeds, or both) prepare for molting sooner than animals missing only one or two legs. (During the premolt period the missing legs are regenerated.) In a study of environmental conditions influencing ecdysis, Bliss (*7*) noted that under unfavorable conditions over a 6-month period during which only one of nine control animals molted, five of five animals lacking all but two walking legs molted. We describe here a systematic study of the effect of the removal of appendages (walking legs, or chelipeds, or both) on the initiation of molting. We have also compared these effects with those of ecdysterone injected into either normal animals or animals with a number of limbs removed.

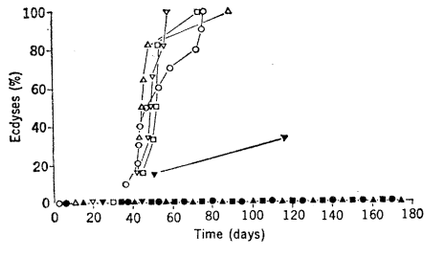

Fig. 1. Graph showing percent of animals which underwent ecdysis after the injection of ecdysterone in the presence of (missing only one walking leg, L^+) or absence of eight (L^-) walking legs. Sixteen animals received 0.1 ml of 0.5*M* NaCl (○, L^-; ●, L^+); 12 animals received 6 μg/g ecdysterone (□, L^-; ■, L^+); 12 animals received 18 μg/g ecdysterone (△, L^-; ▲, L^+); 12 animals received 6 μg/g ecdysterone on days 0, 1, and 3 (▽, L^-; ▼, L^+). Treatment occurred on day zero.

Gecarcinus lateralis, the animals used in this study, came from the Bermuda Biological Station. In the field, they live in deep burrows and ordinarily emerge only at night and after a heavy rain. Their normal molting season in nature is not known. Over the years we have observed that crabs brought into the laboratory and kept in community tanks undergo ecdysis 5 to 10 months after arrival. In a large group of crabs kept in individual containers, some molt sooner than 5 months after their receipt (*7*). However, we find that for adults the intermolt period is 180 to 300 days after adaptation to the animal-room conditions. Regardless of the time of year in which they are collected, we have seen them undergo ecdyses in all months of the year, although relatively few ecdyses occur in June or July.

All experiments were performed on animals weighing 20 to 40 g. If the eyestalks of such animals had been removed, they would have reached ecdysis in 6 to 8 weeks (*5*). All animals on their arrival in the laboratory were induced to autotomize (*8*) one walking leg; its removal does not influence the length of the molting cycle and the regeneration of this limb is a convenient and easily monitored external sign of an approaching ecdysis (*7, 9*). Some animals were induced to autotomize other limbs as well (Table 1). Ecdysterone (*10*) at a final concentration of 1 mg/ml was dissolved in a drop of ethanol and brought to volume in 0.5*M* NaCl. The amount of this solution injected into an animal ranged from 0.18 to 0.54 ml and was less than 1.5 percent of the animal's weight in all cases.

Data from a number of experiments are summarized in Table 1, and the complete data from one experiment (group 3) are shown in Fig. 1 so that the synchronous behavior of one popu-