THE INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON THE INDUCTION OF DIAPAUSE IN DIATARAXIA OLERACEA L. (LEPIDOPTERA)

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(INTRODUCTION)

Many insects, like other organisms, pass through a resting period during some stage in the life cycle in order to survive adverse conditions. In insects this resting period may occur during either the egg, larval, pupal or adult stages, and it is noteworthy that even nearly related species may show little or no similarity either in the stage at which dormancy occurs or the physiological state of the dormant period. Thus in the lepidopterous family Agrotidae the dormant period may occur during the larval stage of Agrotis segetum Schiff., and during the adult stage of Plusia gamma L. These species become quiescent during conditions of low temperature in winter, but on the advent of warm conditions they continue to develop without delay. However, other Agrotidae may show diapause—a physiological stage of arrested development which is not terminated immediately merely by increasing the temperature. Thus, Euxoa nigracans diapauses as an egg, whereas Diataraxia oleracea L. diapauses as a pupa. In view of such diversity in mode of surviving adverse conditions it is perhaps not unexpected that the factors inducing diapause should differ according to the insect species. A review of work on this subject has been given by Wigglesworth (1939) and Dickson (1949), and thus it is sufficient to say here that in many insects behaviour with regard to diapause is inherited, while in others diapause may be partly or wholly induced by adverse environmental conditions such as low temperatures, unsuitable nutrition and low moisture content in the food.

For several insects it has been shown already that the length of the photoperiod may influence diapause. Marcovitch (1924) showed that artificial reduction of day length to 7.5 hr. caused four species of aphis to produce males and oviparous females which laid diapause eggs, while under long-day conditions only viviparous parthenogenetic females were produced. Similar results were reported by Davidson (1929) and Wadley (1931).

Dickson & Sanders (1945) showed that diapause in Grapholitha molesta Busck., was affected by temperature and photoperiod during the larval stage, and the results of further detailed studies on this species are reported by Dickson (1949), who showed that high or low temperatures during the larval feeding period prevented occurrence of diapause. At medium temperatures larvae reared in the absence of light did not enter diapause, but as the photoperiod was increased to more than 3 hr.
the percentage diapause increased and reached 100% at about 11–12 hr. of light per 24 hr. At about 13 hr. the percentage of larvae entering diapause dropped suddenly to practically zero and remained at this level with further increases of light. *Carpocapsa pomonella* showed a similar response to photoperiod.

Preliminary observations on the influence of photoperiod on diapause were reported by Way, Hopkins & Smith (1949), who showed that at 24° C. a photoperiod greater than 16 hr. per 24 hr. during the larval stage was sufficient to prevent diapause in pupae of *Diataraxia oleracea* L. The photoperiod also influenced diapause in *Pieris brassicae* and *Mamestra brassicae*.

The present work was carried out to determine the effects of both light and temperature on diapause in *Diataraxia oleracea*. Particular attention has been paid to a determination of the period during larval development when the photoperiod is operative as a factor influencing diapause.

**MATERIAL AND METHODS**

*D. oleracea* L., the tomato moth, is a species belonging to the family Agrotidae. Under natural conditions the adults are active during June and July, and there is one and sometimes a partial second generation each year. In artificially heated glasshouses, the adults begin to emerge from overwintering pupae in January and February and there are generally three generations per year (Lloyd, 1920; Speyer & Parr, 1947).

All experiments were carried out in constant temperature rooms or cabinets. Larvae were reared in light-proof cages, each of which was artificially illuminated from above by a 60 W. tungsten filament bulb. A large glass trough full of water prevented radiant heat from the bulb causing increase in temperature in the cage. The light intensity to which the larvae were subjected was recorded as 25 f.c. (foot candles). This represents an approximate mean, largely because it was not possible to maintain larvae on the food plant at a fixed distance from the light source. Mention will be made in the text whenever different light intensities were used.

The larvae were reared on cut foliage of various brassica varieties—generally cabbage or brussels sprouts. A dish of dry peat was provided for pupation and each day the newly formed pupae were removed and kept at 24° C. until emergence of the adults. The exact length of the pupal period of each individual was recorded.

**EXPERIMENTAL**

(a) Mode of diapause of *Diataraxia oleracea*

In a valuable study of the habits of *D. oleracea*, Lloyd (1920) found that individuals varied greatly in the length of the pupal stage which lasted from 16 to 300 days. Two categories were distinguished: the short-period pupae from which adults emerged 16–50 days after pupation, and the long-period pupae from which adults emerged after 90–300 days. Although in these studies temperature conditions varied widely, Lloyd was able to show that low temperatures were not required to break diapause in the long-period pupae.
The present investigations have been carried out at constant temperatures. At 24° C. (±0·5° C.) adults emerged from short period pupae 14-30 days after pupation, 86% emerging between 17 and 21 days. Emergence of adults from long-period pupae began about 50 days after pupation and continued for a further 100 days. This distinction between the long-period or diapause pupae and the short-period or non-diapause pupae was always well defined except in experiments at 24° C. where the larvae were reared in continuous darkness. Under such conditions some adults emerged from the pupae during the intervening period 30-50 days after pupation (see Fig. 3).

(b) The influence of photoperiod at 24° C.

Lloyd (1920) found that under glasshouse conditions where the mean monthly temperature varied from 21·5 to 22·9° C. (range 11-34° C.), pupae obtained between May and July were primarily non-diapause while in April and September all were of the diapause type. Table 1 shows the percentage pupae entering diapause during each month between April and September (Lloyd, 1920) compared with the approximate mean day length per month determined from the average number of hours between sunrise and sunset (1950, for London Area).

<table>
<thead>
<tr>
<th>Average hours—sunrise to sunset</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage diapause pupae</td>
<td>100</td>
<td>1'2</td>
<td>0'0</td>
<td>2'3</td>
<td>66'4</td>
<td>100</td>
</tr>
</tbody>
</table>

Clearly the significance of day length as expressed above is limited, since the actual day length depends on the sensitivity of the larva to light of low intensities at the beginning and end of the day. Moreover, irrespective of the time of the year, ‘day length’ would be reduced when the sky is overcast or when the larvae are shaded by foliage of the food plant.

It would appear that if day length during the larval stage is important, a photoperiod of at least 15·5 hr. per 24 hr. is required to prevent onset of diapause, and it is noteworthy that experiments carried out at 24° C. have shown that using artificial light a photoperiod of 16 hr. or more during the larval stage of *D. oleracea* largely prevented diapause in the resulting pupae (Way *et al.* 1949).

The critical photoperiod was determined by rearing batches of about fifty *D. oleracea* larvae at a constant temperature of 24° C. under photoperiods varying from 0 to 24 hr. per 24 hr. using artificial illumination of 25 f.c. Fig. 1 shows the percentage pupae entering diapause in each treatment, and it can be seen that a photoperiod of 15 hr. is critical (cf. Table 1). The sharp drop in percentage of pupae entering diapause which occurs as the photoperiod during the larval stage is increased to more than 14 hr. occurs also in *Grapholitha molesta* Busck. in which (at 24° C.) a photoperiod of 13-14 hr. per 24 hr. is critical (Dickson, 1949).
Fig. 1 shows that, although photoperiods of 4-14 hr. during the larval stage induce 100% diapause, photoperiods of less than 4 hr. may prevent diapause in up to 20% of the pupae.

(c) The influence of temperature on induction of diapause

Experiments were carried out to determine the effects of rearing larvae at 30 and 34°C using photoperiods which induce diapause at 24°C. The prepupae* and pupae from the larvae reared at 34°C, and the pupae from all the other treatments were kept under identical conditions at 24°C.

Fig. 2 shows the effect on the length of the pupal stage, of rearing larvae at 24, 30 and 34°C, using a standard photoperiod of 8 hr. light (25 f.c.) per 24 hr. It can be seen that pupae resulting from larvae reared at 24 and 30°C were all of the diapause type, the length of the pupal period varying from 49 to 147 days. However, when the larvae were reared at 34°C, 49% of the resulting pupae were non-diapause (pupal period 16-21 days), while the other 51% were of the diapause type (pupal period 54-135 days).

A further experiment was carried out in which larvae were reared under the same range of temperatures but in darkness (except for a few minutes per day). Fig. 3 shows that at 24°C, 15% of pupae were non-diapause and 85% were of the diapause or intermediate type. However, at 30 and 34°C all pupae were non-diapause.

Thus, although conditions of illumination are such that at 24°C they induce diapause, diapause may be prevented if the temperature during the larval stage is raised to 30 or 34°C.

Further experiments were carried out to determine the effect of temperatures

* At 34°C, mortality at ecdysis from the prepupa to the pupa is practically 100%, although the larvae develop satisfactorily at this temperature (Way, Smith & Hopkins, 1950).
lower than 24° C, and Table 2 shows the percentages of diapause pupae resulting from batches of larvae reared at 12, 15, and 18° C. using a photoperiod (16 hr. per 24 hr.) which at 24° C, prevents diapause. Only small numbers of insects were used, and furthermore larval development was not satisfactory at 12 and 15° C, but it is clear that low temperatures tend to induce diapause even under long photoperiods.

This behaviour would appear to be advantageous to the insect, and yet it is remarkable that diapause in the *G. molesta* larva (at any rate in California, U.S.A.) is prevented by a low temperature (Dickson, 1949).
Table 2. *The percentages of diapause pupae resulting from larvae of Diataraxia oleracea reared at low temperatures and with a photoperiod of 16 hr. per 24 hr.*

<table>
<thead>
<tr>
<th>Temperature during larval stage (°C.)</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pupae</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Pupae in diapause (%)</td>
<td>92</td>
<td>92</td>
<td>44</td>
<td>0</td>
</tr>
</tbody>
</table>

* Two of these were of intermediate type and emerged after 38 and 39 days.

(d) *The influence of light intensity on diapause*

It has been shown that when larvae of *Diataraxia oleracea* were subjected to illumination of 25 f.c. for a photoperiod of 16 hr. per 24 hr. at 24° C. the resulting pupae were non-diapause. However, when the photoperiod was reduced to 12 hr., diapause was induced. To determine whether light intensity and light energy influenced diapause, batches of 20-30 larvae were reared at 24° C. using photoperiods of 12 and 16 hr. per 24 hr. and at three light intensities—220 f.c. (1000 W. tungsten lamp), 25 f.c. (60 W. tungsten lamp), and 1 f.c. (15 W. tungsten lamp)—a large jacket of running water did not entirely absorb radiant heat from the 1000 W. lamp since a thermometer with blackened bulb placed in the rearing cage showed a temperature of 25–26° C. compared with the external temperature of 24° C.

Fig. 4. The influence of light intensity and length of photoperiod during the larval stage on the duration of the pupal stage of *D. oleracea*. Temperature during larval and pupal stages, 24° C.

The data in Fig. 4 and Table 3 show that at a photoperiod of 12 hr. all pupae were of the diapause type (pupal period 50–147 days) irrespective of the light intensity (1–220 f.c.). However, at a photoperiod of 16 hr. an intensity of 1 f.c. was sufficient to prevent diapause.
Influence of photoperiod and temperature on diapause

Table 3. Comparison of the influences of light energy and light duration during the larval stage on diapause in pupae of Diataraxia oleracea

<table>
<thead>
<tr>
<th>Light intensity (f.c.)</th>
<th>1</th>
<th>1</th>
<th>25</th>
<th>25</th>
<th>220</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratios of total light energy (f.c. x photoperiod)</td>
<td>1</td>
<td>1.3</td>
<td>25</td>
<td>33</td>
<td>220</td>
</tr>
<tr>
<td>Length of photoperiod (hr. per 24 hr.)</td>
<td>12</td>
<td>16</td>
<td>12</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Pupae entering diapause (%)</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The results suggest that light intensity and light energy during the larval stage have no influence on diapause which is influenced solely by light duration, though it is clear that there must be a minimum intensity (less than 1 f.c.) below which the D. oleracea larva is insensitive to light.

(e) Period of larval stage during which the photoperiod is operative

Detailed experiments were carried out in which larvae of D. oleracea reared at 24°C were transferred from a diapause-preventing photoperiod (16 hr.) to a diapause-inducing photoperiod (8 hr.) and vice versa at various stages in larval development. It was found that larvae transferred from an 8 hr. to a 16 hr. photoperiod at the beginning of the moulting sleep at the end of the penultimate instar became non-diapause pupae. Conversely, larvae transferred from a 16 hr. to an 8 hr. photoperiod at the same stage of development became diapause pupae. Thus, the photoperiod is not operative as a factor influencing diapause until after the beginning of the moulting sleep.

At 24°C the moulting sleep prior to ecdysis to the last instar lasts about 2 days, and the last instar about 9–10 days of which the first 5–6 days are spent in feeding and the last 4 days in formation of a cocoon and as a prepupa. Batches of about fifty larvae reared at 24°C were transferred from a photoperiod of 16 hr. (diapause-preventing) to one of 8 hr. (diapause-inducing): (1) at the end of the penultimate instar at the beginning of the moulting sleep; (2) towards the end of the moulting sleep 0–12 hr. before ecdysis to the last instar; (3) after 1 day in the last instar; (4) after 3 days in the last instar; (5) after 5 days in the last instar.

Table 4 shows that diapause is not prevented if larvae of D. oleracea are reared under a diapause-preventing photoperiod only during the period prior to the moulting sleep into the last instar. However, a diapause-preventing photoperiod during the first 36–48 hr. of the moulting sleep is sufficient to prevent diapause in 81% of the pupae.

Further experiments were carried out in which larvae reared under a diapause-inducing photoperiod (8 hr.) were transferred to a diapause-preventing photoperiod (16 hr.) at various stages in their development: (1) at the end of the penultimate instar; (2) after 3 days in the last instar; (3) after 5 days in the last instar. Fig. 5 shows that pupae from treatment 1 were all of the non-diapause type, from treatment 2
were 50% diapause and from treatment 3 were 100% diapause. Thus, the diapause-preventing photoperiod is not operative after the 5th day of the last instar and only partially effective after the 3rd day of the last instar.

Table 4. The percentage of diapause pupae resulting from larvae of Diataraxia oleracea reared under a diapause-preventing photoperiod and transferred to a diapause-inducing photoperiod during various stages of the last instar of Diataraxia oleracea. Temperature, 24°C. Light intensity, 25 f.c.

<table>
<thead>
<tr>
<th>Duration of photoperiodic conditions (hr. per 24 hr.)</th>
<th>No. of pupae</th>
<th>Percentage pupae entering diapause</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 (diapause-preventing)</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>From beginning of moulting sleep to end of penultimate instar</td>
<td>26</td>
<td>19</td>
</tr>
<tr>
<td>From within 12 hr. before ecdysis to last instar</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>From end of 1st day of last instar</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>From end of 3rd day of last instar</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>From end of 5th day of last instar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To summarize: at 24°C the photoperiod is operative as a factor influencing diapause only during the period between the beginning of the moulting sleep at the end of the penultimate instar and the 3rd–5th day of the last instar.

It is of interest that when larvae were subjected to the diapause-preventing photoperiod throughout the whole of the last instar the mean length of the pupal
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stage was 21 days, whereas the mean length of the pupal stage of non-diapause pupae resulting from larvae reared in a diapause-inducing photoperiod for the first 3 days and a diapause-preventing photoperiod for the remainder of the last instar was 25 days. Thus a delay of 5 days before subjecting the last instar to a diapause-preventing photoperiod caused an increase of 4 days in the length of the pupal period (see Fig. 5). This suggests that, under diapause-preventing conditions, the initial stages of adult differentiation begin during the moulting sleep prior to ecdysis to the last larval instar. On this basis adult differentiation at 24 °C. takes about 32 days, of which the first 12 days occur during the larval period and the last 20 days during the pupal period.

(f) The influence of the food plant

To determine whether the effect of the photoperiod might be a secondary one due to the influence on the larva of photoperiodically controlled substances in the food plant, larvae were reared under a diapause-preventing photoperiod (16 hr. at 24 °C.), the food plant being raised under a diapause-inducing photoperiod (8-12 hr.) and changed in the larval rearing cages twice each day in such a way that the larvae never consumed food plant which had been subjected to a photoperiod greater than 12 hr. The resulting pupae were all of the non-diapause type. Similarly, several experiments were carried out in which larvae reared under a diapause-inducing photoperiod were given food plant which had been reared under a diapause-preventing photoperiod. All the resulting pupae entered diapause.

Therefore diapause in *D. oleracea* is not influenced by photoperiodically controlled substances in the food plant. The photoperiod as a factor influencing diapause must act directly on the larva.

DISCUSSION

Bodine (1932) and Salt (1947) have suggested that diapause is induced by formation of a diapause factor or hormone which causes development to be arrested. Diapause continues as long as the factor is present, but the factor may be slowly dissipated over a period of time or rapidly destroyed by exposure to low temperature thus enabling development to proceed.

Dickson (1949) has suggested that in *Grapholitha molesta* a diapause hormone may be produced during the larval stage by a two-phase reaction—a light-induced phase requiring a photoperiod of between 7 and 15 hr. and a darkness-induced phase requiring a dark period of between 11 and 16 hr. However, the present work with *Diataraxia oleracea* has shown that over 80% diapause occurred when the larvae were reared in the absence of light at 24 °C. Thus the theory of a light and dark reaction does not appear to be valid. However, on the basis that a diapause hormone is responsible for the arrest of development, it might be said that when the larva of *D. oleracea* is subjected to a photoperiod of less than 15 hr. a hormone is secreted which inhibits growth and induces diapause, while at photoperiods greater than 15 hr. the hormone is not secreted and development proceeds normally.
However, it is not easy to reconcile this theory with the evidence that: (1) a single diapause-preventing photoperiod during the moulting sleep, prior to ecdysis to the last instar of *D. oleracea*, was sufficient in 81% of the cases to cause development to proceed normally even though the larvae were subjected to a diapause-inducing photoperiod for the rest of the instar; (2) a diapause-inducing photoperiod up to the 3rd day of the last instar did not induce diapause in 50% of pupae providing the larvae were subjected to a diapause-preventing photoperiod for the 4th and 5th days of the last instar. Similarly, in *Grapholitha molesta* (Dickson, 1949) a diapause-inducing photoperiod (presumably causing secretion of the diapause hormone) during the whole of the last 8–13 days of the larval feeding period was incapable of inhibiting growth and inducing diapause even though the larva was subjected to a diapause-preventing photoperiod for only the first 4 days of the larval period. Moreover, a diapause-inducing photoperiod for the first 4 days did not inhibit the effect of a diapause-preventing photoperiod to which the insect was subjected for the last 8–13 days. Now it would seem that if the diapause-inducing photoperiod caused the production of a hormone which inhibited further growth, diapause should have been induced in all the above experiments.

Clearly it is necessary to examine these results with regard to the theory of diapause put forward by Wigglesworth (1934), who showed that arrest of growth due to lack of hormones occurred in *Rhodnius prolixus* and suggested the diapause may result from the absence of hormones necessary to maintain growth rather than to the presence of hormone which inhibits diapause. On the basis of this theory, it may be said that pupae of *Diataraxia oleracea* enter diapause when the hormone which initiates adult differentiation is absent. However, if the last instar larva, reared at 24° C., is subjected to a photoperiod greater than 16 hr. the adult growth hormone is liberated and diapause is prevented. Once production of the hormone has been initiated, the diapause-preventing conditions are no longer required because development does not cease even if the larvae are afterwards subjected to conditions which tend to induce diapause.

The theory that the absence of growth-promoting hormones is responsible for diapause has been strongly supported by Williams (1946), who studied pupal diapause in several lepidoptera and found no evidence that diapause resulted from the presence of a factor inhibiting adult differentiation. Williams showed that the factor or hormone responsible for adult development was secreted by the pupal brain, and by means of transplants he demonstrated that diapause was due to the failure of the brain to supply this factor. The presence of a growth-promoting hormone in lepidoptera was also demonstrated by Kopec (1922), who showed that metamorphosis in *Lymantria dispar* was induced by a hormone produced by the larval brain. If the brain was removed before the hormone had been secreted, development was arrested and a state comparable to that of diapause was induced.

Thus it seems probable that diapause in the pupa of *Diataraxia oleracea* is due to the absence of a hormone responsible for adult differentiation rather than to the presence of an inhibiting hormone. As regards the physiological effect of the photoperiod it is suggested that a photoperiod of 16 or more hr. per 24 hr. prevents
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diapause because the prolonged stimulation of the last instar larval brain by light falling on the photoreceptors induces formation of the adult growth hormone. This condition would be analogous to that occurring in certain birds and mammals where several investigators (see Rowan, 1938) have shown that an artificial increase of the photoperiod corresponding to the change from winter to spring conditions caused the eyes to stimulate the anterior pituitary gland by nervous paths (Hill & Parkes, 1933; Le Gros Clark, McKeown & Zuckerman, 1937) to produce a hormone which induced sexual development.

Perhaps constant high temperatures during the larval stage of \textit{D. oleracea} by increasing nervous activity also induce the brain to secrete the growth-promoting hormone. The fact that low temperatures tend to cause diapause even in the presence of long photoperiods supports this theory, since it might be expected that low temperatures would reduce nervous activity to such a level that even prolonged stimulation from the photoreceptors would not activate the brain sufficiently to cause secretion of the growth-promoting hormone.

It is remarkable that if the larvae are reared in darkness, diapause is partly prevented at 24°C and completely prevented at 30–34°C. This phenomenon is even more marked in \textit{Grapholitha molesta} (Dickson, 1949), where at 24°C diapause is completely prevented at photoperiods less than about 6 hr. per 24 hr. It is of interest that darkness has a similar effect on sexual activity of certain birds and mammals. Thus, although sexual activity in ferrets (Hill & Parkes, 1934) and house sparrows (Ivanova, 1935) is suppressed by a short photoperiod and induced by a long photoperiod, it is also induced by rearing in darkness (23.5 hr. per 24 hr.) or by covering the eyes with an opaque hood.

**SUMMARY**

The induction of diapause in the pupa of \textit{Diataraxia oleracea} is influenced by temperature and photoperiod during the larval stage. Low temperatures and short photoperiods tend to induce diapause while high temperatures and long photoperiods tend to prevent diapause.

Diapause is not influenced by light intensity during the larval stage providing the intensity is above a certain minimum.

Diapause is prevented at high temperatures (30–34°C) if the larvae are reared in darkness.

The photoperiod is operative as a factor influencing diapause only between the beginning of the moulting sleep prior to ecdysis to the last instar and the 3rd–5th day of the last instar. A single diapause-preventing photo-period during the moulting sleep is probably sufficient to prevent diapause.

Diapause in \textit{D. oleracea} is not influenced by photoperiodically controlled substances in the larval food plant.
REFERENCES