Studies on the Light Controlling Flower Initiation of *Pharbitis Nil*.
VI. Effect of Natural Twilight

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Received September 9, 1959

Many investigators consider that the photoperiod affecting flower initiation begins at the time at which luminosity becomes 1–10 lux and ends at the time at which luminosity is also 1–10 lux. This conclusion is based upon the fact that the light of this intensity inhibits the dark process of photoperiodic induction, if the light of this intensity is used as a source of an illumination for a light-break of the dark process or as a supplementary light. In *Pharbitis Nil*, too, plants subjected to light of 1–10 lux for 16 hours or more can not initiate a flower primordium, and those subjected to complete darkness for 16 hours or more can do so readily. But as has been reported previously, the first process of the inductive dark period is believed to be a relatively light-stable one and can proceed even under the illumination of 10–50 lux as easily as in the darkness. It has also been reported that the last process of the inductive dark period is also relatively light-stable but this is less stable than the first one. Thus, the critical light intensity which inhibits floral induction varies with the phases of the dark period.

Plants grown in natural daylight receive twilight at the first and the last phase of the dark period, and in both of them the processes inducing flowering are relatively stable to light. Therefore, twilight length must be taken into consideration to define the natural day length for the photoperiodic induction.

In the present investigation, the critical light intensities in the first and the last phases of the dark period for the photoperiodic induction are examined with *Pharbitis* seedlings under natural daylight condition.

**Material and Methods**

Material used was seedlings of *Pharbitis Nil*, strain "Violet". Photoperiodic behaviour of the seedling of this plant was reported recently by Kujirai and Imamura. Seeds were treated with conc. H₂SO₄ for 40–50 minutes, washed in running water for about one day, and spread on moistened sand. Two days after the treatment with H₂SO₄, germinating seeds were selected for uniformity and sown in 30×20×10 cm³ wooden boxes filled with garden soil. In each box 60 plants were placed in 4 rows, and grown under continuous illumination supplemented with incandescent light at night. Two days after the sowing, cotyledons expanded, and one day later the inferior individuals were removed, and the seedlings were subjected to the experimental treatment.

To eliminate the error due to the individuality of the boxes, experiments were designed in such a way that the rows of plants receiving different treatments were growing side by side in one box in randomized order. Light was excluded when desired by covering the plants with light-tight tin boxes.

All experiments were undertaken in a greenhouse, in which the temperature was 25±2° during the treatment. After the treatments plants were kept under continuous
illumination supplemented with incandescent light of 500 lux at night. About two
weeks later, plants were harvested and dissected under a binocular microscope.

Experiments and Results

Experiment 1. On March 16 and 17, 1959, plants of 4 boxes were treated as
follows:

The first lot of the plants consisting of four rows was subjected to natural day-
light, but they were covered from 7:00 p.m., when the luminosity was 0 lux, to
5:20 a.m. of the next morning, when the luminosity was also 0 lux. Remaining six
lots of the plants, each lot consisting of two rows, were darkened from the times at
which luminosities were 500, 200, 100, 50, 10 and 1 lux to the times at which lumino-
sities were 500, 200, 100, 50, 10 and 1 lux, respectively. Thus, the first of the seven
lots was subjected to natural daylight and received a dark period of shorter duration
than the other six lots. Similar treatments were repeated next day.

From the time at which luminosity was 500 lux to the time at which luminosity
was 0 lux, the luminosity of natural daylight was measured by a Mazda lux meter
with one minute intervals. The changes in luminosity with time are shown in Fig. 1.

Results are shown in Table 1. Plants subjected to natural day length initiated
1.9 flower primordia per plant in the average. The plants darkened from the times
when the luminosities were 1, 10, 50, 100, 200 and 500 lux to the times having the
corresponding luminosities of the next morning initiated 2.4, 2.4, 2.8, 3.1, 3.8 and 4.6
flower primordia per plant, respectively. If the first and the last phase of the inductive
dark period proceed under light of 1 lux or less as easily as in complete darkness,
flowering response of the plants subjected to natural night length and those darkened
from the time at which luminosity was 1 lux to the time having the same luminosity
in the next morning have to initiate flower buds to the same extent. But this was
not the case.

Thus, it appears that light of only 1 lux suppresses the flowering response to
some extent. It had been reported previously\(^3\), however, that the first process of
the inductive dark period can proceed under light of 10–50 lux as easily as in darkness
in this plant. It is conceivable that the difference of the photoperiodic responses
between the plants subjected to natural day length and those darkened from the time
at which luminosity was 1 lux to the time of the same luminosity in the next morning

<table>
<thead>
<tr>
<th>Luminosity of daylight at the start and the end of dark treatment in lux</th>
<th>Duration of dark period</th>
<th>No. of plants dissected</th>
<th>% of plants with flower buds</th>
<th>No. of flower buds per plant</th>
<th>% of plants with terminal flower bud</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>March 16</td>
<td>March 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10(^h) 20'</td>
<td>10(^h) 20'</td>
<td>48</td>
<td>97.9</td>
<td>1.9</td>
</tr>
<tr>
<td>1</td>
<td>11(^b) 25'</td>
<td>11(^b) 17'</td>
<td>25</td>
<td>96.0</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td>11(^h) 42'</td>
<td>11(^h) 39'</td>
<td>27</td>
<td>100</td>
<td>2.4</td>
</tr>
<tr>
<td>50</td>
<td>12(^b) 00'</td>
<td>11(^b) 57'</td>
<td>22</td>
<td>95.4</td>
<td>2.8</td>
</tr>
<tr>
<td>100</td>
<td>12(^h) 11'</td>
<td>12(^h) 08'</td>
<td>20</td>
<td>100</td>
<td>3.1</td>
</tr>
<tr>
<td>200</td>
<td>12(^h) 28'</td>
<td>12(^h) 18'</td>
<td>21</td>
<td>100</td>
<td>3.8</td>
</tr>
<tr>
<td>500</td>
<td>13(^h) 33'</td>
<td>13(^h) 14'</td>
<td>25</td>
<td>100</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Table 1. Effect of twilight on photoperiodic responses of Pharbitis seedlings.

Plants were darkened from the varying times of the evening at which
luminosities were 0–500 lux to the corresponding times of
the next morning. (Treated on March 16 and 17, 1959)
was due to the effect of morning twilight.

Evidence was already available that the last process of the inductive dark period was relatively light stable but less so than the first one⁷). In the previous experiment, however, light sensitivity of the last phase of the inductive dark period was examined during the 12th to 16th hour of the dark period. The process taking place during the 12th to 16th hour of the dark period is relatively light-stable, but the

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Fig. 1. Luminosity of natural daylight in the evening and in the morning.
process taking place during the 10th to 11th hour of the dark period—this corresponds to the last phase under natural conditions—is considered to be highly light-sensitive. In the next experiment, the effect of twilight in the evening and in the morning was investigated separately.

Experiment 2. On April 18 and 19, 1959, two groups of plants each consisting of 4 boxes were treated as follows:

Group 1: Four rows were subjected to natural daylight but from 7:20 p.m., at the time luminosity was 0 lux, they were covered with light-tight tin boxes. Six lots of plants each consisting of two rows were darkened from the times at which luminosities were 500, 200, 100, 50, 10 and 1 lux in the evening. All light-tight covers were removed at 5:15 a.m. the next morning. Similar treatments were repeated again next day. The luminosities in the evening twilight are shown in Fig. 1.

Table 2. Effect of twilight in the evening and in the morning on photoperiodic responses of *Pharbitis* seedlings.

<table>
<thead>
<tr>
<th>Group</th>
<th>Luminosity of daylight at the start or at the end of dark treatment</th>
<th>Duration of dark period</th>
<th>No. of plants dissected</th>
<th>% of plants with flower buds</th>
<th>No. of flower buds per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>9:55' 9:55'</td>
<td>54</td>
<td>29.6</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10:19' 10:19'</td>
<td>29</td>
<td>31.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10:27' 10:30'</td>
<td>28</td>
<td>17.9</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10:35' 10:40'</td>
<td>28</td>
<td>32.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10:40' 10:43'</td>
<td>25</td>
<td>24.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>10:46' 10:47'</td>
<td>19</td>
<td>44.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>11:03' 11:17'</td>
<td>29</td>
<td>93.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

As shown in Table 2, plants subjected to natural daylight in the evening, and those darkened from the times at which luminosities were 1, 10, 50, 100 and 200 lux initiated flower primordia to the same extent. Plants darkened from the time at which luminosity was 500 lux initiated significantly more flower buds than others.

Similar experiments which are not represented here, were undertaken on March 29 and 30, 1959, and gave similar results.

These results show that the first phase of the dark process inducing flower primordia can proceed under natural daylight of 1–200 lux as readily as under darkness. Sunset on April 18 occurred at 6:31 p.m., at the time luminosity was about 200 lux. The inductive dark process is considered, therefore, to proceed from the time of sunset or thereabout.

Group 2: All the plants were covered at 6:30 p.m. Four rows were returned
to natural daylight at 4:30 a.m. the next morning, at the time luminosity was 0 lux. Another 6 lots each consisting of 2 rows were subjected to natural daylight from the time at which luminosities reached 1, 10, 50, 100, 200 and 500 lux in the morning. Luminosities in the morning twilight are shown in Fig. 1.

As shown in Table 2, with delayed removal from darkness, the number of flower primordia initiated was increased. The plants subjected to natural daylight in the morning from the time at which luminosity was 0 lux initiated only 0.1 flower bud per plant, but those kept in the dark until the time at which luminosity was 1 lux initiated 0.6 flower bud per plant. Thus, only 1 lux of natural daylight inhibits the last phase of the dark process. Similar experiments were undertaken on March 29 and 30, 1959, and gave similar results. It appears that the last phase of 10- to 11-hour dark period is very sensitive to natural daylight, and that the difference of the flowering responses between the plants subjected to natural day length and those placed in the dark from the time at which luminosity was 1 lux to the corresponding time of the next morning in Experiment 1, is attributable to the effect of morning twilight.

Luminosity at the beginning of civil twilight is about 1 lux. Therefore, it is considered that the inductive dark period ends before the beginning of civil twilight.

Discussion

From the present experiments, it appears that the inductive dark period in *Pharbitis* plants begins when the luminosity is about 100–200 lux—this corresponds to the time of sunset—and ends at or before the beginning of civil twilight.

Some preliminary experiments which are not presented here showed that the first two hours of 12-hour dark period can proceed to some extent even under the light of 200-1000 lux, and that the last two hours of 12-hour dark period can also proceed to some extent under the illumination of 0.5–10 lux. In these cases the flowering responses are decreased with increasing light intensities.

Plants cultured under natural daylight are subjected to light whose intensity decreases gradually in the evening, and increases gradually in the morning. The inductive dark process is also assumed to begin and end gradually under these conditions. Therefore, the beginning or the end of the inductive dark period can not be determined clearly. But generally it may be said that the inductive dark period begins at the time of sunset and ends at the beginning of civil twilight.

The luminosity at the sunset or the beginning of civil twilight varies considerably with the weather. Some data obtained at Kyoto are presented in Table 3. In

<table>
<thead>
<tr>
<th>Date (1959)</th>
<th>March 16</th>
<th>March 17</th>
<th>March 30</th>
<th>March 31</th>
<th>April 1</th>
<th>April 18</th>
<th>April 19</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weather</strong></td>
<td>cloudy</td>
<td>cloudy</td>
<td>cloudy</td>
<td>rain</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>① Astronomical day length</td>
<td>12° 58'</td>
<td>12° 00'</td>
<td>12° 29'</td>
<td>12° 30'</td>
<td>12° 33'</td>
<td>12° 09'</td>
<td>13° 10'</td>
</tr>
<tr>
<td>② Length from the beginning of civil twilight to sunset</td>
<td>12° 23'</td>
<td>12° 25'</td>
<td>12° 53'</td>
<td>12° 55'</td>
<td>12° 58'</td>
<td>13° 34'</td>
<td>13° 36'</td>
</tr>
<tr>
<td>③ Biological day length for flowering of <em>Pharbitis</em></td>
<td>12° 04'</td>
<td>12° 10'</td>
<td>12° 17'</td>
<td>12° 20'</td>
<td>13° 05'</td>
<td>13° 33'</td>
<td>13° 31'</td>
</tr>
</tbody>
</table>

| | +6' | +10' | -11' | -10' | +32' | +24' | +21' |
| | -19' | -15' | -36' | -35' | +7' | -1' | -5' |
this table, biological day length for flowering of this plant was regarded as the period from the time at which luminosity was 1 lux in the morning to the time at which luminosity was 200 lux in the evening. On clear days the biological day length is longer than the astronomical day length by some 20–30 minutes, and it is approximately equal to the length of time from the beginning of civil twilight to sunset. On cloudy days, biological day length is nearly equal to astronomical day length.

The time at which luminosity is 200 lux in the evening is earlier than sunset by some 30 minutes on cloudy days, but the difference between the time at which luminosity is 1 lux and the beginning of civil twilight varies with the weather only a little and rarely exceeds 15 minutes. Thus, the weather affects mainly the end of biological day length.

From these points of view, the biological day length for *Pharbitis* plants at the summer solstice is about 14.5–15 hours at Kyoto. The critical day length of *Pharbitis Nil* is also about 15 hours5,6). *Pharbitis* plants cultured in the field initiate flower primordia late in June or early in July, at which time the day length is longest. Before June, the day length is shorter than the critical day length of *Pharbitis* plants. Why do *Pharbitis* plants cultured in the field under natural conditions not initiate flower primordia until the end of June or beginning of July? It appears that the night temperature is too low before June. Optimum night temperature for flower initiation of *Pharbitis* plants is about 25°, and the photoperiodic response is reduced with lowered temperature at night.

*Pharbitis* plants subjected to fully inductive short photoperiod initiate a terminal flower bud and stop further growth. But plants cultured under natural day length are subjected to nearly critical photoperiod every day in June and July, and under these conditions flower buds are initiated successively in the leaf-axils, and no terminal flower bud is formed. Thus, under natural conditions, *Pharbitis* plants grow vigorously and initiate lateral flower buds.

Whether the results obtained here can or cannot be applied to other plants is uncertain, and remains to be examined.

**Summary**

Effect of natural twilight on photoperiodic responses of *Pharbitis* seedlings was examined.

1) The inductive dark process proceeds from the time at which luminosity is about 100–200 lux.
2) The inductive dark process is inhibited by morning twilight of 0–1 lux.

Generally, it may be said that the biological day length for flowering of this plant begins at the beginning of civil twilight in the morning and ends at the time of astronomical sunset.

On cloudy days, however, the biological day length becomes shorter than that on clear days by some 30 minutes and becomes nearly equal to the astronomical day length.

Grateful acknowledgment is given to Prof. S. Imamura for his suggestions and criticisms.

**References**

摘 要

アサガオの花芽形成を支配する光条件について
VI. 自然薄明の影響

滝 本 敦・池 田 勝 彦

自然条件下で、アサガオ子葉の日長応応に有効な日の長さを決める目的で実験を行なった。
すでに発表したように、暗期反応の初期段階は比較的光に安定で、夕方 100〜200 ルックスになった時
（大体日没の時）より暗期反応がはじまるものと考えられる。これに対して、暗期反応の後期段階（暗期
開始後 10〜11 時間目）は非常に光に敏感で、1 ルックス以下の光でかなり抑制され、大体朝 1 ルックスに
なった時（大体常用薄明起時）に暗期反応が終了するものと考えてよさそうである。

すなわち日長応応に有効な日長は天文日長より約 30 分長い。しかし昼天の日には天文日長と、日長反応
に有効な日長はほぼ等しくなる。 （京都大学農学部応用植物学研究室）