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Flowering Responses of the Long-day Plant Lemna gibba G3[1]

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Summary. *Lemna gibba* L., strain G3, exhibits a qualitative long-day flowering response with a critical daylength on a 24-hour cycle of about 10 hours. Evidence is presented that the onset of daughter frond formation in a given frond inhibits the activity of the flowering meristem. Consequently, flower induction can only occur in fronds smaller than about 0.05 to 0.07 mm long. Although a minimum of 1 long day seems to be sufficient to induce the formation of flower primordia, at least 6 long days are required to obtain mature flowers since long days are also required for the early stages of flower development. The critical night length on 24, 48 and 72-hour cycles is respectively 14, 16, and 18 to 22 hours. The close similarity between the critical night length for the different cycle lengths is explained in terms of an inhibitory effect of darkness both on flower initiation and flower development. A 10-hour dark period is more inhibitory to flowering on a 36-hour cycle than on 24, 48, 60 or 72-hour cycles. It is suggested that darkness inhibits flowering through the formation of a light-labile flower inhibitor which acts to inhibit the functioning of the flowering stimulus.

Studies on the physiology of flowering have been carried out with a large number of different plants, but generally speaking the processes which control flowering are much better understood in short-day plants than in long-day plants. One reason for this situation is that most long-day plants grow rather slowly, require several to many long days for flower induction and do not exhibit maximum photoperiodic sensitivity until they reach an age of 1 to several months or more. Thus detailed information concerning the various factors which interact in the control of flowering has been obtained for only a few long-day plants such as *Lolium temulentum, Hyoscyamus niger* and *Silene armeria* (16). In view of the somewhat limited information available on the physiology of flowering of long-day plants, a detailed study was undertaken into the flowering response of the long-day plant *Lemna gibba* strain G3.

The first demonstration of unequivocal control of flowering in the Lemnaceae was by Kandeler (12, 13) who showed that several strains of *Lemna gibba,* including strain G3, exhibited a long-day flowering response. Kandeler (12) indicated that the critical daylength for *L. gibba* G3 was between 10 and 12 hours, but did not give any supporting evidence. Oota (18) reported that the critical daylength was about 12 hours but also failed to give any supporting evidence. Hillman (8) referred to a single experiment which suggested that at least 4 long days were necessary to initiate flowering, and that flower primordia would abort under short-day or non-inductive conditions. Umemura et al. (19) also showed that a minimum of 3 or 4 days of continuous light was necessary to obtain flowering. However, Nakashima (17) presented indirect evidence which suggested that a minimum of 2 long days was sufficient to obtain flower induction.

The present paper deals with general aspects of the flowering response of *L. gibba* G3. Particular emphasis will be given to a precise determination of the critical daylength on different cycle lengths and the effect of relative lengths of light and darkness on both flower initiation and flower development. The influence of light quality and intensity on flowering will be presented elsewhere (6).

Materials and Methods

Plant Material. The aquatic flowering plant *Lemna gibba* L., strain G3 was used in the present study. A general description of *Lemna* and its mode of growth and flowering has been given by Hillman (10). When flowering occurs the flower is always formed in the minus reproductive pocket which in *L. gibba* G3 is the left-hand one when one looks at the dorsal surface of the frond with the proximal end pointing toward the observer. The flower is located just proximal to the small frond primordium that is always present as the flower primordium de-

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velops. Thus it is probably best to think of the flower as an axillary structure which arises from a separate meristematic area located just proximal to that which gives rise to frond primordia. Daughter fronds continue to be produced after flower formation, but at a slower rate than if flowering had not occurred. Apparently any 1 frond can flower only once during its lifetime (10).

Culture Conditions. The basic medium used for all experiments is a slight modification of the M medium of Hillman (9). It differs from M medium only in the addition of 30 μM ethylenediaminetetraacetic acid (EDTA) and is designated as E medium. Triple distilled water (twice redistilled from glass) was used to prepare all media, and its conductivity never exceeded 0.15 mg/l as NaCl equivalents.

For most work the plants were grown in model MB-54 growth chambers (Percival Refrigeration and Manufacturing Co.). One chamber was maintained on a short-day cycle consisting of 9 hours light followed by 15 hours darkness (9L:15D), while the second chamber was kept on continuous light. In each chamber the light source consisted of 4 cool-white VHO fluorescent lights (Sylvania F48T12-CW-VHO) supplemented with 4 25-watt incandescent bulbs. The light intensity, as measured with a ft-c meter (Weston Elec. Inst. Corp., model 703, type-3A) and a 1.0 neutral-density filter (Kodak Wratten), was kept between 600 to 700 ft-c at plant level. It was found that for optimal results the fluorescent lights, which account for approximately 95 % of the light intensity measured by the ft-c meter, should be replaced after about 1000 hours use, since with older fluorescent lights there was a significant decrease in the flowering response (5).

The air temperature in the chambers during the light period was maintained at 28 $\pm$ 1° as measured by a thermometer at plant level. Temperature recordings were also made in the liquid cultures under these conditions by using a recording Tele-thermometer (Yellow Springs Instrument Co., Inc., model 44) with a liquid probe. They indicated that the temperature of the medium in the light was always between 28 to 29°, while in the dark it was always between 26 to 27°. However, at any 1 time the temperature variation was less than 0.2° in individual cultures and only about 0.1 to 0.3° higher in crowded cultures than in cultures with just a few plants.

For certain experiments it was necessary to give dark treatments during the day. For this purpose the cultures were placed in ventilated but light-tight cabinets in a constant temperature dark room maintained at 25 $\pm$ 1°.

Experimental Procedure. The plants were grown in 125 ml Erlenmeyer flasks with 50 ml of E medium. Stock cultures for starting experiments were grown on short-day conditions on E medium plus 600 mg/l bactotryptone and 100 mg/l yeast extract to insure detection of any contamination. They were usually 2 to 3 weeks old when actually used. A single 4-frond colony was used to start each experimental culture. Such colonies were selected as much as possible for uniformity, and care was taken that the smallest of the 4 fronds was clearly visible without a fifth frond, no matter how small, being visible. A typical 4-frond colony is illustrated at the top of figure 1.

Although there were only 4 visible fronds present in each flask at the start of an experiment, careful dissection with a binocular microscope at 30x magnification indicated that there were at least 45 to 55 fronds already formed in a typical 4-frond colony. Consequently, each flask of an experiment was actually started with a population of approximately 50 fronds, all of which were vegetative. A highly diagrammatic expanded diagram of the total number of fronds that were seen in a typical 4-frond colony is presented in figure 1. The different size circles have been used to indicate relative frond size. The smallest fronds that could be identified were 0.01 to 0.02 mm in length, but daughter fronds could only be seen in fronds longer than about 0.06 to 0.07 mm. The broken lines indicate the actual physical relationships between the various frond generations present in the 4-frond colony.

The method used for the evaluation of flowering was basically the same as that used by Hillman (8). All fronds in a culture were transferred to a petri dish and examined under a dissecting microscope at 10x magnification (30x magnification was used occasionally when the flower primordia were very small). For the determination of total frond number all fronds, no matter how small, which visibly projected beyond the margin of their mother frond were counted. For the determination of flowering, 100 or

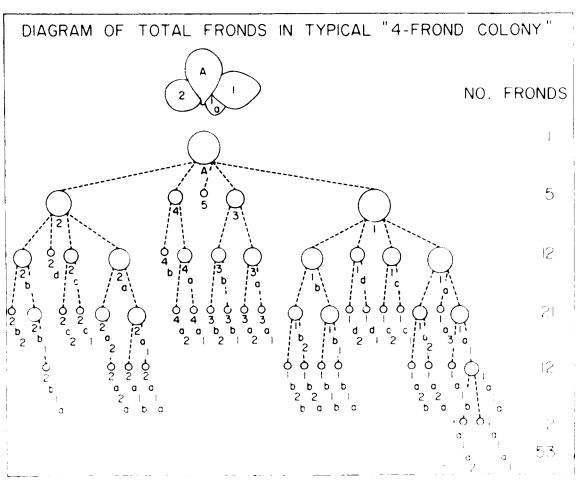


FIG. 1. Diagrammatic expanded diagram of the total number of fronds that can be seen in the typical 4-frond colony illustrated at the top of the figure. The different size circles have been used to indicate relative frond size. The broken lines indicate the actual physical relationships between the different fronds. The diagram is presented from the dorsal view with the proximal end of the frond directed toward the bottom of the figure.

more commonly 200 of these fronds selected at random were dissected and examined for the presence of flowers or flower primordia. A frond was considered to be flowering if a recognizable flower primordium could be seen. Under certain conditions flower primordia on some fronds ceased to develop and aborted leaving a brownish mass of tissue where the flower primordium should have been located. Although different parts of the flower primordium could sometimes still be distinguished, these fronds were always considered to be vegetative. The flowering percent (FL %) of the culture was determined by dividing the number of fronds with flowers or flower primordia by the total number of fronds examined and multiplying this value by 100.

In evaluating flowering it has proved valuable to determine not only the FL % of the culture but also the total number of vegetative fronds (#VF) in the culture. Since this is an absolute value it tends to vary more from culture to culture than does the FL %. However, providing the growth rate for different treatments is approximately the same, there is an inverse relationship between the FL % and #VF values with the change in the #VF often being more dramatic than the corresponding change in the FL %. Furthermore, the #VF for the long-day control has often proved to be a more sensitive indicator of the level of flowering in a particular experiment than has the FL % (5).

Most experiments were terminated after 11 to 14 days. In some experiments the last 3 or 4 days consisted of continuous light for all treatments except the short-day control. This procedure increases growth and thus slightly increases the FL % for a given culture, but does not result in any measurable flowering by itself since the FL % was always zero for 8 short day-4 long day (8SD–4LD) controls (5). All experiments were repeated at least once with good qualitative agreement between replicate experiments. Short-day (9L:15D) and long-day (continuous light) controls were included in each experiment. Three cultures were used for each treatment, and the variation between cultures was usually very small. Consequently, all experimental values presented in the results will be the average of 3 cultures, except in rare cases where 1 of the 3 cultures became contaminated and was not counted.

Results

Progress of Flowering on Continuous Light. Umemura et al. (19) reported that when *L. gibba* G3 was placed on continuous light a minimum of 5 days was necessary to obtain measurable flowering. A similar finding was obtained in this study (fig 2). After 5 long days only a few small flower primordia were counted, and mature flowers with visible emergent anthers did not appear until after 10 long days. The #VF, which started at 4, showed a gradual rise over the first 9 days, but then showed no further increase for the duration of the experiment.

At the start of this experiment an average of 45 fronds could be counted in each 4-frond colony. About 30 of these fronds were less than 0.06 to 0.07 mm long and thus were too small to have any recognizable daughter fronds. Blodgett (2) carried out histological studies on an unspecified species of *Lemna* and showed that a frond first starts to form a daughter frond when it is approximately 0.05 mm long. Thus it would seem probable that close to half of the fronds present at the beginning of the experiment had not yet started to form daughter fronds.

Although the first measurable flower primordia were seen after 5 long days, flower primordia were first seen after 4 long days in fronds too small to count (i.e., not projecting beyond the margin of their mother frond; see Materials and Methods). At that time there were 23 visible fronds all of which were vegetative. In addition, there were 17 other fronds too small to count but large enough to determine as being vegetative or flowering, and of these 9 had flower primordia (9/40 = 25 %). This number of 40 corresponds very closely to the number of 45 fronds originally counted at the start of the experiment. Since there was a total of about 65 fronds that could be counted after 1 long day, it would seem clear that a single long day was sufficient to induce flowering in at least some of the fronds present during the first day.

Most of the fronds actually present at the start of the experiment were not induced to flower. Thus it would appear that flower induction can only occur in fronds smaller than some critical size, and once a frond has passed this size it loses the capacity to initiate flower formation even when grown under optimal flowering conditions. Presumably the majority of the fronds present at the start of the experiment had either passed the critical size by the time the experiment started or did so before the inductive stimulus from the first long day could take effect. Since over half of the fronds initially present were smaller than 0.06 to 0.07 mm long but only about 25 % were induced to flower, the critical size for flower induction is less than about 0.07 mm. In this regard *L. gibba* G3 is similar to *L. perpusilla* 6746 where flower induction occurs only in fronds less than about 0.08 mm long (8).

A period of 5 to 6 days elapses from the time a frond is induced to flower until it becomes large enough to be counted. Thus changes in the flowering response that occur on a particular day are actually a reflection of changes that occurred approximately 6 days earlier. In the experiment illustrated in figure 2 there was no further increase in the #VF after the eighth or ninth day. Therefore, it can be concluded that the level of flower induction was essentially 100 % after the second or third day.

Even under optimal conditions flower induction is usually not quite 100 %, as shown in figure 2, and a more typical experiment is presented in table I. The increase in the #VF from the 8 to the 21 long-day treatment was only from 55 to 77, while the number

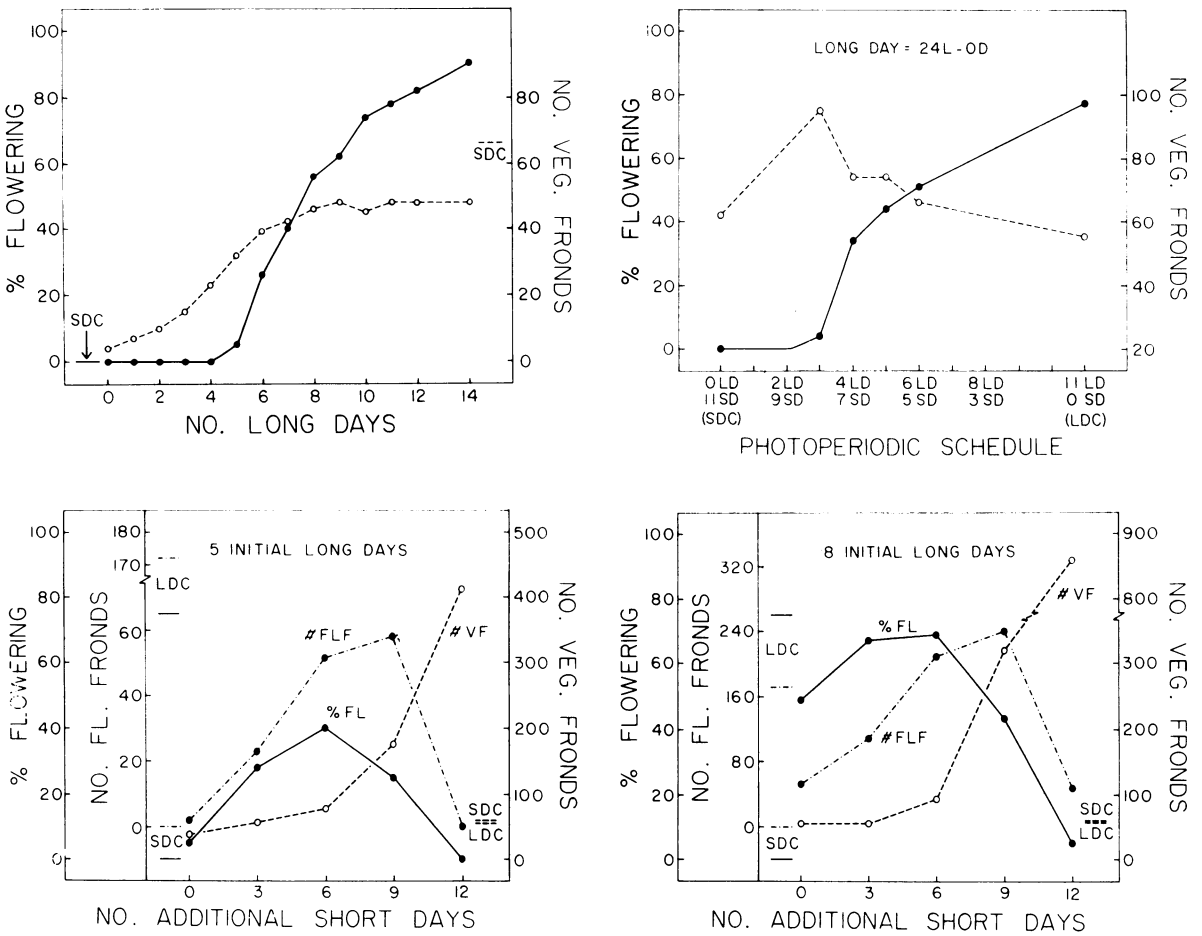


FIG. 2. (top left). Progress of flowering over 14-day period on continuous light. On each successive day 3 flasks were removed and examined for flowering. The short-day control was counted after 11 days. Solid line, FL %; dashed line, #VF.

FIG. 3. (top right). Effect of different number of continuous-light long days on the flowering response in an 11-day experiment. Solid line, FL %; dashed line, #VF.

FIG. 4. (bottom left). Effect of giving 5 initial long days followed by 0 to 12 short days on the flowering response. The short- and long-day controls were counted after 11 days.

FIG. 5. (bottom right). Effect of giving 8 initial long days followed by 0 to 12 short days on the flowering response. The short- and long-day controls were counted after 11 days.

of fronds counted increased from 109 to 1898. From these results it can be calculated that after the second day flower induction was approximately at 98 % level.

Number of Long Days Required for Flower Induction. Indirect evidence presented above suggested that a single long day was sufficient to induce flowering. However, Umemura et al. (19) reported that a minimum of 3 or 4 long days was needed to obtain flower induction. Their experiments involved giving 1 to 8 long days (continuous light) followed in each case by 8 short days (10L:14D).

Table I. *Progress of Flowering over 21-Day Period on Continuous Light*

No. long days	FL %	#VF
5	5	37
8	49	55
11	75	57
21*	96	77

*Based on only 1 flask.

Similar experiments have also been carried out in this study (fig 3). A minimum of 3 long days was required to obtain some flowering but from 1 experiment to the next the FL % of the 3LD:8SD treatment never exceeded 5 % and often was zero. For a significant level of flowering it was necessary to give 4 long days, and with the increase from 3 to 4 long days there was always a sharp rise in the FL % and a corresponding drop in the #VF.

At first sight this experiment would seem to contradict the results of the earlier experiment where the

progress of flowering was followed for 1 to 14 days on continuous light. However, one important point is that with the 4LD:7SD treatment mature flowers were never formed, and all the flowers that were seen were still very small primordia. A fully mature flower may be 3 mm long (from base of ovary to stamen) and 1 mm wide (ovary width). A flower that is just starting to emerge from the minus pocket is approximately 0.8 mm long and 0.4 mm wide. However, in the experiment illustrated in figure 3 the largest flower primordium seen with the 4LD:7SD treatment was 0.16 mm long and 0.21 mm wide (at this stage the stamens account for most of the flower primordium so they are the basis of this measurement). Clearly, the development of these flower primordia was inhibited to a considerable extent. Furthermore, in some fronds there was a small mass of brownish tissue where a flower primordium would normally be located. This observation suggested that in these fronds flower primordia had been formed, but had then aborted, and the brownish tissue was the remnant of the flower primordium.

Long Day Requirement for Flower Development. The possibility that long days are needed both for flower initiation and flower development was examined in the following manner. Plants were subjected to an initial treatment of 5 or 8 long days and then examined after 0 to 12 additional short days. When 5 initial long days were followed by short days (fig 4), the FL % and the actual number of flowering fronds (#FLF) continued to increase through 6 to 9 short days, but then both dropped to zero after 12 short days. The largest flower primordium seen was about 0.2 mm long and 0.3 mm wide. Signs of aborted flower primordia were first apparent after 6 short days, and after 9 short days there were over 50 fronds which contained the remains of aborted flower primordia.

When 8 initial long days were followed by short days (fig 5) the results were similar to those presented above, except that about 50 mature or emergent flowers were formed. Some of these were only slightly emergent and often had only 1 normal stamen, while others were fully emergent and appeared completely normal. With almost no exceptions the only flowers that were seen after 12 short days were these old emergent flowers. However, in every case the flower parts had died, and all of the ovules had shrunken to small brownish masses.

All flowers which did not develop to maturity were smaller than about 0.2 mm long and 0.3 mm wide. These dimensions were the maximum size for flower primordia when only 5 initial long days were given. Thus it seems that, providing a flower passes this critical size, it can develop more or less to maturity even on short days. In the above experiment there were no emergent flowers and only a few flower primordia larger than the above critical size after 8 long days. However, after 6 additional short days essentially all flowers that were destined to develop to maturity had done so. Apparently flower development did not start to be arrested at the above critical size until after several short days had elapsed. Furthermore, these studies indicate that more than 5 long days are needed to obtain mature flowers, and preliminary work indicates that a minimum of 6 long days is sufficient to obtain at least a few emergent flowers.

The results of the above experiments explained why the flower primordia seen after 4LD:7SD treatment were always quite small. They also suggested that it might be possible to demonstrate flower induction after just 2 or even 1 long day by examining plants after only a few subsequent short days instead of waiting for 9 to 10 short days. When 2 long days were followed by 4 or 5 short days a few flower primordia were seen in fronds too small to count, but after 9 short days they all had aborted and disappeared. The flower primordia that were seen were extremely small with the largest being about 0.06 mm long and 0.08 mm wide. These results provide direct evidence for flower induction after 2 long days and agree with the findings of Nakashima (17). When a single long day was followed by short days flower primordia were never seen, presumably because any flower primordia that were formed, aborted before they ever became large enough to be detected microscopically. It might be possible to demonstrate the formation of flower primordia after a single long day by using histological techniques, but such studies have not been undertaken. Thus direct evidence for flower induction after a single long day is still lacking.

Critical Daylength Determinations. Flowering has never been observed on the 9L:15D short-day schedule, even when cultures were grown for 28 days (table II). This observation indicates that in a population of plants there is apparently no tendency toward flower induction in old crowded cultures. Thus it is clear that *L. gibba* G3 behaves as a strictly qualitative long-day plant.

The critical day-length is between 9.5 and 10 hours for a 24-hour cycle (fig 6). With longer photoperiods the FL % rises rapidly and approaches the level of the long-day control with the 16L:8D treatment. In this experiment flowering was not quite optimal and since there was considerably more growth on continuous light than on the 16L:8D schedule (346 fronds versus 199, respectively), the higher #VF for the long-day control is a reflection of the increased growth and does not imply a higher level of flower induction with the 16L:8D schedule.

Experiments have also been performed to determine the critical day-length on 36, 48 and 72-hour

Table II. *Flowering Response after 28 Short Days*

Photoperiodic treatment	No. days	FL %	#VF
Continuous light (LDC)	11	76	61
9L:15D (SDC)	11	0	61
9L:15D	28	0	1085

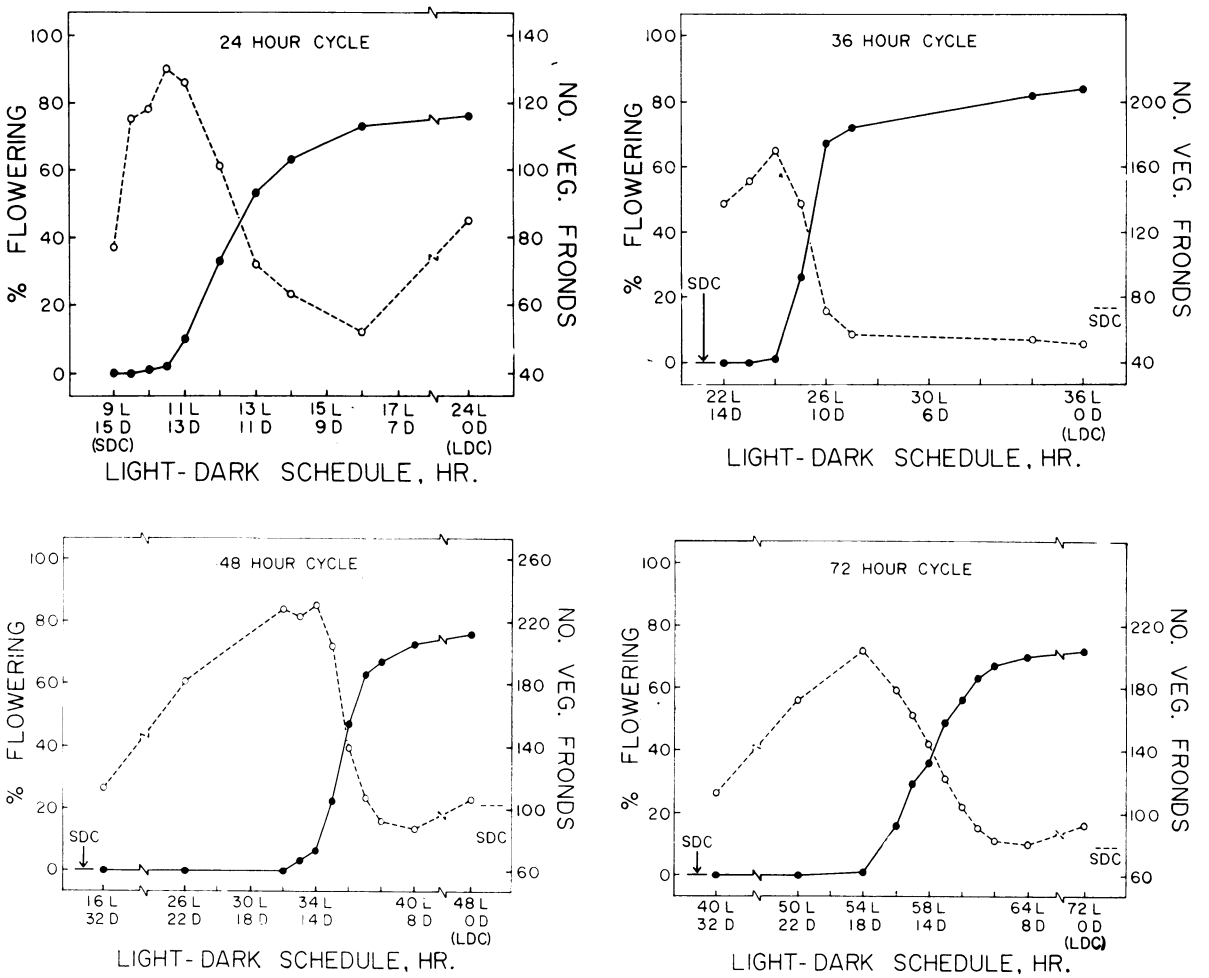


FIG. 6. (top left). Determination of the critical daylength for flower induction on a 24-hour cycle. The duration of the experiment was 12 days with the last 3 days continuous light for all treatments except the short-day control. Solid line, FL %; dashed line, #VF.

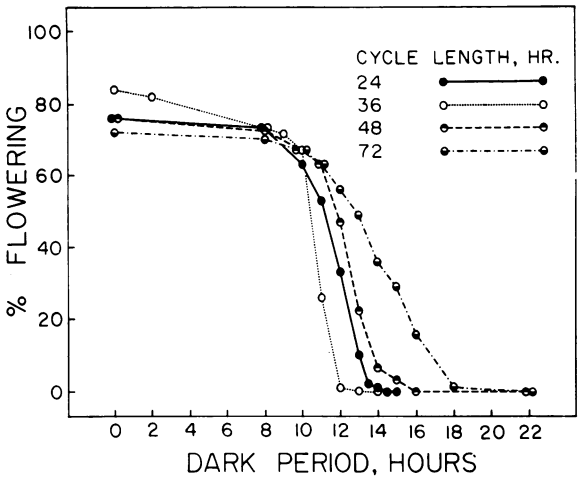
FIG. 7. (top right). Determination of the critical daylength for flower induction on a 36-hour cycle. The duration of the experiment was 12 days with the last 3.5 days continuous light for all treatments except the short-day control. The light period for the first cycle was 9 hours shorter than indicated. Solid line, FL %; dashed line, #VF.

FIG. 8. (bottom left). Determination of the critical daylength for flower induction on a 48-hour cycle. The duration of the experiment was 13 days with the last 3 days continuous light for all treatments except the short-day control. Solid line, FL %; dashed line, #VF.

FIG. 9. (bottom right). Determination of the critical daylength for flower induction on a 72-hour cycle. The duration of the experiment was 12 days with the last 3 days continuous light for all treatments except the short-day control. Solid line, FL %; dashed line, #VF.

cycles, and the results are presented in figures 7 to 9. The critical daylength increases with cycle length; however, the critical night length is quite similar for each of the different cycles. In figure 10 the FL % results for the 4 different cycle lengths from figures 6 to 9 have been plotted against dark period length. The critical night length increases slightly with longer cycle lengths for the 24, 48 and 72-hour cycles. However, the critical night length for the 36-hour cycle, instead of falling between that of the 24 and 48-hour cycles, is almost 2 hours shorter than that of the 24-hour cycle.

These results indicate that darkness exerts a strong inhibitory effect on flowering. The importance of dark inhibition has been further demonstrated in a different kind of experiment (fig 11). Plants were given 2 long days, followed by various treatments during the third day, then 2 additional long days and finally 7 short days for a 12-day experiment (2LD-Day 3-2LD-7SD). During the third day plants were given light-dark cycles from 24L:OD to 3L:21D to examine the effect of increasing amounts of darkness on flowering. As the length of the dark period during the third day was increased, there was a marked



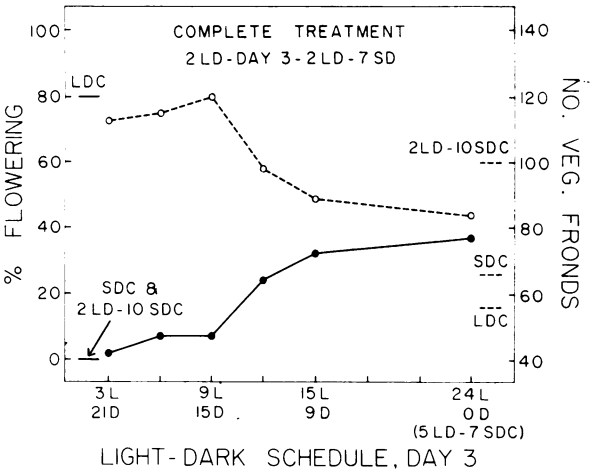


FIG. 10. (left). Comparison of the critical night length on 24, 36, 48, and 72-hour cycles.

FIG. 11. (right). Inhibitory effect of darkness on flowering when given during the third day of a 2LD-Day 3-2LD-7SD schedule. Short- and long-day controls were counted after 12 days. Solid line, FL %; dashed line, #VF.

decrease in the FL % and a corresponding increase in the #VF. Thus, the results of this type of experiment agree with those of the earlier experiments by showing that darkness definitely exerts a strong inhibitory effect on flowering.

Discussion

Kandeler (12) originally reported that the flowering response of *L. gibba* G3 was long-day dependent. However, in a later paper Kandeler (14) failed to obtain flowering on a 10L:14D regime with a short red-light treatment 10 hours after the start of the dark period. Consequently, he considered that the flowering response of *L. gibba* G3 was not strictly photoperiodically controlled, and thus referred to it as a day-neutral plant (15). However, results to be published elsewhere (6) demonstrate conclusively that *L. gibba* G3 is sensitive to short interruptions of the dark period by red light. Furthermore, the results of the present study clearly indicate that *L. gibba* G3 is not a day-neutral plant since, although it will grow on photoperiods as short as 1 or 2 hours, it will not flower unless the daily photoperiod is longer than about 10 hours. This response defines *L. gibba* G3 as a long-day plant regardless of its sensitivity to various light interruption treatments (11, 16, 20).

The critical daylength of 9.5 to 10 hours for a 24-hour cycle is 2 to 3 hours shorter than that reported by other workers (12, 18). The most likely explanation for this difference is that conditions for flower induction were not optimal in the other investigations. It has been observed that with a reduction in the level of flower induction there is a slight increase in the critical daylength for a 24-hour cycle (Cleland, unpublished results). Furthermore, Kandeler (12) obtained results with *L. gibba* G1 which suggested that a slight increase in the temperature could cause an increase in the critical daylength by about 2 hours. Thus it appears that the critical daylength of *L. gibba* is fairly sensitive to environmental conditions, and only under optimal conditions is a critical daylength of 9.5 to 10 hours obtained for *L. gibba* G3.

There appears to be a good correlation between the maximum frond size allowing flower induction (0.05–0.07 mm long) and the size of a frond when daughter-frond production first begins (approximately 0.05 mm long). This correlation suggests that once the first daughter frond has been formed in the minus pocket it in some way inhibits the activity of the meristematic area that could normally form a flower in this minus pocket. Consequently, the parent frond is no longer capable of flower formation even though a mature frond more than likely can form the flowering stimulus.

A finding which confirms the preliminary result of Hillman (8), is that long days are required not only for the formation of flower primordia but also for at least the early stages of their development into mature flowers. Although the results are still somewhat preliminary, the evidence suggests that unless a flower primordium passes a certain size (approximately 0.2 mm long and 0.3 mm wide) by the time of transfer to short days, or very shortly thereafter, it will not develop past this stage and will eventually abort and disappear.

At present there is no evidence to suggest what may be special about the above size. However, it would seem to correspond to some critical stage in the development of a flower primordium. Since the flower primordium lacks any visible chlorophyll it is obvious that its growth is dependent upon photosynthetic assimilates and growth factors obtained from the mother frond. Some of these substances may be similar to those that stimulate the growth of young fronds of *L. minor* (1). In addition, the early growth

of the flower primordium after induction is clearly dependent upon some additional factor that is only formed by flowering fronds. It is tempting to postulate that this factor is, in fact, the flowering stimulus, but it is equally possible that it is some other substance whose formation is dependent upon the presence of the flowering stimulus. Support for the involvement of the flowering stimulus in flower development is provided by preliminary experiments which indicate that on a 24-hour cycle the critical daylength for flower initiation and flower development are quite similar, although not necessarily the same. Furthermore, attempts to define conditions that would permit flower development but not flower initiation have so far not been successful. This close similarity in the photoperiodic requirements for flower initiation and flower development suggests that the flowering stimulus is required both for the initiation of flower primordia and also for their development, at least until they reach the above critical size.

The similarity in the critical night length for different cycle lengths, contrasted with the widely different critical daylengths, can be explained in terms of an inhibitory effect of darkness on flower development. For instance, on a 48-hour cycle with a 32L:16D regime flower primordia are presumably formed during the first 24 hours of continuous light. During the second 24 hours, the concluding 16-hour dark period would prevent any further flower initiation. In addition, it would also arrest the growth of the flower primordia that were formed during the first day and cause them to abort. The same explanation applies to the 72-hour cycle, except that in this case possibly a slightly longer dark period would be needed to cause all of the flower primordia that were formed during the first 2 days of continuous light to abort.

In some long-day plants such as *Hyoscyamus,* flowering is obtained with a 9-hour photoperiod if the accompanying dark period is quite short or very long, but not if it is of intermediate length (3, 4, 7). In *L. gibba* G3, because darkness inhibits both the initiation and early development of flower primordia, flowering is absent on any light-dark schedule in which the dark period is longer than about 14 to 18 hours. Thus experiments similar to those carried out with *Hyoscyamus* are not possible. However, the influence of cycle length on flowering has been shown for *L. gibba* G3 by keeping the dark period shorter than 14 hours and varying the length of the light period. When plants were grown on various cycle lengths from 24 to 72 hours with a 10-hour dark period, the FL % = 70 to 74 for the 24, 48, 60 and 72-hour cycles. However, the FL % = 41 for the 36-hour cycle. The influence of cycle length on flowering was also shown by the finding that the critical night-length was shorter for the 36-hour cycle than for the other cycle lengths (fig 10).

The above results indicate that the relative time of dark treatment can be more important than the frequency of dark treatment. For instance, on a 12L:12D regime (24-hour cycle) the plants receive a 12-hour dark period each day, and the level of flower induction is nearly 50 %. With a 24L:12D regime (36-hour cycle) the plants receive 24L:OD; 12D:12L; and 12L:12D over 3 successive days, and thus receive less total darkness than with the 12L:12D regime. Yet the level of flowering is practically zero. These results agree with those reported by Nakashima (17) by showing that a given dark period appears to exert a stronger inhibitory effect on flowering on a 36-hour cycle than on 24, 48, or 72-hour cycles. However, since the flowering response on a 60-hour cycle was as good as on a 48 or 72-hour cycle it seems clear that the interaction of 2 dark periods which leads to an enhanced inhibitory effect on flowering only extends over a relatively short light period.

The mechanism by which darkness inhibits flowering in long-day plants is not known. One possibility is that darkness in some way interferes with the translocation of the flowering stimulus in the phloem (16). However, since the vascular tissue in a *Lemna* frond is greatly reduced and the distances involved are less than 1 cm (10), it seems very unlikely that the phloem is of any real importance for the movement of the flowering stimulus within the frond.

A second explanation for the inhibitory effect of long dark periods on flowering is that the flowering stimulus is unstable during the dark. Although this possibility can not be eliminated and may very well be of some importance, it seems rather unlikely that it could be the sole basis for the dark inhibition effect. The sharp break between dark periods that completely inhibit flowering (14.5 hrs and longer) and those that permit some flowering (14 hrs and shorter) argues against the dark decay of the flowering stimulus as the sole basis for the dark inhibition effect.

It seems more probable that the sharp break between inductive and non-inductive light-dark cycles results from the interaction of the flowering stimulus with a light-labile flower inhibitor that is formed during the dark period. Since there is no flowering on a 50L:22D regime even though the photoperiod is clearly long enough to result in the formation of the flowering stimulus, it would appear that the flower inhibitor (i.e., darkness) acts to inhibit the functioning of the flowering stimulus, as well as presumably inhibiting its formation. At present there is no information on the nature of the presumed flower inhibitor, but it is hoped that future work may provide some information on this question.

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