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# Gibberellin and CCC Effects on Flowering and Growth in the Long-day Plant Lemna gibba G3[1]

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*Abstract.* The application of gibberellic acid ($GA_3$) to the non-rosette long-day plant *Lemna gibba* G3 at concentrations from 0.1 to 100 mg/l did not induce flowering on short days and inhibited flowering on long days at concentrations of 1 mg/l and higher. On both short and long days $GA_3$ concentrations above 1 mg/l caused a decrease in frond size and fresh and dry weight, but an increase in the rate of frond production and thus an increase in the # VF (number of vegetative fronds). Identical results were obtained when gibberellin $A_7$ was used instead of $GA_3$.

The addition of the plant growth retardant CCC [(2-chloroethyl) trimethylammonium chloride] to the culture medium on long days resulted in almost complete inhibition of flowering at $10^{-3}$ M. Vegetative growth was also inhibited to some extent. With CCC at $10^{-3}$ M the simultaneous addition of $GA_3$ resulted in partial reversal of flower inhibition with 0.3 mg/l $GA_3$ being optimal. The inhibition of vegetative growth as measured by fresh and dry weight was also partially reversed by $GA_3$, but the threshold concentration for reversal of flower inhibition was at least 10 times lower than that for inhibition of vegetative growth.

These results are interpreted as indicating that gibberellins are important for flowering in the non-rosette long-day plant *L. gibba* G3, but apparently are present in non-limiting concentrations on short days.

Gibberellic acid ($GA_3$) is known to cause stem elongation and flower induction in many rosette long-day plants (11, 12). Induction of flowering by $GA_3$ has also been reported in the non-rosette long-day plant *Lolium temulentum* (6), the long-short-day plant *Bryophyllum daigremontianum* (22), and the short-day plant *Impatiens balsamina* (14). However, in each of these last 3 cases flower induction, whether by photoperiodic treatment or $GA_3$ application, is accompanied by considerable stem elongation. In contrast, with the exception of *Lolium* and *Impatiens,* gibberellin treatment does not result in flower induction in caulescent or non-rosette long-day plants and short-day plants (all of which are caulescent) (12) and may even prove inhibitory to flowering in a few cases (8, 19, 21). On the basis of such results it appears that endogenous gibberellins may be important for flowering at least in those plants in which flower induction is accompanied by considerable stem elongation.

In recent years further evidence for the involvement of gibberellins in the flowering process of at least some plants has been obtained through the use of the plant growth retardants CCC [(2-chloroethyl) trimethylammonium chloride] and AMO 1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride) which are thought to act primarily by inhibiting endogenous gibberellin biosynthesis (5, 7, 10, 15, 18). The application of CCC or AMO 1618 to the long-day rosette plant, *Samolus parviflorus,* the long-short-day plant, *Bryophyllum daigremontianum,* and also the short-day plant, *Pharbitis nil,* when grown on inductive photoperiods, resulted in complete or nearly complete inhibition of flowering with only a small accompanying inhibition of growth (1, 23, 24). In each case the simultaneous application of $GA_3$ reversed the inhibition of both flowering and growth, but a 10 to 50-fold higher concentration of $GA_3$ was required to reverse the inhibition of growth than was needed to reverse the inhibition of flowering.

These results provided further evidence that gibberellins might be important for flower induction in long-day rosette plants and in the long-short-day plant *Bryophyllum.* In addition, the results with *Pharbitis* suggested that even in plants for which $GA_3$ treatment does not result in flower induction, gibberellins might be involved in the flowering process. In view of these results it was felt that similar experiments on a non-rosette long-day plant might provide additional information on the role of gibberellins in flowering.

The non-rosette long-day plant used in this study was *Lemna gibba* G3 which previously has been

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shown to exhibit a qualitative long-day flowering response with a critical daylength of about 10 hr (3). The present paper reports the effect of $GA_3$ and the plant growth retardant CCC on flowering and growth in this particular non-rosette long-day plant.

## Materials and Methods

The aquatic flowering plant *Lemna gibba* L., strain G3 was grown aseptically in 125 ml Erlenmeyer flasks with 50 ml of E medium. The E medium is based on the M medium of Hillman (9) and differs only in the addition of 30 $\mu$M ethylenediaminetetraacetic acid (EDTA). All experiments lasted 11 days and were carried out in model MB-54 growth chambers (Percival Refrigeration and Manufacturing Company) at a temperature of 28 ± 1° and a light intensity of 600 to 700 ft-c at plant level. The light source consisted of 4 cool-white VHO fluorescent lights (Sylvania F48T12-CW-VHO) supplemented with four 25 watt incandescent bulbs. Short-day conditions consisted of 9 hr light followed by 15 hr of darkness (9L:15D), while long-day conditions consisted of continuous light. A more detailed description of the medium, culture conditions and general experimental procedures used in this study has been given earlier (3).

The total frond number of a culture was determined by counting all fronds, no matter how small, which visibly projected beyond the margin of their mother frond. For the evaluation of flowering the flowering percent (FL %) 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. Flowering was also evaluated by determining the total number of vegetative fronds (# VF) in a culture. Providing the growth rate for different treatments is approximately the same, there is always 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 %.

CCC was dissolved in the medium and used at concentrations from $10^{-7}$ to $10^{-3}$ M. It was donated by the Agricultural Division of the American Cyanamid Company, Princeton, New Jersey. $GA_3$ (K salt, 75 %, Nutritional Biochemical Company) was dissolved in the medium and used at concentrations from 0.001 to 100 mg/l (corresponds to actual molarity of $2.17 \times 10^{-9}$ to $2.17 \times 10^{-4}$ M). When CCC or $GA_3$ were used they were present in the medium for the full duration of the experiment.

It is well known that gibberellins are somewhat unstable to heat (20). Hillman (8) investigated the effect of autoclaving for 10 min at 15 psi on the $GA_3$ activity for the growth of dwarf pea seedlings. His results indicated that autoclaving the $GA_3$ with the medium resulted in a loss in activity of about 1 order of magnitude. It has been assumed that autoclaving has a similar effect in the present study. However, the concentrations of $GA_3$ listed are those that were actually incorporated in the medium and do not represent estimates of the effective concentrations.

It has also been reported that CCC may be somewhat unstable to high temperature treatment (2). Consequently, each of the experiments to be presented has been repeated with the $GA_3$ and CCC added to previously autoclaved medium by sterile filtration. In each case there was complete qualitative agreement with the results obtained when $GA_3$ and CCC were autoclaved with the medium. Therefore, it is clear that the results to be presented are due to the $GA_3$ and CCC in the medium and not to any breakdown products that might possibly have resulted from the autoclaving.

## Results

The application of $GA_3$ to *L. gibba* G3 when growing on short days did not result in flower induction at any of the concentrations that were tried (Fig. 1). At higher concentrations $GA_3$ inhibited growth somewhat as indicated by a slight drop in the fresh and dry weights with a minimum at 10 mg/l $GA_3$. Furthermore, at $GA_3$ concentrations of 10 mg/l and higher the fronds were noticeably smaller and less gibbous than in the short-day control. However, at these higher $GA_3$ concentrations, the rate of frond production was increased, and there was an increase in the # VF.

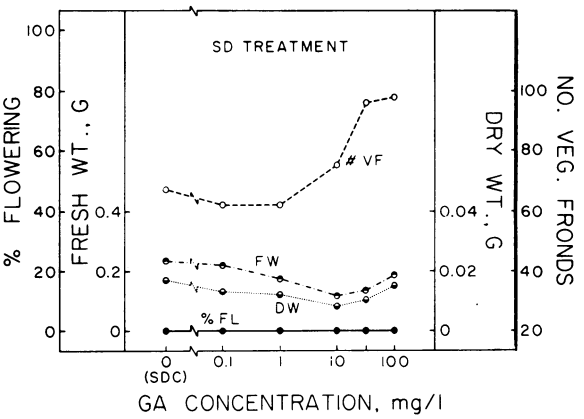


FIG. 1. Influence of $GA_3$ on flowering and growth on short days.

The application of $GA_3$ to plants growing on long days had an inhibitory effect on flowering at concentrations of 1 mg/l and above (Fig. 2). At these higher concentrations there was also a definite inhibition of growth with a substantial decrease in fresh weight resulting from the fronds being rather small and only slightly gibbous. The dry weight, however, showed only a slight decrease at higher $GA_3$ concentrations.

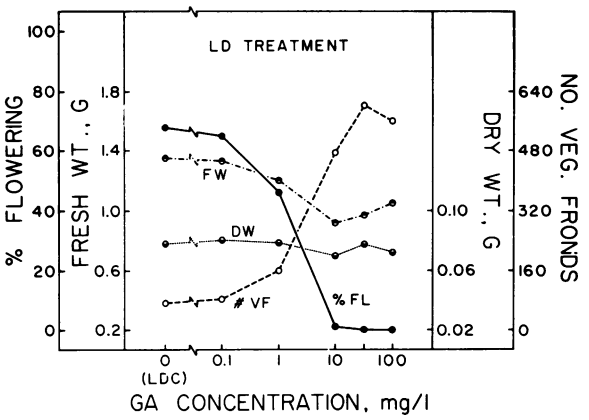


FIG. 2. Influence of $GA_3$ on flowering and growth on continuous light.

From the above results it would appear that the only influence exogenously applied $GA_3$ has on the flowering response of *L. gibba* G3 is to be inhibitory at concentrations over 1 mg/l. However, in the short-day plant *Pharbitis nil* Zeevaart (24) showed that although gibberellin treatment on long days did not induce flowering, the inhibition of flowering on short days by CCC could be overcome by the simultaneous application of $GA_3$ to the plants. Consequently, it appeared that gibberellin was important for flowering in *Pharbitis* but was present in nonlimiting concentrations on long days.

In view of the above results with *Pharbitis,* it was decided to try similar experiments with *L. gibba* G3 using CCC. When plants were grown on long days in the presence of CCC there was only a slight inhibition of flowering at concentrations as high as $10^{-5}$ M (Fig. 3). However, at a CCC concentration of $10^{-4}$ M flowering was strongly inhibited, and with an increase to $10^{-3}$ M only 2 very small flower primordia were seen in a total of 552 visible fronds examined for the 3 flasks.

Growth was also inhibited to some extent by CCC. This was shown both by the decrease in fresh and dry weights at $10^{-4}$ and $10^{-3}$ M CCC and also by the tendency at these concentrations for the fronds to remain attached together in 1 or 2 large clumps instead of separating into numerous 2 to 8-frond colonies as in the long-day control.

Attempts were made to reverse the inhibition of flowering caused by $10^{-3}$ M CCC by the simultaneous addition of various concentrations of $GA_3$ to the culture medium (Fig. 4). Clearly $GA_3$ can effect partial reversal of the flower inhibition caused by CCC. The optimum $GA_3$ concentration of 0.3 mg/l corresponds to an actual molarity of $6.5 \times 10^{-7}$ M (the effective concentration was probably less than $10^{-7}$ M $GA_3$; see Materials and Methods).

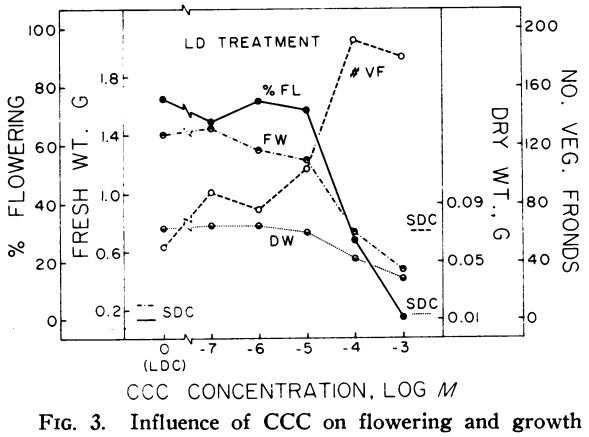


FIG. 3. Influence of CCC on flowering and growth on continuous light.

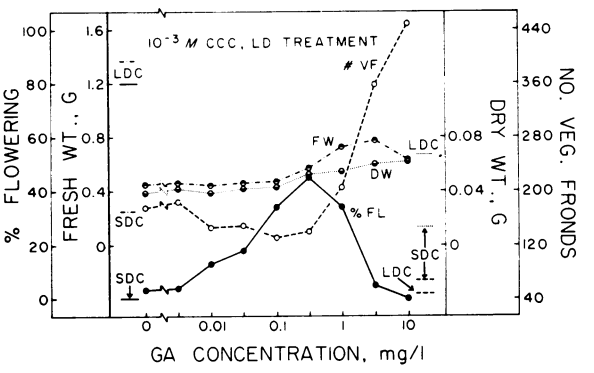


FIG. 4. Effect of $GA_3$ on reversing the inhibition of flowering and growth caused by $10^{-3}$ M CCC. All experimental treatments included $10^{-3}$ M CCC in addition to the indicated concentration of $GA_3$.

The $GA_3$ also partially reversed the growth inhibition caused by CCC. However, the threshold for the reversal of growth inhibition was 0.1 to 0.3 mg/l $GA_3$, whereas the threshold for the reversal of flower inhibition was about 0.01 mg/l $GA_3$. Furthermore, the optimum $GA_3$ concentration for flowering was 0.3 mg/l, while the optimum for growth was 1 or 3 mg/l. From these results it seems clear that CCC is able to inhibit flowering independent of

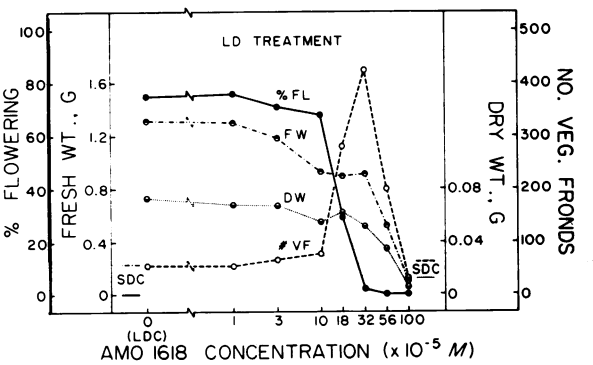


FIG. 5. Influence of AMO 1618 on flowering and growth in continuous light.

its effect on growth, and thus the ability of $GA_3$ to cause partial reversal of flowering suggests that a certain level of endogenous gibberellin is needed to obtain flowering in *L. gibba* G3.

When AMO 1618 was used instead of CCC, inhibition of flowering was also obtained (Fig. 5). However, several attempts to reverse the inhibition of flowering caused by $3.2 \times 10^{-4}$ M or $1.8 \times 10^{-4}$ M AMO 1618 with $GA_3$ have been completely unsuccessful.

## Discussion

The results of the present study indicate that *L. gibba* G3 is like most other non-rosette long-day plants that have been examined in that the application of $GA_3$ to plants growing on short days does not result in flower induction. This result is further substantiated by preliminary findings which indicate that treatment with gibberellin $A_7$ ($GA_7$), which often proves more effective for flower induction than $GA_3$ (13), does not lead to flower induction when tested over the dosage range of 0.1 to 30 mg/l.

A conflicting report for *L. gibba* G3 has been published by Oota (17). He claimed that $GA_3$ enhances flowering and that $10^{-5}$ M $GA_3$ reduces the critical daylength by about 2 hr. However, the results are based on a very small flowering response, and the reported differences are extremely small and thus of questionable significance. In addition, on a 12L:12D regime low concentrations of $GA_3$ have no effect on flowering, while concentrations of 1 mg/l and higher inhibit flowering (Cleland and Briggs, unpublished results). Consequently, it is the opinion of the present authors that the results of Oota do not support his conclusions and thus do not contradict the results of the present study.

In this study the only effect exogenously applied $GA_3$ had on flowering was to prove inhibitory at concentrations of 1 mg/l and higher. In preliminary work $GA_7$ was also found to inhibit flowering on long days at concentrations of 1 mg/l and higher. In the short-day plant *Lemna perpusilla* 6746 $GA_3$ also proved inhibitory for flowering (8). The mechanism of this inhibition remains unknown. However, in *Fuchsia* it appears that gibberellin-induced inhibition of flowering occurs at the receptor meristem (19). Therefore, by analogy it may be that the high gibberellin levels inhibit flowering in *Lemna* by interfering with the expression of the flowering stimulus rather than with its formation.

In addition to the effects on flowering $GA_3$ had some rather striking effects on growth. On both short and long days at concentrations of 1 mg/l $GA_3$ and higher, there was a decrease in the frond size and fresh and dry weight, but an increase in the rate of frond production which resulted in an increase in the # VF. Similar results were obtained by Hillman (8) for *L. perpusilla* 6746 and by Kato for the short-day plant *L. paucicostata* (see 20). Thus these growth effects of $GA_3$ may prove typical for most species of *Lemna*.

The failure of $GA_3$ to produce a complete reversal of the CCC inhibition of flowering is open to several possible explanations. First of all if $GA_3$ was less effective than the endogenous gibberellin(s) for stimulating flowering, then application of $GA_3$ might only produce a partial reversal of the CCC inhibition of flowering. Secondly, CCC may affect processes other than the biosynthesis of endogenous gibberellins, and thus $GA_3$ or any other gibberellin might only be expected to cause partial reversal of the CCC inhibition of flowering. One process that may be affected by CCC is the auxin metabolism. Norris (16) has shown that CCC results in a reduced level of auxin in wheat seedlings. Cleland (4) showed that AMO 1618 inhibited the growth of *Avena* leaf sections, and that this inhibition was partially reversed by auxin, but not by $GA_3$. Thus it is possible that the application of auxin to *L. gibba* G3 along with 0.3 mg/l $GA_3$ would have resulted in a further reversal of the CCC inhibition of flowering.

The failure of $GA_3$ to produce any reversal of the inhibition of flowering caused by AMO 1618 suggests that AMO 1618 inhibits certain processes in addition to gibberellin biosynthesis. However, attempts to reverse the AMO 1618 inhibition of flowering by substances other than gibberellins have yet to be made.

The results of the present study indicate that endogenous gibberellins are important for flowering in *L. gibba* G3 but apparently are present in non-limiting concentrations on short days. However, the results do not indicate whether gibberellins may play some direct role in the flowering process *per se*, or simply influence flowering in some indirect fashion. Furthermore, since the CCC and $GA_3$ were present throughout the 11 day experiment, it is not clear whether gibberellins influence flowering through an effect on flower induction or some later stage of flower development.

The results of the present study are similar to those obtained with *Samolus, Bryophyllum,* and *Pharbitis* where gibberellin reversal of CCC or AMO 1618 inhibition of flowering has also been reported (1, 23, 24). However, the significance of the results with these 4 plants for a better understanding of the flowering process in general is somewhat questionable since Cathey and Stuart (2) tested the effect of CCC and AMO 1618 on a great number of plants and obtained inhibition of flowering in only a few cases. Furthermore, in the long-day rosette plant *Silene armeria* a very high dose of AMO 1618 sufficient to completely block bolting on long days had absolutely no effect on flower initiation (Cleland and Zeevaart, unpublished results.) Therefore, it would appear that the extent to which gibberellin influences flowering may vary considerably from plant to plant and only in a few cases can an actual dependence upon gibberellin for flowering be demonstrated.

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