Photoperiodic Responses of Two Cestrum Species and Non-interchangeability of Their Flowering Hormones¹,²,³

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Introduction

Recently Lincoln et al. (2) isolated a fraction from the leaves of induced Xanthium strumarium L. which, when applied to the leaves of noninduced plants of X. pensylvanicum Wallr., caused formation of flower primordia. With this discovery it becomes more important that we examine various species to determine, if possible, whether a single flowering hormone is present in Angiosperms, or a series of such hormones. This report offers some evidence in this area.

Evidence concerning photoperiodic response of Cestrum nocturnum L. has been previously reported by Sachs (4). In his work it was demonstrated that C. nocturnum is a long-short day (LSD) plant; that the hormone is synthesized in mature leaves and is transported through the stem to the potential floral buds both acropetally and basipetally. This present investigation gives evidence that C. nocturnal responds with flower formation to a long day (LD) treatment as well as to a LSD treatment.

Materials & Methods

Plant Material. Two species, C. diurnum and C. nocturnum, were used for this study. They were grown from seeds gathered in the Los Angeles area from plants maintained for ornamental purposes. Seeds were germinated under LD conditions, the seedling appearing within 10 to 14 days. Plants were transplanted to 8 ounce cups when they had their first pair of true leaves and later to quart size pots when necessary.

Growth Conditions. Temperatures for all experiments were 23° day and 19° night. Plants maintained on LD conditions were given 18 hours of natural and supplemental light and on short day (SD) 8 hours of natural light; supplementary light was from fluorescent tubes and had an intensity of ca. 20 ft-c. Those experiments in which the length of the LD and/or the quality of supplementary light might have affected the reported results were repeated with a 16-hour LD and supplemental light supplied by a combination of fluorescent tubes and incandescent bulbs with an intensity of ca. 200 ft-c.

Graft combinations. In some experiments graft combinations with C. diurnum and C. nocturnum as scions, and C. diurnum as stock were made. These plants were grafted by using the inarching method, severing the scion from its root system after the graft union had been established. Intergrafts were produced in the same manner. The same plant was used for several experiments by simply cutting back both shoot systems and allowing new ones to develop. Further, all experiments involving grafted plants were repeated with different plants and different ages of graft unions.

Both species used in these experiments produce axillary flower clusters, generally with from 7 to 15 flowers per cluster. These are most readily initiated in the axes of leaves but also occur on old wood after leaf abscission has occurred. In both species all axillary buds are potential flower sites. Quantitative measurement is based on the percentage of flower clusters formed relative to the total number of potential floral sites. Statements of bloom infer that more than 25% of the sites initiated flower buds. Flower buds were considered formed when visible with a hand lens.

Results

Photoperiodic Responses of C. diurnum. Seedlings were grown under LD conditions and, starting with 16-week old plants, a single plant was placed on SD conditions each week for 12 consecutive weeks. Plants placed on SD after having reached an age of 20 weeks or more produced floral primordia.

Single-stemmed plants, grown continuously on LD, were either partially or completely defoliated and placed under SD conditions. Defoliated plants did not produce floral primordia, while those plants on which one or more leaves were retained did, demonstrating that leaves are necessary for perception of the SD treatment. Two-branched plants, grown on LD, were placed on SD after all of the leaves had been removed from one of the branches: flower
clusters were produced on both branches, indicating that the hormone moved from the branch with leaves to the defoliated one.

Six-month old plants, grown continuously on LD, were placed on different numbers of SD starting with a single SD. All plants given two or more SD produced floral primordia, none of those given a single SD responded with flower formation.

Eight plants were grown continuously on LD for 24 months without pruning. At the end of that time they were placed on SD and produced 615 ± 150 flower clusters from 2650 ± 540 floral sites. Prior to placing these plants on SD, 5 of them had not initiated floral buds while 3 had sporadically done so as follows: 1 plant, 6 flower clusters at 16 months; 1 plant, 1 flower cluster at 20 months; and 1 plant, 3 flower clusters at 14 months, 2 at 16 months, 6 at 20 months, and 6 at 22 months.

Four plants have been growing on SD for 12 months and at this time have about 1500 floral sites each. Two of these have not produced flower clusters. One has produced a single cluster at 10 months and another 15 clusters during the 9 to 12 month period.

These experiments show that seedlings 4 months old or older of C. diurnum respond to LSD treatment with a minimum of 2 SD, and that flowering hormone is synthesized in the mature leaves and is transported acropetally and basipetally to the floral sites. The data obtained so far show that C. diurnum reacts as a LSD plant, but when grown under LD or SD conditions for long periods a few flower clusters are sporadically formed.

Photoperiodic Responses of C. nocturnum. Seedlings were grown under LD conditions, and starting with 12-week old plants, a single plant was placed on SD each week for 15 consecutive weeks. Plants placed on SD after having reached the age of 19 weeks or more bloomed. A group of 12 plants was continued on LD. These plants bloomed between the 26th and 31st week. In all cases the total number of flowers was about the same as in those on plants that had received a SD induction. However, the appearance of the floral primordia occurred over a period of about 1 month while in those that received a SD induction the floral primordia all appeared at about the same time.

A similar experiment with seedlings sown 3 months later was conducted in the following manner. Three blocks of 12 plants each were selected: two blocks were grown on normal LD, and the third grown on LD with shading to cut the maximum light intensity to about 600 ft-c. One of the blocks maintained on normal light conditions was transferred to SD 22 weeks after sowing. All 12 plants produced floral primordia in from 5 to 7 days. The plants in the other two blocks, retained on LD, produced floral primordia in from 28 to 40 weeks: with 16 of the plants blooming before the 34th week. There was no difference in the number of plants blooming in any one given period between shaded and non-shaded plants, although the shaded plants exhibited the vegetative characteristics usually found in plants with insufficient light.

A single plant, given a SD inductive period and returned to LD conditions for purposes of seed production, bloomed continuously and profusely for 12 months. During this time the plant was not pruned in any way.

The experiment was terminated after 1 year. Five other plants treated in a similar manner have not shown a tendency for continuous bloom. In each case they have had a periodic bloom flush typical of the species when maintained on LD.

Finally, a group of 18 plants were maintained on LD conditions and allowed to bloom. When the first flower of a bloom flush opened on each plant, that plant was cut back to a short stump just above the soil line, with no mature leaves retained on the plant. It was again allowed to bloom and the process repeated. These plants bloom in from 12 to 28 weeks after being cut back. At the time of writing this paper some of them have completed 6 such bloom cycles. No rhythm or other regularity has been noted in this periodic bloom to date.

These experiments show that seedlings, 5 months old or older, of C. nocturnum respond to LSD treatment while seedlings 7 to 10 months old and older, bloom on continuous LD, and that the quantity of light, 600 ft-c or more does not materially effect the floral response. Sachs (4) has shown C. nocturnum on SD for 18 months without flowering. Further,
there appears to be a factor for continuous bloom present in some individuals of this species.

Specificity of the Flowering Hormone. Grafted plants comprised of *C. diurnum* and *C. nocturnum* were treated the following ways with the listed results (fig 1). When established plants were maintained on LD conditions the *C. nocturnum* shoot bloomed in the same manner as single plants of the species (fig 1A). In one plant, maintained continuously on LD for 18 months, the *C. nocturnum* shoot bloomed at the following times: 3 months, 10 months, 14 months, and 17 months. During this time the *C. diurnum* partner did not form floral buds, as was the case in all plants continued on LD conditions.

Plants were given SD treatment with the leaf tissue of both species intact (fig 1B). In all cases both shoots bloomed profusely and in a manner similar to single plants of that species.

Plants were given SD treatment with the leaf tissue of one or the other species completely removed (fig 1C & D). In two experiments all of the axillary buds were carefully removed from the donor, in one case just prior to the short-day stimulus, and in the other 4 days after the SD stimulus when the buds had started growth. In the plants with the buds intact the donor bloomed normally. In all cases no floral primordia were formed on the tissue of the species whose leaves had been removed (table I).

As a final experiment intergraft of *C. nocturnum* were placed between two shoots of *C. diurnum* under LD conditions (fig 1E). After these were well established, one of the branches of *C. diurnum* was defoliated and the plant given a SD treatment. Both the intact and defoliated branches of *C. diurnum* produced floral buds regardless of the presence or absence of leaves on the interstock. In cases where no leaves were retained on the *C. nocturnum* intergraft, there were no floral buds; but when the leaves were retained, in one case an intact side branch 40 cm long, *C. nocturnum* tissue also produced floral buds (table II).

These experiments indicate the synthesis of different flowering hormones in these two species which will not initiate floral buds in the other species but can be readily transmitted through its tissue.

**Discussion**

A survey of the literature to date shows that transfer of a flowering hormone has been observed on more than 25 graft combinations of various plants. These include combinations of LD, SD, and day neutral (DN) species or varieties. From this evidence it follows that various plants will respond to the same flowering hormone and this hormone may be synthesized as a result of different photoperiodic treatments in various species, or that a plant may respond to more than one flowering hormone.

In an early review of this subject, Lang (1) presents work on grafting to that time and concludes that the immediate effect of day length is localized in the leaf and that it results in the formation of a floral stimulus which is alike in LD and SD plants. He further states that this interchangeability is not conclusive proof of identity.

In a recent review Salisbury (3) finds no evidence in addition to that presented by Lang (1) to alter the general idea concerning the problem of hormone identity. In his review there is a tendency to refer to it as a single hormone, although no claim of such is made.

Recently Zeevaart (5, 6) reported on a series of highly successful grafting experiments on transmission of the floral stimulus. His work included the successful transmission between various plants requiring SD, LD, and LSD stimuli for hormone synthesis. In particular, his work showing that on LD conditions, *Nicotiana tabacum* var. Delcrest, a DN plant, could not induce flowering in either *N. tabacum* Maryland Mammoth, a SD plant, or *N. sylvesteris*, a LD plant, but could act as a successful intergraft between the two while *N. sylvesteris* stimulated *N. tabacum* Maryland Mammoth to bloom
becomes an important key in seeking the true condition. He concluded that it seems improbable that the floral stimulus in *N. tabacum* var. Delcrest and *N. tabacum* var. Maryland Mammoth are identical and cautioned against assumption of an identical flowering hormone in all plants.

The experiments reported in this present investigation indicate the presence of different flowering hormones synthesized in the mature leaf under the influence of the same treatment. LSD; and in this respect support statements by Lang (1) and Zeervaart (6,7) cautioning against assumption of identity of the flowering stimulus. One approach to the problem, however, has not been tested in this work, i.e., the reduction of the size of the acceptor scion to a few or a single floral site to concentrate any material crossing the graft from the donor. Such experiments are being conducted.

Recently Lincoln et al. (3) have succeeded in extracting a mixture from the leaves of Helianthus that produced floral primordia in Xanthium when applied to its leaves. They conclude that this strengthens the belief that there is a common chemical substance, or closely related series of chemical substances, governing the transition to flowering in many plants. This work on Cestrum, pointing toward a group of flowering hormones is not, in this respect, in disagreement with the above work.

Finally the questions are raised: if a single hormone can be synthesized as a result of more than one stimulus, and if a plant can respond to more than one hormone. *C. nocturnum* may offer a clue to the answers of these problems since floral primordia are initiated in this species as a result of two different environmental patterns: a continuous 16 or 18 hour photoperiod, or the change from a 16 or 18 hour photoperiod to an 8 hour photoperiod. Investigation through this approach is being carried on.

**Summary**

Photoperiods and interchangeability of flowering hormones were investigated in *Cestrum diurnum* L. and *C. nocturnum* L. *C. diurnum* responded to long-short day treatment with a minimum of 2 short days; the flowering hormone was shown to be synthesized in the mature leaves, moving both acropetally and basipetally. *C. nocturnum* responded to both long day and long-short day treatment with plants of 5 months maturity or older responding to long-short day treatment and plants of 7 to 10 months maturity or older responding to long day treatment. Grafted plants, comprised of the above two species, were grown under long day conditions. Plants maintained on long day for a sufficient length of time had the *C. nocturnum* scion bloom normally without affecting the *C. diurnum* scion. When one of the species was defoliated and the grafted plant placed on short day conditions, the scion whose leaves were retained bloomed while the other did not. An intergraft of *C. nocturnum* was placed between two shoots of *C. diurnum* and one of the shoots defoliated. The plant was placed on short day conditions and both the foliated and defoliated branches bloomed. These data suggest different flowering hormones in these two species with the hormone of one not capable of instigating flowering in the other.

**Literature Cited**