

CONTROL OF DAILY FLOWERING TIME IN *TALINUM* (PORTULACACEAE)

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**ABSTRACT**--The limestone cedar glade endemic *Talinum calcaricum* Ware (Portulacaceae) and its sandstone outcrop ecological vicariad *Talinum mengesii* exhibit very constant daily flowering time and duration (1200 to 1800 h) throughout the summer growing season in their respective habitats. Experiments were conducted to test for characteristics of an endogenous circadian rhythm in flowering. In both species, phase shifting of the light-dark cycle produced an equal phase shift in flowering time. Neither the daily rhythm nor flowering synchrony persisted for even 24 h in either constant light or constant darkness, but normal rhythm was re-established the first day after return to normal light-dark cycles. Actual flowering time was not directly related to changes from dark to light or light to dark, and normal 24-h flowering cycles were maintained with or without changes in light intensity during the day, with or without changes in temperature during the day, and at 8-, 12-, or 16-h photoperiods. Though the failure of the diurnal cycle to persist at all in constant light or constant dark argues against a classical circadian rhythm, the extreme accuracy and synchrony of the flowering clock under a variety of conditions argues that a very accurate daily clock exists, one with remarkable similarity to the non-classical clock previously shown to time leaf movement in the related genus *Portulaca*.

The small perennial leaf succulent *Talinum calcaricum* Ware (Portulacaceae) is endemic to the limestone cedar glades of middle Tennessee and adjacent northern Alabama and southwestern Kentucky (Ware, 1967; Baskin and Baskin, 1991). It flowers all summer long (mid-May to late September) in the narrow ecotone of very shallow soil (1 to 10 cm) between bare limestone and the denser, grass-dominated vegetation of deeper soil (Quarterman, 1950, 1989; Ware, 1969b; Somers et al., 1986). Its bright purplish-pink flowers, borne on flexible wiry stems, stand well above the thicker-stemmed, leaf-bearing lower portion of the plant. These conspicuous flowers have no doubt led to one of its local common names, "rock pink," while the similarity of its succulent terete (cylindrical) leaves to those of the cultivated moss-rose (portulaca) are responsible for its other local common name, "rock moss." The name "fameflower" listed for the genus *Talinum* by botanical manuals is apparently used only by individuals who have learned that name from such manuals. On the sandstone outcrops of northern Alabama, *Talinum mengesii* Wolf, a similar species with larger, paler pink flowers, plays a similar ecological and esthetic role to that played by *T. calcaricum* in the cedar glades (Ware, 1967, 1969a, 1991; Ware and Pinion, 1990).

Not only do these two species flower during the hottest, driest part of the summer in their respective rock outcrop communities, but their ephemeral flowers are open during the hottest part of the day (in both species 1200 to 1800 h). Both species are remarkably constant in the time of opening and closing of their flowers each day. Other botanists have also commented on the remarkable constancy of daily flowering time in *Talinum*. Holzinger (1900) noted that flowering time was so reliable it could be used as a taxonomic character in the genus. He reported that flowering time alone could be used to separate *Talinum rugospermum* (flowering from 1530 to 1800 h) from the very similar *T. teretifolium* (flowering from 1200 to 1500 h). He urged collectors to note

the time of flowering on herbarium labels for the benefit of future users of those herbarium specimens, but his advice seems to have generally been ignored or overlooked. Lebrun (1965) confirmed that timing and duration of daily flowering was characteristic of each species and population of *Talinum* he collected in Africa, with each maintaining its characteristic flowering time when all were grown under the same conditions. The isolated populations of *T. calcaricum* in Franklin Co., Alabama, begin flowering 2.5 h later than those in Tennessee but then close at the same time (Ware, 1967). Plants grown from seeds in the greenhouse maintain this difference in time and duration of flowering, confirming that, in this species also, the timing of flowering is genetic.

The regularity of flowering time in *T. calcaricum* and *T. mengesii* and its genetically controlled nature suggest that an endogenously timed diurnal cycle, or circadian rhythm, might be involved in the control of flowering. It is already known that the length of the growth season in *T. calcaricum* and *T. mengesii* is internally controlled (Ware, 1972), so clearly both species possess some kind of internal clock mechanism. A series of experiments was conducted to test the diurnal flowering cycle of these two species for characteristics of an endogenous circadian rhythm.

## MATERIALS AND METHODS

*Phase Shift*--Ten plants of *T. calcaricum* and 10 of *T. mengesii* were placed in a growth chamber on a 16L:8D cycle (with a 27° to 10°C thermoperiod). The light period began at 0600 h (approximately local dawn). This was the "normal" cycle. Ten plants of each species were placed in a second growth chamber with similar photoperiod and thermoperiod but with the light period shifted 6 h out of phase (beginning at 1200 h). Flowering time was recorded for the next 4 days. To standardize recording of flowering time for groups of plants in this

experiment and others, a flower was counted as open when its petals were at an angle with the pistil  $>45^\circ$ , and flowering time for the group was recorded when at least half the flowers had exceeded the  $45^\circ$  condition (flower opening normally continues until the angle is about  $90^\circ$ ).

In a second experiment, 10 plants of *T. calcaricum* were again placed on the normal cycle, and a second 10 plants of that species were kept in a growth chamber 12 h out of phase with the first. Flowering time was recorded for 1 week. At the end of the week, the out-of-phase plants were removed from the growth chamber at 2100 h (4 h before they should have flowered) and transferred to the dark (nighttime) greenhouse. Their flowering time was recorded the next day.

**Effect of Constant Dark or Light**—Ten plants of each species were transferred to a constant light at  $27^\circ\text{C}$  for 1 week, and daily flowering times were recorded. Ten plants of each species were also transferred to constant darkness at  $10^\circ\text{C}$  for 1 week, and daily flowering times were recorded. The delicate petals of *T. calcaricum* and *T. mengesii* were too weak to trip a mechanical movement sensor, so that the growth chambers had to be opened to check for flowering. The growth chambers were in a partially enclosed space that could not be darkened, so brief interruptions of the dark regime (usually  $<30$  sec) took place when the growth chamber was opened to observe any flowering response. These interruptions were usually made at night, so that light intensity could be kept very low. However, on the second and third day of constant darkness, a very brief interruption of darkness occurred in mid-afternoon when a visual check for flowering was made.

**Effect of Change for Dark to Light**—To determine whether flowering cycles were set directly by changes from dark to light or light to dark, plants of *T. calcaricum* were grown throughout their lives at 8-, 12-, and 16-h photoperiods with a  $27^\circ$  to  $10^\circ\text{C}$  thermoperiod, and the timing of opening and closing of the flowers in each photoperiod was recorded once the plants matured.

## RESULTS

**Phase Shift**—In *T. calcaricum* and *T. mengesii*, the plants on the normal photoperiod regime flowered at approximately 1300 h, the time they would be expected to flower in nature. In both species, the plants on a photoperiod cycle 6 h out of phase with the normal regime flowered 6 h later than those on the normal regime. In the second experiment, *T. calcaricum* on a photoperiod cycle 12 h out of phase with the normal cycle flowered 12 h later than those on the normal cycle (at 0100 h instead of the normal 1300h). After a week on this regime, the out-of-phase plants were removed (during the light phase, 4 h before they should have flowered) to the (unlighted) greenhouse at 2100 h. None of the plants flowered in the dark greenhouse that night, but flowers opened at the normal time (1300 h) the next day, without a readjustment period.

**Constant Light or Dark**—In the constant light and constant dark experiments, results were the same for both species, and the events described here apply to both species. Plants transferred to a dark growth chamber from the greenhouse in the morning (3 h before they should have flowered) opened in darkness that afternoon, though 1 h later than usual. After 24 h in darkness, however, flowering synchrony had been lost. Flowers opened thereafter around the clock. Most of the flowers did not open fully ( $>45^\circ$  but  $<90^\circ$ ) and began closing before 6 h had elapsed. After 4 days in darkness, the plants were removed to light at 1500 h, and, 4 h later, flowers opened on several plants synchronously. The next day the plants flowered at the normal time.

Plants transferred to a constant-light growth chamber flowered as expected the first day, but, after 24 h, synchrony had been lost, and flowers were opening around the clock. Here also, most of the flowers did not open fully and began closing before 6 h had elapsed. After a week

in continuous light, the plants were given an 8-h dark period, and they flowered in synchrony about 6 h after the dark period ended.

**Change from Dark to Light**—Table 1 presents a summary of the relationship of flowering time in *T. calcaricum* to the change from dark to light on three different photoperiods. The flowers on the 8-h photoperiod were still fully open when the light period ended, 2.5 h after anthesis, and they closed in the dark before the usual flowering time of 6 h had expired. The plants in the other two photoperiods had normal 6-h-long flowering periods. However, there was no clear relationship between the time of flower opening and either the change from dark to light or the previous night's change from light to dark.

## DISCUSSION

Since the beginning of a cycle of an endogenous rhythm is usually regulated by the change from dark to light or light to dark, the shift of flowering time in *T. calcaricum* and *T. mengesii* in response to (and by the same number of hours as) a shift of the timing of the light-dark cycle is what would be expected if their flowering was controlled by an endogenous cycle set by the change from light to dark or vice versa. However, the diurnal rhythm and synchrony among plants was lost after only 24 h in either constant darkness or constant light, rather than persisting for several days as is the usual case with endogenous rhythms (Sweeney, 1987). Furthermore, the plants returned to a normal flowering time just 1 day after being removed from constant darkness or constant light, without any readjustment period to reset a diurnal clock. The complete loss of synchrony of flowering time in *Talinum* in constant light and constant dark argues against the notion of an ordinary endogenous circadian rhythm controlling flowering in this genus (Sweeney, 1987). Further, the single brief interruption of the constant darkness in mid-afternoon on the second and third days would have been sufficient to entrain an endogenous rhythm and keep the flowering synchronized in many plants (Salisbury and Ross, 1991), but this did not happen. On the other hand, Karve and Jigajinni (1965, 1966) reported a daily rhythm in leaf movement in *Portulaca grandiflora* in which the cycle became very weak after only 24 h in constant darkness and in which out-of-phase movements seemed to adjust themselves to the original timing immediately upon being returned to the original light-dark cycle. They interpreted the quick return to the original timing as indicating an underlying endogenous clock resistant to phase shifting even as the activity it timed (leaf movement) became irregular. If their interpretation is correct, then it might be reasonable to conclude that flowering in *Talinum* is controlled by the same sort of endogenous clock that controls leaf movement in the related genus *Portulaca*. It is not a classical endogenous rhythm as defined by Sweeney (1987), however.

The ease in shifting the flowering time by light-dark phase shift, the quick loss of synchrony in flowering in constant light or constant darkness, and the quick return to synchrony the first day back on alternating light-dark cycles suggest that change from dark to light (or

TABLE 1. Effect of photoperiod on flowering time in *Talinum calcaricum*.

Photoperiod (h)	Time of light period (h)	Flowering time (h)	Hours after light began	Hours after last dark period begin
8	0900-1700	1430	5.50	21.50
12	0900-2100	1345	4.75	16.75
16	0600-2200	1245	6.75	14.75

light to dark) could be the essential cues to timing of flowering in *Talinum*. This could be true whether the clock is a classical circadian one, or an "hourglass" clock, i.e., one that begins timekeeping when stimulated and runs only until the timed activity is completed (Bunning, 1973). An "hourglass" clock probably controls the length of the summer growth period in these two species of *Talinum* (Ware, 1972).

However, neither the experimental data presented nor the behavior of the plants in nature support the change from dark to light (or vice versa) as a simple clock-setting agent for flowering. An examination of Table 1 shows that while daily flowering time remained on a constant 24-h cycle on all photoperiods, there was no consistent relationship across photoperiods between flowering time and the change from dark to light or between flowering time and the change from light to dark at the end of the previous cycle. In nature, daily flowering time remains constant all summer (1300 h), instead of being either earlier or later as it would if a clock were set daily by the light-dark change at dawn or dusk. The timing mechanism in *Talinum* must be unaffected by, or must compensate for, the changing day lengths during the flowering season. Since "hourglass" clocks do not have this characteristic, some support is provided here for a circadian mechanism which could adjust to changing daylength (Bunning, 1973).

There is some suggestion in nature that changes in light intensity during the daylight period may give a necessary cue for proper timing and synchrony of flowering in *Talinum*. Both in the field and in the greenhouse, anthesis is sometimes delayed an hour or even two on overcast or rainy days, when there is less increase in light intensity toward 1300 h. The flowering period is cut short in such a case, since closing then occurs at the normal time or even slightly early. The flowers may never open fully on a heavily overcast day but stop when about three fourths open and remain in that condition until closing. If the next day is bright, flowers open at the usual time. Since all the changes in light intensity during the day mentioned earlier result in parallel temperature changes, temperature change might be invoked as a possible cue for timing of flowering. In fact, temperature is known to control timing in some genera of Portulacaceae (Iwanami and Tsugi, 1962).

However, the experimental data do not support either light intensity or temperature change as essential clues to timing of flowering. The less complete opening of flowers in constant darkness or constant light and the shortening of the length of the flowering period were somewhat similar to the behavior of the flowers in nature on heavily overcast days. However, in the growth chambers on 12- and 16-h photoperiods, light intensity and thermoperiod were constant throughout the light period; yet, flowering constancy and synchrony was maintained in *T. calcaricum* despite the lack of change in light intensity or temperature, and the flowering period was of normal length. Therefore, while change in light intensity (or associated temperature change) during the light period may affect flowering, neither light intensity change nor temperature change during the light period are essential in setting flowering time or controlling synchrony.

While flowering asynchrony in constant darkness and constant light argue against a classical circadian rhythm in these two species of *Talinum*, the remarkable synchrony of flowering of plants and constancy of the length of the daily flowering period reveal that these plants have a very accurate daily clock of some kind. Neither the time of change from dark to light or from light to dark, nor changes in light intensity or temperature during the day, nor the duration of light period accurately predicts the beginning of the daily flowering time in experimental or natural conditions. The mechanism of clock setting is not based solely on any one of these simple changes, though all of them clearly interact to control the timing and duration of flowering in *Talinum*.

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