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# Synchronous pulsed flowering in passion flower (*Passiflora incarnata*)

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**Daily observations of the number of flowers blossoming per day of passion flower, *Passiflora incarnata* reveal synchronous pulsed flowering. There seems to be no direct correlation between the observed temporal oscillations in flowering and environmental factors like the change in temperature, humidity and photoperiod. These temporal oscillations are characteristic of an open nonlinear dynamical system and are likely to arise from endogenous factors like the concentration of a florigen and an anti-florigen. A modified Lotka model is shown to mimic the monthly periodicity and the annual recurrence.**

**Keywords:** Florigen, Lotka model, passion flower, *Passiflora incarnate*, temporal oscillations.

PLANTS are generally classified into gymnosperms and angiosperms. The latter yield flowers, which may result in fruits and seeds. Some plants are seasonal and some are perennial. In the latter category, flowering may occur seasonally or may occur over a period of time, each year. Flower opening in many plants follows the circadian rhythm. It has impact on the lives of pollinators and on the production of seeds, which in turn has enormous commercial and societal implications. Therefore, it becomes important to follow and understand the cycle of flowering in plants.

Phenology of flowers has been the subject of several investigations over the years. For example, Gentry[1] found evidence for steady state flowering, cornucopia flowering (flowering over a period of a month or so) and 'big bang' or 'burst' flowering (with synchrony) in some of the species in Central America. Augspurger[2] studied the flowering in 20 individual plants in 6 different shrub species. In addition to counting the number of newly opened flowers every day in each plant, she looked for synchrony, if any, in each set of 20 flowers. Although she noticed a large variation in the number of flowers per individual plant in some of the species, she concentrated on the mean number of flowers per plant. While reporting on the phenology and control of flowering in tropical trees, Borchert[3] emphasized on the difference between trees and herbaceous plants. He also emphasized on flower induction, early differentiation of flower buds, development of flower buds and the onset of anthesis. He pointed out that the environmental factors played little role in flower induction and anthesis. Kochmer and Handel[4] analysed the monthly data on flowering for a large number of species in North and South Carolina and Japan (comparable latitude) and concluded that there was a bimodal distribution in flowering time and it was more dependent on phylogenetic constraints than on competition for pollinators. Toy[5] followed flowering in certain trees in West Malaysia over a period of 75 days. There was some intra-species synchrony; otherwise, there was sequential flowering between species.

Newstrom *et al.*[6] classified the flowering patterns as continual, subannual, annual and supra annual. Each category of flowering was further classified as regular or irregular. Unfortunately, they did not present any time series analysis that could have revealed the underlying nonlinear dynamical behaviour. Wright and Calderon[7] examined weekly phenological data on flowering season length for 217 tropical species and concluded that there was no basis for water stress hypothesis (i.e. flowering precedes development of leaves, which requires more water) or for the shared pollinator hypothesis (i.e. species flowering at about the same time would share the same set of pollinators). Sakai[8] found certain irregular patterns

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in general flowering and found certain periodicities in annual production of seeds in lowland mixed dipterocarp forests of South-east Asia. Bawa *et al.*[9] pointed out certain generalizations about flowering and its relation to dry and wet seasons in tropical rain forest trees, but they did not focus attention on individual plants. Chang-Yang *et al.*[10] examined decade long (weekly) data on temporal variation in flowering and seed production in a subtropical rain forest and concluded that while extreme weather conditions could cause considerable change in the pattern, it was difficult to discern the effect of usual fluctuations in weather conditions on flowering.

Michalski and Durka[11] observed synchronous pulsed flowering in *Juncus* family and presumed that it must be to enhance outcrossing. Munguía-Rosas and Sosa[12], on the other hand, found the pulsed flowering in columnar cactus, *Pilosocereus leucocephalus* to be asynchronous and related to the external temperature and plant size. Wadgymar *et al.*[13] noted simultaneous pulsed flowering in temperate legume, but could find no correlation with average temperature or water availability.

The present communication reports synchronous pulsed flowering in *Passiflora incarnata* (PI), commonly known as passion flower (Figure 1). PI is a perennial vine that blossoms over an extended period (several months) each year. It sheds practically all its leaves by the peak of winter and the leaves begin to appear by the end of winter and the beginning of spring. The flower buds appear at the nodes of the vine through the formation of a shoot apex.

Passion flower has a characteristic violet colour. There are five petals and five sepals (not shown), five anthers, a trifurcated stigma and a double layered corona, with about one hundred fibrils of alternating bands of violet and white colour right below the anthers. A more detailed description of the flowers of PI and their economic importance can be found elsewhere[14,15]. Krosnick *et al.*[16] have studied recently the variations in floral morphs and their possible implications to seed formation. Shivanna[17] has investigated the pollination aspects of *Passiflora edulis*, a fruit-bearing variety extensively.

The only PI plant available on IISER Mohali campus at that time was chosen for investigation as it was yielding a large number of flowers at certain times of the year. The number of flowers that blossomed each day in that particular plant in the year 2014 was plotted as a function of the number of days from the starting date (21 April 2014) of flowering in that year, as shown in Figure 2. Counting of the number of flowers blossoming each day in the PI plant was straightforward. A fresh flower would open up in the morning and close in the evening. It would not open again the next day and would fall off a few days later. Initially, the number of flowers blossoming each day increased exponentially. After reaching a maximum of about 145 flowers per day, there was a decline in the number, but then the number started increasing again. Monitoring the number of flowers each day as the season changed revealed several pulses with a period of approximately 30 days.

After the initial peak in flowering, the subsequent peaks showed considerable decline, with the flowering ending completely by the onset of winter in December 2014. There were minor fluctuations in the number of flowers yielded by the plant on a day-to-day basis. Overall, one sees damped oscillations, with superimposed noise in the results plotted in Figure 2. Fourier transform of the temporal oscillations yielded a power spectrum that revealed a period of approximately 30 days, as illustrated in Figure 3.

May and Spears[14] had studied flowering in PI from May to August 1983. Although they counted the number of flowers in more than 200 plants (collectively) once a week, their results showed certain (4–5 weeks ~30 days) periodicity in flowering. But, they never discussed it in their paper. The focus of their paper was on reproductive aspects of the plant. They did note that there was no direct correlation between the number of flowers and humidity variations.

Efforts were made to see if there was any correlation between the observed oscillations in the number of flowers yielded per day by the PI plant under study with the variation in the external temperature, humidity and the photoperiod in IISER Mohali campus. There was considerable variation in the minimum and maximum temperature and humidity (recorded by the environment laboratory in IISER Mohali) as the season changed from spring to dry summer to monsoon to autumn (April–November 2014) as illustrated in Figure 4. A comparison of these variations with the change in the number of flowers blossoming per day during the same period showed no noticeable correlation between the number of flowers and environmental conditions. Although there

**Figure 1.** A sample picture of passion flower. Five anthers and a trifurcated stigma are clearly visible. The double layered corona with nearly one hundred fibrils of alternating bands of violet and white colours is also evident. Five petals and five sepals are behind (not visible in the picture). Courtesy: N. G. Prasad, IISER Mohali, 2012.

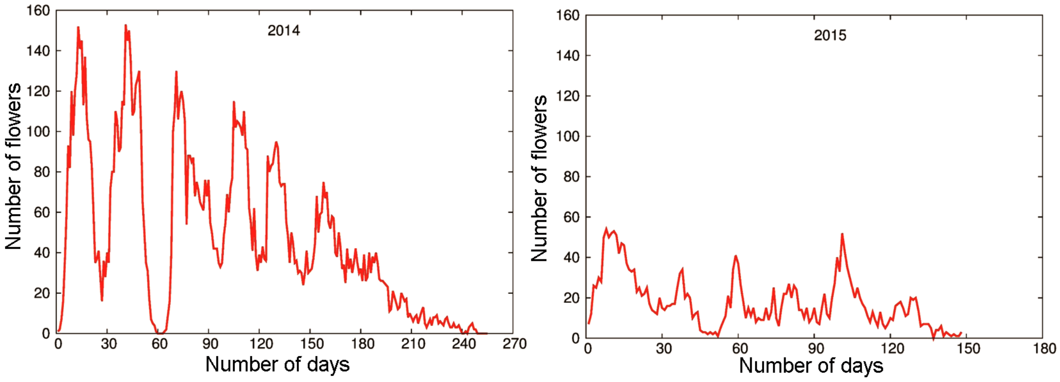

**Figure 2.** Flowering data for *Passiflora incarnata* as the season progresses, as a function of the number of days from the first day of flowering (21 April) for 2014 and 2015.

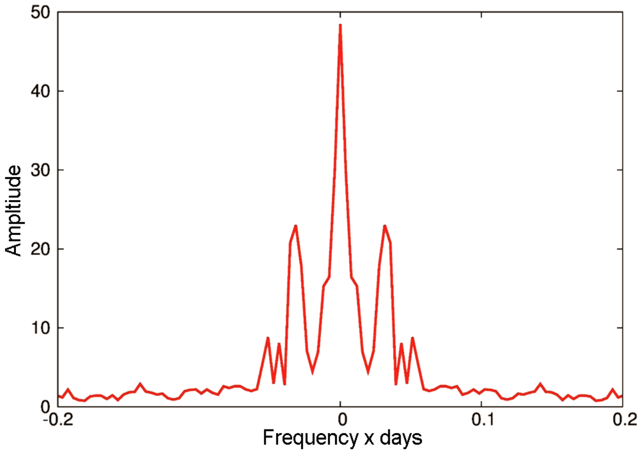

**Figure 3.** Fourier transform of the number of flowers yielded per day by the plant of PI in 2014.

was a noticeable delay in the time of opening of individual flowers on cloudy days, there was no noticeable correlation between the number of flowers blossoming per day and the photoperiod (sunrise to sunset) recorded during the same eight months (Figure 5).

To make sure that the observed trends in the results obtained in the present study are reproducible, the number of flowers from the same plant was monitored in the following year (2015) and the results are included in Figure 2. Flowering started again on 21 April 2015, but the number of flowers was much less in 2015 than that in 2014. Although the oscillations in the number of flowers blossoming each day with a period of approximately 30 days were evident for 2015 also, the day-to-day fluctuations in the number of flowers blossoming were much more in 2015 than those in 2014, presumably because of the smaller numbers. Unfortunately, the plant died after a few months of our monitoring.

Four more plants of PI were acquired in the same year (2015) and they started blossoming in 2016. But the number of flowers yielded by these plants was limited in 2016. The number became substantial only in 2017. The data collected from these plants, plotted in Figure 6, reveal several pulses. Although the number of flowers yielded per day was different for the different plants, the synchronicity between them becomes evident when the data from all four plants were plotted in the form of a stacked area chart as illustrated in the bottom panel in Figure 6. Sequencing was similar between plants 1 and 2, which were co-located and sequencing was similar between plants 3 and 4, which were located about 100 m away from plants 1 and 2.

Most of the papers on the subject try to correlate the phenology of flowering with pollination by wind or insects. The PI plants under study yielded seemingly fully developed flowers. Although there were a large number of flowers, most of them were hermaphrodite and visited by carpenter bees and other bees, the fruit yield was zero. Therefore, it is difficult to interpret the synchronous pulsed flowering in PI in terms of pollination and seed production. We examine temporal oscillations in flowering in PI from a nonlinear dynamical point of view.

Since blossoming each year was preceded by a period of no blossoming for about 5–6 months, one could conclude that that there was an induction period before the onset of oscillations, a characteristic of open nonlinear dynamical systems. The initial growth followed by temporal oscillations is indicative of an autocatalysis/feedback mechanism in oscillatory chemical reactions[18]. The fact that autocatalysis (exponential growth) is controlled subsequently by damping is an indication of an underlying inhibitory mechanism. With the change in season, there is bound to be a change in environmental temperature, humidity, sunlight, etc. and yet the onset of damped oscillations is clearly evident in Figure 2. The fact that temporal oscillations persisted during four different seasons is a clear indication that the mechanism responsible for the oscillations is endogenous and likely biochemical in origin.

As early as in 1936, Chailakhyan had proposed the idea of a florigen (blossom-former), a photo-induced stimulus in the leaves being responsible for inducing flowering at

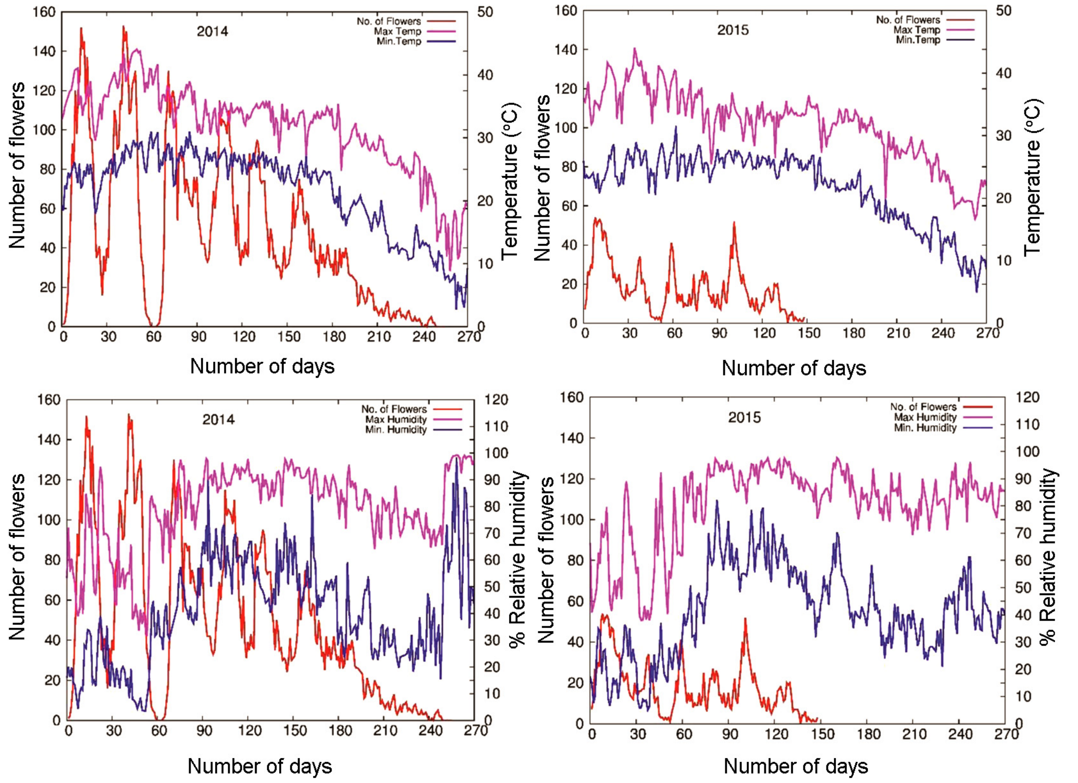

**Figure 4.** Variation in the maximum and minimum temperature and relative humidity each day, compared with the variation in the number of flowers of PI per day as a function of the number of days after the first day of flowering for 2014 and 2015 (reproduced from Figure 2 of this paper).

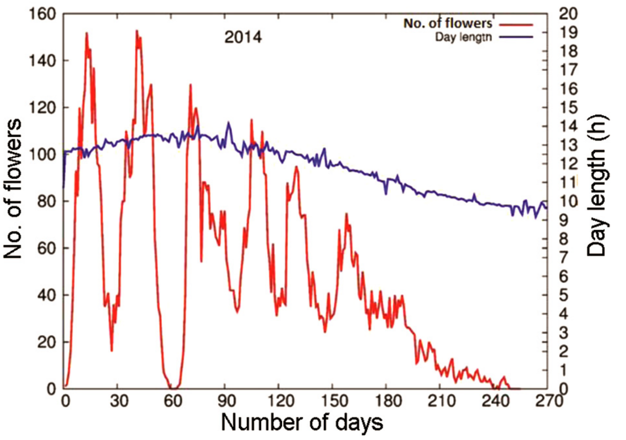

**Figure 5.** A plot of the day length (photo period) as a function of the number of days starting from 21 April 2014 compared with the variation in the number of flowers per day during the same period, for a particular plant of PI, reproduced from Figure 2 of this paper.

the stem apex in chrysanthemums[19]. This observation was critical in distinguishing the vegetative phase from the reproductive. Although the idea of a floral repressor or an anti-florigen was proposed long ago[20], only recently Higuchi *et al.*[21] found experimental evidence for the anti-florigen in chrysanthemums.

It has been shown in *Arabidopsis* and other plants that the day-length-specific induction of the *Flowering Locus T* (*FT*) gene, which encodes a small mobile protein (florigen) is the major output of the photoperiod pathway[22]. It has also been shown[23] that the *FT* gets activated in the leaves and is responsible for flowering at the shoot apex. Nakamura *et al.*[24] have shown that in *Arabidopsis thaliana*, the florigen binds with diurnally oscillating phospholipids that accelerate flowering.

Therefore, it can be concluded safely that in PI also, there is a florigen that induces flowering and an anti-florigen that inhibits it. Unfortunately, to the best of our knowledge, the florigen and anti-florigen have not been identified in PI so far.

The number of flowers produced by a plant would, in all likelihood, be related to the amount of florigen ($F$) produced. This, in turn, must be related to the biochemical ($B$) responsible for the production of $F$. There could be a feedback (autocatalytic) mechanism in the production of $F$ (see R1 below). The growth in $F$ could be controlled by an anti-florigen, an inhibitor ($I$) (see R2 below). Drawing upon Lotka's prey-predatory model[25], one could write down the mechanism for flowering as follows

$$B + F \rightarrow 2F\ (k_1), \tag{R1}$$

$$F + I \rightarrow 2I\ (k_2), \tag{R2}$$

$$F \rightarrow \text{decay products}\ (k_3), \tag{R3}$$

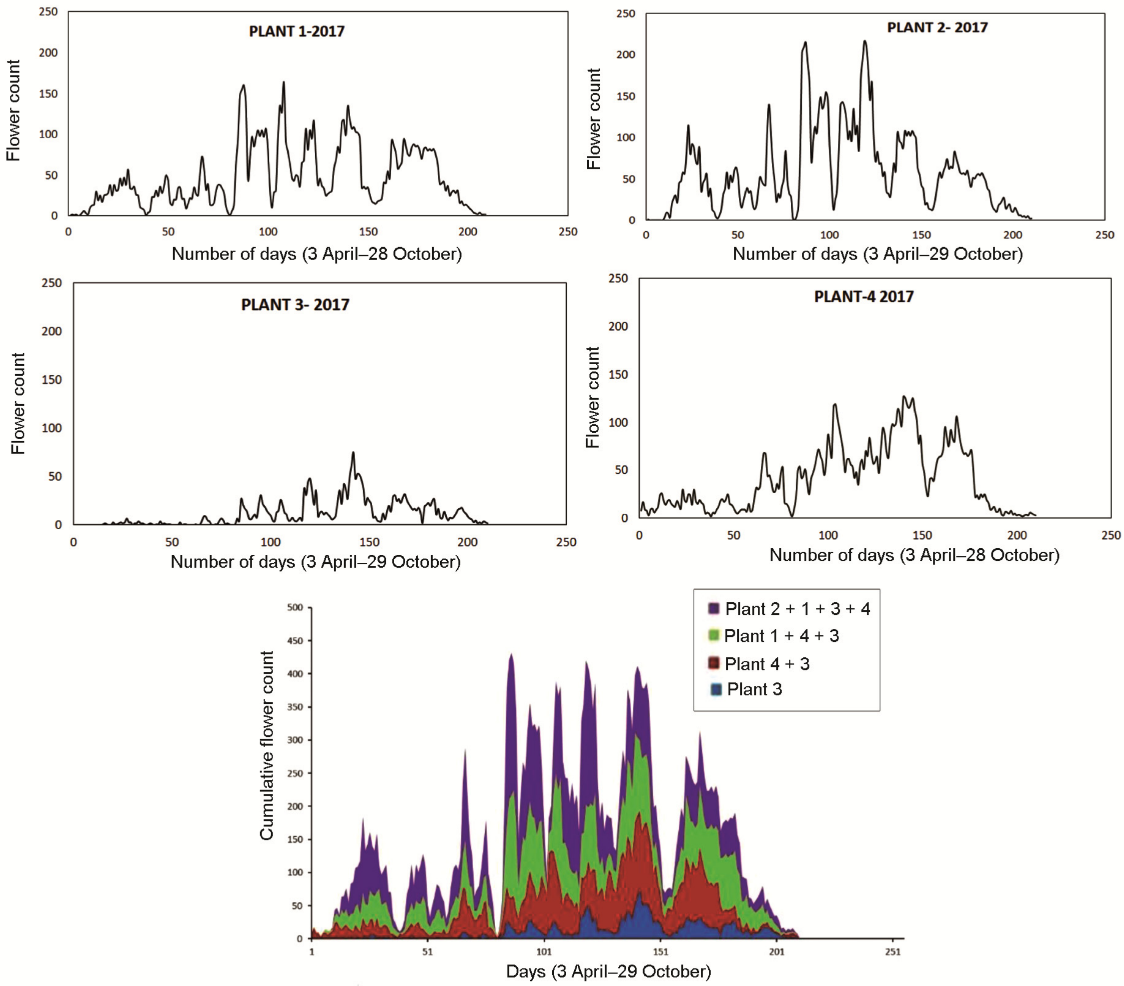

**Figure 6.** Flowering data for four different plants of PI and the cumulative data in the form of a stacked area chart for 2017.

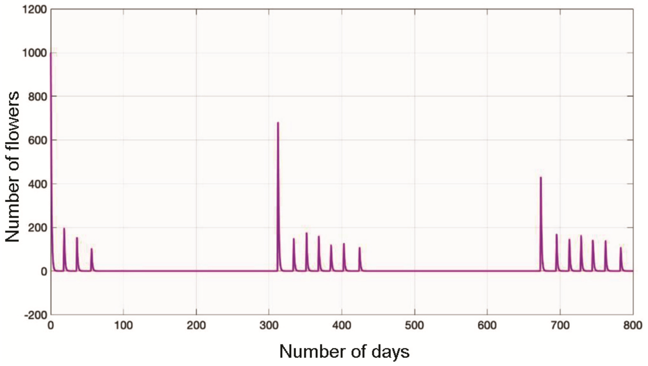

**Figure 7.** Predictions from a modified Lotka model for flowering as a function of the number of days after the starting date of flowering.

$I \rightarrow$ decay products ($k_4$). (R4)

Here we have considered the possibility of the florigen and the inhibitor decaying (or undergoing transformations) through some other mechanism (R3 and R4), which is negligible in comparison.

Clearly,

$$\mathrm{d}F/\mathrm{d}t = k_1[B][F] - k_2[F][I] - k_3[F], \tag{1}$$

$$\mathrm{d}I/\mathrm{d}t = k_2[F][I] - k_4[I]. \tag{2}$$

In the Lotka model, $B$ would be taken to be large enough in concentration that it could be taken to be a constant $B_0$. However, knowing that PI yields flowers year after year and flowering starts more or less on a particular day of the year for each plant, one could take $B$ to be a variable with a period of 365 days: $B = B_0 \cos(2\pi\omega t)$, with $\omega = 1/365$.

One could solve the coupled differential eqs (1) and (2) for a set of ($k_1$, $k_2$, $k_3$, $k_4$) and look for temporal oscillations in $[F]$ to understand the underlying mechanism that is responsible for the temporal oscillations. From a phenomenological point of view, we could consider the fact that the number of flowers doubled every day in the beginning of the flowering season in 2014. Approximating the initial rate as $\mathrm{d}F/\mathrm{d}t = k_1[B][F]$ and taking $[F] = 1$, $k_1[B]$ can be approximated as 0.69. In the spirit of the prey–predator model, [$B$] would be much larger than [$F$] and [$F$] $\gg$ [$I$]. In the same spirit, $k_2$, $k_3$ and $k_4$ would be much smaller than $k_1$.

From a practical point of view, however, we had to make $k_2$ in eq. (2) to be different from that in eq. (1).

By solving the set of differential equations

$$du/dt = a^*u - b^*u^*v, \tag{3}$$

$$dv/dt = c^*u^*v - d^*v, \tag{4}$$

with $u = [F]$, $v = [I]$, $a = 1.5 \cos(\pi t/180)$, $b = 0.1$, $c = 4.0$ and $d = 1.0$, and $u(0) = 1000$ and $v(0) = 0.1$, we could get the results shown in Figure 7. Although the results in Figure 7 do not reproduce the results in Figure 2, it is clear that the essential features of an autocatalysis and an inhibitory pathway in an open system are captured in our model.

To account for the observed temporal oscillations in the flowering of PI quantitatively, there is a need to determine the underlying biochemical pathways and the coupling between them. A more detailed biochemical investigation is clearly needed.

From a commercial point of view, the date of first flowering and the periodicity in flowering have enormous implications for the socio-economics of floriculture.

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