# FLOWERING OF 'TAHITI' LIME

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All angiosperms, including trees, produce flowers as an integral part of the reproductive process. Floral induction is that specific event in an actively growing or quiescent bud which is responsible for the transition from vegetative growth to formation of the flower or flowers which develop from that bud. This event generally occurs in response to an environmental stimulus and results, either immediately or at a later time, in the transition from synthesis of proteins directing vegetative growth to those proteins responsible for development of floral structures. Environmental cues responsible for floral induction have been extensively studied in a number of plant species. Such cues include day length as well as low temperature and water stress. Initiation is the first perceivable change in cells towards development of flowers or flowering structures. Although initiation and subsequent development of floral structures are important aspects of study in the flowering process, this chapter will deal only with those investigations attempting to uncover the mysteries of floral induction, especially as they relate to citrus flowering.

When one considers that nearly all of the fruit consumed by humans is a direct result of the flowering process in trees, the mechanism of floral induction becomes more than just an academic question. Aside from complications due to juvenility, flowering research on trees must consider a number of confounding factors such as the fact that flowering occurs, in many arboreal species, on lateral buds, not the apex. In the case of <u>Citrus</u> species, flowering shoots, as well as vegetative shoots, develop from quiescent, lateral buds during periods of seasonal flushes of growth. Apical meristems, the growing point at the tips of branches of most plants, are not present on the branch tips of citrus. This is because the apical meristem of each developing shoot aborts prior to full elongation and its return to a quiescent state. Thus, there is no transition to flowering from an actively growing vegetative meristem as is found in the case of herbaceous, annual plants. Not all lateral buds produce flowering shoots (henceforth described as either generative shoots, which produce only flowers, or mixed shoots, which produce both leaves and flowers). They may form vegetative shoots instead. Furthermore, typical of many tropical species, citrus growth is not continuous. Vegetative and reproductive growth on individual branches occur several times throughout the year with substantial periods of rest (quiescence) in between. As a result, timing of induction may be separated from initiation by weeks or even months. These problems have traditionally made studies of floral induction of tree species difficult.

We have developed methods over the past few years whereby we can predictably control flowering in <u>Citrus</u> <u>latifolia</u> Tan. ('Tahiti' lime). These methods overcome many of the problems involved with flowering studies in woody plants. 'Tahiti' lime trees cultivated in South Florida produce numerous, periodic flushes of growth year around. These flushes are typically accompanied by flowering, the greatest amount of which occurs in the late fall and early spring flushes. Using small plants produced by air layering (marcottage), we have been able to completely avoid complications brought on by juvenility.

## Marcottage and Citrus Flowering

Air layers, or marcots, are made by cutting away a ring of bark approximately 1-inch wide on 0.5 to 1.0 cm diameter branches of mature trees. The exposed cambium layer is scraped from the xylem with a knife edge to avoid rapid regeneration of tissue which would result in closure of the newly-exposed surface. The ringed area is covered with a fist-sized ball of drained, water-saturated sphagnum moss, over which foil or plastic is placed to reduce desiccation of the sphagnum. After one to two months on the tree, depending upon the season, roots

will have invaded the sphagnum ball. The root-bearing branch is excised from the tree and planted in a pot from which time on it is treated as a typical potted plant. The plants are then preconditioned in a greenhouse for several months prior to experimentation. The resulting plants are small so that they are easily manipulated, and they demonstrate no signs of juvenility which are typical of small, seedling plants. Hundreds of plants can be produced at a time.

We have noted when propagating these marcots that they, at times, produced a profuse flush of flowers soon after planting. Flower production would continue for a period of weeks before the plants returned to a typically vegetative condition. This phenomenon occurred at times when few flowers were forming on the mother trees. We, therefore, examined the effect of marcottage date through the year on the types and amounts of shoots formed to see if this response could be consistently reproduced. The response appeared to be variable throughout the year with enhancement and extension of the normal flowering seasons. Marcots harvested in January and February generally produced, on the average, several fold more generative shoots than did the mother trees which typically flower at this time. Those marcots harvested in November, December, and March, when the amount of natural flowering was small or nil, produced a substantial number of generative shoots per branch (an average of 0.8 to 1.2) in a similar manner to our earlier observations. There also appeared to be a small stimulation of flowering by marcottage during the summer months of June and July. The amount of flowering in marcots was not significantly greater than that occurring in the trees at other times of the year. The flowering response to marcottage thus appears to be seasonal in nature. We have reason to believe that cytokinins, a class of plant hormones which can be produced in the adventitious roots, may be playing a role in the expression of these off-season flowers, but the role of cytokinins and/or any presumptive flower-stimulating hormones which may be involved is at present not clear.

## Environmental Affects on Citrus Flowering

Using marcotted plants that have recovered from the initial flowering flush, we have developed convenient, reproducible methods of floral induction that can be conducted at any time of the year through the application of cold night temperatures or programmed water stress. Lime plants were placed in a growth chamber maintained at 10°C night and 18°C day with a 12 hr photoperiod. Groups of plants were removed to a warm greenhouse weekly to promote rapid new growth. The intensity of the flowering response, as indicated by the newly emerging shoots in the greenhouse, was dependent upon the amount of time the plants had spent in the inductive cold temperature. A period of between one and two weeks appeared to be the minimum time required for induction. The plants increased in flowering intensity the longer they were in the cold temperature for up to two months when they began to flower in the cold growth chamber. Similar results were obtained in plants subjected to two levels of water stress. The longer the plants were in water stress, the more flowering resulted when they were rewatered. Plants subjected to severe water stress produced more flowering shoots than did those undergoing a moderate level. As in the chilling stress experiments, the minimum time needed for induction of flower production was between one and two weeks of water stress.

The opportunity to gain significant new knowledge about floral induction in arboreal species is now available to us through the use of these two different methods of induction. It is important to understand that with two independent means of inducing flowering, we can discern those biochemical and physiological responses that are correlated with the particular stress but not necessarily associated with flowering per se; for example, if we observe a change in one parameter as a result of water stress and no similar changes take place when the plants are treated with chilling temperatures, then we can assume that the parameter in question is not likely involved in the inductive processes of

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flowering. Such an approach has been used in the study of the hormonal control of floral induction.

## Plant Hormones in Citrus Flowering

The observation that gibberellins inhibit flowering in a number of woody perennial species including apple, peach, mango, bougainvillea, and citrus at present is our primary inroad into the study of the hormonal control of floral induction in these plants. Exogenously applied gibberellin must be continuously present to inhibit the development of flowering buds of citrus. Even if applications of gibberellin are continued for up to a year so that flowering is continually inhibited, treated trees flower in concert with controls once gibberellin treatments are terminated. Gibberellin-mediated inhibition of flower development is effective until well after differentiation of sepal primordia. It, therefore, is capable of interrupting floral evocation as well as induction.

Under some conditions, gibberellin-synthesis inhibitors such as cycocel and daminozide have been shown to promote flowering in lemons and oranges in Israel. These observations have led to the suggestion that gibberellin plays a direct role in citrus flowering. It has been suggested that conditions which reduce the levels of overall plant gibberellins are conducive to floral induction, and conditions which promote high levels of gibberellins in the plant inhibit flowering. Attempts to reproduce these results in oranges and limes, however, have failed in other areas of the world including Florida.

Three endogenous gibberellin-like compounds, including two tentatively identified as $GA_1$ and $GA_9$ have been reported in citrus fruits as well as in petals and stamens. A compound tentatively identified as $GA_1$ was reported in high concentration in vegetative shoots, in low concentrations in flowering shoots, and at intermediate levels in mixed shoots. It is probable that the levels observed in these shoots were a reflection of the hormonal component in and possibly production by those developing shoots and not related to the inductive events associated with the formation of these shoots. Ironically, levels of gibberellins in the bark, woody twigs, and leaves of citrus have been observed to increase when exposed to conditions conducive to flowering such as the onset of spring and branch girdling. Clearly, gibberellins are present in citrus trees, and exogenous applications inhibit flowering, but it is still not clear whether gibberellins play an active role in the negative control of citrus flowering in nature.

We have recently examined gibberellin levels in individual leaves and buds during and after exposure to inductive conditions using immunological assay techniques to see if the levels of the hormone are in some way correlated with floral induction. We utilized the two inductive treatments described above and were able to compare the gibberellin response to these treatments on a weekly basis for a month for possible correlation with the flowering response. The levels of gibberellins in the leaves were reduced during the month-long period of cold stress whereas they increased during a similar period of water stress. In both treatments, the levels of gibberellins in the leaves returned to their original levels upon relief of stress. The gibberellin levels of the buds did not significantly change either during or after stress. These results would suggest that flowering is not necessarily associated with changes in endogenous gibberellin levels in citrus.

## Flowering Inducer

Other lines of research which we have been pursuing involve the location of the source of the presumed flowering message. Previous research has shown that cool conditions in the root zone inhibit production of new shoots whereas cool conditions in the canopy promote production of flower-bearing shoots. In contrast, warm conditions in the roots promote shoot production, but warm

temperatures in the canopy cause the new shoots to be vegetative. These observations strongly suggest that the interpreting system of flower promoting temperatures resides in the canopy of the plant.

Evidence derived from experiments conducted in our lab also suggests that flowering control resides in the canopy of the tree. When the apical three nodes of all the branches on small, 'Tahiti' lime plants were clipped and the plants were placed into an environmentally controlled chamber maintained under cool, inductive conditions as described above, the plants responded with a limited amount of flowering. Clipping was necessary to stimulate the shooting response to occur during the experimental period. Most of the new shoots were vegetative. If, on the other hand, only half of the branches were clipped below the third node, a marked promotion of flowering occurred. The branches which had not been clipped produced the highest percentage of flowering shoots while those that had been clipped produced a lower percentage than the non-clipped branches but a higher percentage than those from fully clipped branches. No flowering occurred in plants similarly clipped but placed in a chamber maintained under warm conditions of 25° C night and 28° C day with the same photoperiod. All new shoot growth was vegetative. These experiments have been run several times with consistent results. They suggest to us that a flower-stimulating component appears to be synthesized or at least present in highest amounts distal to the third node. This floral stimulator may be able to move since clipped branches of half-clipped plants consistently produced more flowering shoots than those plants in which all of the branch tips had been removed. Active involvement of an inhibitor in this system seems contraindicated since the presence of the apical three nodes stimulates flower production under inductive conditions, and removal of these inhibits flowering. At present, we have not ascertained whether this putative floral stimulant is newly synthesized or is simply released from a bound form upon exposure to inductive conditions.

Interestingly, each branch appears to manage its own capacity to flower under inductive conditions; for example, seldom will both generative and vegetative shoots occur on the same branch. Often, one observes mixed shoots with generative or mixed shoots with vegetative shoots, but the great majority of individual branches express either generative or vegetative shoots on a branch of 'Tahiti' lime. This response has been verified in several field studies. When we clipped branches of 'Tahiti' lime to stimulate shooting at a time near the normal flowering period (late fall through early spring), we observed that the clipped branches produced only vegetative shoots while subtending side branches produced flowering shoots. These observations furnish additional evidence that a flower-stimulating component may reside in the apical nodes of C. latifolia. It is not clear if the presumptive stimulus is formed in the apical leaves or in the branch apex.

These observations have been extended in another type of experiment. Small plants derived from air-layers were either severely pruned (to within 4 inches from the soil level), moderately pruned (all green wood removed), or not pruned. Pruning was carried out in the severely pruned set of plants first, followed several months later by the moderately pruned set of plants. This difference in pruning time allowed regrowth of the pruned plants such that the stature of the plants from all three treatments were similar at the time the experiment was conducted. Exposure to inductive conditions began when all plants were quiescent (no active growth occurring). The plants from all treatments were half clipped to stimulate development of new shoots and placed in the inductive conditions previously described. The pruning history of the plants made a marked difference upon their flowering behavior. None of those plants flowered that had been previously severely pruned. They produced only vegetative shoots. A few flowers were formed on a few branches of the moderately pruned treatment, but the majority of new shoots formed on the non-pruned plants were generative. Thus, when pruning has occurred, several subsequent flushes of vegetative shoots are apparently required to replace the ability to flower under inductive conditions. This

observation is consistent with the concept that as more wood is cut away by pruning, more of the flowering potential or stimulus is also removed. Hence, heavily-pruned plants require more new flushes to gradually replace the original level of flowering stimulus required to induce flowering under inductive conditions than those plants with less wood removed. These observations also lead to the suggestion that the putative floral stimulus appears to transfer its potential activity to the new apex furnished by each successive flush.

We have observed that a variable number of 'Tahiti' lime cuttings produce flowers instead of vegetative shoots. The percentage of flower-producing cuttings vary according to season and previous history. Flowering without production of any leaves in cuttings has been observed to last for up to a year. In every case, flowers formed from the lateral buds fell off after a few weeks, and formed new flowers which repeated the process. It is assumed that the controlling hormonal messages must be continuously present in these cuttings because they continue to produce the same types of shoots from undifferentiated buds whether they be flowering or vegetative.

## Conclusion

Collectively, the observations cited above suggest that an identifiable floral stimulating complex is present in or near the apex or its subtending leaves of C. latifolia. The presumptive stimulus is manipulated by temperature, water status, and pruning. It appears to be long lived in certain cuttings. We have an effective floral inhibitor, e.g. gibberellin, but it may not be operating in the plant to inhibit flowering from occurring in nature. These observations give us the opportunity to better understand flowering mechanisms in all citrus species.