OVERVIEW OF FLOWER BUD INDUCTION, FLOWERING AND FRUIT SET

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Flowering is a critical step in fructification. No flowers mean no fruit, and when flower number is low crop load may be limited by the number of flowers formed. In most cases, however, citrus trees form a number of flowers exceedingly higher than the final number of fruits harvested, which usually is a very low percentage of the initial flower number. As in other species which form a large amount of flowers, fruit set rather than flower number is the parameter which usually determines yield in citrus.

Apart from their importance in the determination of crop yield, some events occurring during flower formation and set affect fruitlet development and final fruit size and quality, having an additional effect on returns. The study of these processes has not only an academical interest but also an applied aspect. The purpose of this discussion is to review basic knowledge available on the regulation of flowering and fruit set and the way as these processes can be manipulated to the advantage of the grower.

The Floral Biology of Citrus

Citrus trees usually have several flushes of growth during the year. The number of flushes and their importance are determined by cultivar characteristics, crop load and climate. The newly formed shoots arise from lateral resting buds and may form either leaves (vegetative shoots), flowers (generative shoots also called leafless inflorescences), or both flowers and leaves (mixed-type inflorescences). No recognizable flower primordia are found in the resting buds. The earliest signs of flower morphogenesis are detectable at the onset of bud sprouting, and are ensued by the uninterrupted development of the flower organs leading to anthesis.

Under subtropical climate conditions most citrus cultivars have three main flushes of growth. The main one occurs in late winter or early spring (spring flush). Two additional ones occur at the end of the June drop (summer flush) and late in September (autumn flush). In most cases, only vegetative shoots are formed in the summer and the autumn flushes of growth. Inflorescences are formed in the spring flush of growth as a response to the low winter temperatures.

The shoots developed during the flushes of growth arise mainly from the axillary buds present in the vegetative shoots formed in previous flushes of growth. Bud behavior in spring depends critically both on the time the buds were formed (bud age) and in the position of the bud along the shoot.

In the Washington Navel orange, the highest rate of bud sprouting is found in the buds formed during the autumn flush of growth (5-month-old buds at the time of spring sprouting). The rate of sprouting decreases with bud age (Figure 1). Very few shoots emerge from buds older than one year

at the time of sprouting. Irrespective of bud age, there is a gradient of bud sprouting along the shoot. Sprouting is highest in the apical positions, decreasing steadily towards the base of the shoot.
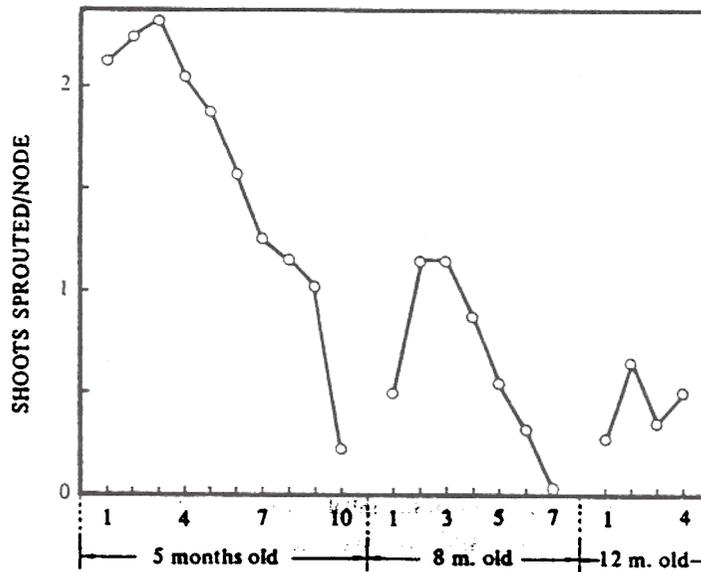
Figure 1. The influence of bud age and position along the shoot on bud sprouting in spring in the Washington navel orange. For each age the buds have been numbered from de apex of the shoot (position 1). From Guardiola et al.(1977).

Differences are very marked as well as regards to the nature of the shoots formed. Young buds (5-month-old) form a much higher proportion of generative shoots (inflorescences) than older buds (Table 1). As for shoot formation there is a marked gradient of flower formation along the shoot, highest towards the shoot apex.

Table 1.   The influence of bud age on the pattern of sprouting in the Washington navel orange (From Guardiola et al. 1977).

Bud age at sprouting (months)	Shoots formed (% of total)		
	Vegetative	Mixed-type	Generative
5	6.7	50.0	43.5
8	14.7	67.8	18.0
12	57.0	53.9	4.2

When the summer flushes of growth are intense most of the flowers are borne on the axillary buds formed in them, and the buds formed during the previous spring make a minor contribution to flower formation. However, when the summer flushes are scarce most of the flowers are formed on the previous spring shoots, but total flower number per tree is lower.

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The sprouting behavior is somewhat different for satsuma mandarin. As described above for Washington navel, there is a marked gradient in bud sprouting and flowering along the shoots, highest in the apical positions. However, the rate of bud sprouting in spring is similar for spring formed buds (12-month-old) and for the younger buds formed during the summer and the autumn flushes of growth. Further, the spring buds flower more profusely than the younger ones. These younger buds make a minor contribution to spring flowering, particularly when crop load is high.

Therefore, the extent of spring flowering is determined by three parameters which are not independent:

1) the number and the age of the buds present on the tree;

2) on the extent of bud sprouting in spring, and

3) on flower induction and development

It is in this framework that the effect of the fruit and environmental conditions on flowering is to be discussed.

Flower Induction

The term induction as defined initially applied at the triggering of the flowering process by environmental factors resulting in a change in the pattern of development of the buds which become committed to flower. As some plants do not require any specific environmental signaling to flower, the term autoinductive was coined to describe them.

Citrus trees are capable to grow and flower under a wide range of environmental conditions, ranging from cold semicontinental climates to very hot tropical areas. No single indispensable inductive stimulus for flowering seems necessary and citrus is considered to be autoinductive. However, flowering is markedly enhanced by moderately low temperature treatments and by water stress, and under appropriate conditions an inductive effect may be demonstrated. The response to these factors is however complex and involves several effects.

<u>Low temperature releases bud dormancy</u>. The floral response to low temperature treatment is quantitative, the intensity of flowering increasing with the duration of the cold treatment. A major effect of low temperature is the release of bud dormancy, allowing the buds to sprout when returned to temperature conditions favorable for growth.

A typical response is shown in Figure 2, which depicts the sprouting behavior of potted satsuma trees forced to sprout at 22/13 °C at different moments during the winter rest period. Bud sprouting increased linearly with the duration of exposure to the inductive outdoor conditions, and

this effect showed some specificity. The trees barely sprouted when forced by mid September. By late November there was a significant increase in the formation of both vegetative and flower shoots. A further duration of the cold treatment increased the number of developing inflorescences but had no further effect on the number of vegetative shoots.
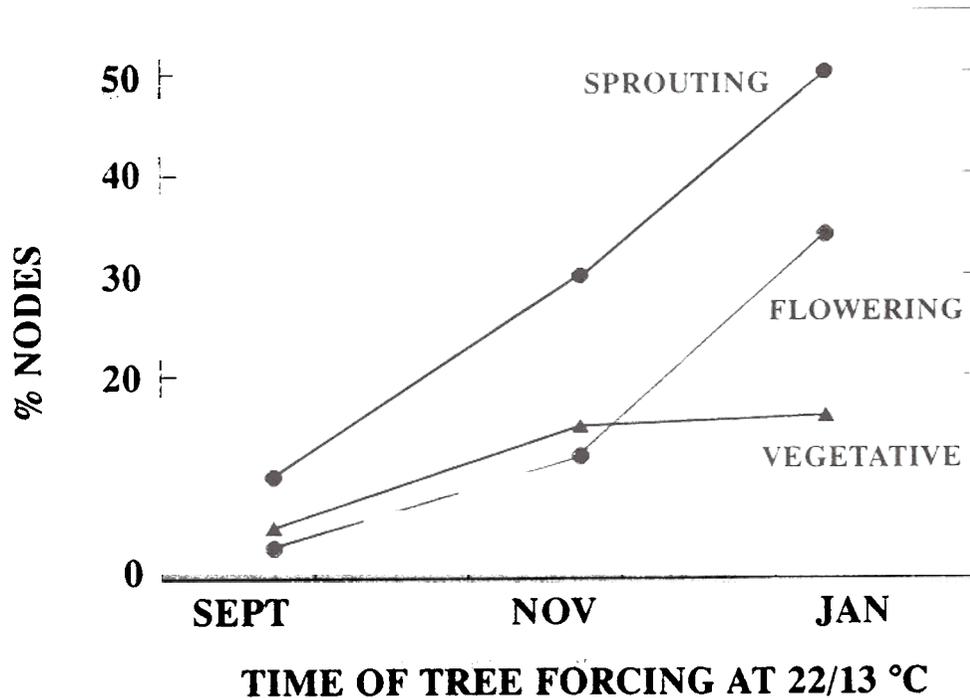
Figure 2. Bud sprouting and flowering in potted fruit-bearing satsuma trees forced to sprout at 33/13°C at different dates. From Garcia-Luis et al.(1992).

While the effect on the release of bud dormancy is clear-cut, evidence for an inductive effect turning potentially vegetative buds into flowering ones is lacking. These results could be interpreted assuming that bud dormancy is weaker in vegetative than in flower buds, and that the buds were committed in their pattern of development (vegetative or flowering) at an earlier date.

Low temperature also induces flower formation. The dormancy of citrus buds is very weak and imposed by the influence of other organs (paradormancy). Isolated buds readily sprout in vitro, and in this way floral induction and morphogenesis can be studied without the interference of bud dormancy.

In this way an inductive flowering effect of low temperature could be demonstrated. When buds from non-chilled satsuma trees are forced to sprout at 25/13 °C they barely flower, but flower formation occurs after incubation at low temperature (Table 2). Buds from chilled trees flower irrespective of temperature of incubation. This demonstrates that low winter temperatures have an inductive effect, and that are perceived by the buds themselves. The effective temperatures are higher

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(10-20°C) than those required to break bud dormancy in deciduous trees. Lower temperatures, in the range 0-4°C, not only fail to induce flowering but also inhibit flower formation by induced buds.

Table 2. The influence of the temperature in incubation on flower formation by buds from unchilled (November 5) and chilled (December 22) Satsuma trees (From García-Luis et al. 1992).

Tree condition and temperature of incubation	% of buds	
	Sprouting	Flowering
Non-chilled trees		
Incubated at 25/13°C	100	8
Incubated at 17/10°C	96	49
Chilled trees		
Incubated at 25/13°C	98	40
Incubated at 17/10°C	100	51

An inductive effect of low temperature is also readily shown in non-fruiting trees. The bud dormancy of these trees is very weak and most of the buds sprout readily when forced at high temperatures (25-13°C), but most of the formed shoots are vegetative (Table 3). A low temperature treatment turns most of the developing shoots into inflorescences. This demonstrates the inductive effect of temperatures on entire trees. Also, it shows that bud dormancy is much stronger in the fruiting trees and seems to be imposed by the fruit.

The flowering response to water stress is similar to the response to temperature. A quantitative relationship exists between the duration of stress and floral intensity. As a result of stress there is a release of bud dormancy, but also an inductive flowering effect seems evident.

Table 3. The comparison of the effect of a low temperature (17/10°C) treatment for 53 days on bud sprouting and flowering by fruiting and non-fruiting potted Satsuma trees (From García-Luis et al. 1992).

Tree condition and temperature treatment	% of nodes		
	Sprouting	Flowering	Vegetative
Fruiting trees			
Forced at 25/13°C	9 ± 2	5 ± 1	4 ± 2
Induced at 17/10°C, then forced	34 ± 8	11 ± 5	19 ± 5
Non-fruiting trees			
Forced at 25/13°C	33 ± 6	8 ± 5	22 ± 6
Induced at 17/10°C, then forced	38 ± 9	37 ± 7	1 ± 0.3

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The Effect of the Fruit on Flowering

The inhibitory effect of the fruit on flower formation in spring is well documented and is the cause of the alternate bearing behavior of some citrus cultivars. This inhibitory effect arises in part through the repression of the summer and the autumn flushes of growth. Both the number and the length of the shoots formed during these flushes are inversely related to crop load. As shown above, the lateral buds from these shoots make a major contribution to the spring flush of growth and hence to flowering in some cultivars. A reduction in the number of buds in one component, but not unique, of the effect of fruit on flowering.

The fruit directly influences bud behavior. A direct effect on bud behavior is usually a major component of the fruit effect on flowering. This direct effect of the fruit on the buds is shown by the influence on the number of flowers formed per available node. In this way we measure the effect of the fruit on flowering independently of the effect on bud formation. This parameter measures the combined influence of the fruit both in bud sprouting as well as in flower induction and development.

The direct influence of the fruit on bud behavior is time dependent. As shown in Figure 3, which depicts the flowering behavior of field-grown adult satsuma trees defruited at different times along the year, an early removal of the fruit results in a marked increase of spring flowering. The longer the fruit stays on the tree, the lower next year's flowering. Therefore, for early maturing cultivars an early harvest should result in an increase in return bloom.

Two aspects of this effect must be noted

a) Most of the inhibitory effect of the fruit on flowering is exerted during the autumn months (September to November) and probably earlier, long before the commercial maturity of the fruit.

b) At least in satsuma mandarin, the flowering inhibition by the fruit during the low-temperature inductive winter conditions is very weak. It should be kept in mind that at this time this cultivar is overmature, and other cultivars may behave differently.

The effect of the fruit on flowering parallels effects on bud sprouting. An additional insight on the effect of the fruit on flowering is obtained when compared to the effect on bud sprouting. As shown in Figure 4 which depicts results obtained in two separate experiments with Navelina sweet orange, bud sprouting in spring is inversely related to crop load, although the inhibitory effect of the fruit on bud sprouting is clearly higher in one of the orchards. When flower formation is plotted against bud sprouting a common straight line fits well the experimental points from both experiments (Figure 5). The differences in inhibition of flowering by fruit results from the differential effect on bud sprouting. At least for this cultivar there is no evidence of any specific effect of the fruit on flower induction.

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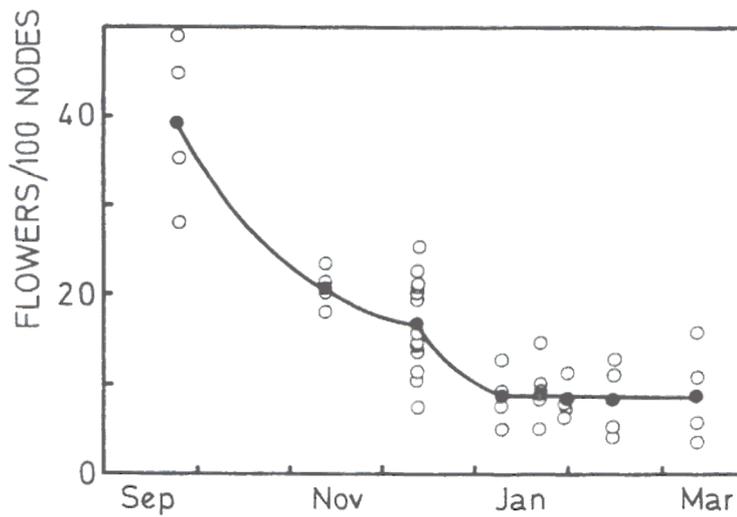
Figure 3. The influence of the time of fruit removal on spring flowering in satsuma mandarin trees. From García-Luis et al. (1986).

% BUD SPROUTING

PREVIOUS CROP (FRUIT/SQ. METER)
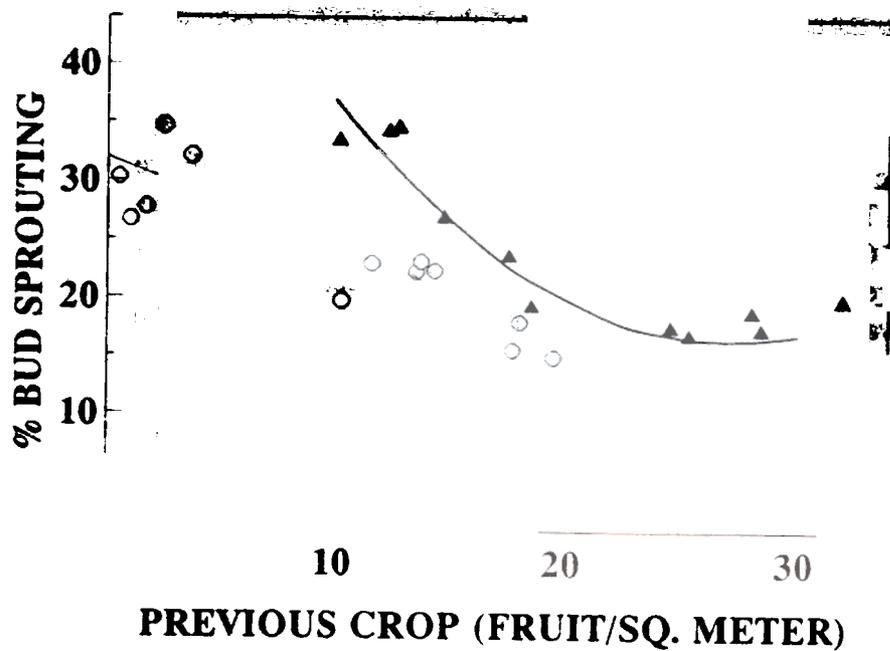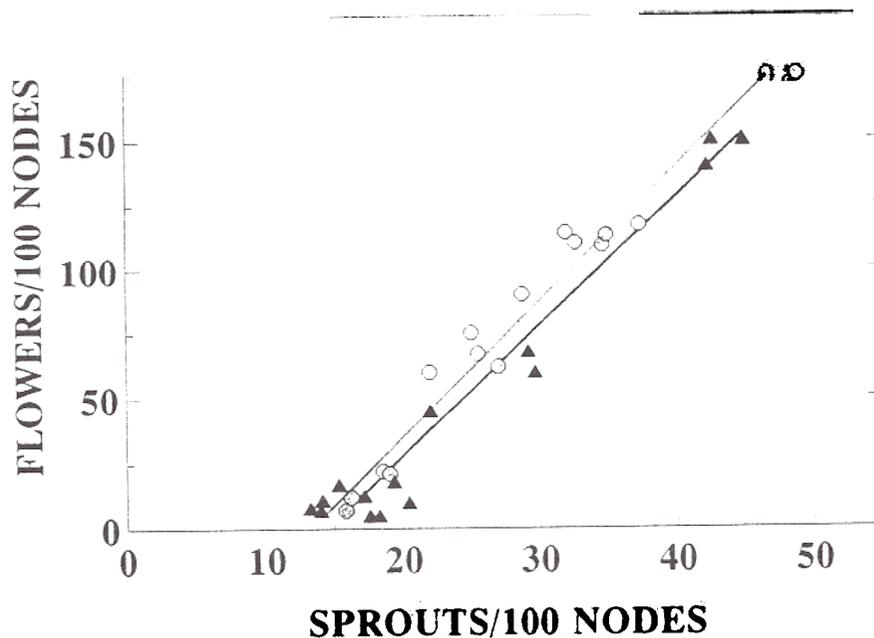
Figure 5. The relationship between bud sprouting and flowering in Navelina orange. Data from the trees in Figure 3. From Becerra and Guardiola (1984).

The Characteristics of the Spring Flush

The basic parameters of the spring (flowering) flush of growth are related to the flowering level of the trees. The relationship between flowering and bud sprouting and shoot characteristics is shown in Figure 6 for satsuma mandarin trees grown under a wide range of climate conditions and with wide differences in crop load. With minor differences a similar relationship is found in most species and cultivars. An increase in flowering results from an increase in bud sprouting, particularly by buds forming generative (leafless) inflorescences. Only in the low flowering range there is an inverse relationship between flowering and vegetative growth as shown by the reduction in the number of vegetative shoots as flower number increases. The number of mixed-type inflorescences, which in most cases make a major contribution to crop load (see below), are hardly affected by flower number. Only in the very low range of flowering there is a reduction in their number.

The characteristics of the spring flush are largely determined by the combined effect of climate and fruit on flower induction and bud sprouting. Deviations from these relationships may come from the effect of additional environmental factors and through the effect of cultural practices.

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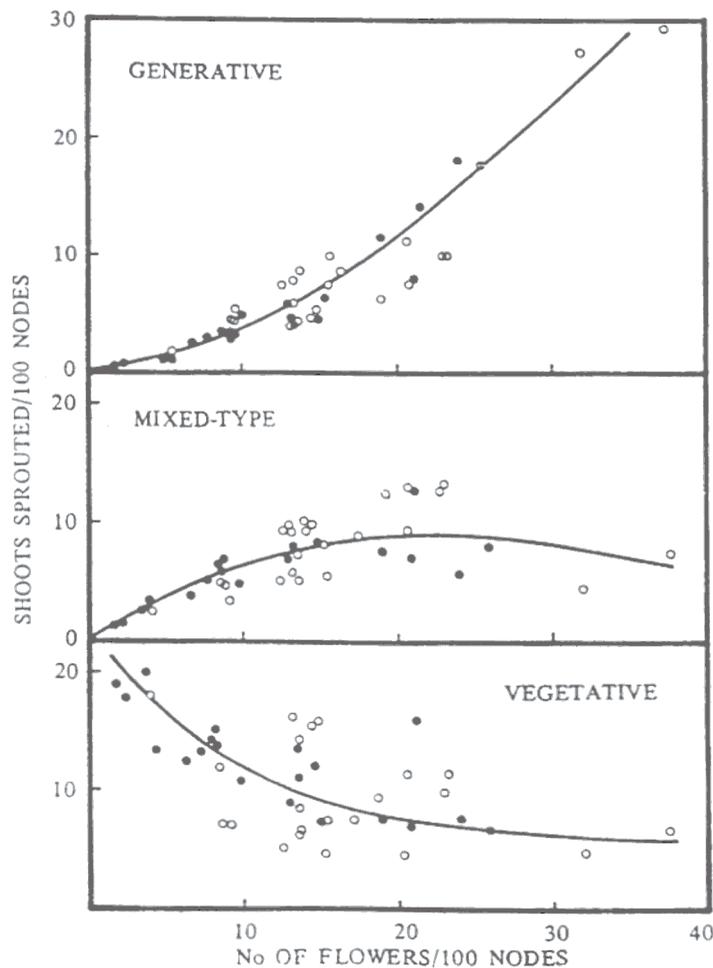
Figure 6. The relationship between flowering intensity and shoot formation during the spring flush of growth. Data from satsuma mandarin. From Guardiola (1981).

High temperatures during bud sprouting have been reported to increase the leafiness of the inflorescences and to inhibit flowering. This observation is of scientific interest as it demonstrates that buds are not committed irreversibly to flower until the early stages of flower initiation. Also, it helps to explain the influence of climate in some aspects of fruit set and growth. It has no practical use except for greenhouse-grown citrus, as for field-grown trees temperature is beyond the grower control.

Girdling increases flower number when performed prior to the winter inductive conditions. Potentially is a useful technique to increase flower number, but its effectiveness is markedly lower in overloaded trees which are harvested late in season after bud sprouting. The most clear-cut results are obtained with early cultivars.

Gibberellic acid applications inhibit bud sprouting and spring flowering. Appropriately timed, these applications have been reported also effective to suppress off-season flowering.

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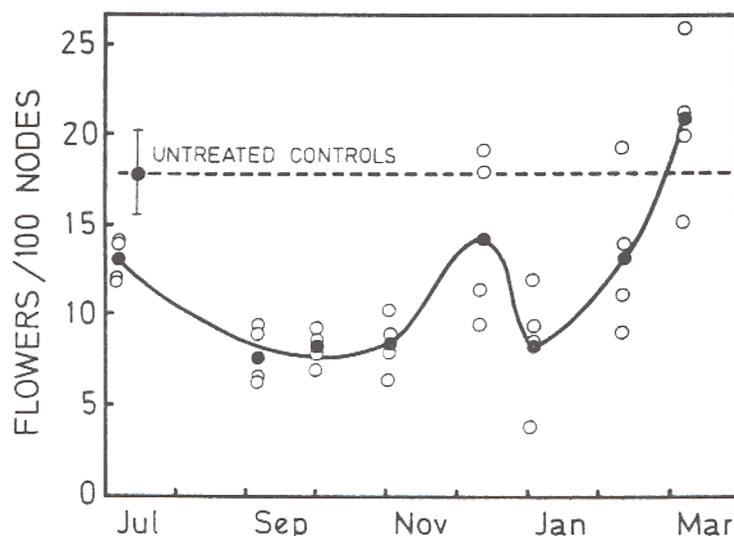
Figure 7. The influence of the time of a full coverage spray gibberellic acid (10 ppm) application on spring flowering in satsuma mandarin. From Garcia-Luis et al.(1986).

Flower Development and Anthesis

The morphological development of the flowers follows without interruption the initiation of the flower primordia. The rate of development depends markedly on temperature and also on flower intensity. A shift of several weeks in the beginning of anthesis may be observed from year to year for the same location. Also the duration of anthesis may vary from a few weeks to about two months. This duration is markedly affected by cultivar characteristics.

Flowers of most citrus species are perfect, having both stamen and pistil. Staminate flowers having only a rudimentary pistil are formed sometimes in a variable proportion. Unfavorable climatic conditions and mineral deficiencies have been reported to increase their number.

Fruit Set

Fruit set is the developmental stage which mark the transition of a flower (ovary) into a young fruit which shall grow until maturity. A failure in set results in abscission, which may occur at different times, sometimes weeks after the abscission of the petals and the stamens and when the ovary has made significant growth. Set, and therefore abscission, depends on cultivar characteristics, flowering intensity, climate and tree management. Within a tree, leafy inflorescences set invariably more fruit than leafless ones. Usually the number of fruits set is much smaller than the number of flowers formed.

<u>Seed production and fruit set</u>. As a rule, most citrus cultivars require seed development to set fruit. Fruit set depends upon pollination, pollen tube growth, sexual fertilization and seed development. Pollination (the transfer of pollen to the stigma) is greatly aided by insects, in particular honey bees. Citrus pollen is heavy and sticky and is not blown by wind.

Many citrus cultivars are self-compatible. Exceptions are some pummelo types and some mandarin and hybrids. When these self-incompatible cultivars are self pollinated the pollen tube grows slowly due to the presence of inhibitors in the style, although their pollen is viable and effective on other cultivars. In these cases, fruit set is dependent upon pollination with a compatible cultivar. Mixed plantings of compatible cultivars are recommended, and the presence of enough number of honey bees should be ensured.

<u>Parthenocarpy</u>. A few citrus cultivars are parthenocarpic and thus are able to produce seedless fruits. Outstanding examples of this behavior are navel oranges, satsuma mandarins, several selections of clementine mandarin and Tahiti lime. The formation of seedless fruits depends both upon the absence of seed formation and the capacity of the cultivars to set parthenocarpic fruit.

Navel oranges and satsuma mandarin are mostly pollen sterile. In addition most of the embryo sacs abort, hence they produce almost no seeded fruits even if cross-pollinated. Clementine mandarin and some mandarin hybrids are self-incompatible but have viable embryo sacs. Seeded fruits are formed when cross-pollinated, a circumstance which should be prevented to obtain seedless fruits.

The capacity of the different cultivars to set parthenocarpic fruit differs widely. In some cases the amount of fruit set is well below the holding capacity of the tree, and an increase in yield may be obtained through an increase in set. Further, parthenocarpy is markedly affected by climate and growth conditions, and cultivars reported in some places as weakly parthenocarpic as Nova tangelo and Fino clementine set readily seedless fruits in Spain.

While seedlessness is a highly favorable commercial trait, particularly for fruit to be sold in the fresh market, seedless cultivars yield as a rule less than seeded cultivars. Further, some of them are very sensitive to nutritional and environmental stresses which may cause an excessive post-bloom drop and reduced yields. Fruit set may be increased either by the application of gibberellic acid at flower opening or girdling the trees at any time from bloom until the end of drop. In some cultivars similar results are obtained with these two systems. Other cultivars, however, show a weak response to gibberellic acid.

On the other hand, when well managed the parthenocarpic cultivars behave as regular bearers. Alternate bearing, which is common in many seedy cultivars, is not frequent in seedless ones.

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Flowering and Fruiting Relationships

As stated earlier, flower formation is the first step of fruiting and indeed an essential one. The relationship between flower intensity and fruiting is very complex as it involves flower number, flower quality, which is related to flower number, and nutritional competition among the developing fruitlets, which act as sinks throughout their development. Some basic aspects, based mainly on research done on parthenocarpic cultivars are discussed below. The behavior of seeded cultivars is significantly different in some aspects.

Flower number and fruit set. In most cases, the percentage of flowers which set and develop into a mature fruit is very small, ranging from 0.1 to 3%. Only in low flowering trees, and always in those cultivars forming a low number of flowers, higher Figures for the percentage of set are found.

The percentage of set is inversely related to flower number, and decreases rapidly as flower number increases in such a way that the number of fruits set remains nearly constant for a wide range of flower number as shown in Figure 8 for clementine mandarin. Only when flower number is too low or exceedingly high, flowering has a significant effect on yield.

The relationship between flowering and fruiting for three different orchards of the parthenocarpic Navelina orange which differ in their capacity to set fruit and show marked differences in flowering intensity among trees is shown in Figure 9. At any number of flowers, the low yielding orchard (Picassent) had a lower percentage of set than the high yielding orchards (Quart and Vila-Real). However, the curves relating flowering intensity and fruiting are remarkably similar for the three orchards and similar to those found for other parthenocarpic cultivars. In the low flowering range (less than 20 flowers per 100 nodes) an increase in flower number resulted in an increase in yield. For the high yielding orchards, yield was maximal and unrelated to flower number when this parameter ranged between 20 and 70 flowers per 100 nodes, with a clear trend towards lower yields as flowering increased beyond these values. A decrease in yield was apparent also in the low yielding orchard as flowering increased, where this effect was more marked than in the high yield orchards.

A similar response has been reported for Washington navel. Under the Spanish conditions, maximum yield is obtained when flower number per tree ranges between 19,000 and 70,000. Beyond this value in flower number there is a sharp decrease in yield.

In seedy cultivars the behavior is different. Noteworthy is the behavior of Murcott, which may set such a heavy crop as to cause the death of the tree. In other seedy cultivars, excessive fruit set arising from a high flower number often leads to alternate bearing.

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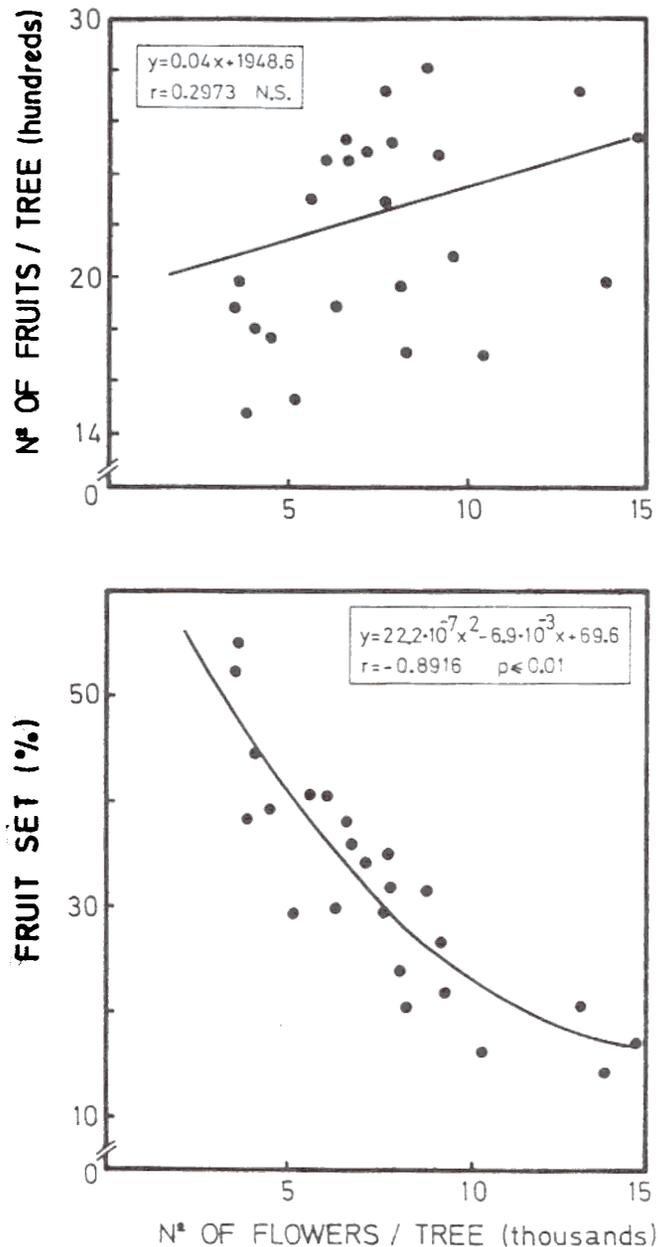
Figure 8. The influence of flower intensity on the percentage of set and final fruit count in clementine mandarin. In the range of 3 to 15 thousand flowers per tree, flower number had no effect on crop yield. From Guardiola (1992).

Unfruitfulness may arise in parthenocarpic cultivars as a result of excessive flowering. This situation results in a low set of fruit (Figure 9) which, in turn, allows the formation of several summer flushes of growth and the build-up of carbohydrate reserves. The high number of available bud sites linked to the weak inhibition of flowering by the few existing fruits (Figure 4) results in a very high flower intensity the following spring. The process is repeated and the trees may enter a cycle of unfruitfulness (Figure 10). The reduction of flowering by the application of gibberellic acid in early winter may break that cycle and bring the trees into a regular bearing habit. A medium intensity

flowering results in a medium yield and allows the formation of enough bud sites to ensure a medium intensity flowering the following year.
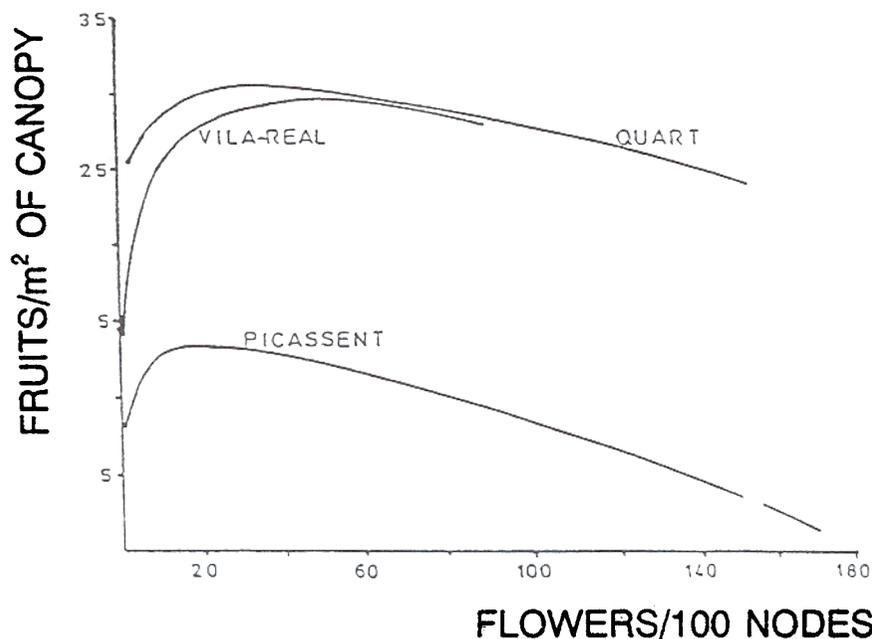
Figure 9. The relationship between flower intensity and fruit count in two high-yielding (Vila-Real and Quart) and a low-yielding (Picassent) Navelina orange orchards. Data from Becerra and Guardiola (1984).

Figure 10 also depicts the behavior of alternate cultivars. In these cultivars, an intense spring flowering results in a heavy crop load which suppress the summer flushes of growth hence reducing markedly bud site formation. A nearly flowerless spring flush with a light crop follows. Bud site formation occurs as a result of several intense summer flushes of growth. An intense flowering follows the following spring, thus starting an alternate bearing cycle. This situation may be handled by reducing flower formation through the application of gibberellic acid plus intense pruning during the high--flowering year. Pruning removes the apical bud sites in the shoots and thus markedly reduces flowering.

Flowering effects on fruit size. Fruit size is inversely related to crop load, and this effect is considered to be the result of competition among the developing fruits for a limited supply of metabolites. To increase fruit size we rely on fruit thinning. The removal of some of the developing fruits is usually performed manually after June drop but has a limited effect on final fruit size.

The inverse relationship frequently reported between fruit number and final fruit size is often weak, far beyond the variability usually found in agricultural experimentation. The main cause for this great variability is that this correlation only considers those events which take place after June drop. In the range of flower number which has no influence on crop yield, final fruit size is usually better related to flower number than to final fruit number. This is shown in Figure 11 for the same clementine mandarin trees whose fruit set behavior was presented in Figure 8. Similar results have

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FIRST YEAR

	PROFUSE FLOWERING	PROFUSE FLOWERING	MEDIUM FLOWERING
Competition during set	Very high	Very high	High
% set	High	Very low	Low
Depletion of tree reserves	Very high	Very high	High

	HEAVY CROP	LIGHT CROP	MEDIUM CROP
Vegetative shoots developed	Very few	Many	Some
Accumulation of reserves	No	Many	Some
Inhibition of floral induction	Very strong	Weak	Important

SECOND YEAR

	SPARSE FLOWERING	PROFUSE FLOWERING	MEDIUM FLOWERING
Competition during set	Low	Very high	High
% Set	Very high	Very low	Low
Depletion of tree reserves	Low	Very high	High

	LIGHT CROP	LIGHT CROP	MEDIUM CROP
Vegetative shoots developed	Many	Many	Some
Accumulation of reserves	Many	Many	Some
Inhibition of floral induction	Very Weak	Weak	Important

THIRD YEAR

PROFUSE FLOWERING	PROFUSE FLOWERING	MEDIUM FLOWERING

Figure 10. Sequence of events leading to an alternate bearing cycle and to unfruitfulness as compared to the behavior of regular bearer trees. From Guardiola (1992).

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been found for other orange and mandarin cultivars in the medium range of flowering. In most cases, the events occurring before the June drop have usually more effect on final fruit size than post-June drop competition.

There are two main factors related to flowering that affect fruit size:

a) An increase in flower number results in the formation of smaller flowers, and final fruit size seems related to ovary size at anthesis.

b) The developing fruitlets compete for metabolites, and an intense competition early in development may impair the later growth of the fruit.

A significant increase in fruit size may be obtained in some cultivars reducing flower number. This reduction increases flower size and also the size of the inflorescence leaves. Further, chemical thinning, which may be performed before the end of June drop, is invariably more effective than hand thinning to increase fruit size.
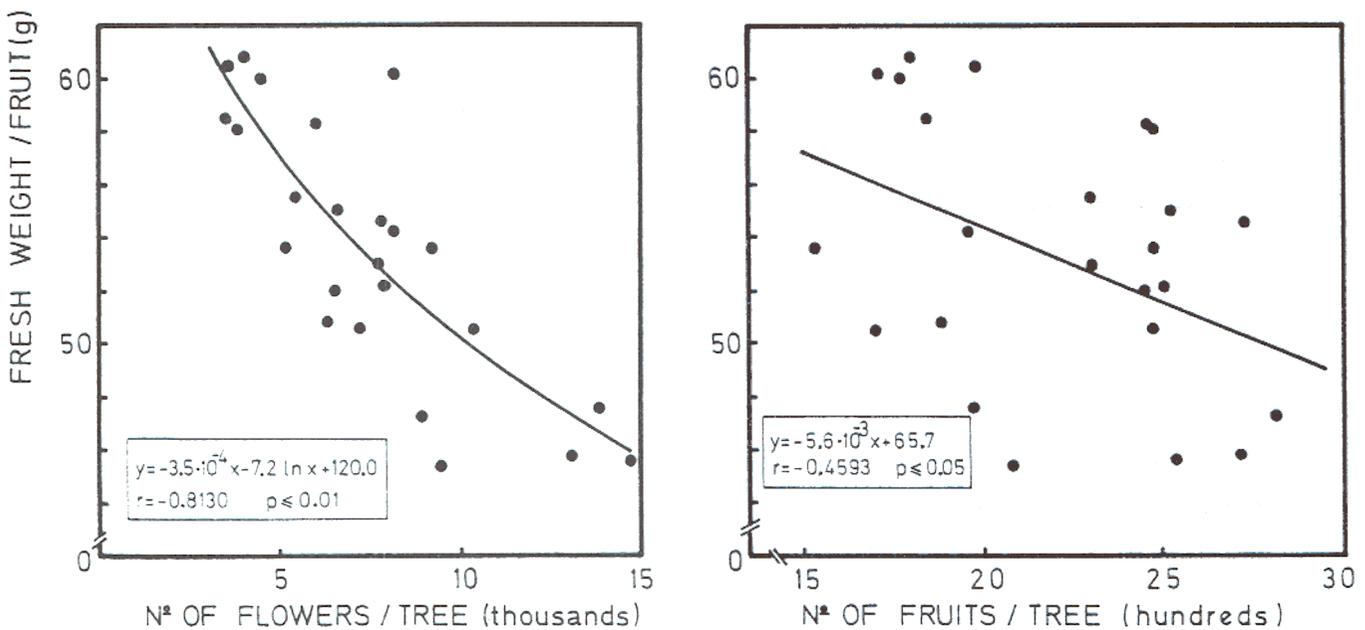
Figure 11. The relationship between flower number and fruit number to final fruit size in clementine mandarin. Data from the same trees in Figure 8, in a range of flower intensity which has no effect on yield. From Guardiola (1992).

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Flowering and Crop Value

For many years the studies on citrus flowering were considered of academic interest without much practical application since in most cases it was considered that natural flower formation was high enough to ensure a maximum crop, and only in the cases of alternate bearing was considered that flowering was limiting yield. As I have shown, the actual situation is more complex. Excess flowering is not the result but sometimes is the cause of unfruitfulness. Also fruit size, a major parameter of fruit quality, is partly determined at flowering. Flower quality, which is inversely related to flower number, determines also the response to fruit setting treatments. The manipulation of flowering is now considered as a standard practice for the production of high quality fruit for the fresh market. Unfortunately we are able to reduce flower number, but the available techniques to increase flower number are not reliable. The development of the appropriate technique would permit to obtain flowers at the grower's whim and for some cultivars to produce out of season highly priced fruit.

Flowering influence on yield parameters

	INCREASE IN FLOWER NUMBER →		
	Low -range flowering		Excess flowering
% SET	DECREASES	DECREASES	DECREASES
FRUIT No	INCREASES	SMALL EFFECT (INCREASES)	DECREASES
FRUIT SIZE	DECREASES MARKEDLY	SLIGHT DECREASE	
YIELD	INCREASES	NO EFFECT	DECREASES

Figure 12. Summary effects of flower intensity on yield parameters. From García-Luis (1995).