

## SEED DEVELOPMENT IN CITRUS

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Because seed development is necessarily a part of the development of fruit, it is important to consider the processes responsible for seed development and some of the reasons for the lack of seeds in some cases. We will then turn attention to the actual growth and development of the seed within the fruit. Lastly, we need to consider the horticultural significance of seed production and especially some of the unique features of citrus seeds which make them both an asset and a liability for growers and research scientists.

**Citrus Flower and Fruit Morphology.** There are 2 main types of bloom in citrus. Leafy bloom occurs when flowers form on and with new vegetative growth in the spring. The vegetative growth of the elongating shoot is transformed into a terminal inflorescence of 1 or more flowers about the time lateral shoots emerge from bud scales. Other flowers are produced directly in leaf axils of previous growth flushes and are termed bouquet bloom.

As a vegetative growth point is transformed into a flower bud, it becomes broadened and flattened, forming a floral apical meristem. Each flower part is formed from the outside in (acropetally). The sepal (button) primordia are formed first, then those of the petals, the anthers and finally the carpels which will form the segments. The female flower parts (pistil) are composed by fusion of a whorl of approximately 10 carpels. The cavities formed by fusion of the carpels are called locules. Each locule contains 2 vertical rows of ovules (Fig. 1), that can develop into seeds.

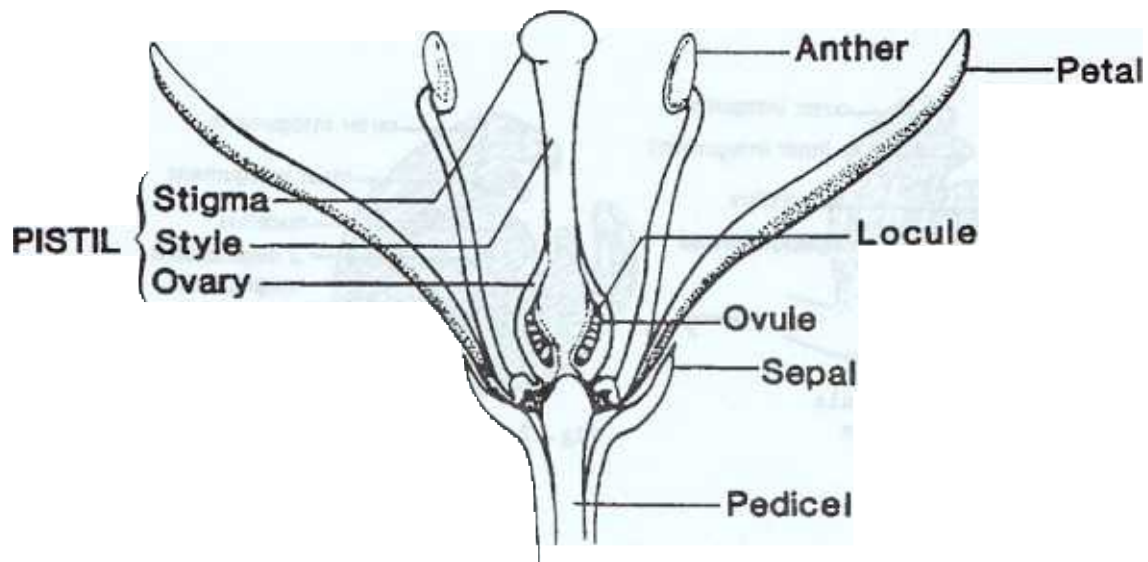


Fig. 1. Diagrammatic portrayal of open citrus flower.

The ovules are arranged in rows near the central axis (axile placentation). The inner integument is differentiated first. The outer integument is developed later. Both integuments take part in the formation of the seed coat and the micropyle which provides the opening through which the pollen tube enters the ovule.

The First Steps to Seed Formation: Megasporogenesis and Embryo Sac Development.

Within the ovule, the archesporial cell forms near the apex of the nucellus before the integuments are completely developed. The cell is distinguished from those surrounding it by its larger size and larger nucleus. The archesporial cell divides to form a tapetal cell and an embryo sac mother cell (megasporeocyte). The tapetal cell divides rapidly and the megasporeocyte is soon surrounded by tissue near the center of the nucellus. The megasporeocyte undergoes reduction division, producing 4 haploid cells with only one set of chromosomes per cell. These cells, the megaspores, are arranged in a longitudinal row in the nucellus. Three of the 4 megaspores degenerate and the remaining megaspore develops into the megaspore mother cell and later, the embryo sac. (Fig. 2).

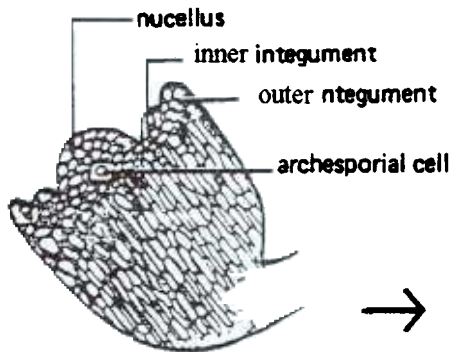


Fig. 2a. Developing ovule with archesporial cell.

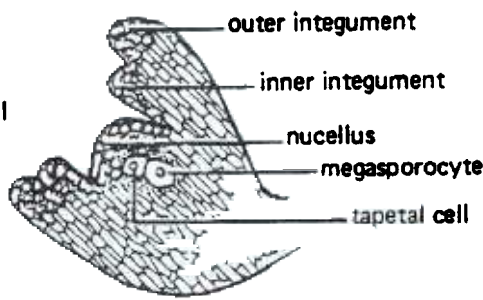


Fig. 2b. Developing ovule with megasporeocyte.

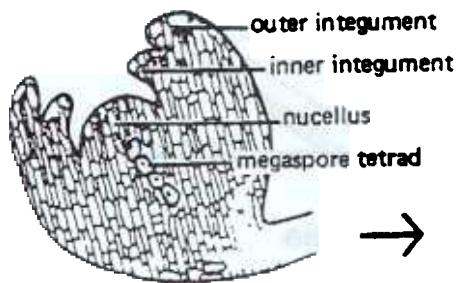


Fig. 2c. Developing ovule with megaspore tetrad.

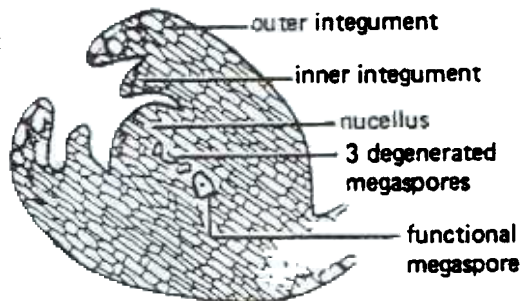


Fig. 2d. Developing ovule with megaspore mother cell.

Megasporogenesis.

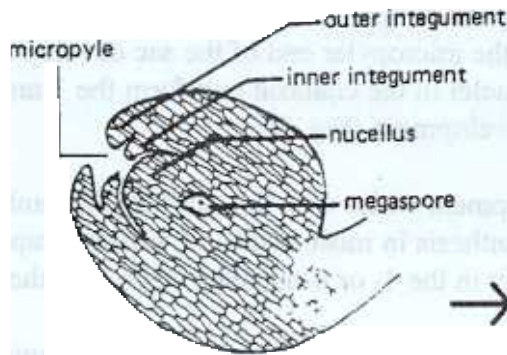


Fig. 3a. First stage of embryo sac development in ovule - megaspore mother cell.

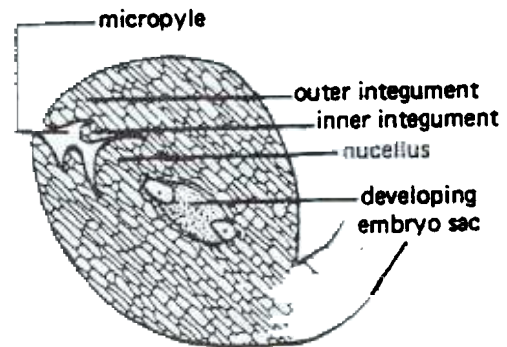


Fig. 3b. Two-celled stage of embryo sac development in ovule.

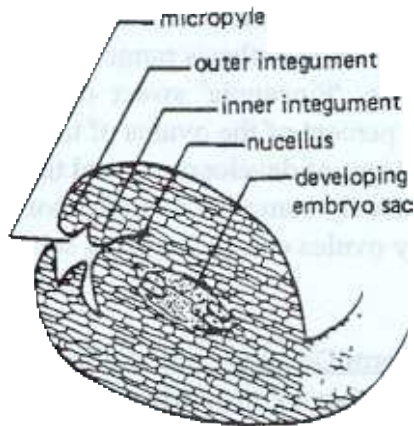


Fig. 3c. Four-celled stage of embryo sac development in ovule.

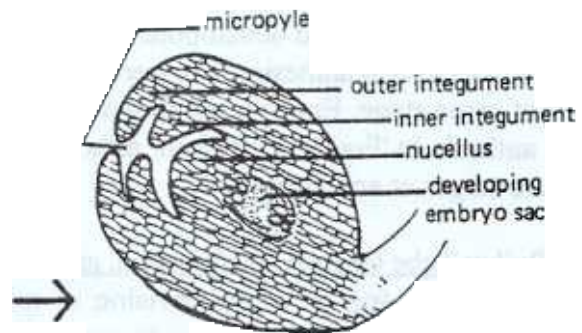


Fig. 3d. Eight-celled stage of embryo sac development in ovule.

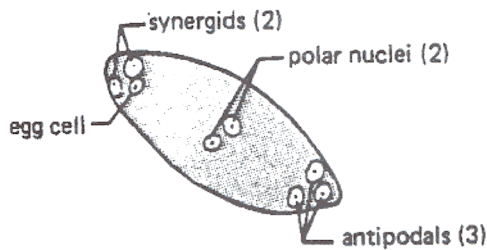


Fig. 3e. Detailed drawing of 8-nucleate embryo sac.

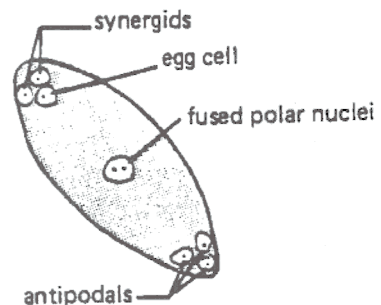


Fig. 3f. Mature 8-nucleate embryo sac.

Fig. 3. Embryo sac development.

The functional megaspore divides to form 2 daughter nuclei which migrate to opposite ends of the sac and remain there until the next nuclear division. Meanwhile, the cytoplasm is reduced to a thin layer in the periphery of the embryo sac, except around the nuclei. The 2 daughter nuclei divide again, producing 4 nuclei which form 2 pairs at opposite ends of the sac. The nuclei divide again to form the final 8 nuclei. The 4 nuclei in the micropylar end of the sac develop into the egg, 2 synergids, and upper polar nucleus. The 4 nuclei in the chalazal end form the 3 antipodals and lower polar nucleus to complete embryo sac development (Fig. 3).

The time required for embryo sac development varies with the cultivar. The embryo sacs are reported to be in the 2- or 4-nucleate stage at anthesis in most ovules of 'Foster' grapefruit, while 'Pineapple' sweet orange embryo sacs are mostly in the 4- or 8-nucleate stage at anthesis.

Maturation of the embryo sac reportedly occurs several days prior to petal opening (anthesis) in 'Foster' grapefruit, at anthesis in 'Pineapple' sweet orange and several days after anthesis in 'Washington' navel sweet orange and 'Satsuma' mandarin. The process of embryo sac formation varies from ovule to ovule within the same fruit. Formation of the mature embryo sac with respect to floral development is apparently cultivar-dependent and may also be influenced by other external and internal factors. Megaspore or embryo sac development at anthesis ranges from the megaspore tetrad stage through the fully developed embryo sac. 'Pineapple' sweet orange embryo sacs apparently undergo a rapid development. Thirty-four percent of the ovules of this cultivar contain mature embryo sacs at anthesis, 28% were in various stages of development, and the remaining 38% aborted at some stage. Embryo sac formation is reportedly complete in some ovules several days prior to anthesis in 'Foster' grapefruit, although many ovules contain embryo sacs in the 1-, 2- or 4-nucleate stages at anthesis.

**Pollen Tube Growth, Fertilization, and Endosperm Development.** Pollen grains develop in the anthers, following reduction division of microsporocytes to produce microspores with the haploid chromosome number. Insects vector the pollen to the stigma, where they germinate to produce a pollen tube that grows through the style to the ovary. This tube penetrates the micropyle of the developing ovules, and the 2 sperm nuclei are discharged into the embryo sac. One fuses with the egg nucleus and the second nucleus fuses with the 2 polar nuclei and forms a triploid endosperm nucleus. This double fertilization apparently occurs simultaneously. The zygote goes into a resting stage after fertilization for several weeks before dividing (Fig. 4).

The endosperm nucleus divides successively many times, without cell wall development, to produce a multinucleate endosperm. Free nuclear endosperm can be seen up to 67 days after pollination. This endosperm material becomes cellular, with 3 layers of cells on the micropylar end and a single layer of cells further down the chalazal end. The endosperm provides a source of nutrition that is absorbed as the embryo develops; it is consumed by 100 days after pollination.

**Sterility.** Gametic sterility is the inability to produce functional pollen or embryo sacs. Male sterility may result from imperfect development of stamens but usually is caused by defective pollen development. Pollen sterility is found in varying degrees in many cultivars of the genus Citrus. The 'Washington' sweet orange is essentially void of pollen. Satsuma mandarin pollen also degenerates

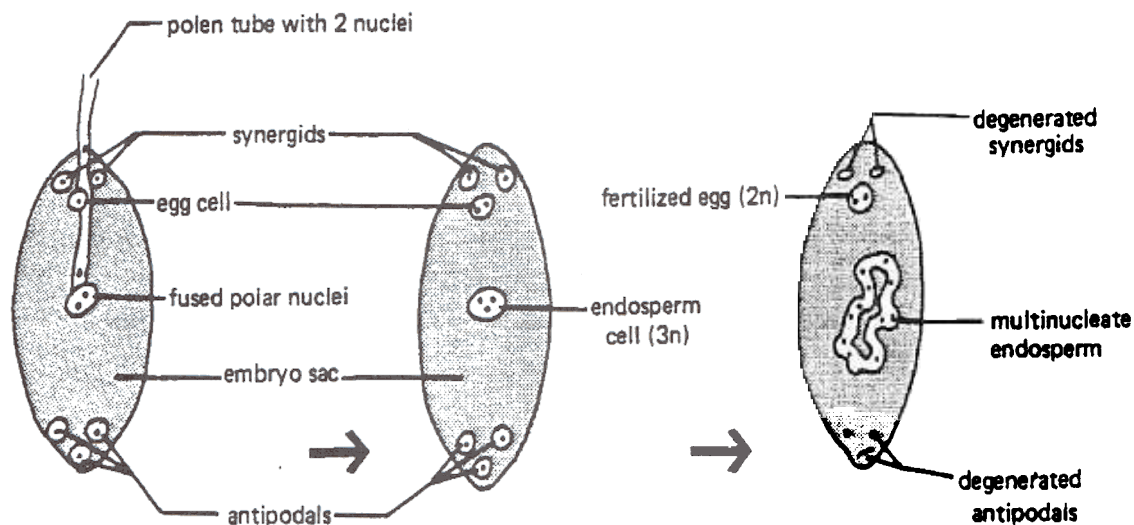


Fig. 4. Fertilization and endosperm development within the embryo sac.

during formation, leaving only a few normal grains at anthesis. Cross-pollination slightly increases seed set of commercially seedless citrus cultivars; their relative seedlessness is associated with low pollen viability and meiotic anomalies resulting from chromosomal structural changes.

Female sterility may result from aborted female flower parts or defective embryo sac development. Studies of 'Satsuma' mandarin and 'Washington' sweet orange provide classic examples of female sterility. The megaspore mother cell of 'Washington' often aborts before division. The cell becomes long and narrow and is compressed upward by the surrounding nucellar tissue. Generally, the megaspore mother cells pass through normal divisions, producing the linear tetrad of megaspores. Soon after the normal degeneration of the 3 megaspores nearest the micropyle, the functional megaspore shrivels and aborts. Embryo sacs often abort in other stages of development. Many of the embryo sacs exhibit delayed development which indicates the egg is not mature and viable for fertilization at the proper time. Embryo abortion is another form of sterility. Functional gametes (male and female parts) may be produced and fertilization occur, yet the embryo may fail to develop. The occurrence of empty seed coats in fruit suggests embryo abortion.

Citrus normally has 18 chromosomes but various other multiples of chromosome levels. The Tahiti, or Persian, lime for example is a triploid citrus cultivar (i.e.  $2n = 3x = 27$ ). When triploid citrus attempt to undergo meiosis, gametes (eggs and pollen) are produced with unbalanced numbers of chromosomes because, quite simply, 27 is not evenly divisible by 2. Citrus breeders are attempting to exploit this form of sterility to develop seedless cultivars. Unlike other cultivars such as 'Clementine' which are seedless by virtue of self-incompatibility, but which will bear seeded fruit when cross-pollinated by other citrus, triploids should be seedless regardless of pollination by other cultivars. One method to develop triploids is to induce embryogenesis in endosperm cultural in vitro;



although it has been accomplished, the limited number of successes makes this method unattractive. The most commonly used approach has been to cross normal monoembryonic diploids with pollen from tetraploids ( $2x \times 4x \rightarrow 3x$ ). Although embryos are produced from such interploid hybridizations, seed development is halted because the normal embryo to endosperm ratio is not achieved. (i.e. 3:4, rather than 2:3). Tissue culture of rescued embryos is necessary to recover plants from these crosses. This is now done routinely by many breeding programs.

**Incompatibility.** Sterility prior to fertilization is characterized by non-functional gametes, but with gametic incompatibility the pollen and ovules are functional and failure to produce fruit with seed results from a physiological hindrance to fertilization. This is usually manifested in citrus by slow growth of the pollen tube down the style.

There are 2 basic types of gametic incompatibility mechanisms, sporophytic and gametophytic. The sporophytic type is characterized by the inhibition of pollen germination. Gametophytic incompatibility is genetically controlled and pollen tube growth down the styles is retarded.

**Zygote Development.** Development of the zygotic (sexual) embryo in dicotyledons follows a somewhat regular order. The first division of the zygote is usually transverse with respect to the long axis. This is usually followed by further transverse divisions, and in some parts of the embryo, vertical divisions. Gradually, the embryo assumes a club-like form. The distal part of the embryo then becomes the center of active cell division and develops into a spherical structure. This sphere is called the embryo proper, and the stalk at the proximal end, the suspensor. Subsequent changes produce a distally flattened structure with bilateral symmetry. This flattening precedes the initiation of the 2 cotyledons. The embryo assumes the shape of a heart as the cotyledons are formed. Further divisions organize root and shoot meristems and rudiments of other organs (Fig. 5).

The fertilized zygote in citrus undergoes a resting period of several weeks before division. This period is 21 to 28 days in trifoliate orange and 50 days in 'Foster' grapefruit. First signs of zygote division in 'Orlando' tangelo occur 40 days after pollination. A 4-celled suspensor can be seen 5 days later and future growth of the embryo follows the classical pattern. The sexual embryo remains attached by the suspensor at the micropylar tip of the developing seed. The terminal end of the embryo flattens 9 weeks after pollination and a fork-like embryo develops 12 weeks after pollination as the cotyledons are differentiated. The endosperm develops as the sexual embryo is forming and later deteriorates. The nucellus also shrinks away, leaving only vestiges to contribute to seed coat formation.

**Seed Development.** The ovule develops into the seed following fecundation. The endosperm enlarges at the expense of the nucellus. Nucellar embryos may develop on stalks attached to the nucellus before the egg cell divides. These enlarging embryos and the zygotic embryo gradually replace the endosperm, the latter being digested by the developing cotyledons. An inner seed coat or tegmen is formed from the inner integument, the inner parts of the outer integument, and the remains of the nucellus and endosperm. The chalaza forms a cap over the distal end of the seed and the brown-to-reddish or purple epidermal cells form the colored area adjacent to the nucellus. The

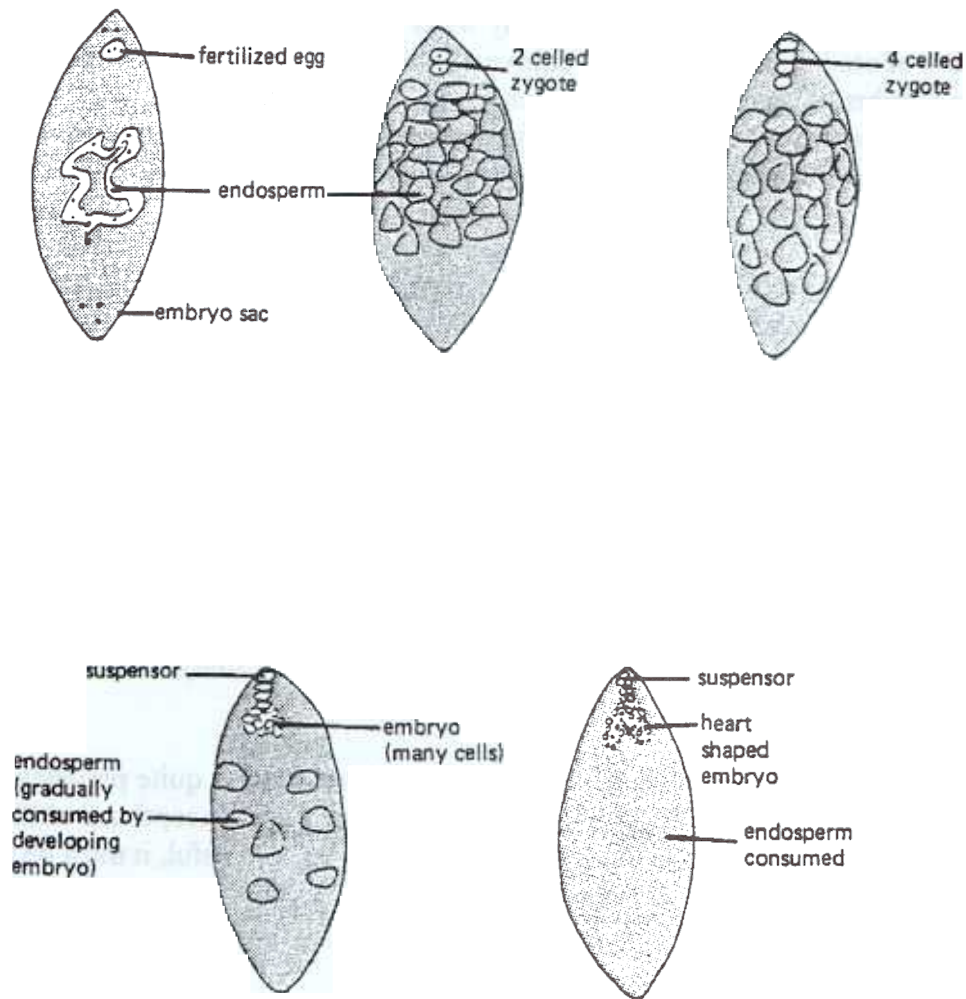


Fig. 5. Development of the fertilized citrus egg into a zygote and embryo.

testa, or outer seed coat, is composed primarily of the outer epidermis of the ovular wall. The epidermal cells form a secondary wall which makes up a woody, cream- or yellow-colored tough covering. The testa is often quite wrinkled or ridged and extends beyond the rest of the seed at one or both ends to form a beak or flat plate which may extend along the length of the seed.

Within the tegmen and the testa are 1 or more embryos. They form a solid, rounded mass in which the root initials (radicles) normally point toward the micropylar end. A seed with 1 embryo usually has 2 cotyledons of similar size and shape. The cotyledon size and shape often varies greatly if more than 1 embryo is present. The embryos in polyembryonic cultivars are usually crowded into the micropylar end. The cotyledons are white, cream or green and constitute most of the mature seed.

## Horticultural Significance of Seed Production

Seed production in citrus fruit impacts in many ways. The yield or number of fruit produced per tree is a function of seed numbers for many cultivars. The size of individual fruits is controlled to a degree by the number of seeds per fruit. Parthenocarpy, or the ability to produce fruit without seeds, is an important quality of many of our citrus cultivars. Polyembryony, the production of multiple embryos within a seed, has far-reaching impacts on the entire citrus industry. Each of these are discussed below.

**Parthenocarpy.** Many citrus cultivars produce acceptable yields of fruit with little or no seed in the fruit. Perhaps the best example of this is the 'Tahiti' lime which is almost completely seedless and still produces good crops. Such a cultivar would be considered strongly parthenocarpic. At the other extreme would be 'Wilking' mandarin, which sets no fruit when pollen is excluded. Between the 2 extremes are the moderately parthenocarpic cultivars. Often, the yield of citrus cultivars in this group will improve as more seeds are set as the result of better pollination or cross-pollination. An example of a cultivar in this group would be the 'Orlando' tangelo, which benefits greatly from cross-pollination.

Seedy cultivars may also possess strong parthenocarpy but this characteristic is unimportant and unnoticed. Therefore, parthenocarpy is not a consideration for us to study in the production of normally seedy citrus fruit.

Seedless fruit are another matter. The tendency to parthenocarpy is quite prevalent among citrus, and the horticulturally successful parthenocarpic cultivars possess exceptional ability to set fruit without seeds. If a cultivar incapable of producing seeds is to be successful, it must be strongly parthenocarpic. Probably many seedless types have arisen in the past which were of little value because of the lack of parthenocarpy.

Cross-pollination is desirable on many moderately parthenocarpic cultivars to increase yields. The numerous tangerine hybrids are excellent examples and the value of interplanting is well established. Cross-pollination increases seed set by overcoming incompatibilities in these cultivars. Fruit set may also be increased in weakly or moderately parthenocarpic cultivars by other means. The use of growth regulators such as gibberellic acid is an established and recommended practice and girdling has been shown effective, as well.

**Fruit Set.** Normal fruit set is the result of the stimulus of sexual fertilization and the production of seeds. Parthenocarpic cultivars represent an exception to this that is especially noticeable in incompatible or sterile cultivars where very few seeds are produced. Non-parthenocarpic cultivars will set few if any fruit if fertilization and seed production do not occur. Therefore, enhancement of seed production is often important in maximizing fruit set and yield.



Citrus flowers in self-fertile cultivars are usually self-pollinating since the structure of the flower allows this to happen. Increased bee activity assists this process and is known to help increase fruit set, especially in self-incompatible cultivars where cross-pollination is necessary.

**Fruit Size.** In some cultivars, correlations between seed number and fruit size have been reported for at least 8 cultivars. With cultivars plagued by small sizes, cross-pollination is important to produce fruit of an acceptable commercial size.

**Polyembryony.** The formation of multiple embryos is quite common in many citrus cultivars. These "extra" embryos may be the result of multiple zygotic embryos or twinning of a single zygotic embryo. The predominant cause of multiple embryo formation, however, is nucellar embryony, the development of vegetative embryos from the nucellus. Development of adventive embryos from nucellar tissue is extremely interesting since they are not the product of sexual fertilization. They are outgrowths of the nucellus and are therefore the product of vegetative reproduction, not sexual reproduction as is the case with zygotic embryos. This asexual reproduction has very important consequences in citrus.

Cells destined to develop into nucellar embryos can be seen in nucelli even before floral anthesis. Apparently pollination is essential to stimulate the development of nucellar embryos. It is not clear whether actual fertilization is essential but certainly excluding pollen will result in a greatly reduced fruit set. Perhaps the stimulus of pollen tube activity and the release of growth regulators accompanying such activity is all that is necessary to promote the growth of nucellar embryos in many citrus types.

Except in cultivars which rarely or never produce nucellar embryos, the zygotic embryo will have to compete with one or more nucellar embryos for space within the seed. The result of this competition depends upon the number of embryos within the seed, their time of development, their location and vigor. The lower the number of embryos per seed, the larger the average size of the embryos and the greater probability of survival. When zygotic embryos fail to survive in all or most of the seeds, the resultant seedlings will largely be from nucellar material and therefore genetically identical to the female parent plant. Such cultivars would be referred to as "highly nucellar," producing a large proportion of nucellar plants compared to zygotic ones.

Three factors are mentioned which contribute to the development of nucellar embryos at the expense of zygotic: (1) nucellar embryos grow off more rapidly within the seed, thereby getting a "head start" on the zygotic embryo, (2) the zygotic embryo is located unfavorably in the apex of the embryo sac and may receive fewer nutrients and is more subject to crowding pressure, and (3) zygotic embryos are usually genetically weaker than their nucellar counterparts because of inbreeding depression.

Polyembryony is extremely important for 3 reasons. These are of great horticultural significance and will be discussed individually in the paragraphs that follow.

Virus diseases are a serious concern for citrus production. The process of nucellar embryony cleanses plant material of the citrus viruses. The mechanism of the process is unclear but well documented. Plant material arising from nucellar seedlings will be free of citrus viruses, even if the parent plant is infected. The benefits of nucellar, virus-free budwood are well known. In cultivars where nucellar embryos are not produced, freedom from viruses must be attained in other ways such as searching for disease-free material, or heat treatment of infected sources.

Nursery operations are very dependent upon nucellar embryony because the process allows for vegetative seed reproduction. It is unique to be able to grow plant material in this way, and citrus nurserymen are lucky to be able to capitalize on this phenomenon. Eliminating off-size and off-type variants in a seedling population ensures a very high percentage of nucellar seedling material which is genetically identical to the desired female parent plant.

Plant breeders are often frustrated by nucellar embryony since it greatly complicates their work. The high degree of nucellar embryony common in many citrus cultivars means it is very difficult to obtain hybrid seedlings. This is why all the early breeders used monoembryonic female parent plants or utilized readily-observed genetic markers such as the trifoliate leaf characteristic of Poncirus to able to discern hybrid seedlings.