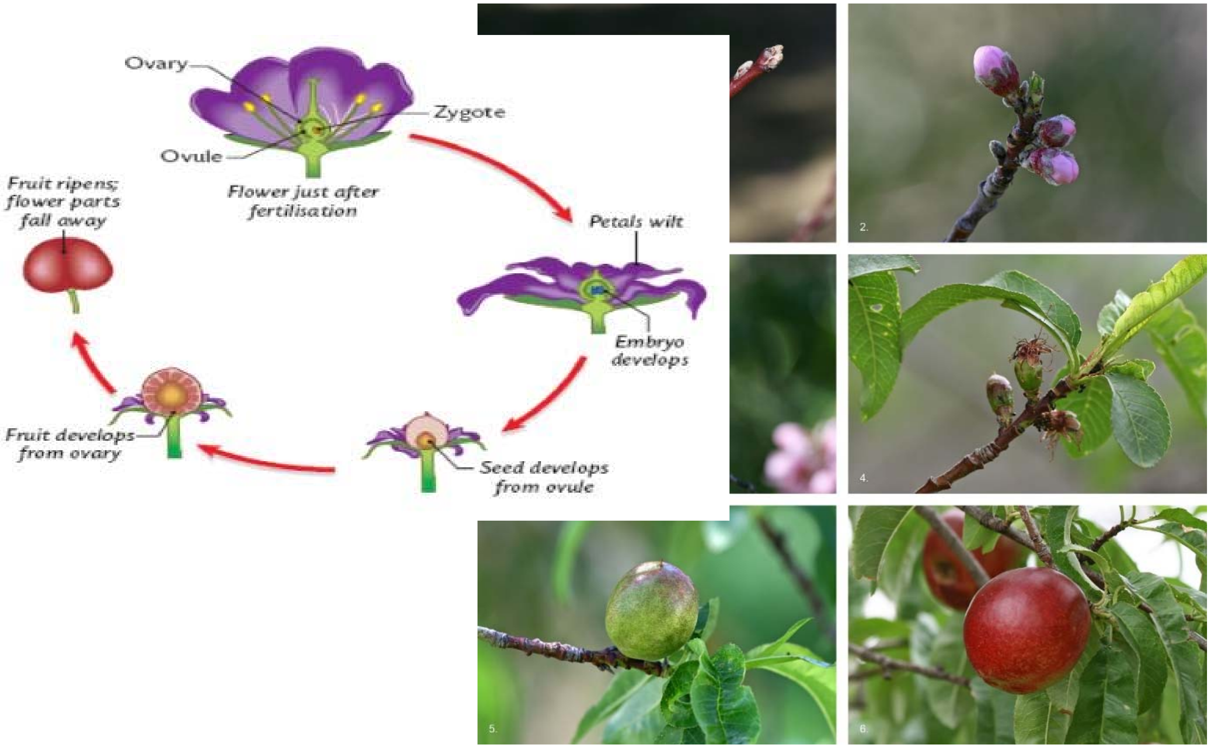
In the Name of God

the Merciful the Compassionate

Flower Formation



Book Review

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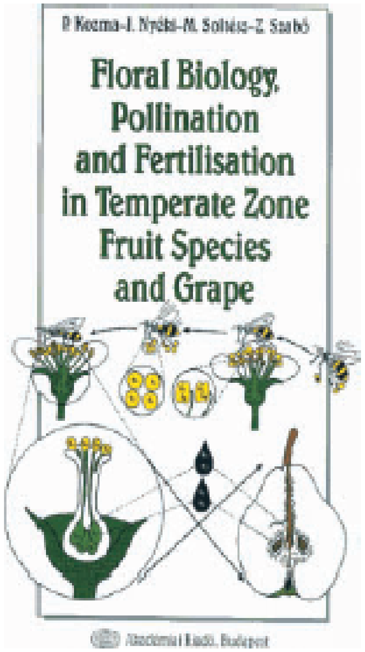


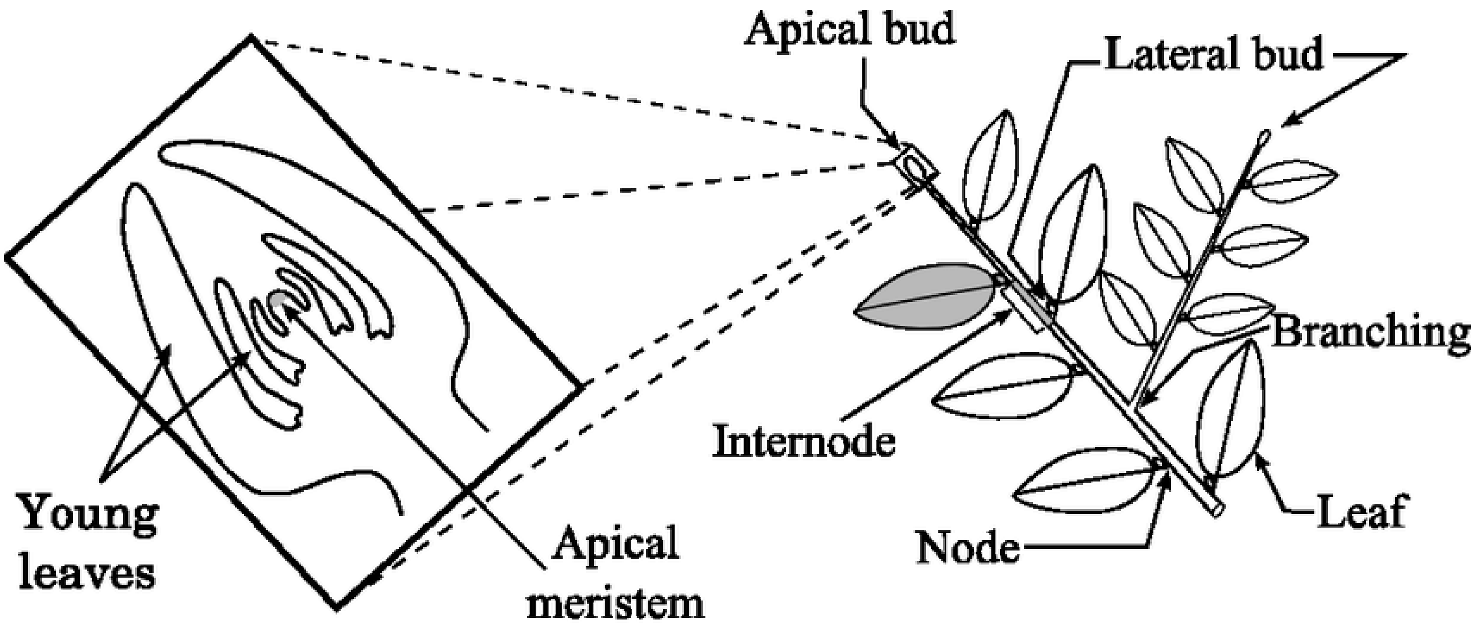
Floral biology, pollination and fertilisation in temperate zone fruit species and grape. Kozma P, Nyéki J, Soltész M, Szabó Z. 2003.
Budapest: Akadémiai Kiadò.$98(hardback).
621 pp.

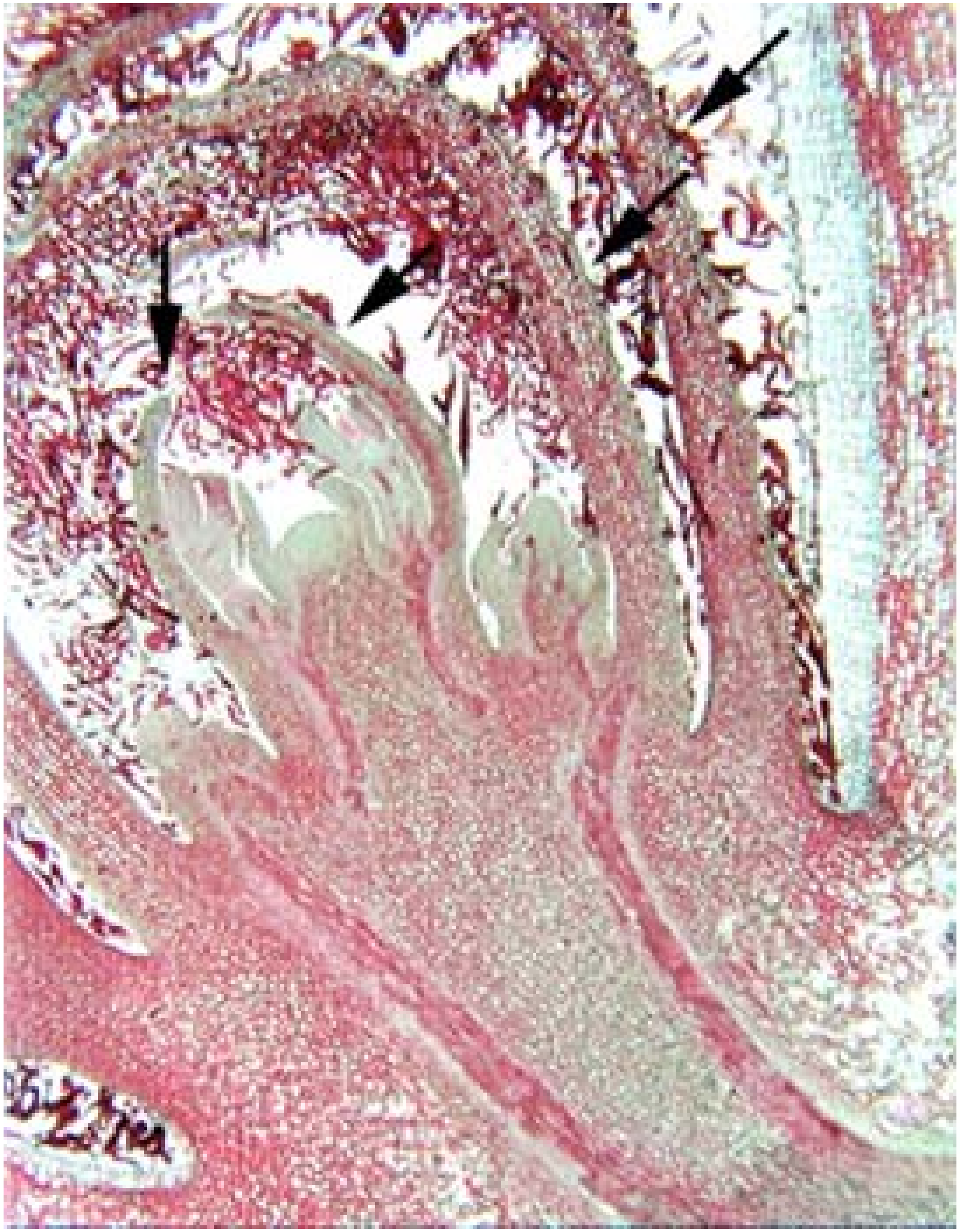
The primary focus of this book is to discuss the floral biology of the fruit crops including apple, grape, *Ribes*, *Rubus*, strawberry, the stone fruits and several nut species. The emphasis is on horticultural aspects of reproductive biology and pollination ecology. The strong point of the book is the amount of information provided on the anatomy and morphology of floral structures, the different patterns of floral development and the types of crossing relationships in fruit crops. It begins with five overview chapters concerning the hormonal aspects of floral development, the morphology of pollen, stigmata and anthers, and descriptions of the types of floral injury. There is then a lengthy (150 pages) discussion of flower types in grapes, followed by a chapter on plantation design. The book then launches into a series of chapters describing individual crops, which vary somewhat in content due to multiple authors, but all provide comprehensive information on bloom phenology, levels of self-fertility and varietal compatibility groups. There is a wealth of information on types of mating systems and extensive lists are given on the fertility and inter-fertility of the common varieties grown. The book ends with a general discussion of bee pollination and the conditions that influence its effectiveness. The information provided in this book is heavily slanted towards the Hungarian and European literature on floral biology, but overall it is an excellent survey of literature not available anywhere else in English. Its English is a bit stilted as is often the case when chapters are translated, but in general it is understandable. The book is recommended to individuals interested in the planning of large-scale commercial plantations of temperate fruit crops, and those who are interested in detailed descriptions of the anatomy and morphology of plant reproductive structures.

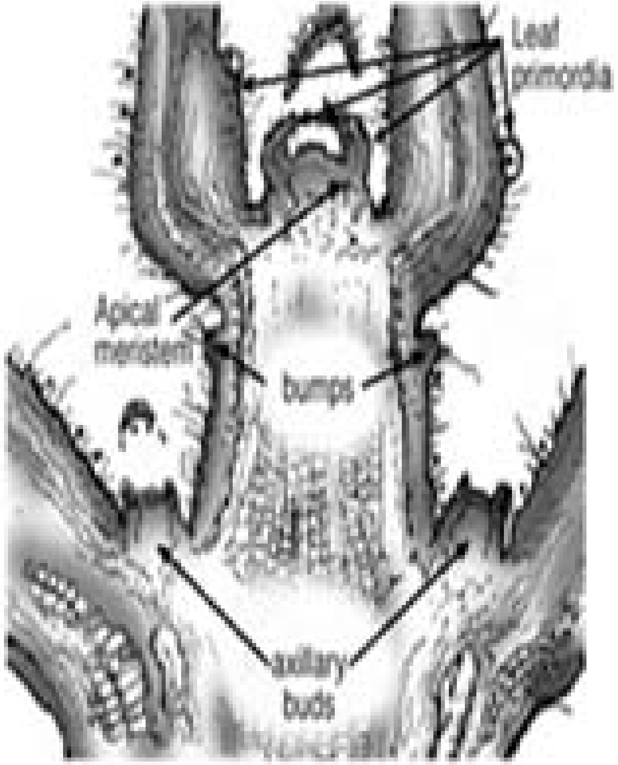
J. F. Hancock

Flower formation

- vegetative development preceding flower formation







plastochron

The function of endogenous hormones in flower formation

- Gibberellins

Alternate bearing: GA3/GA4   8:1

Regular bearing: GA3/GA4   1:5

- Indole acetic acid
- Cytokinins, ethylene, Abscisic acid

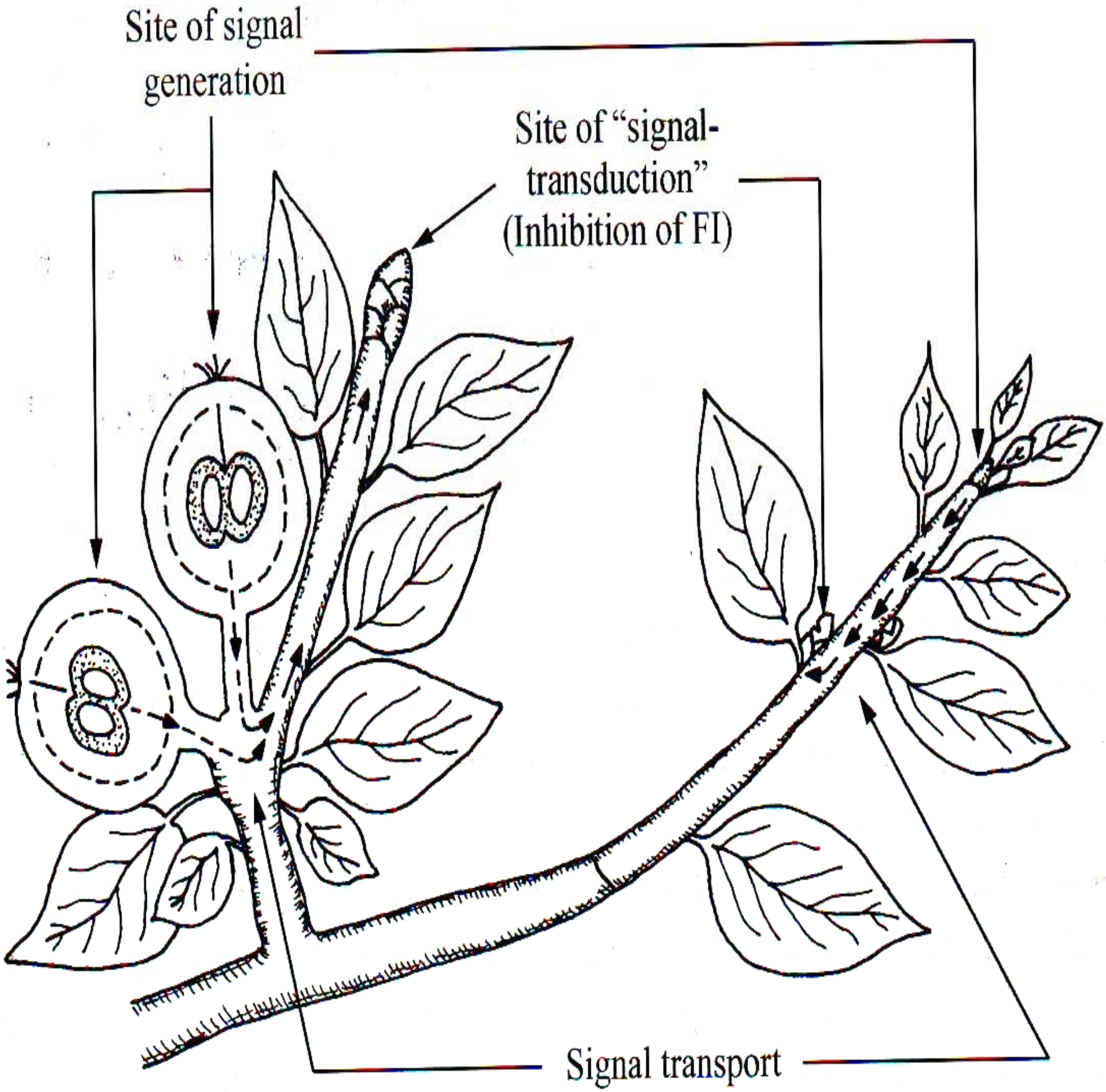
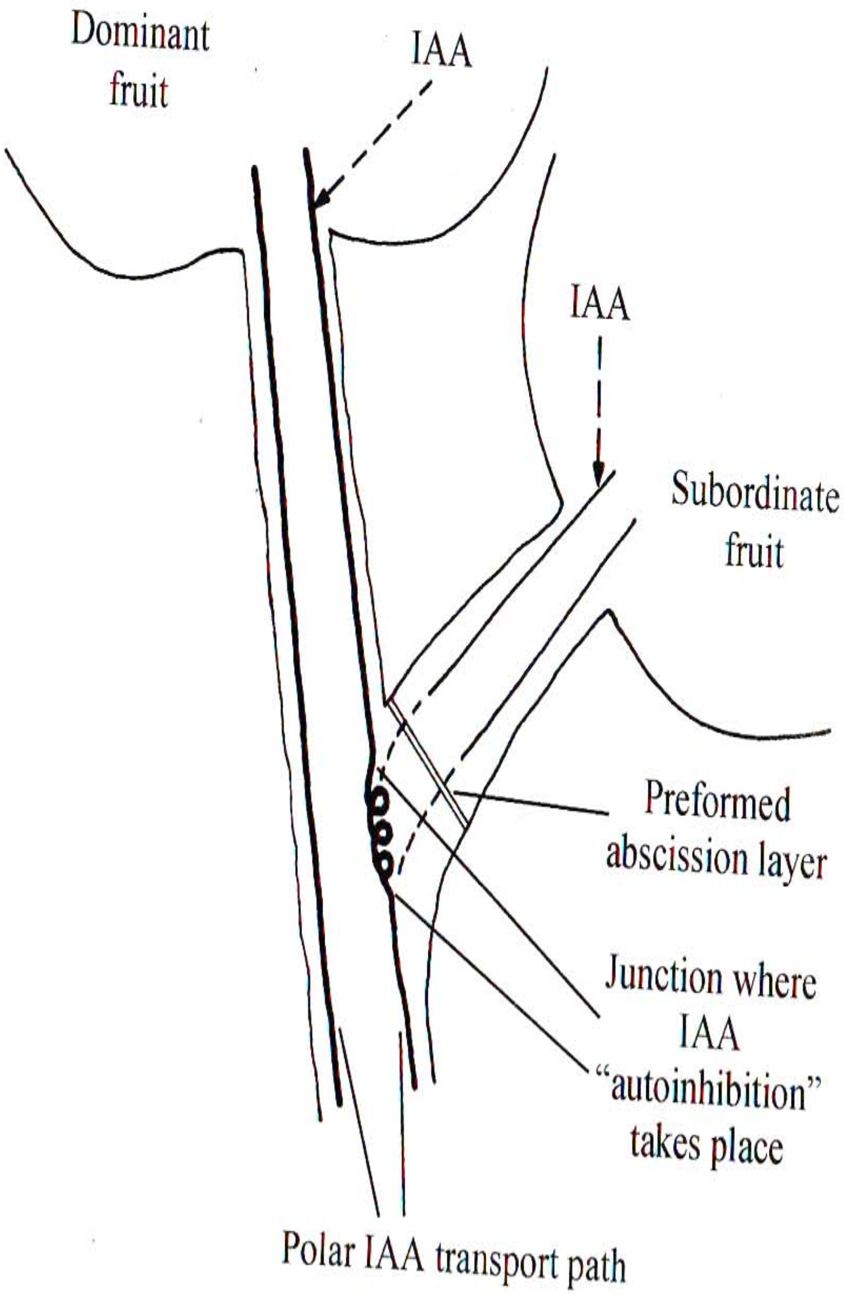


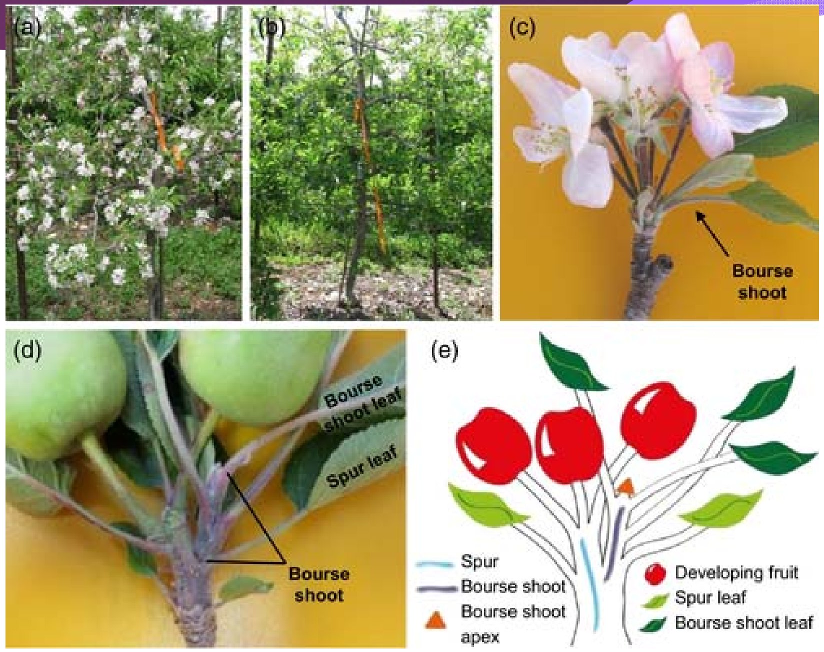
Fig. 2.1. The scheme of the hormonal signal inhibiting flower induction (FI) (after Callejas & Bangerth, 1997)

Fig. 2.2. The polar IAA transport from dominating and subordinate fruit with autoinhibition at "junctions". The reduced IAA transport in the pedicel of dominated fruit (thin and dashed lines) reduces IAA concentration in the abscission zone of that fruit (after Bangerth, 1990)



The presence of fruit as a particular factor

- Effect of fruit load



(a, b) Pictures of two adjacent ‘Top Red’ trees taken at the same day (27 April 2011), one with many flowers (a), the other with very few flowers (b).

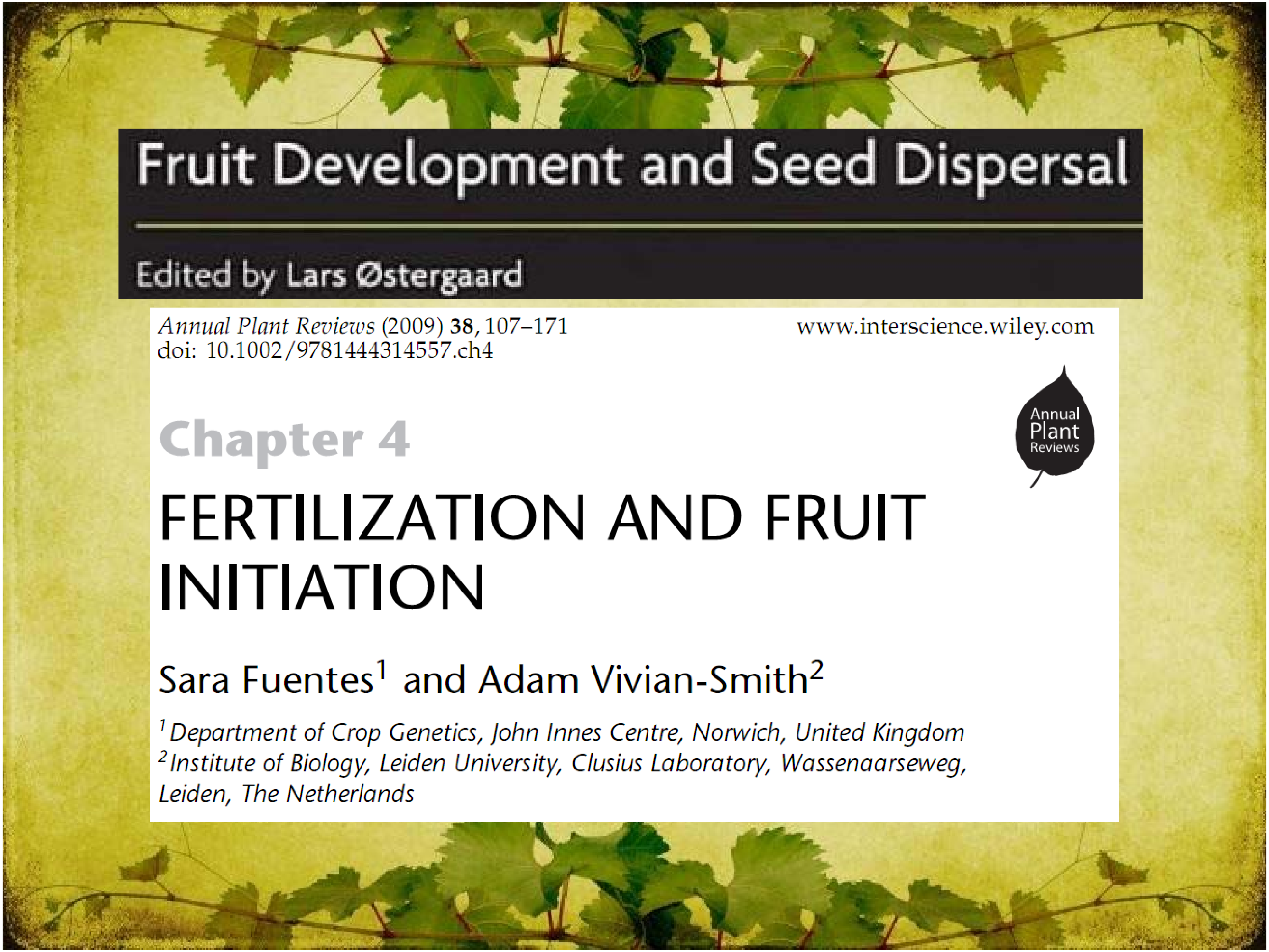
(c) A spur with an inflorescence at full bloom; a Bourse shoot (BS; arrow) with two to three open leaves can be seen.

(d) Spur with three developing fruit (one was removed) and a BS at 43 DAFB.

(e) Sketch of picture in (d). The BS apex will likely remain vegetative due to the presence of adjacent fruit. In the absence of fruit it may terminate with an inflorescence that will reach anthesis in the subsequent spring.

The transcription of a hitherto repressed information to a new structure, the flower bud

The cessation of the repression of genes responsible for flower formation



Fruit Development and Seed Dispersal

Edited by Lars Østergaard

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Chapter 4

FERTILIZATION AND FRUIT INITIATION

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In this chapter, we examine the role of female receptivity in fruit set and the key pathways and genes that control fruit initiation together with their complex relationship with fertilization, and with flower maturation. We present data that reinforce the idea that fruit initiation occurs in a very short period of time, characterized by hours and minutes, and not necessarily days. The contribution of various phytohormones such as auxin and gibberellins is also examined, as is the molecular genetic study of parthenocarpy as a tool to interrogate the early and immediate steps in fruit initiation. Through the course of understanding the molecular basis of fruit initiation, the evolution of the angiosperm fruit structure is also addressed, since extensive conservation of candidate regulatory genes exists.

5. Fertilization

5.1 Signal transduction before fertilization

- Pollen-tube growth in stigma, style and ovule is guided by gradients in γ-aminobutryic acid (GABA).
- Pollen tubes utilize a GABA-transaminase, (encoded by the *POP2* gene) to provide pollen directionality by degrading the GABA stimulant.

two other processes regulating pollen-tube guidance:

- diffusible ovule-derived attractants from unfertilized ovules

and

- repellents from fertilized ovules.

Auxin, together with calcium produced in synergids, had long been hypothesized as pollen-tube chemotropic attractants

pollen-tube guidance (cont.)

- **The synergids and the central cell**

also play a role in the guidance of the pollen tube prior to fertilization.

- In contrast to the GABA gradient, these appear to be short-range recognition and developmentally regulated.

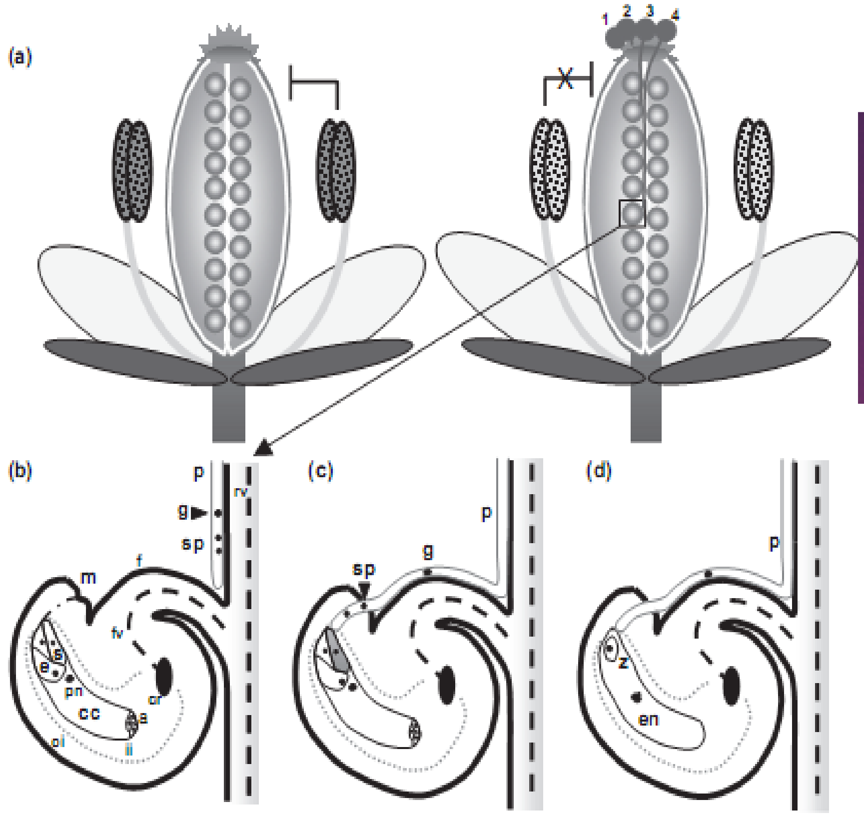


Figure 4.1 Pollination and fertilization in *Arabidopsis* and Brassicaceae. (a) Flower at anthesis stage (left) and during pollination (right) showing several rows of ovules within the pistil. During pollination, the stages of adhesion and hydration (1), recognition and pollen tube emergence (2), tube growth (3) and guidance (4) are shown (right). When anthers dehisce, the inhibitory stimulus for fruit development is removed (see the cross symbol). (b) A pollen tube containing two sperm cells is guided to the ovule micropyle by signals emanating from a fertile female gametophyte and the surrounding sporophytic tissue. (c) The tube tip enters the micropyle of the ovule and unites with a synergid cell that degenerates upon fusion. Two sperm cells migrate to combine with the egg cell and polar nuclei of the central cell, respectively. (d) Synergid cells degenerate and the diploid zygote and triploid endosperm begin development. The outer and inner integuments undergo cell expansion and division to form the seed testa. a, antipodal cells; cc, central cell; cr, chalazal region; e, egg cell; en, endosperm; f, funiculus; fv, funiculus vascular tissue; g, generative cell; ii, inner integument; m, micropyle; p, pollen tube; pn, polar nucleus; oi, outer integument; rv, replum vascular tissues; s, synergid cell; sp, sperm cells; z, zygote.

2 Double fertilization

Double fertilization, first described by Guignard in 1899, involves one sperm cell uniting with the egg cell, while the second sperm cell fuses with the central cell and undergoes karyogamy with the polar nuclei.

- Double fertilization begins upon fusion of the tube tip with a synergid cell. At this point, the pollen tube stops growing and discharges the two sperm cells.
- In some species, synergid degeneration occurs well before pollen tube arrival (Raghavan, 2003) while in *Arabidopsis* it has been reported that synergid cell death occurs upon pollen tube contact (Sandaklie-Nikolova *et al.*, 2007).

- Upon discharge of the sperm cells into the degenerating synergid:

1- Migration of sperm cells to the egg and central cells occurs (Faure *et al.*, 2002; Weterings and Russell, 2004; Ingouff *et al.*, 2007).

Movement towards their respective nuclei is facilitated by remnant F-actin coronas and microtubules (Ye et al., 2002; Raghavan, 2003).

2- Following double fertilization, development of a diploid zygote and triploid endosperm is initiated.

3- The remaining synergid eventually deteriorates (Kasahara et al., 2005)

4- The integuments expand and divide to accommodate the developing embryo and endosperm.

5- In *Arabidopsis* and other angiosperms, the integuments differentiate post-fertilization to form the seed coat or testa that protects the seed and facilitates the transfer of nutrients and photoassimilates to the seeds.

5.3 Signal transduction during fertilization

- After compatible fertilization, rapid changes in membrane-bound calcium occur.
- A complex signalling network is involved in the coordination of double fertilization
- An early signalling event is the generation of a calcium influx following the fusion of the sperm and egg cell.

Characterization of the *Arabidopsis* *aca9* mutant has further contributed to the understanding of the role played by calcium signalling during fertilization. *ACA9* encodes a Ca2+ pump that is primarily expressed in pollen. Mutant *aca9* pollens not only display reduced pollen-tube growth but are also defective in sperm cell release

5.3.1 Roles of the egg cell and central cell in fruit initiation

- The role of the endosperm and egg cell in the control of seed development has been extensively reviewed.
- first observed in the *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*).
- It is widely accepted that upon fertilization auxin originating fromthe seed is generated.
- In *cdc2a* mutant pollen, a single sperm cell is produced and is able to fertilize the egg cell.

5.3.2 Roles of the integuments in fruit initiation

- selection of male gametes arriving at the ovule
- Providing nutritive support to the developing zygote and endosperm,
- signal transduction that directly stimulates fruit initiation.

Cells remain mitotically active throughout the female receptive period. However, soon after fertilization, the integument cells begin expansion and the mitotic index further increases

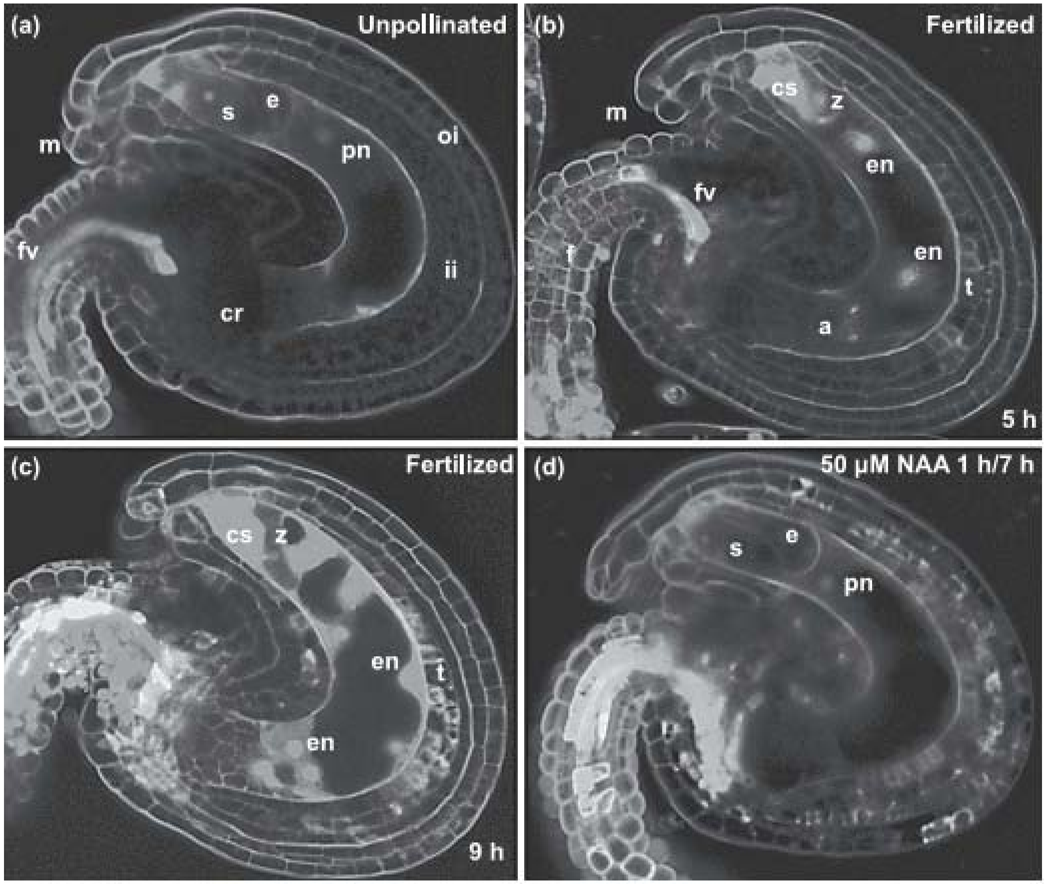


Figure 4.2 Confocal laser scanning microscopy (CSLM) images of unfertilized and fertilized ovules of *Arabidopsis* expressing the synthetic auxin-responsive reporter gene *DR5rev::eGFP*. (a) Unfertilized anthesis ovule with minimal GFP expression.
(b) Post-fertilized ovule at 5 h. The first nuclear endosperm division has occurred and G expression is observed in the endothelium, the chalazal domain and adjacent to the funiculus vascular strand. (c) Ovule after the third endosperm division (9 h post-fertilization) with eight endosperm nuclei and an elongated zygote. Strong GFP expression occurs in the endothelium, the chalaza and funiculus. Weaker expression is observed in the outer integument. (d) Treatment of detached pistils with NAA (50 μM) for 1 h, with subsequent washing for 7 h, induces strong GFP activation in the funiculu and chalaza, and moderate activation in the inner integument and weaker expression i the outer integument. a, antipodal cells; cc, central cell; cr, chalazal region; e, egg cell; en, endosperm; f, funiculus; fv, funiculus vascular tissue; g, generative cell; ii, inner integument; m, micropyle; p, pollen tube; pn, polar nucleus; oi, outer integument; rv, replum vascular tissues; s, synergid cell; sp, sperm cells; t, endothelium; z, zygote. (For colour version of this figure, please see Plate 2 of the colour plate section.)

6 Hormonal cues during fruit initiation

In 1936, Gustafson discovered that application of synthetic auxins to emasculated flowers of several different plant species resulted in parthenocarpic fruit development and, thus, established the initial linkage between fruit initiation and plant-growth regulators (Gustafson, 1936). At present, three main types of plant-growth regulators are recognized as having phytohormonal properties that can potentially induce fruit setting and fruit development (Gillaspy *et al.*, 1993). Application of auxin, gibberellins or cytokinin, either alone or in combination, has been shown to trigger parthenocarpy across a wide variety of plant species (Gustafson, 1936; King, 1947; Srinivasan and Morgan, 1996; Vivian-Smith andKoltunow, 1999; Ozga *et al.*, 2002, 2003). Application of optimal combinations of

6.1 Auxin

- Auxin appears to have a primary role during fruit initiation since the genetic analysis of wild-type *Arabidopsis* fruit initiation with gibberellin biosynthesis and perception mutants shows that auxin-mediated differentiation underlies other signalling pathways.

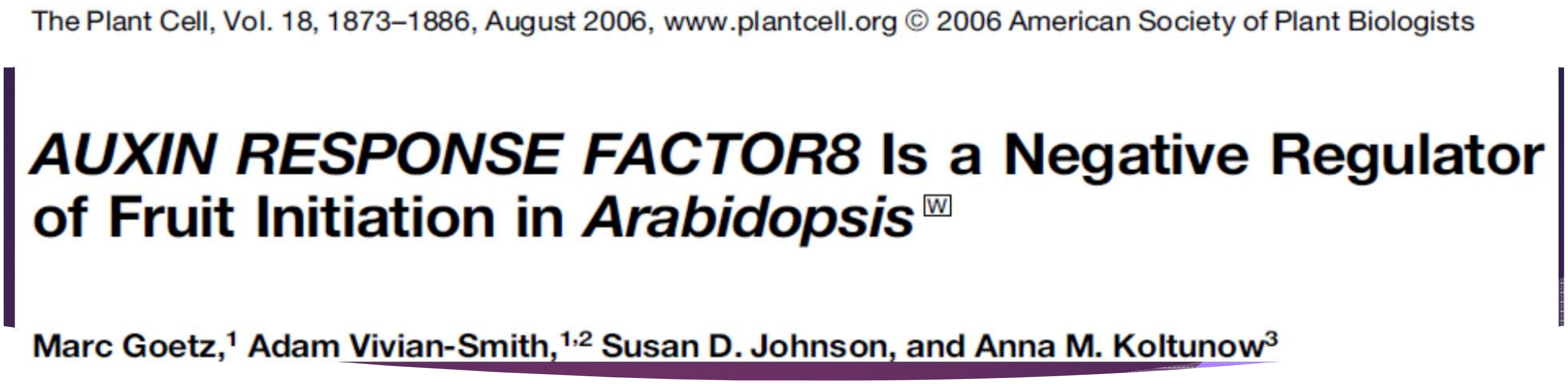
- transcriptional profiling during fruit initiation also shows directionality in phytohormonal responses with auxin preceding gibberellin responses at 12–14 h period post-fertilization.

- The use of the transgenic *DEFH::iaaM* construct in a broad range of species additionally suggests a universal role for auxin in triggering fruit set.

Accordingly, auxin-mediated signalling is an early response in the Arabidopsis ovule (Fig. 4.2). Auxin-responsive reporters show transcriptional activation 2–3 h post-fertilization expression, when the nuclear endosperm has undergone only one division.

6.1.1 Auxin-mediated transcriptional activation

- Several genetic lesions in the auxin pathway conferring autonomous fruit initiation have been isolated. Each of these mutants appears to work within the auxin-mediated transcriptional network. The *Arabidopsis* genome encodes 22 functional auxin response transcription factors (ARFs) and 29 Aux/IAA interacting proteins, and each gene appears to have strong sequence conservation in other plant genomes.
- The ARFs are a family of Aux/IAA interacting proteins that contain a DNA-binding domain (DBD) which recognizes auxin response elements (AuxREs) in DNA sequences.
- *FWF/ARF8* transcripts are naturally downregulated within 24 h post-pollination.



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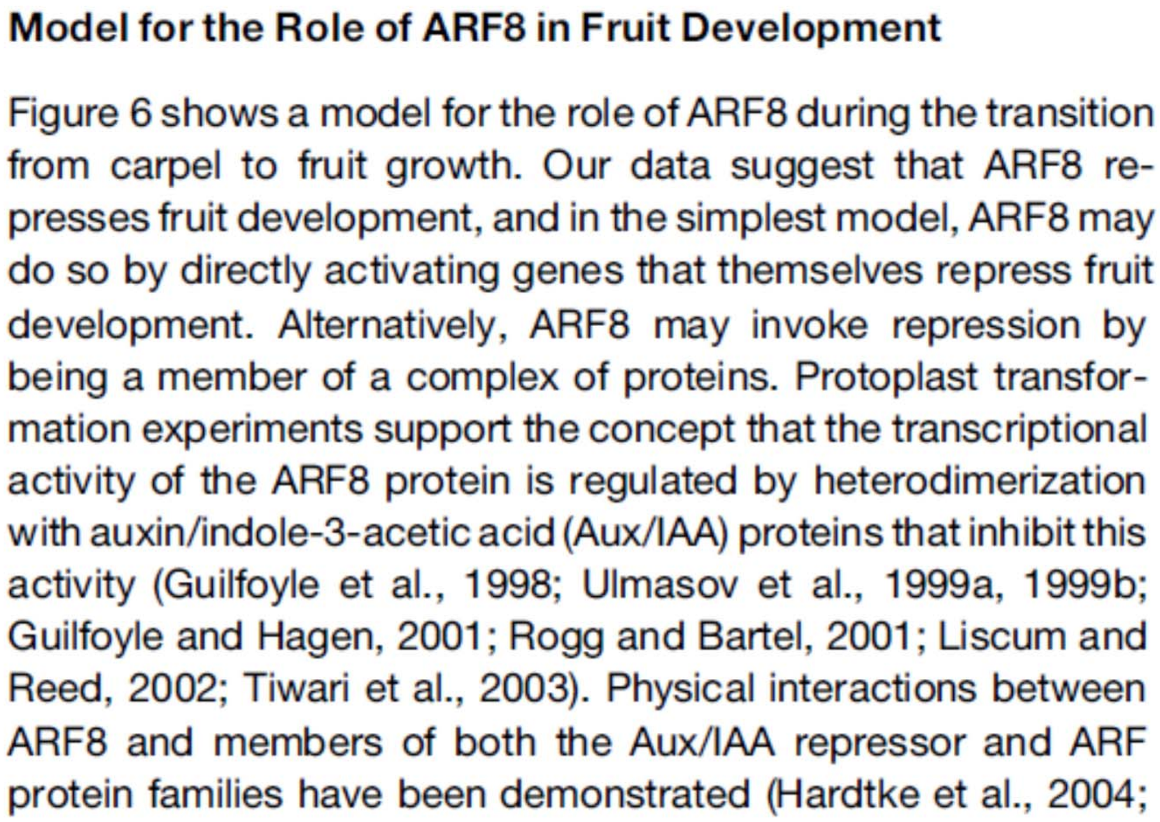
AUXIN RESPONSE FACTOR8 Is a Negative Regulator of Fruit Initiation in *Arabidopsis* W

Marc Goetz,[1] Adam Vivian-Smith,[1,2] Susan D. Johnson, and Anna M. Koltunow[3]

ARF8:GUS expression is switched off soon after fertilization has occurred in wild-type plants, indicating that a fertilization signal deactivates ARF8. The removal of ARF8 activity after fertilization might abolish a developmental block that represses fruit growth and allows initiation of seed and fruit developmental programs.

In unfertilized ovules, expression of the ARF8:GUS marker persists and staining is observed throughout the ovule, indicating that the negative regulation through ARF8 is kept active. These expression patterns are therefore consistent with ARF8 acting as a negative regulator of fruit initiation, and collectively they indicate a central role for the ovules in mediating positive and negative signals involved in fruit development.



Model for the Role of ARF8 in Fruit Development

Figure 6 shows a model for the role of ARF8 during the transition from carpel to fruit growth. Our data suggest that ARF8 represses fruit development, and in the simplest model, ARF8 may do so by directly activating genes that themselves repress fruit development. Alternatively, ARF8 may invoke repression by being a member of a complex of proteins. Protoplast transformation experiments support the concept that the transcriptional activity of the ARF8 protein is regulated by heterodimerization with auxin/indole-3-acetic acid (Aux/IAA) proteins that inhibit this activity (Guilfoyle et al., 1998; Ulmasov et al., 1999a, 1999b; Guilfoyle and Hagen, 2001; Rogg and Bartel, 2001; Liscum and Reed, 2002; Tiwari et al., 2003). Physical interactions between ARF8 and members of both the Aux/IAA repressor and ARF protein families have been demonstrated (Hardtke et al., 2004;

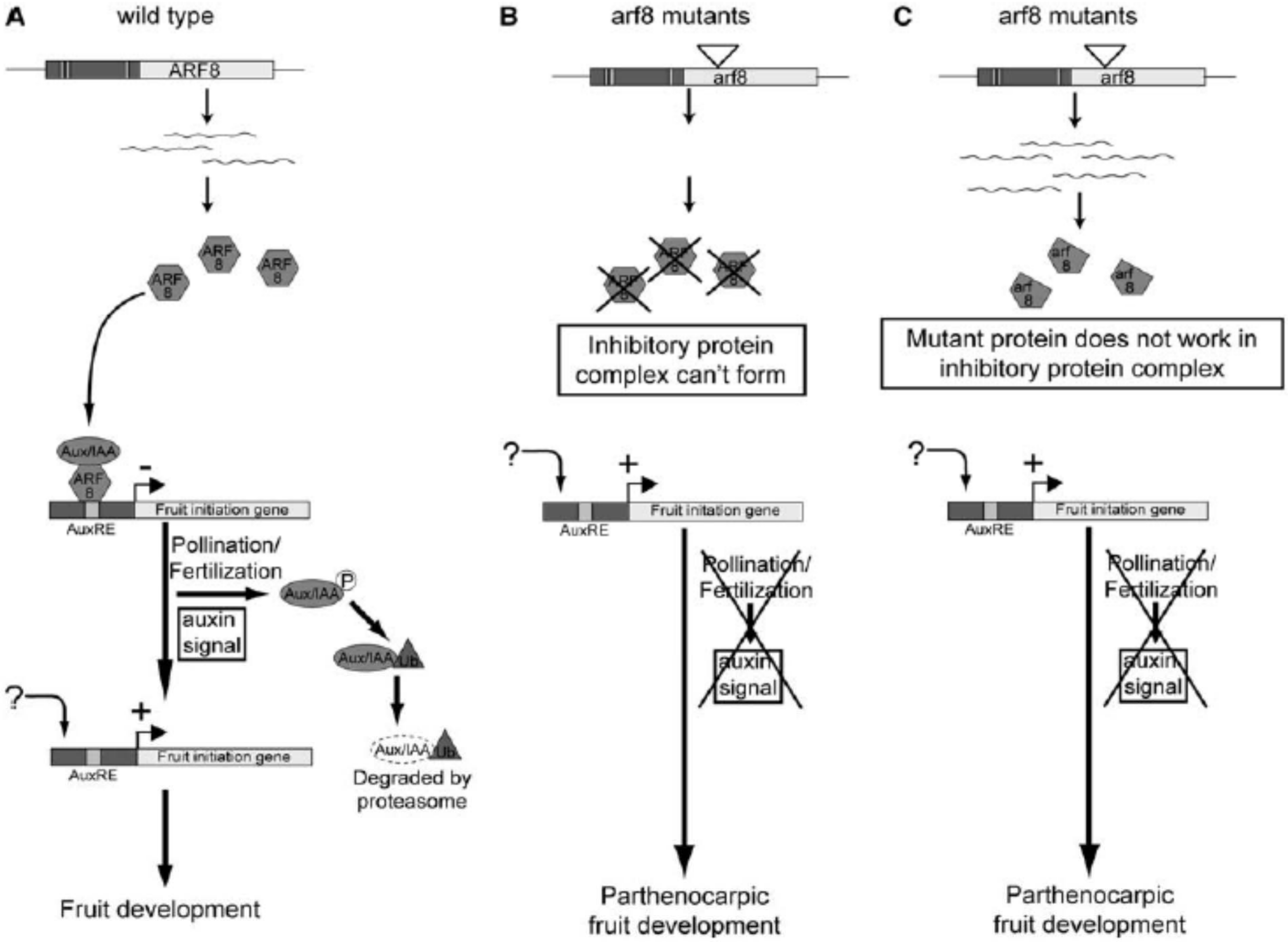


Figure 6. Model for the Role of *ARF8* in Fruit Development.

6.1.2 Auxin biosynthesis and transport

- Three important processes that regulate auxin action in the flower and fruit are its biosynthesis, transport and catabolism.

iaaM is involved in the conversion of tryptophan to indole-3-acetamide, which is the hydrolyzed to IAA.

In an experiment, the *iaaM* gene from *Pseudomonas syringae* pv savastonoi was placed under the control of the placental and ovule-specific promoter DEFH9. Eggplants and tobacco transformed with this construct had parthenocarpic fruit development...