Fifty Years of Citrus Developmental Research: A Perspective

Eliezer E. Goldschmidt

Institute of Plant Sciences and Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Received for publication 20 Feb. 2013. Accepted for publication 15 May 2013.

Abstract. An overview of the conceptual and methodological changes in citrus developmental research during the past half century is provided. Beginning with the hormonal era, the extensive use of plant growth regulators (PGRs) is described and the presumed role of gibberellins in various stages of the reproductive processes is emphasized. Introduction of the source-sink concept opened the way for alternative, carbohydrate-mediated explanations of productivity and alternate bearing. The potential role of ethylene in citrus abscission and fruit ripening is reviewed. The recent adoption of genetic–molecular approaches paves the way for deeper understanding of citrus developmental enigmas.

For a long time, morphological observations, microscopes, and simple chemical analyses were fruit tree researchers’ major tools. However, then, toward the middle of the 20th century, horticulturists were enchanted by the plant hormone concept. The hormonal thinking dominated citrus developmental physiology for a long time; rooting, vegetative growth, flowering, fruit development, ripening, and abscission were all attributed to one specific plant hormone or to the perplexing “hormonal balance” concept. Synthetic growth regulators were exploited in every possible way to solve practical problems. The emergence of the source-sink concept (≈1980) partially replaced the hormonal hypothesis. The source-sink hypothesis claims that carbohydrate levels control the major stages of the fruiting process—flowering, fruit set, and fruit enlargement—and are, therefore, responsible also for the “alternate bearing” syndrome. Although the effects of girdling and fruit thinning seem to support the source-sink concept, direct control of flowering and fruit set by carbohydrate levels has not been unequivocally demonstrated. However, then, rather soon, came the molecular–genetic era, forcing us all to acquire a new language and follow its logic. We can now identify the genes responsible for developmental phenomena. Citrus research is still a few steps behind Arabidopsis but here we are, still doing our best to solve the remaining mysteries of citrus.

The purpose of the present article is to provide an overview of the conceptual changes that took place in the area of Citrus developmental physiology during the recent half century or so and highlight major achievements. Corresponding changes must have taken place in other fruit tree crops. In the background of this specific area are the far-reaching developments in biological theory and, of no lesser significance, the emergence of new techniques and methodologies. While plant physiological research gradually adopted the new experimental tools, the acquisition of new methodologies by horticulturists was a slow, painstaking process. A schematic illustration of these conceptual changes is provided in Figure 1. Please note that the “source-sink” concept “invades” the scenery but does not fully replace the “hormonal paradigm.” The elliptic “genetic–molecular era” is wider than the circle of the “hormonal paradigm,” indicating its broader biological significance.

The Hormonal Era

The dawn of this half century period roughly coincides with the emergence of the “hormonal paradigm,” which appeared to provide an explanation for all plant developmental phenomena. The hormonal concept, which had been developed with a few model systems (Avena coleoptiles, pea seedlings), was rapidly adopted by horticulturists. It is quite amazing that although so little was actually known about plant hormones, the hormonal theory became so dominant, pushing aside alternative explanations such as nutritional carbon/nitrogen ratios (Zheng, 2009). The introduction of synthetic PGRs opened the way for a multitude of horticultural treatments in an attempt to solve every developmental problem and cure every physiological disorder. The implementation of PGRs in citrus culture began very soon after the adoption of the hormonal paradigm. In fact, the American physiologist W.C. Cooper was the first to demonstrate that treating woody cuttings with a synthetic auxin promoted root formation; these early experiments with lemon cuttings (Cooper, 1935) initiated the use of synthetic auxins as universal rooting agents. The pioneering work of C.W. Coggins, Jr., and his team in California laid the foundation of a range of still prevailing citrus pre- and post-harvest PGR treatments (Coggins and Held, 1968; Lovatt, 2012).

The review of El-Otmani et al. (2000), although not completely up to date, demonstrates the extensive, worldwide use of PGRs in citrus culture. It also contains references to a large number of experimental field studies conducted in Israel, Spain, the United States, Brazil, Japan, and elsewhere to define the specific chemicals, concentrations, and conditions for optimal orchard treatments.

The Productivity Complex

It is not easy to distinguish among the physiological problems of flowering, fruit set, and tree productivity because they are so closely intertwined. Flowering of Citrus is a subbranch of the general flower induction enigma. The work of Monselise and Haley (1964) was one of the first to show that GA inhibited the flowering of citrus, whereas GA antagonists promoted it (Monselise et al., 1966). Gibberellins were assumed therefore to play an inhibitory role in the natural regulation of citrus flowering; the environmental cues, drought and cool temperatures, presumably interfere with the biosynthesis of gibberellins, thereby enabling the formation of flower buds (Goldschmidt and Monselise, 1972; Monselise, 1985).

Closely related to the regulation of flowering is the shoot type/inflorescence phenology. Following various attempts to define citrus shoot types (Lenz, 1966; Sau er, 1951), the distinction among the three shoot types—vegetative, mixed-type, and reproductive (Goldschmidt and Monselise, 1972)—became widely accepted. GA and GA antagonists can modify the shoot type distribution toward the vegetative or the reproductive end, respectively (Goldschmidt and Monselise, 1972; Greenberg et al., 1993). Furthermore, fruit set follow-ups invariably showed that most fruit are borne on mixed-type inflorescences.
suggesting a role (nutritional, hormonal, or water transport; Erner et al., 2000) for inflorescence leaves in the fruit set process (Moss et al., 1972). Initial set was found to be high on leafless inflorescences (especially after fall girdling; Erner, 1988), but during the subsequent fruit drop period, the proportion of fruit on leafless inflorescences decreased in favor of the mixed-type inflorescences (Goldschmidt and Monselise, 1977). The importance of GA became evident also in another reproductive event. Quite early, Soost and Burnett (1961) showed that GA could replace the pollinator trees in ‘Clementine’ orchards, opening the way for the current use of GA as an inducer of parthenocarpic, seedless mandarins and their hybrids (Monselise and Goldschmidt, 1982). Thus, fruit enlargement is correlated with fruit size (Goldschmidt and Monselise, 1977). Fruit thinning reduces the number of fruit per unit leaf area per tree, thereby enabling each fruit to increase its size, presumably as a result of improved supply of photosynthate. The intensity of fruit thinning is negatively correlated with fruit size (Goldschmidt and Monselise, 1977). Thus, fruit enlargement is most closely dependent on the supply of photosynthate (Goldschmidt, 1999). Most orange and grapefruit cultivars have an effective self-thinning mechanism that adjusts the number of remaining fruit to a tree’s fruit bearing potential. The natural thinning mechanism, i.e., fruitlet abscission, appears to be induced by a shortage of carbohydrates (Garcia-Luis et al., 1988; Mehouachi et al., 1995).

As already mentioned, alternate bearing is common in mandarins and mandarin hybrid cultivars. In extreme cases, trees collapse after a heavy crop (“on”) year; this collapse is clearly associated with severe depletion of carbohydrate reserves, particularly in roots (Goldschmidt and Golomb, 1982; Smith, 1976). Carbohydrates seem therefore to play a role in the alternate bearing syndrome (Li et al., 2003a; Monselise and Goldschmidt, 1982), but other nutritional and hormonal factors are certainly involved.

The source-sink hypothesis of citrus productivity is schematically presented in Figure 3. Carbohydrate levels seem to play a role in floral induction, fruit set, fruitlet abscission, and fruit enlargement but they are not the sole factor and direct evidence for their involvement in some of these stages is still missing. The interplay of plant hormones and carbohydrates, in particular, requires further study. There is a general understanding that plant hormones and source-sink mechanisms must interact in the execution of plant performance. However, how this is accomplished is still debatable (Baker, 2000; Peleg et al., 2011; Wardlaw and Moncur, 1976).

**Abscission, Ripening, and Senescence**

Although the previous section had GA as its hormone champion, ethylene is the major player, although not the only one, in the present section. Ethylene has been used as a citrus and banana degreening agent since the 1920s but its general function as a plant hormone was discovered much later. Abscission of plant organs is a natural process, regulated by a complex interaction between
auxin that delays abscission and ethylene that promotes it (Taiz and Zeiger, 1998). In citrus, we need means to regulate abscission in both opposite directions; the natural shedding of young fruitlets must sometimes be amplified (=fruit thinning) but excessive fruit drop may cause up to total loss of the crop. With mature fruit too, there is a need to prevent preharvest drop, for which 2,4-D is commonly used (El-Otmani et al., 2000) but, on the other hand, we want to “loosen” the fruit to facilitate mechanical harvesting. Following the early work of Addicott et al. (1949) who developed the citrus explant system, Goren and coworkers conducted thorough physiological studies of citrus leaf and fruitlet abscission, elucidating the anatomical, hormonal, and enzymatic aspects of the process (Goren, 1993). A unique event is the abscission of the style, which occurs in all citrus fruits shortly after fruit set, except for certain cultivars of citron (Citrus medica L.).

The synthetic auxin, Picloram (4-amino-3,5,6-trichloro-picolinic acid), prevents style abscission (Goldschmidt and Leshem, 1971), a treatment used in Israel to ensure the persistence of the style in citrus fruits used for the feast of Tabernacles (Nicolosi et al., 2005). A tremendous amount of work was invested in the calibration and fine-tuning of fruit thinning treatments for each cultivar and growth area. Various synthetic auxins that bring about evolution of ethylene are most commonly used (El-Otmani et al., 2000). Synthetic auxins are also used to increase fruit size in C. reticulata hybrid cultivars. Such treatments must be applied within a narrow time window, when the fruit thinning capacity has already faded away but the fruit is still growth-responsive to exogenous synthetic auxins. Endless efforts have been invested, mainly in Florida, in screening and adoption of fruit-loosening chemicals. Recent research by Burns and coworkers has focused on CMNP (5-chloro-3-methyl-4-nitro-1H-pyrazole) as a promising fruit-loosening agent (Alferez et al., 2007). High concentrations of 2,4-D are applied in the packing house, often together with GA, to avoid loss of the calyx (which is also an abscission process), maintain rind firmness, and reduce postharvest decay (El-Otmani et al., 2000).

Citrus fruits have a unique pattern of ripening, often called “maturation” (Spiegel-Roy and Goldschmidt, 1996). According to classic definitions citrus, fruits are “non-climacteric,” revealing a slow decline in respiration and a gradual increase in sugar/acid ratio, not promoted by ethylene. On the other hand, the degreening response to exogenous ethylene suggests that ethylene is somehow involved in the maturation process. Evidence accumulated during the last decades (Aharoni, 1968; Goldschmidt et al., 1993; Katz et al., 2004) enforces the view that the extremely low amounts of endogenous ethylene evolved by citrus fruits have nonetheless a significant physiological role in fruit maturation. This conclusion is in line with a recent review that questions the classic distinction between climacteric and non-climacteric fruits (Paul et al., 2012).

During citrus fruit maturation, rind and pulp behave in most respects as separate entities (Iglesias et al., 2007; Monselise, 1977). The flavedo, which was up to now a green photosynthetic tissue, undergoes senescence; the chlorophyll is degraded and the chloroplasts are transformed into carotenoid-rich chromoplasts. This is a partially reversible environmentally, nutritionally, and hormonally controlled process (Goldschmidt, 1988), which involves ethylene-induced upregulation of chlorophyllase (Azoulay-Shemer et al., 2008) and carotenoid build-up as well as other molecular events. These changes are reversed by the senescence antagonists GA and cytokinins; such an antagonism was also demonstrated with other fruits (Goldschmidt et al., 1977).

The degreening-regreening events of citrus fruit peel and the regulatory systems involved are illustrated in Figure 4. Maturation of the pulp, on the other hand, consists primarily of the developmentally regulated decline in acid and accumulation of sugars, processes that are barely affected by ethylene or other PGRs and are still insufficiently understood (Katz et al., 2011). The profound difference between peel and pulp can best be demonstrated by the differential regulation of the same biochemical process: carotenoid biosynthesis. Ethylene has a dramatic effect on peel carotenoid biosynthesis (Rodrigo and Zacarias, 2007) but its effect on pulp carotenoids is negligible (Matsumoto et al., 2009). Also, peel and pulp carotene genesis respond differently to temperature regimes (Tao et al., 2012).

Both acceleration of peel coloration by ethylene and delay of peel senescence by GA...
are used extensively in citrus culture, pre- and post-harvest (El-Ottmani et al., 2000). GA also reduces albedo apoptosis phenomena such as creasing (Embleton et al., 1973; Monselise et al., 1976). Synthetic auxins reduce creasing as well (Greenberg et al., 2006).

The Genetic–molecular Era

Establishing a causal relationship between an endogenous hormonal factor and a presumably related morphological or physiological process proved to be difficult since the early years of the hormonal era (Jacobs, 1959) and became increasingly so with the expanding list of plant hormones and their interactions. Indirect, correlative evidence was the most one could attain in physiological experiments. One type of evidence required according to Jacobs (1959) was called "excision"—"Remove the source of the chemical and demonstrate subsequent absence of formation of the structure... The chemical is sometimes removed by selecting genetic mutants." Indeed, mutants became eventually a major tool in plant hormone studies; gibberellin research, in particular, was based from the outset on mutants, which enabled the deciphering of gibberellins’ biosynthetic pathway and physiological activity (Ross et al., 1997). With the growing consent that all plant developmental events are genetically programmed, there was but a short step from the use of mutants in physiological studies to their implementation in molecular–genetic studies. Furthermore, genetic engineering techniques enabled the design of new types of mutants, which became indispensable for deeper, direct understanding of the underlying developmental and metabolic mechanisms. The main drawback from the point of view of a citrus physiologist was that most if not all of this impressive progress has been achieved with model plants, primarily Arabidopsis.

Although the biological principles were formulated during the 1960s, it took a long time until the genetic–molecular language was adopted by plant scientists and even longer until the molecular approaches were applied to crop plants. Fruit trees, including citrus, were probably the last to enter the molecular era; heterozygosity, scarcity of point mutations, difficulties in transformation, and lack of genetic maps delayed and restricted progress in this field of research. Yet, during the last decade or so, there has been an increasing number of studies aimed at elucidation of the distinct molecular basis for citrus developmental phenomena; flowering, fruit set, alternate bearing, fruit maturation, pigment changes, and flavonoid biosynthesis are all currently under molecular scrutiny. Some progress has been achieved using peel color mutants (Alos et al., 2008). Transformed citrus plants revealed reduced juvenility (Pena et al., 2001) and other transgenic plants were used to demonstrate the role of gibberellins in citrus stem elongation, similarly to that previously shown in model plants (Fagoaga et al., 2007). The molecular evolution of citrus carotenoids (Alquezar et al., 2009) and flavonoids (Frydman et al., 2013) has been elucidated in detail; these are just a few examples. The recent deciphering of most if not all of the citrus genome (Talon and Gmitter, 2008) provides the basis for more advanced molecular studies of citrus development in the near future. Extensive review of this research is beyond the scope of the present article. It must be realized, however, that current molecular–genetic research does not negate the earlier physiological work. Rather, the genetic evidence is imposed on top of the hormonal findings, providing them with explicit molecular explanations. As an example, the long known inhibition of citrus flowering by GA is still valid, but now a molecular explanation of this phenomenon can be sought (Albrigo and Chica, 2012; Goldberg-Moeller et al., 2013). Thus, past, present, and future citrus research efforts form a continuum, reflecting the endless human quest for knowledge and comprehension.

Literature Cited

Addicott, F.T., R.S. Lynch, G.A. Livingston, and J.K. Hunter. 1949. A method for the study of foliar abscission in vitro. Plant Physiol. 24: 537–539.
Aharoni, Y. 1968. Disipation of oranges and grapefruit harvested at different stages of development. Plant Physiol. 43:99–102.
Albrigo, L.G. and E. Chica. 2012. Flowering gene regulation in Citrus sinensis during floral bud induction and initiation: Water deficit effects. HortScience 47(suppl):S189–S190 [ASHS Annual Conference abstract].
Alferez, F., G.Y. Zhong, and J.K. Burns. 2007. A citrus abscission agent induces anoxia- and senescence-related gene expression in Arabidopsis. J. Exp. Bot. 58:2451–2462.
Alos, E., M. Roca, D.J. Iglesias, M.I. Mingez-Mosquera, C.M. Damasceno, T.W. Thannhauser, J.K.C. Rose, M. Talon, and M. Cernos. 2008. An evaluation of the basis and consequences of a stay-green mutation in the navel negra citrus mutant using transcriptomic and proteomic profiling and metabolite analysis. Plant Physiol. 147:1300–1315.
Alquezar, B., L. Zacarias, and M.J. Rodrigo. 2009. Molecular and functional characterization of a novel chromoplast-specific lycopene b-cyclase from Citrus and its relation to lycopene accumulation. J. Exp. Bot. 60:1783–1797.
Azoulay-Shemer, T., S. Harpaz-Saad, E. Belausov, N. Lovat, O. Krokhin, V. Spicer, K.G. Standing, E.E. Goldschmidt, and Y. Eyal. 2008. Chlorophyllase dynamics at ethylene-induced leaf abscission in Citrus and its relation to lycopene accumulation. J. Exp. Bot. 59:2451–2462.
Baking, D.A. 2000. Long-distance vascular transport of endogenous hormones in plants and their role in source: Sink regulation. Isr. J. Plant Sci. 48:199–203.
Bevington, K.B. and W.S. Castle. 1985. Annual growth pattern of young citrus trees in relation to shoot growth, soil temperature and soil water content. J. Amer. Soc. Hort. Sci. 110: 840–845.
Coggins, C.W. and H.Z. Hield. 1968. Plant-growth regulators, p. 371–389. In: Reuther, W., L.D. Batchelor, and H.J. Webber (eds.). The citrus industry. Vol. 2. University of California Press, Berkeley, CA.

Cooper, W.C. 1935. Hormones in relation to root formation on stem cuttings. Plant Physiol. 10: 789–794.
deWitt, C.T. 1986. Modeling agricultural production. Chron. Horticult. 26:53–55.
El-Ottmani, M., C.W. Coggins, M. Agusti, and C.J. Lovatt. 2000. Plant growth regulators in citrus culture: World current uses. Crit. Rev. Plant Sci. 19:395–447.
Embleton, T.W., W.W. Jones, and C.W. Coggins, Jr. 1973. Aggregate effects of nutrients and gibberellic acid on 'Valencia' orange crop value. J. Amer. Soc. Hort. Sci. 98:281–285.
Emer, Y. 1988. Effects of girdling on the differentation of inflorescence types and fruit set in 'Shamouti’ orange trees. Isr. J. Bot. 37:173–180.
Fagoaga, C., F.R. Tadeo, D.J. Iglesias, L. Huerta, I. Llisio, A.M. Vidal, M. Talon, L. Navarro, J.L. Garcia-Martinez, and L. Pena. 2007. Engineering of gibberellin levels in citrus by sense and antisense overexpression of a GA 20-oxidase gene modifies plant architecture. J. Exp. Bot. 58:1407–1420.
Frydman, A., R. Liberman, D.V. Huhman, M. Carmeli-Weissberg, M. Sapir-Mir, O. Riph, L.W. Sunner, and Y. Eyal. 2013. The molecular and enzymatic basis of bitter/non-bitter flavor of citrus fruit: Evolution of branch-forming rhamnosyltransferases under domestication. Plant J. 73:166–178.
Garcia-Luis, A., F. Fornes, and J.L. Guardiola. 1995. Leaf carbohydrates and flowering formation in Citrus. J. Amer. Soc. Hort. Sci. 120:222–227.
Garcia-Luis, A., F. Fornes, A. Sanz, and J.L. Guardiola. 1988. The regulation of flowering and fruit set in Citrus: Relationship with carbohydrate levels. Isr. J. Bot. 37:189–201.
Goldberg-Moeller, R., R. Shalom, L. Shilizerman, S. Samuels, N. Zur, R. Ophir, E. Blumwald, and A. Sadka. 2013. Effects of gibberellin treatment during flowering induction period on global gene expression and the transcription of flowering control genes in Citrus buds. Plant Sci. 198:46–57.
Goldschmidt, E.E. 1988. Regulatory aspects of chloro-chromoplast interconversions in senescing Citrus fruit peel. Isr. J. Bot. 37:123–130.
Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. HortScience 34:1020–1024.
Goldschmidt, E.E., Y. Aharoni, S.K. Eilati, J. Rov, and S.P. Menselise. 1977. Differential counter-action of ethylene effects by gibberellin A3 and N6-benzyladenine in senescing citrus peel. Plant Physiol. 59:193–195.
Goldschmidt, E.E., N. Aschkenazi, Y. Herzano, A.A. Schaffer, and S.P. Menselise. 1985. A role for carbohydrate levels in the control of flowering in citrus. Sci. Hort. 26:159–166.
Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate bearing citrus trees: The significance of reserves for flowering and fruiting. J. Amer. Soc. Hort. Sci. 107:206–208.
Goldschmidt, E.E., M. Huberman, and R. Goren. 1993. Probing the role of endogenous ethylene in the degreening of citrus fruit with ethylene antagonists. Plant Growth Regulat. 12:35–39.
Goldschmidt, E.E. and B. LeShem. 1971. Style abscission in the Citron (Citrus medica L.) and other citrus species. Morphology, physiology and chemical control with Picloram. Amer. J. Bot. 58:14–23.
Goldschmidt, E.E. and S.P. Monselise. 1972. Hormonal control of flowering in citrus trees and other woody perennials, p. 758–766. In: Carr, D.J. (ed.). Plant growth substances 1970. Springer Verlag, Berlin, Germany.

Goldschmidt, E.E. and S.P. Monselise. 1977. Physiological assumptions towards the development of a citrus fruiting model. Proc. Intl. Soc. Citriculture 2:668–672.

Goren, R. 1993. Anatomical, physiological and hormonal aspects of abscission in citrus. Hort. Rev. 15:145–182.

Goren, R., M. Huberman, and E.E. Goldschmidt. 2004. Girdling: Physiological and horticultural aspects. Hort. Rev. 30:1–36.

Greenberg, J., E.E. Goldschmidt, and R. Goren. 1993. Potential and limitations of the use of paclobutrazol in citrus orchards in Israel. Acta Hort. 329:58–61.

Greenberg, J., I. Kaplan, M. Fainzack, Y. Egozi, and B. Giladi. 2006. Effects of auxins sprays on yield, fruit size, fruit splitting and the incidence of creasing of ‘Nova’ mandarin. Acta Hort. 727:249–254.

Harty, A.R. and J. van Staden. 1988. The use of growth retardants in citriculture. Isr. J. Bot. 37:155–164.

Iglesias, D.J., M. Cercos, J.M. Colmenero-Flores, M.A. Naranjo, G. Rios, E. Carrera, O. Ruiz-Rivero, I. Lliso, R. Morrilon, F.R. Tadeo, and M. Talon. 2007. Physiology of citrus fruiting. Braz. J. Plant Physiol. 19:333–362.

Jacobs, W.P. 1959. What substance normally controls a given biological process? I. Formulation of some rules. Dev. Biol. 1:527–533.

Katz, E., K.H. Boo, H.Y. Kim, R.A. Eigenheer, B.S. Phinney, V. Shulaev, F. Negre-Zakharov, A. Sadka, and E. Blumwald. 2011. Label-free shotgun proteomics and metabolite analysis reveal a significant metabolic shift during citrus fruit development. J. Exp. Bot. 62:5367–5384.

Katz, E., P.M. Lagunes, J. Riov, D. Weiss, and E.E. Goldschmidt. 2004. Molecular and physiological evidence suggests the existence of a system II-like pathway of ethylene production in non-climacteric Citrus fruit. Plants 219:243–252.

Koch, K.E. 1996. Carbohydrate modulated gene expression in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:509–540.

Kosola, K.R. and D.M. Eissenstat. 1994. The fate of surface roots of citrus seedlings in dry soil. J. Expt. Bot. 45:1639–1645.

Lenz, F. 1966. Flower and fruit development in ‘Valencia Late’ oranges, as affected by type of surface roots of citrus seedlings in dry soil. J. Expt. Bot. 17:461–467.

Li, C.Y., D. Weiss, and E.E. Goldschmidt. 2003a. Effects of carbohydrate starvation on gene expression in citrus root. Plants 217:11–20.