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Why are Small Hass Fruit Small?

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ABSTRACT

This paper proposes a paradigm shift for studies aimed at resolving the Hass avocado small fruit problem. Research to date, has not provided a convincing solution to the problem although several strategies have been proposed. The major reason being limited information on the physiology of avocado fruit growth and without this information, it is unlikely that orchard management strategies will successfully manipulate the crop to permanently reduce the incidence of small fruit. Physiological investigation has revealed a potential relationship between fruit size, cell division, in situ *cytokinin synthesis, activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and carbohydrate status and a working hypothesis is described.*

INTRODUCTION

The Hass small fruit problem has been the subject of intense investigation by South African growers and researchers for more than a decade. A survey of available literature reveals the following information: firstly, between 5 and 20% of the total crop in any season, can be considered 'small fruit' (i.e. fruit unsuitable for profitable export marketing). Furthermore, the proportion of small fruit may reach 40% in crops borne on older, stressed trees particularly when cultivated in warm, dry conditions. Secondly, the major cause of small Hass fruit is accepted as being genetic although the incidence of small fruit is exacerbated by climate and edaphic factors, and cultural practice. Attempts to resolve the problem have thus far included crosspollination, utilization of plant growth regulators, summer girdling, pruning, late hanging of fruit and mulching of the root zone (Wolstenholme & Whiley, 1995). Despite these efforts, a solution to the Hass small fruit problem has not been forthcoming.

In tomato, the current model system for studying fruit growth, final fruit size is determined by a number of internal and external factors. Thus, the number of cell divisions is recognised as the most significant internal factor contributing to tomato fruit size (Bohner & Bangerth, 1988; Ho, 1992; Ho & Hewitt, 1986). Since the period of cell division in the ovary is complete within two weeks of anthesis, potential fruit size in this species is determined by cell division activity in the pre-anthesis ovary and once fixed, actual fruit size is a consequence of cell enlargement (Ho, 1996). In avocado, fruit size is also a consequence of cell number and size. However, unlike tomato, cell division is not restricted to anthesis but continues throughout fruit ontogeny albeit at a slower rate during the latter stages of development. Analysis of the growth kinetics of small and

normal Hass fruit has revealed that fruit set of normal (large) fruit occurs 2-3 days earlier than that of small fruit (Zilkah & Klein, 1987). These authors concluded that conditions within the tree were unlikely to change drastically within such a short interval and suggested that the two distinct populations of fruit arose as a result of prevailing climatic conditions at the time of either pollination, anthesis or fruit set.

Seemingly, but not necessarily, conflicting evidence has recently been obtained in our studies. A detailed analysis of changes in cell size and number throughout the course of Hass fruit development showed that the small fruit phenotype first appeared 55-60 days after fruitset coincident with maximum humidity, temperature and irradiance in the orchard. Thus, prevailing climatic conditions could exert a profound effect on final Hass fruit size by impacting on the cell division cycle activity at that particular stage of fruit development (i.e. 55 60 days after fruitset).

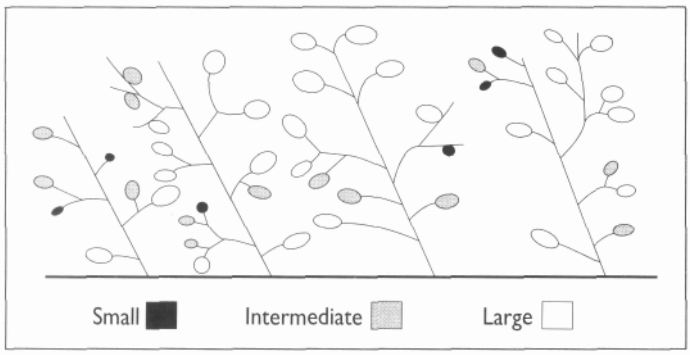
In an attempt to derive a solution to the small fruit problem we have established a research programme aimed at elucidating the metabolic control of Hass avocado fruit growth and development. The basis of our rationale is that without an understanding of the problem, it is almost impossible to derive a solution.

One of the most striking anomalies in plant physiological research is the disparity between students of field and tree crops in their approach to the study of plant growth and development. Agronomists have made extensive use of seedlings to study physiological aspects of vegetative growth in order to improve plant vigour, photosynthetic activity, photosynthate partitioning and yield. By comparison, horticulturalists have concentrated their efforts on ripening and senescence and post-harvest physiology. This dichotomy is largely a consequence of the type of plant product being generated and the economics involved. While it is clear that there exists a wealth of knowledge on the physiology of maize, wheat, barley and rice cultivation, the same cannot be said of crops such as avocado. Obvious reasons for this include the phenology of the species and the general inaccessibility of this and similar crops, to the broader plant science community. However, the current situation is changing rapidly. The advent of plant molecular biology means that processes occurring in previously inaccessible species can now, through the technique of transgenic expression, be studied in the comfort of one's own laboratory, far removed from the specific region of cultivation. Undoubtedly this will remain the technology of choice as we enter the next century and millenium. If we are lax in harnessing this technology, we are bound to lose whatever competitive edge is currently ours. Thus, there is an urgent need for a paradigm shift with regard to plant science research in South Africa. In the light of these comments, I pose the question: Why are small Hass fruit small?

COMMENT ON SOME RECENT OBSERVATIONS OF PHENOTYPICALLY SMALL FRUIT

The small fruit syndrome or phenotype, is charactersided by early seed coat senescence which is, in many instances, associated with 'pedicel ring neck'. Although there is a range of fruit size on any tree, casual observation reveals no pattern with respect to the distribution of small fruit (figure 1). It is therefore difficult to accept that overall tree stress is the most (only) significant contributing factor. Growth in

phenotypically small fruit is limited by cell number and not cell size. Furthermore, the small fruit phenotype can be chemically induced by abscisic acid (ABA) and mevastatin, an inhibitor of isoprenoid biosynthesis (Cowan *et al,* 1997). In both cases these inhibitors were injected via the pedicel into individual fruit in the presence of an active the effect of both exogenous ABA and mevastatin, it might have been expected that root-derived cytokinin would act similarity. However, this did not appear to be the case eliminating a role for root-derived cytokinin in the control of fruit growth. Also, phenotypically small fruit, like ABA and mevastatin-treated fruit showed a substantial increase in endogenous ABA and a reduction in activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), the enzyme affected by mevastatin. In attempting to crystallize this information we concluded that an interaction between ABA, cytokinins and HMGR was responsible, at least in part, for induction of phenotypically small fruit.



transpiration stream. Since co-treatment with cytokinin negated

Figure 1

Pattern of distribution to small, intermediate and large fruit on fruit bearing branches of Hass avocado trees at Everdon Estate, KwaZulu-Natal during the 1995/96 season.

HMGR catalyses the formation of mevalonic acid (MVA), the precursor to all isoprenoid compounds including ABA and cytokinin, from hydroxy-methylglutaryl coenzyme A. Together with MVA and cytokinin, this enzyme plays a major role in regulating cell division. Modulation of enzyme activity is achieved through (de)phosphorylation of HMGR, a process most likely involving a protein kinase referred to as HMGR kinase. Recently, evidence has been obtained to suggest that the protein is a member of the *sucrose nonfermenting-1 (SNF-1) protein kinase* family (Barker *et al.,* 1996). This suggests that carbohydrate status could play a major role in regulating the activity of HMGR and hence cell division cycle activity and final fruit size in Hass avocado. In fact we have demonstrated that intercellular sucrose movement in seed coat and mesocarp tissue is prevented in phenotypically small fruit and in fruit pretreated with ABA by 'gating' or 'plugging' of the plasmodesmata, the ports of symplastic solute flow (Cowan *et al,* 1997). Interestingly, cytokinin reverses this ABA-induced response. Therefore, interaction between HMGR activity, isoprenoid status (specifically the cytokinin/ABA ratio) and carbohydrate flux seems likely in avocado and may represent part of a metabolic sequence which, if adversely affected, results in expression of the small fruit

phenotype.

TOWARDS AN INTEGRATED MODEL OF METABOLIC CONTROL OF AVOCADO FRUIT GROWTH

Based on our accumulated data, we propose the following model to explain the emergence of phenotypically small Hass fruit in response to environmental perturbation (figure 2). Clearly cell division is a major factor in the determination of final fruit size. Both the plant stress hormone, ABA, and water stress retard the cell division cycle activity (Meyers *et al,* 1994; Artlip *et al,* 1995), whereas cytokinins promote this event (Jacobs, 1995) and do so by regulating the G_2 to M transition, i.e. the transition of cells from the stage following DMA replication to mitosis (M). Similarly, withdrawal of cytokinin causes cessation of the cell cycle and cells accumulate in M, S (period of DMA replication) and Gj (Mader & Hanke, 1996). An imbalance in cytokinin/ABA ratio, through reduced cytokinin synthesis or increased ABA, will therefore impact on cell division activity and final fruit size. Assuming cytokinin is derived *in situ* by isoprenylation of purine (Binns, 1994), inhibition of isopentenyl pyrophosphate (IPP) synthesis will limit the amount of dimethylallyl pyrophosphate (DMAPP) available for cytokinin synthesis.

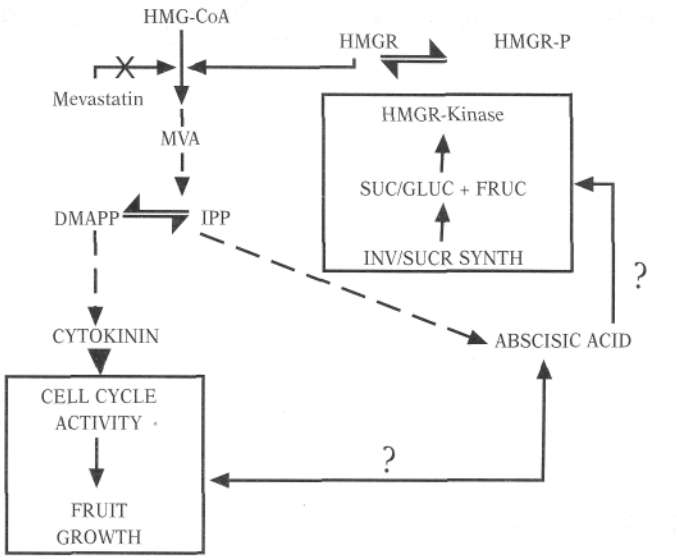


Figure 2

Hypothetical scheme depicting the interrelationship between cytokinin, abscisic acid, HMGR activity and carbohydrate status in the metabolic control of Hass avocado fruit growth., Abbreviations: DMAPP, dimethylallyl pyrohosphate; FRUC, fructose; GLUC, glucose; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; INV, invertase; IPP, isopentenyl pyrophosphate; MVA, mevalonic acid; SUCR, sucrose; SUCR SYNTH, sucrose synthase

IPP is formed from MVA, the product of the reaction catalysed by HMGR. This enzyme is regulated by phosporylation and is a member of the SNF-1 gene family (Barker *et al,* 1996), suggesting that it is regulated by carbohydrate status.

Carbohydrate-modulated enzymes are controlled by a hexose sensor comprising phosphorylated glucose and fructose and a putative plasma membrane signal (Koch, 1997). The concentration of each component is determined by the amount of carbohydrate hydrolysed on import to sink cells. Metabolism and utilization of hexose is a function of invertase and sucrose synthase. So expression and activity of these enzymes is crucial in the control of fruit size. In fact, it has recently been demonstrated that sucrose accumulation, in the absence of active acid invertase, correlates with small fruit in tomato (Klann *et al,* 1996).

CONCLUSIONS AND FUTURE PROSPECTS

This paper calls for a paradigm shift in studies aimed at solving the Hass avocado small fruit problem. To this end I have asked the question: Why are small Hass fruit small? rather than attempting to find a short term solution to the problem by asking, as so many others have done: How to solve the small fruit problem? I contend that without knowledge of what contributes to the regulation of fruit growth and development, we are unlikely to ever find a solution to the small fruit-phenotype in Hass. It is obvious that there is limited information on the physiology of fruit growth, particularly that of avocado. However, the information presented in this paper suggests that with the correct scientific approach a solution, based on a sound understanding of the physiology involved, can be found.

To date, we have concentrated our efforts on metabolic events occurring within fruit, more specifically, the effect of growth regulators on HMGR activity. Clearly these studies need to be expanded to include a detailed investigation of the contribution of imported carbohydrate to the metabolic control of growth. Several projects have recently been initiated with this objective in mind. In one project, attempts are being made to cement the relationship between carbohydrate composition and activity of HMGR in the control of avocado fruit growth. Another project aims to elucidate the effect of ABA and cytokinin on carbohydrate transport into the developing structure. Yet another project is examining the competence of fruit tissue to synthesize ABA in order to determine the source of carbon from which it is preferentially produced.

The tissue of origin of ABA in avocado trees is likely to be the leaves, particularly during times of water deficit and coincident high irradiation. Such factors, and an associated increase in ABA concentration, may impact on the mobilization of sucrose from the affected leaves causing a change in the composition of translocatable carbohydrate at or in sink tissue. These changes might be expected to impact on the expression and/or activity of invertase and sucrose synthase to alter the properties of the hexose sensor. Thus, down regulation of HMGR, cytokinin synthesis, cell division cycle activity, and the appearance of the small fruit phenotype occurs concomitantly with increased ABA concentration.

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