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CUKE DEVELOPMENT IN CONSISTENTLY LOW PRODUCING TREES OF THE 'FUERTE' AVOCADO WITH SPECIAL REFERENCE TO SEED ABORTION

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INTRODUCTION

The mechanism of fruit development is normally set in motion by two consecutive stimuli. Auxins and gibberellins produced during pollen tube growth act as the primary stimulus that initiates fruit development (Roth, 1977; Lee, 1987), but a second stimulus is required to maintain fruit growth. This secondary stimulus emanates from the developing seed and especially the endosperm (Luckwill, 1959; Lee, 1987) that starts growing rapidly and immediately after fertilization, producing high levels of auxins. Other phytohormones like gibberellins and cytokinins may also be involved (Lee, 1987). How seeds influence fruit development is not clearly understood, but literature suggests that whether a fruit aborts or matures depends on its ability to chemically inhibit neighbouring fruits, or on its sink strength (Lee, 1987). High metabolic activity of the developing embryo and endosperm and the consequent production of phytohormones in these tissues create strong sinks for photosynthate and mineral nutrients. On the other hand, fruits without seeds or with abortive seeds, constitute weak sinks that ultimately fail to develop, unless the plant has the ability to synthesize phytohormones like gibberellins (Coombe, 1960; Hayashi *et al.,* 1968; Ivahori *et al.,* 1968; Khalifa, 1966; Lodhi *et al.,* 1969) or auxins in the ovary wall itself (Roth, 1977). The avocado (*Persea Americana* Mill.) may represent one such plant, as small amounts of various phytohormone-like substances have been found in the mesocarp of the 'Fuerte' avocado and 'seedless' fruits frequently occur on 'Fuerte' and 'Ettinger' trees.

These underdeveloped fruits, commonly referred to as 'cukes' because of their cucumber-shaped appearance, are usually not entirely seedless, but contain an embryo and endosperm during the earliest stages of fruit development. Reports on cuke structure (Blumenfeld & Gazit, 1974; Tomer *et al.,* 1980) describe the arrest of ovule growth, the degeneration and ultimate destruction of the embryo sac and chalazal part of the nucellus and integuments and the proliferation of the micropylar part of the outer integument, so that this part of the seed coat sometimes covers the degenerated tissues. Tomer *et al.,* (1980) suggested that the micropylar part of the outer integument keeps growing, because the avocado seed coat contains a high level of phytohormones that may exert a strong sink effect for photosynthates. This suggestion does not explain why the chalazal part of the same structure then aborts, together with the developing embryo and endosperm, resulting in the formation of a cuke instead of a normal fruit. No attempt was made in the above-mentioned studies to relate seed structure to ovule structure. During the course of the present investigation it became clear that basic characters of the avocado ovule and seed, such as the pachychalaza, hypostase and multicellar archesporial tissue have so far not been taken into account in literature dealing with avocado fruit development. As previous investigations were executed on F.A.A.-preserved, paraffin-imbedded material, sectioned at 15µ, we reasoned that better fixation and sectioning procedures and a more detailed study of seed abortion may provide the necessary information for determining the causal effects of cuke formation in the avocado.

MATERIAL AND METHODS

Developing cukes, varying between 0.75 cm and 3 cm in length, were collected at Westfalia in the Duiwelskloof district during the first week of October 1991 from five trees, that had previously been classified as consistently low producers (hereafter referred to as E-type trees). The trees flower profusely, have earlier first dates for flowering as well as a longer flowering period than consistently high producers of normal fruits (hereafter referred to as A-type trees) and shed most developing fruits at an early stage. The few fruits that are retained, develop into cukes. When normal fruits do occur, they are not dispersed among the cukes, but occur on separate branches.

Cukes were fixed in Carnoy for 24 hours and preserved in 70% ethanol. Flowers collected earlier during the season from the same trees had been treated accordingly. One hundred fruits as well as one hundred open flowers from the abovementioned five trees were dissected to determine the presence of seeds/ovules. For detailed embryological studies, additional flowers and early post-fertilization stages of the flowers were collected two weeks later from the same trees, fixed and stored in a phosphate-buffered solution (pH 7.4) of 5 percent formaldehyde that contained 0.5 percent caffeine to improve the fixation of phenolic-containing cells (Mueller & Greenwood, 1977). Carnoy and formaldehyde-fixed flowers and fruits were classified according to age/size in seven stages and processed for ultimate serial sectioning in glycol methacrylate (GMA) at 3-5µ, using conventional procedures. At least 15 series were obtained from each stage. Perfectly median (Fig. 1) as well as transmedian longitudinal sections of all stages were made from GMA-embedded material. Sections were stained with PAS/toluidine blue, but some received special staining to test for lipids, proteins, starch, lignin and cutin.

The vasculature of ovules was determined by clearing the phenolic-containing tissues of 75 ovules from three E-type trees in a 50% v/v aqueous solution of household bleach (Javel) for 30 to 60 minutes, rinsing briefly in water and staining in a saturated solution of phloroglucinol in a 20% (v/v) aqueous solution of HCl.

RESULTS AND DISCUSSION

Macromorphology of the ovule

Of the flowers 96% had pistils of apparently normal size and structure. In 4% of the flowers the solitary ovule was borne externally on the ovary. Each of the normal pistils contained a single, pendulous, anatropous, bitegmic and crassinucellate ovule. In the extreme apical part of the locule, the ovule that fits tightly into the locule, is attached to the ventral, abaxial surface of the carpel by a short, overarching funicle (Figs. 1 & 2) so that the raphe is dorsal. The outer integument is multi-layered, not discernable as a separate entity on the raphal side and slightly shorterthanthefour-layered inner integument. The latter integument forms the micropylar canal and becomes broader in this region (Fig. 3). A meristematic zone in the chalaza, i.e. the tissues below the place of attachment of both integuments

(Figs. 1,2,4,5 & 6), is responsible for the formation of a pachychalazal seed where the seed coat consists of a pachychalazal, highly vascularised basal part covering most of the seed and a small, integumentary part around the micropyle.

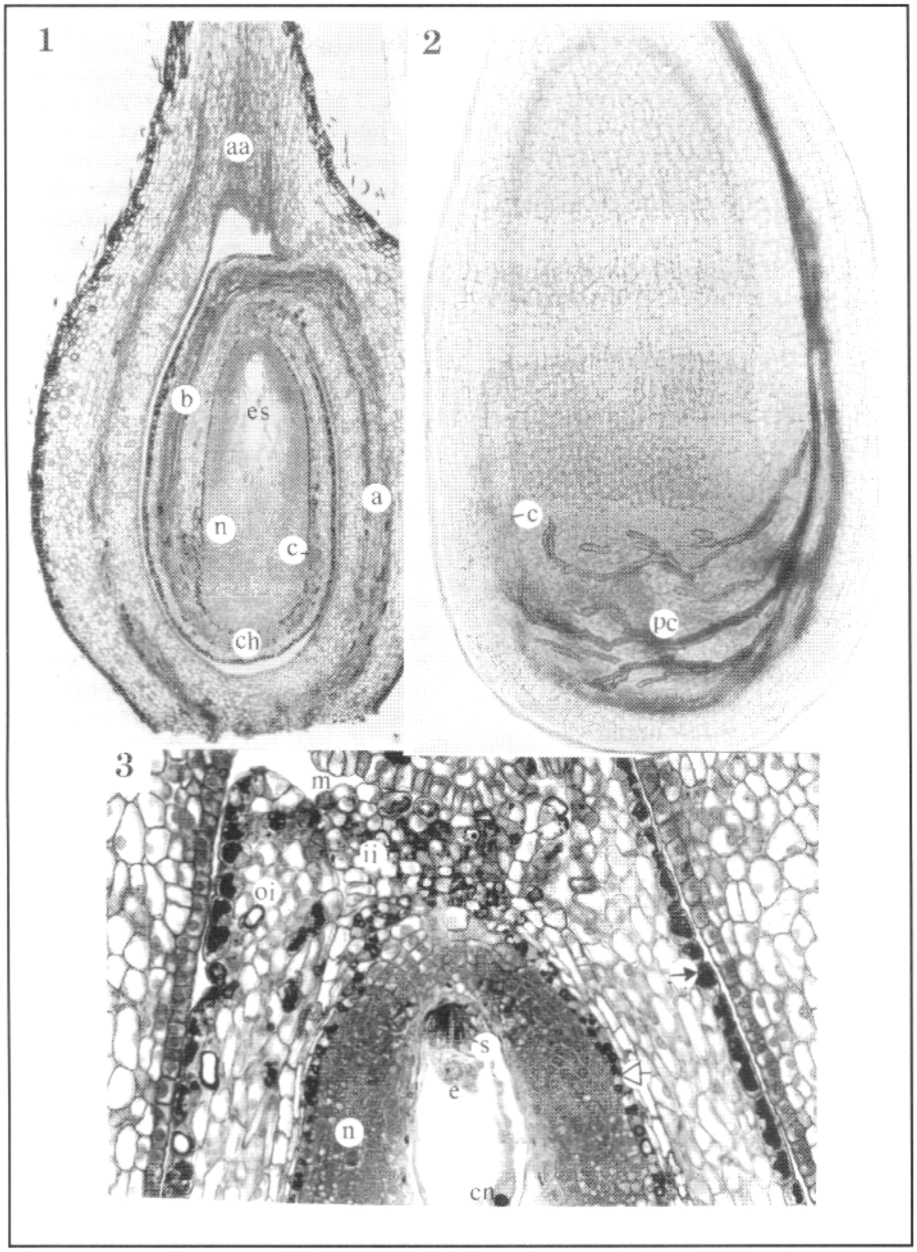

FIG. 1 - 3 Orientation, structure and vasculature of ovule. **1.** Median longitudinal section of ovary of female stage flower. **2.** Whole-mount of cleared ovule, stained with phloroglucinol to illustrate the vasculature of an ovule in a flower bud. **3.** Longitudinal section of micropylar ovular region, illustrating the structure of the integuments and embryo sac. a, ventral carpel trace; aa, base of style; b, dorsal raphe; c, base of integuments; ch, chalaza; cn, nucleus of central cell; e, egg cell; es, embryo sac; ii, inner integument; m, micropyle; n, nucellus; oi, outer integument; pc, vascular strands in pachychalaza.

Cuke formation

Cukes develop from female sterile, cryptically male flowers on consistently low yielding 'Fuerte' trees. A hypostase that has, as yet, not been reported for the avocado, is present in the chalazal tissue of the mature ovule and aborting seed (Figs. 5 & 6). This layer seems to play a rolé in the degeneration of the peripheral nucellar tissue and the non-development of a intercalary meristem (Fig. 4) responsible for the extension of the pachychalaza. The ultimate cause of cuke formation, however, seemingly lies in the disturbance of the polarity of the primordial nucellar tissue. Additional embryo sacs and non-functional megaspores or megaspore mother cells (Fig. 5) that develop in the nucellus, effect the collapse of the chalazal region of the dominant embryo sac. Degeneration of these embryo sacs and megaspores causes the formation of nucellar cavities (Fig. 7) that isolate the embryo sac from the chalazal flow of nutrients. If fertilization has already taken place, a globular embryo and a limited amount of endosperm tissue are formed (Fig. 6). Because the endosperm is starved of nutrients, the formation of this tissue is curtailed at an early stage and embryo development ceases. A meristematic zone that initiates from the inner layers of the outer integument, directly opposite the place where the vascular supply to the chalaza terminates (Figs. 5,6 & 8), causes abnormal growth in the outer integument. It is suggested that, due to the absence of meristematic activity in the chalazal region of the embryo sac and the non-developing pachychalaza, resources are redistributed towards the stronger sink, i.e. the outer integument. Consequently, this part of the seed coat proliferates, while the embryo sac and pachychalaza degenerate. In spite of the abortion of the seed, the pericarp of the cuke continues to develop, possibly because sufficient phytohormones are produced by the cell activity in the outer integument to support minimal pericarp development. A crude model of the structure of the ovary and ovule as well as the possible processes involved in fruit development and cuke formation, is presented in Fig. 9.

CONCLUSIONS

Cukes develop from flowers that are unable to produce viable seeds, *i.e.* are female sterile, because endosperm development is curtailed at an early stage and the embryo stops growing. Female sterility has previously been reported for the avocado (Tomer & Gottreich, 1978; Tomer, Gottreich & Gazit, 1976; Sedgley & Griffen, 1980). Due to the limited number of ovules sectioned for this study, aid, as well as to the University of Pretoria we cannot claim that all flowers on E-type trees are female sterile, but our results have shown that cuke formation is an indication of the presence of female sterile flowers on avocado trees.

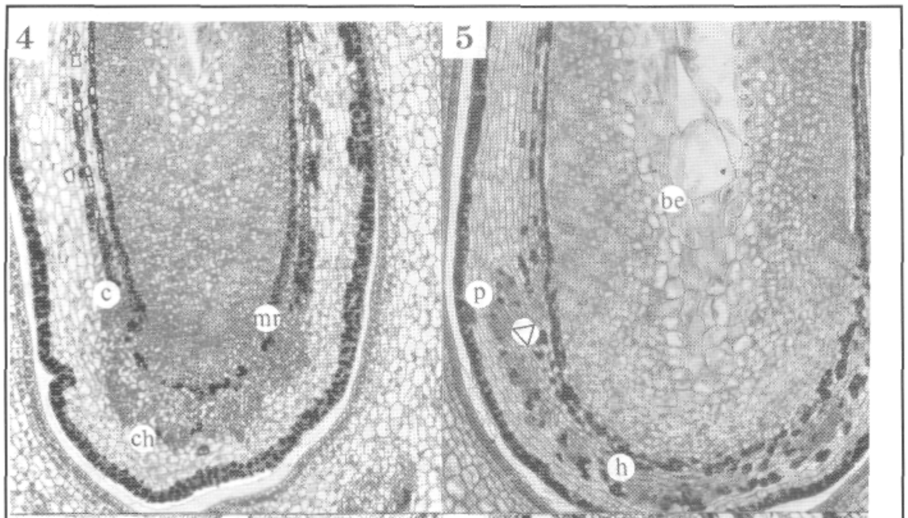

FIG. 4 & 5 Chalazal regions of **4**, female stage flower and **5**, male stage flower. be, additional megaspore mother cell at base of embryo sac; c, base of integuments; ch, chalaza; h, hypostase; mr, intercalary meristem *responsible for development of pachychalaza; p, periclinal divisions in* basal part of outer integument.

FIG. 6 & 7 Aborting seed in 0.6-cm-long fruitlet **(6)** which had developed from an abortive ovule **(7)**. ca, cavities around aborted embryo sac; es, aborted endosperm; g, embryo aborted in the globular stage; h, hypostase, consisting of cup-shaped zone of darkly stained cells; p, advanced stage of periclinal cell divisions in basal part of outer integument.

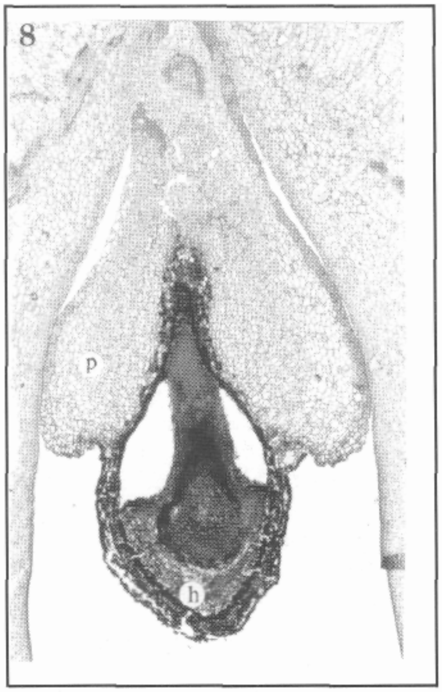

FIG. 8 Longitudinal section of cuke, measuring 1 cm in length. h, *hypostase*, *forming part of* dead and shrunken pachychalazal section of the seed coat; p, proliferated part of integumentary section of seed coat.

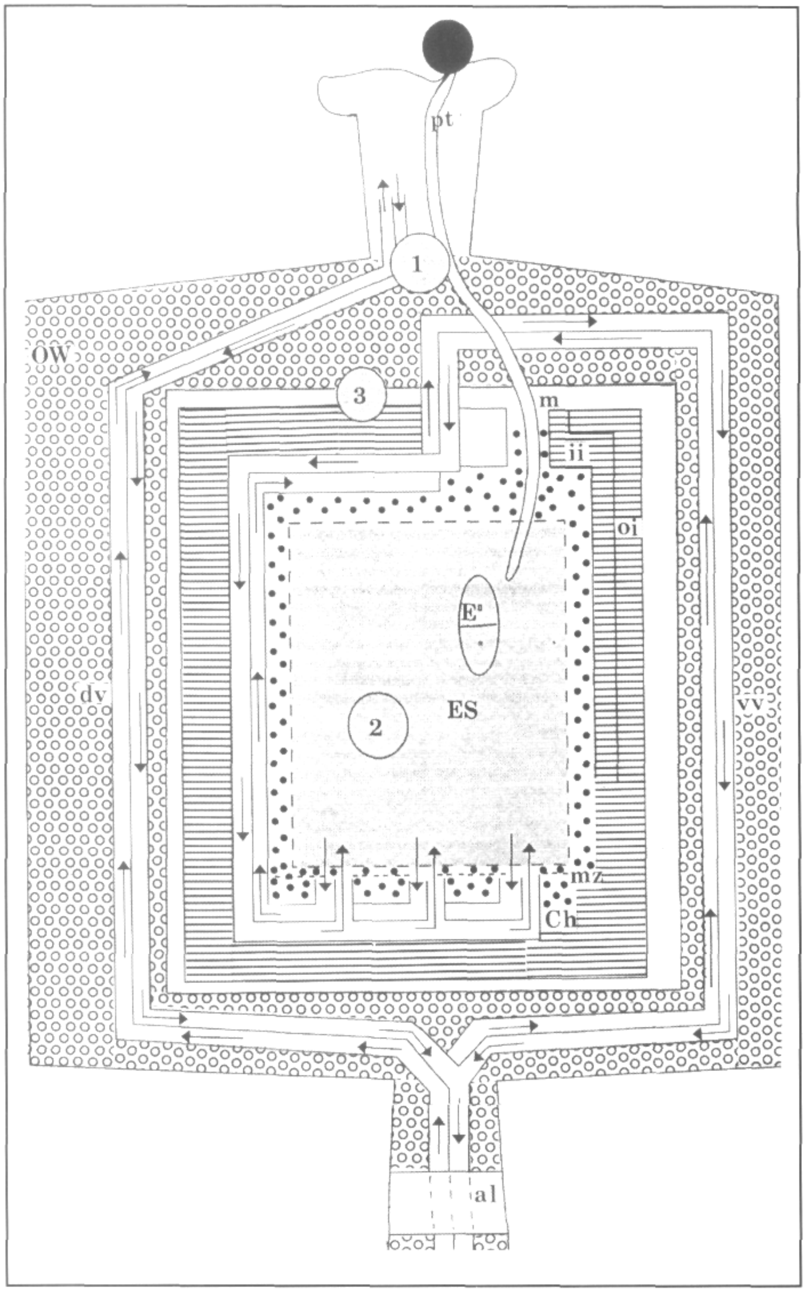

FIG. 9 Model of a longitudinal section of an ovary, based on Figures 1, 2 and 3.

1 - 3, centres of phytohormone production ('pumps'), switched on in this order, firstly by pollen tube growth (1), embryo and endosperm development (2) and seed coat development (3).

a-arrows, direction of flow of photosynthates and minerals to the sinks, created by cell activity in the ovary wall and ovule.

b-arrows, direction of flow of phytohormones from the 'pumps'. Phytohormones stimulate fruit development and prevent abscission (fruit drop).

al, abscission layer; ch, chalaza (end of vascular tissue) where nutrients are deposited into the nucellus and transported to the embryo sac; dv, dorsal vein of vascular tissue; e, embryo; es, embryo sac containing endosperm; ii, inner integument; oi, outer integument (continues to develop in cuke while chalazal part dies); ov, ovary wall (forming the skin and flesh of the fruit); pt, pollen tube; vv, ventral vein.

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