OVULES OF 'FUERTE' FLOWERS WITH REDUCED MALE FUNCTION

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ABSTRACT

The microscopic structure of ovules of 'Fuerte' flowers with anthers that appeared normal, but had a substandard pollen yield and seemingly defective pollen grains, was investigated. The results showed that flowers with functional ovules only occurred on the high-producing tree included in the study and that such ovules comprised twenty percent of the ovules investigated. Many flowers, collected from the high-producing as well as two low producing trees in the same orchard, tended towards sexual neutrality. A possible influence of such neuter flowers on the production of the three trees is discussed

INTRODUCTION

Annual fruit yield records of 'Fuerte' trees at Westfalia showed that the trees can be graded as A, B, C, D and E-producers with A-trees as the highland E-trees as the low-producers in the orchards. Causal factors for this variation in yield did not seem to lie in single cultural or physiological differences among the trees (Smith *et al.* 1993). and it was suggested that differences in the flowers on the trees might play a role in the variation in fruit yield. A study of the flowers on A-trees and E-trees was therefore launched during the 1992-93 flowering season.

The first results of this study have recently been published and showed that 'Fuerte' trees in the orchards indeed differed in pollen production and quality. This resulted from the fact that the trees produced variable numbers of flowers with anthers that looked normal, but their pollen yield was substandard and the grains seemed defective (Steyn 1993). The flowers therefore had reduced male function. It was assumed that these flowers had normal female organs, although information on the ovules of these flowers was not available. These data have since been collected and form the basis of the present report.

MATERIAL AND METHODS

During the 1992-93 season, three trees comprising a high-producer with a yearly yield of 800 1000 kg and two low-producers from the same orchard, bore large numbers of flowers with reduced male function. After the anthers of the flowers had been investigated, the ovaries were stored individually in labelled containers (Steyn 1993). From these containers, ovaries of flowers with reduced male function were selected and processed for serial sectioning in glycol methacrylate according to conventional methods. Ovaries of male-phase flowers collected from the aforementioned high-producer were treated in the same way. Median longitudinal sections of the ovules were

stained in periodic acid-Schiff's reagent (PAS) primarily to reveal the presence of starch in the ovular tissue and counterstained with toluidine blue to indicate the position of phenolic-containing cells.
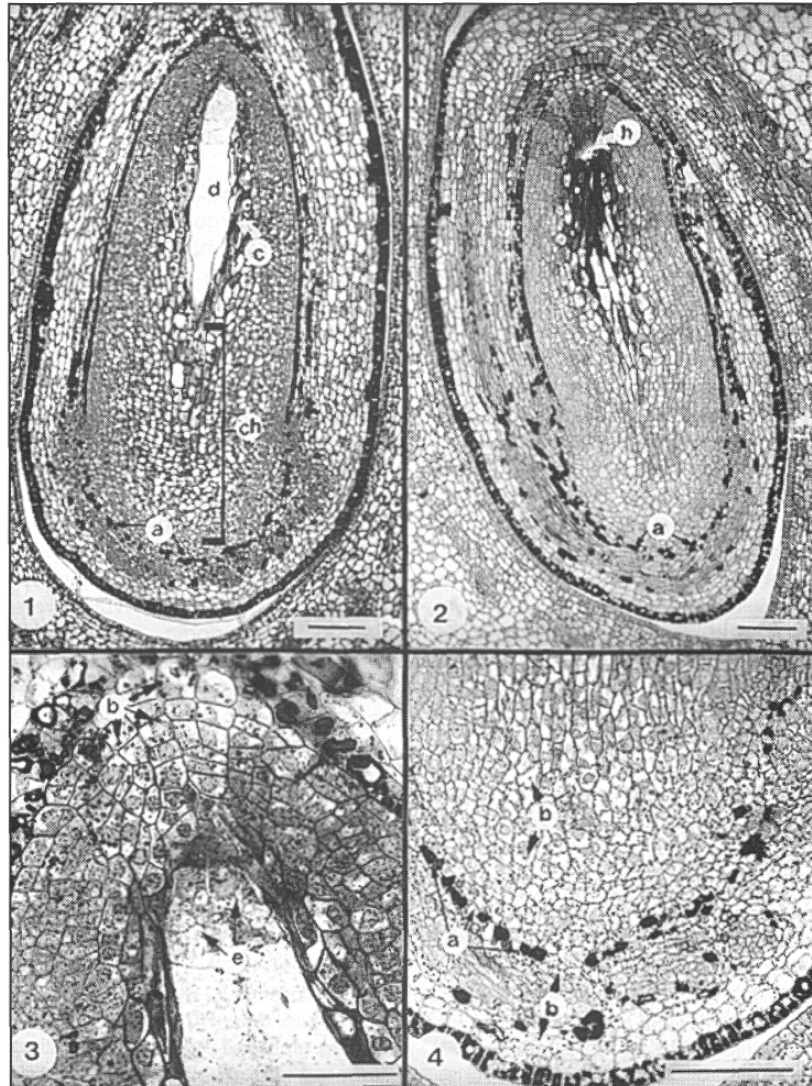

FIG.1
Functional ovule of a high-producing tree. Note a, hypostase; c, thick-walled nucellus cells; ch, orderly arranged chalazal nucellus; d, single embryosac. Scale bar 100µ.

FIG.2
Non-functional ovule of low-producing tree. Note a, hypostase; h, degenerated embryosac. Scale bar 100µ.

FIG.3
Micropylar region of same ovule as Fig. 1 showing b, starch grains; e, egg apparatus. Scale bar 50µ.

FIG.4
Chalazal region of same ovule as Fig. 2 indicating b, starch grains on both sides of a, hypostase. Scale bar 100µ.

RESULTS

Flowers with reduced male function had pistils of normal structure and size (Steyn 1993) with well-developed ovules (Figs. 1 & 2). The solitary, pendulous, anatropous and bitegmic ovules with their short, overarching funicles had large nucelli that bordered onto well-developed chalazal regions. A cup-like hypostase layer with groups of

phenolic-containing cells seperated the vascular bundles from the chalazal parenchymatous tissue (Figs. 1, 2 & 4).

FIG.5
Micropylar region of ovule of high-producing tree. Note f, second embryosac-like structure obstructing the base of d, dominating embryosac surrounded by c, thick-walled nucellus cells. Scale bar 100μ.

FIG.6
Ovule of low-producing tree. Note d, mature embryosac and k, disorganised chalazal nucellus. Scale bar 100μ.

FIG.7
Chalazal region of same ovule as Fig.5 with g, incrustations of callose in c, thick-walled nucellus cells. Scale bar 50μ.

FIG.8
Micropylar region of ovule of low-producing tree. Note h, crushed embryosac and i, second, immature embryosac. Scale bar 100μ.

Ovules of female phase flowers:

PAS-staining revealed an abundance of photosynthates in the ovular tissue. Numerous starch grains occurred in the parenchyma of the integuments, the nucellus tissue and in the chalazal parenchyma. Starch grains were especially abundant in the apical nucellar cells (Fig. 3), in the cells on both sides of the hypostase layer (Fig.4) and in the central

nucellar cells.

The central nucellar tissue was a conspicuous feature of all ovules. It formed a sheath around the embryo sac and consisted of elongate cells with irregularly thickened walls that were highly PAS-positive (Fig 5). The elongate shape of the cells, their wall structure and affinity for PAS suggested that the central nucellar tissue consisted of transfer cells (Gunning & Pate 1969), but this will have to be confirmed by an electron microscopic investigation. Such cells are specialized in relation to short distance transport of solutes as the wall outgrowths increase the surface area of the plasma membrane (Pate & Gunning 1972).

The female phase flowers of the high producing tree contained two types of embryo sacs. In twenty percent of the sectioned ovules, a single embryo sac with an extremely thin wall that bordered onto the thick-walled central nucellus cells was present (Fig. 1). Well-formed synergids and an egg cell occurred underneath the nucellar cap cells (Fig. 3) with the fusion nucleus some distance apart from the egg apparatus. In these ovules the chalazal nucellar cells lay in orderly rows. No callose-like incrustations were found in the embryo sac or the cell walls of the chalazal nucellar cells so that the pathway for nutrients from the chalazal vascular bundles towards the embryo sac base seemed unimpeded.

Eighty percent of the sectioned ovules contained a second embryo sac-like structure inside the central nucellar tissue, firmly attached to one side of the dominant embryo sac (Fig. 5). The second structure did not contain an egg cell, synergids or a fusion nucleus, but seemed to have cytoplasm similar to that of the embryo sac. This can only be ascertained by an electron microscopical study. The origin and significance of the second structure is unknown, but it seemed to obstruct the base of the dominant embryo sac. Additional signs that the chalazal flow of nutrients was obstructed in these ovules and that the latter were not functional, was the presence of callose and crystals in the ovular tissue, e.g. in the nucellus cap cells, inside the fertile embryo sac, against the embryo sac wall and in the cell walls of the central nucellar cells bordering onto the embryo sac base (Fig. 7).

Flowers collected from the two low-producing trees did not seem to contain ovules that had a possibility of developing into normal seeds. Mature embryo sacs with well-formed contents were found, but the chalazal nucellar tissue was disorganised (Fig. 6). Structurally, these ovules corresponded to ovules that occurred in flowers with a tendency to form cukes (Steyn et al. 1993). Approximately half of the ovules did not contain mature embryo sacs. In some ovules the embryo sacs had degenerated extremely early (Fig. 2), in others the original embryo sac was squashed at a late stage and a second one had started to develop in the chalazal, but was immature (Fig. 8) and in some of the ovules the embryo sacs were well-developed, but contained only four nuclei, in stead of an egg apparatus and fusion nucleus.

Ovules of male-phase flowers:

Flowers collected during the morning while the high-producing tree was in the male-phase had ovules with collapsed embryo sacs. The egg cell, synergids and fusion nucleus had degenerated and the cytoplasma had contracted. Numerous starch grains were still present in the ovary wall, integuments, nucellus cap and disorganised embryo sac. Two of the ovules sectioned, had been fertilized and the first cell walls were

forming between the dividing endosperm cells. The embryo sacs were single, thin-walled structures. Numerous starch grains occurred in the cytoplasm of the embryo sac and in the central nucellus cells, bordering onto the narrow, chalazal extension of the embryo sac. The occurrence of starch grains in these cells substantiated the aforementioned theory that the thick-walled cells were transfer cells.

DISCUSSION

This study indicated that the three trees differed in the production of functional ovules and that flowers with reduced male function did not necessarily contain ovules that are were capable of developing into normal seeds as had been assumed in an earlier study (Steyn 1993). In fact, functional ovules were only found in flowers collected from the high-producing tree and comprised twenty percent of the ovules investigated. These results compare well with previous findings that the percentage of normal ovules on avocado trees ranged between two and twenty percent (Tomer *et al.* 1976). The latter results were obtained during a light-microscopic study of ca. 700 ovules collected throughout the season from eight trees of different cultivars growing in Israeli orchards (Tomer *et al.* 1976). One-fifth of millions of flowers on a tree is a substantial number of flowers. It seems logical to argue that the presence of these flowers was the reason for the high yield of a ton of fruit on the tree. However, there might have been another factor involved, a factor that had to do with the presence of the numerous flowers that produced abnormal pollen as well as non-functional ovules. Such sexually neutral flowers usually referred to as neuter flowers, have not yet been reported for the avocado, possibly because previous researchers did not examine in detail the anthers as well as the ovule of the same flower. Neuter flowers can contribute in several ways to increase the production of a plant (Lloyd 1979; Zapata & Arroyo 1978). One of these ways involves two inherent characteristics of avocado trees:

Firstly, it has recently been established that 'Fuerte' trees produce between 4000 and 7000 pollen grains for every ovule that is formed on the tree (Steyn 1993). This indicates that the avocado has a strong tendency towards obligate out breeding (Cruden 1977). The flowers of such plants must receive pollen from a genetically different individual to produce viable seed. With regard to the avocado, it means that pollen from another cultivar (crossed pollen) is highly desirable for the formation of normal seeds that, in turn, stimulate the development of well-formed avocado fruits. (Blumenfeld & Gazit 1774; Steyn et al. 1993).

Secondly, the avocado is a self-compatible plant. Such a plant has no physiological barrier in the stigma or style of the female-phase flower (Sedgley and Griffen 1980) to prevent the tree's own pollen (selfed pollen), or pollen from neighbouring trees of the same cultivar from fertilizing the egg cells. If this happens in self-compatible, outcrossing trees like the avocado, seed set is low, because of lethal recessive genes (Hagman 1967). Moreover, selfed pollen can adhere to and block the stigmas or selfed pollen tubes can occlude the styles, thereby interfering with incoming crossed pollen (Bawa & Beach 1981).

It was to prevent this interference of selfed pollen that protogynous dichogamy had evolved in the avocado (Bertin 1993; Lloyd 1986). However, the mechanism cannot prevent self- or intra-cultivar pollination during the daily, transitional period of up to two hours when both male-phase and female-phase flowers are open, or when the usually

synchronized flowering cycle is disrupted by low temperatures (Ish-Am & Eisikowitch 1991; Vithanage 1986), overcast skies and rainy weather (Kubitzki & Kurtz 1984). If under such circumstances an effective way can be found to keep selfed pollen from obstructing incoming crossed pollen, there is a chance that fruit production can be increased.

It is possible that such a situation occurred during the 1992-93 season in the Quantock orchards. The abnormal pollen of the neuter flowers could not interfere with the stigmas of the flowers on the high-producer and the low number of normal pollen grains offered little competition to crossed pollen tubes during their growth towards the egg cells of the twenty percent functional ovules. Consequently, the yield of high-producing tree was exceptional, namely a ton of fruit, but the presence of neuter flowers could not improve the yield of the low-producing trees, because viable embryo sacs were unavailable.

CONCLUSIONS

Manipulating practices that enhance the possibility of cross pollination in the orchards, e.g. the planting of pollenizers or increasing insect visitation in the orchards, have a good chance of increasing fruit production and quality on 'Fuerte' trees, but the challenge of the future might lie in finding ways to keep 'Fuerte' pollen from interfering with cross pollination.

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