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Defective Ovules in Avocado Cultivars[1]

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Abstract. Normal, abnormal, and degenerate ovules of avocado (Persea americana Mill.) are described and illustrated. The frequency of occurrence of these ovule types differed among 4 cultivars ('Fuerte', 'Ettinger', 'Hass', 'Tova') with a total frequency ranging from 15 to 40%. A higher frequency of apparently degenerative ovules increased the proportion considered defective to 80—98%. No relationship could be discerned between the percentage of normal ovules and the yields of 8 trees differing widely in fruitfulness.

The contrast between profuse flowering and a comparatively limited fruit-set in avocado trees raised doubts as to the perfect nature and functionality of all flowers. Schroeder (12) described the normal development of the avocado ovule and some abnormalities, of the embryo sac (10, 11). The present work extends his description of normal and also nonfunctional ovules, the latter including degeneration and abnormalities. The relationship between yield and the ratio of normal to defective ovules was investigated. The investigation was limited to a small number of trees of differing fruitfulness.

Materials and Methods

The selected trees were in trial plots in which yield records have been taken for years. They included one consistently high and one consistently low bearer of 'Fuerte' and 'Ettinger'; two 'Fuerte' trees, half of each girdled; one regularly prolific tree of 'Hass'; and one of 'Tova', a new local cultivar with outstanding fruit-set. The trees of 'Fuerte', 'Ettinger' and 'Hass', were planted in 1957 and are of comparable size. 'Tova' trees were grafted in 1970 and reached a size of about 25% from that of the other cultivars (13).

The protogynous flowers were sampled at the stage of female opening at various dates during the blossoming season. Flowers were collected from outside branches from ground level up to 3 m. The flowers were fixed in FAA. Excised, paraffin-embedded carpels were cut serially in lengthwise sections of 15u thickness, and stained with safranin, fast green. Clearing agents recommended by Herr (3) or NaOH — were used for whole ovule observations.

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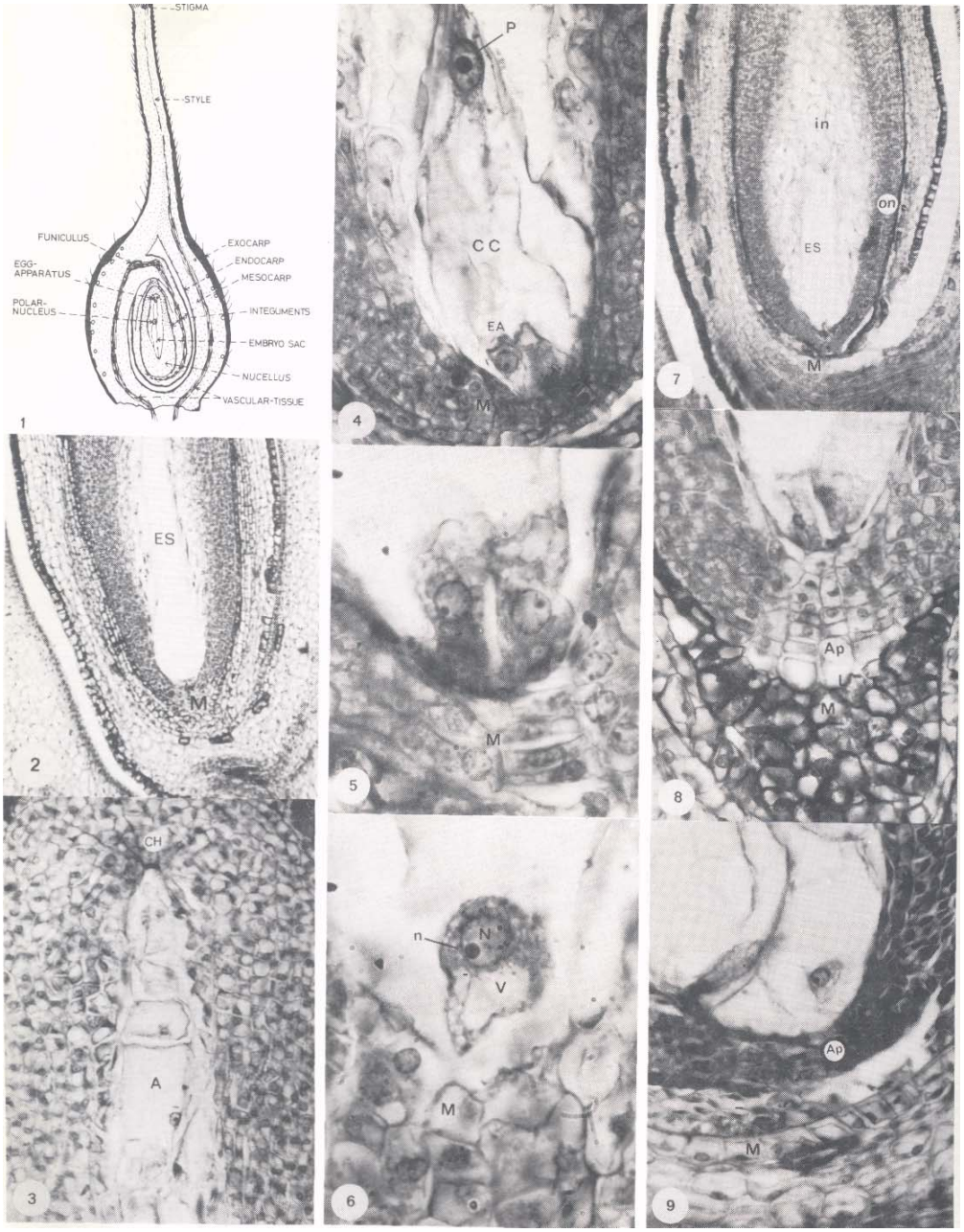
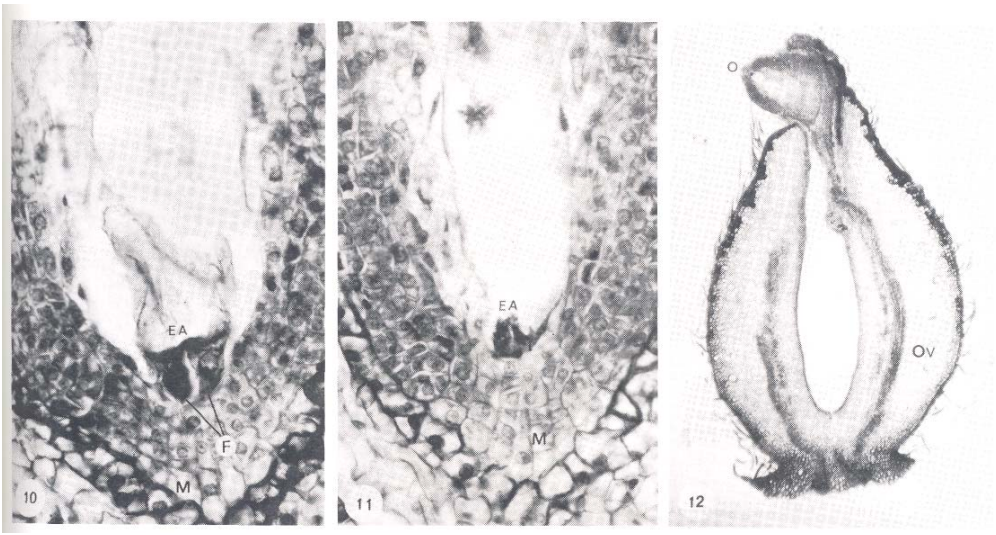
Fig. 1-12.

Fig 1. gynoecium showing the normal position and relative dimensions of the ovule. Fig. 2. An elongated embryo sac widened in the micropylar region and bordered most of its length by loose inner nucellar cells. x125. Fig. 3. Antipodal cells. x300. Fig. 4. Central cell with the polar nucleus embedded in cytoplasm which extends to and surrounds the egg apparatus. x500. Fig. 5. Synergids attached to the micropylar end of the embryo sac, with nuclei in the micropylar half of the cell and the chalazal region filled with one large or several smaller vauoles. x1250. Fig. 6. Egg cell with the conical form of the vacuolated micropylar region. Nucleus in the chalazal cytoplasmic region. x1250. Fig. 7. Nucellus, composed of outer compact cell layers and of inner large, irregular, vacuolated cells. x125. Fig. 8. Protuberant nucelllar apex of 'Fuerte'. x500. Fig. 9. Flat nucellar apex of 'Ettinger'. x500. Figs 10, 11. Micropylar end of the embryo sac containing degenerate egg apparatuses. x500. Fig. 12. An ovule on the carpellary surface.

A = antipodal cells. Ap = apex of nucellus. Ch = chalaza. CC = central cell. F = filiform apparatus. EA = egg apparatus. ES = embryo sac. M = micropylar end. N = nucleus. n = nucleolus. on = outer nucellus. in = inner nucellus. o = ovule. Ov = ovary. P = polar nucleus. V = vacuole.





Results

The normal ovule. The normal avocado carpel bears, on a short funiculus, one ovule which is anatropous, bitegmic, and crassinucellate (1). The two integuments can be separated by clearing agents. The embryo sac (ES) is of the polygonum type. It consists of an egg apparatus, a central cell with one large polar nucleus or, sometimes, two smaller ones in some stage of fusion, and 3 degenerating antipodals. The antipodals are often traceable only with difficulty or not at all (12) (Fig. 1, 2). However, 3 to 6, very large, intact antipodal cells, occupying the chalazal third of the ES, were sometimes present (Fig. 3). Antipodal cells containing nuclei pairs also were observed. The central cell occupies most of the remaining sac space, extending to and surrounding the egg apparatus (Fig. 4). The polar nucleus is located at about one-third of the ES's length from the micropyle. The synergids have a prominent filiform apparatus (thickened wall protrusions) (4) attached to the ES's micropylar end, staining densely with fast green. The nucleus of the synergids is in the micropylar half of the cell, the chalazal half consisting mostly of one large or several smaller vacuoles (Fig. 5). The egg cell has a conical shape, its tip pointing toward the micropyle. The chalazal end of the egg cell protrudes slightly above the synergids, forming a flat summit of the egg apparatus. The nucleus of the egg cell is usually larger than that of the synergids and is located close to the chalazal end of the cell. The micropylar portion of the egg cell is filled with one large or several smaller vacuoles (Fig. 6). Both the central cell in the vicinity of the egg apparatus, and most of the egg apparatus, are devoid of a cell wall.

The nucellus is composed of 3 types of cells: 1) The outer region consists of 6 to 8 rows of small, dense, quadratic cells compactly arranged; 2) The cells of the inner part are large, of irregular form, very vacuolated, and very loosely connected with one another. They surround about three quarters of the ES from the chalaza and are absent in the micropylar portion, where the sac is widened and bordered by the compact nucellus cells (Fig. 7). 3) The third kind of cells constitutes the nucellar apex which is situated between the ES and the micropyle of the integuments, and through the center of which the pollen tube passes to the ES (14). These cells are a continuation of the outer nucellar cells, but they are larger, especially the outermost ones which form a protuberance, and are mostly vacuolated. They are seen in longitudinal sections or in cleared whole mounts as a

column, 4 to 5 cells wide and 6 to 7 cells high (Fig. 8). The apex of 'Fuerte' is the most protuberant because of the exceptionally large outermost cells. The 'Hass' apex is similar but less protuberant, due to smaller cells. In 'Tova' sometimes and in 'Ettinger' as a rule, the greatly reduced protuberance is level with the nucellus tissue. This results from both reduced number and size of cells (Fig. 9).

Ovule degeneration. Degeneration of the ovule occurred in the ES. Apart from the antipodals, degeneration concerned mainly the egg apparatus and, less frequently, the polar nucleus. One, 2 or all 3 cells of the egg apparatus may degenerate. The appearance of the deformed egg apparatus was classified. Schematically, 5 grades of degeneration may be distinguished: 1) The cells are complete, but the shape of some parts deviates from the normal which, together with blurred staining, indicates disintegration. Because there is no established criterion for delimiting normal from degenerate ovules, the definition of grade 1 is arbitrary, representing our best judgment. 2) The cell vacuole is disintegrated, but the rest, including the nucleus, looks normal. 3) The nucleus assumes a shriveled angular form, but the region with the filiform apparatus appears normal. 4) A more advanced grade has an amorphous mass at the locus of the egg apparatus, with almost nothing recognizable except the nucleolus and some remnants of the filiform apparatus. 5) A contracted, formless, dark mass without any structure is seen (Fig. 10, 11). There are variants and intermediate forms of these 5 grades. All stages are accompanied by more or less drastic discoloration. Instead of the well-defined, bright, differential staining of the intact egg apparatus, a blotch of mixed colors marks the degenerate one.

A difference in the pattern of degeneration was found between the cultivars. 'Hass' has a large portion of grade 1 degeneration, and the other cultivars have more severe deformations. 'Ettinger' has the most extreme deformations.

Ovule abnormalities. The frequency of ovule abnormalities is shown in Table 1.

1.) *Twin ovules.* Two ovules in one carpel, one of ordinary size, the other smaller; both may be normal or one or both of them degenerate.

2.) *Twin embryo sacs.* One ovule contains two embryo sacs, usually one of them normal. A rare variant of this anomaly consisted of one ovule containing two egg apparatuses, but only one polar nucleus. This variant type provides the possibility of duplication in an almost mature ES. Another type contained two nucelli enclosed in the same integuments.

3.) *Extra-ovary ovule.* This abnormality (Fig. 12) occurs during the development of the ovary, prior to the closing of the carpel primordia. The ovule, instead of bending inward to the anatropous position, bends outward and becomes located on the carpellary surface (10).

4.) *Intra-ovary abnormal position.* The longitudinal axis of the ovule and that of the ovary are not parallel, but form an angle of up to 45°.

5.) *Additional nuclei.* Additional nuclei, numbering from very few to 20 were found, dispersed or in clusters, in the ES of normal and degenerate ovules, and in ovules lacking an ES having, instead, a circular cavity. Neither their origin nor their function is known (12).

6.) *Immature ovules.* An ES of less than full size contains 1 to 6 nuclei, their position and

grouping indicating an intially normal development which stopped at some stage of megagametogenesis. Also some ES were seen in which an incompletely organized egg apparatus lacking a filiform apparatus was "floating" at some distance from the normal site of attachment.

7.) *Ovules lacking an embryo sac.* This type was the prevalent abnormality and constituted in 'Ettinger' a considerable portion of all ovules.

Frenquency of normal and defective ovules. Table 2 shows the frequencies of normal and defective ovules in relation to the yields of the sampled trees. Defective ovules include those which are apparently not functional, that is, those called "degenerate," as well as some of those called abnormal (extra-ovary ovules, nuclei in cavities, immature ovules, and ovules lacking ES). The data for ovules devoid of an ES are presented separately, because of their considerable number.

Table 1. Frequencies of ovule abnormalities in 4 avocado cultivars.

Variable	Fuerte		Ettinger		Hass		Tova	
	No.	%	No.	%	No.	%	No.	%
Ovules examined	692		1,400		416		431	
Twin ovules	2	0.30	1	0.06	12	2.90	3	0.7
Twin embryo sacs	0		0		7	1.70	19	4.4
Extra-ovary ovules	7	1.00	0		1	0.25	4	0.9
Intra-ovary abnormal position	1	0.15	0		1	0.25	7	1.6
Additional nuclei in ES	7	1.00	6	0.40	1	0.25	4	0.9
Additional nuclei in cavities			1	0.06			7	1.6
Immature ovules	50	7.20	13	0.90	14	3.40	5	1.1
Ovules lacking ES	76	11.00	540	38.50	29	7.00	17	4.0

Table 2. Percentages of normal and defective ovules (1973), and yields (1973/74) of 4 avocado cultivars.

Cultivars	No. of ovules examined	Ovules (%)			Yield	
		Normal	Defective		No. of fruits	Wt (kg)
			Total	No embryo sac		
Fuerte	97	20	80	11	966	191
Fuerte	112	17	83	8	55	13
Fuerte girdled[y]	253	12	88	15	748	190
Fuerte ungirdled[y]	230	17	83	9	220	59
Ettinger	820	2	98	38	373	100
Ettinger	671	2	98	39	2	1
Hass	416	17	83	7	1500	231
Tova	420	20	80	4	260	70

[y]Data from 2 trees, each half girdled and half ungirdled.

Two pairs of 'Fuerte' had very large differences in yield (ratios of 15:1 and 3:1), but the heavier setting trees averaged a slightly lower percentage of normal ovules. For the 'Ettinger' pair the percentage of normal ovules was the same, whereas the fruit yield difference was even greater. Moreover, the extremely low percentage of normal ovules in 'Ettinger' showed that even 2% was enough to produce a satisfactory yield of 100 kg. The

'Hass' tree with the highest yield of all the test trees, has the same percentage of normal ovules as the unfruitful 'Fuerte' trees. The percentage of normal 'Tova' ovules was in the 'Fuerte' range.

To summarize, no relationship was found between the percent of normal ovules and yield.

Flowers were collected throughout the flowering season. When the results were analyzed, wide fluctuations were found in the percentage of normal ovules. Since no consistent trend could be discerned it could be concluded that the adverse weather conditions which occurred at the beginning of the season (avg min temp = 10°C, and lowest absolute min = 6°C in a standard meteorological shelter of plastic material — Thaller type (designed and manufactured by the Israeli Meteorological Service) did not result in a lower percentage of functional ovules.

Discussion

The normal avocado ovules was described by Schroeder (12). Our observations add new information, in that a persistent and well defined nucellus was found in all 4 cultivars studied (Fig. 7), with an apex composed of columns of specialized cells facing the micropyle (Figs. 8, 9). A similar apex was described for the cotton ovule (6). These specialized cells, rarely reported, constitute the passageway for the pollen tube to the ES in avocado (14) and in cotton (7). The basic structure of the avocado egg apparatus and central cell is very similar to that of other species (2, 4, 5, 9). Electron microscope studies (2, 5, 8, 9) demonstrated the delicate nature of these structures and point to the possibility of artifact formation during standard histological procedures. Therefore slight deformations can not with certainty be considered as degeneration. However, with these slightly deformed ovules classified as defective, only 2—20% of all ovules were "normal."

No relationship was found between the percentage of normal ovules and the yield of 8 trees differing widely in fruitfulness (Table 2). Additional tests are needed to determine if this conclusion holds true for other years, and to what extent the trees chosen are representative of their cultivars. In spite of these reservations we believe that our results tentatively indicate that a low percentage of normal ovules is not a factor in low yields of avocado.

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