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Ovule development in seeded and seedless grapes[1])

by

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Introduction

Descriptions of female gametophyte, endosperm and embryo development in seeded grape varieties are numerous and comprehensive (3, 4, 6, 7), but descriptions of the events leading to seed abortion (stenospermy) in seedless varieties are fewer and often incomplete (4, 5, 7). This paper, part of a more complete study (1), examines the sequence of events leading to seed abortion in 3 seedless varieties and their seeded parent, which have not previously been described.

Materials and Methods

Three seedless seedlings from the cross Ontario × Thompson Seedless were selected for study: Himrod, Interlaken Seedless (referred to here as Interlaken) and N. Y. 15302. At maturity the berries of Himrod and Interlaken have soft seeds less than 4 mm in length while N. Y. 15302 has soft seeds which average 5.5 mm in length. As a contrast to the seedless varieties ovules were also examined in the variety Ontario which at maturity has hard seeds averaging 6.5 mm in length. The vines were grown in the vineyards of the New York State Agricultural Experiment Station.

For all 4 varieties ovaries were collected at full bloom (the time when 50 per cent of the calyptras had fallen) and at 2, 8, 14 and 25 days after full bloom. A pre-bloom collection of flower buds was also made. A collection of shatter berries, those which fell from the cluster with a gentle tap of the rachis, was made 9 days after full bloom.

For all varieties ovaries were fixed in either Belling's Modified Navashin fluid (2) or in FAA (70 per cent ethanol 85 ml, neutral formalin 5 ml, and glacial acetic 5 ml). A vacuum pump was used to hasten penetration of the fixative. Tissues were embedded in Tissuemat using a tertiary butanol and ethanol dehydrating schedule (2) and longitudinal serial sections were made at 15 microns thickness. Sections were fixed to the slides with Venning's adhesive (8), stained in Heidenhein's iron alum hematoxylin (2) and mounted in Permount mounting medium. Ovule lengths were measured with a calibrated ocular micrometer.

Results

Following microscopic examination each ovule was classified in one of 8 categories: a) megaspore mother cell or megaspore (Fig. 1), b) degenerate megaspore, c) embryo sac, d) degenerate embryo sac, e) nonfunctional ovule, f) endosperm, g) nucellar degeneration, and h) collapsed ovule. The embryo sac category was sub-

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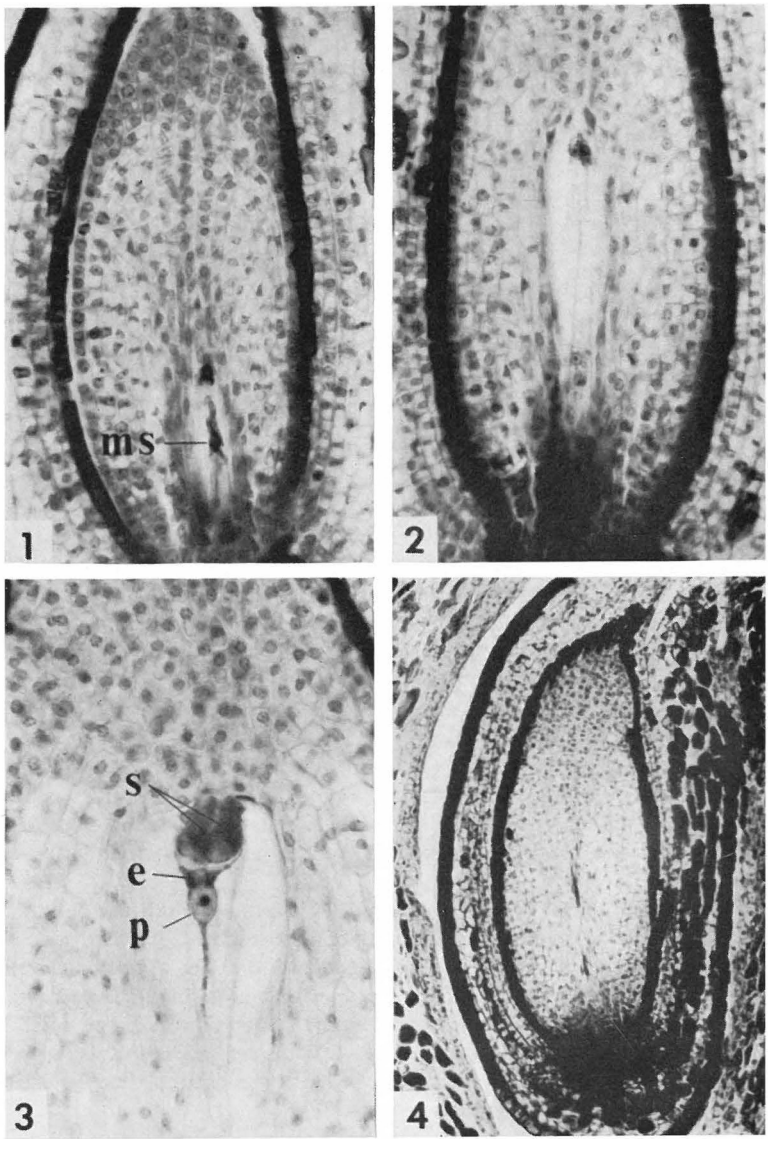

Fig. 1: An enlarged chalazal megaspore (ms) and immediately to its micropylar end degenerating megaspores in an ovule collected from N. Y. 15302 grape 7 days before full bloom. (×285)

Fig. 2: A 4-nucleate embryo sac in an ovule collected from N. Y 15302 7 days before full bloom. (×285)

Fig. 3: A mature embryo sac with fused polar nucleus (p), egg (e) and 2 synergids (s) in an ovule of N. Y. 15302 collected at full bloom. (×400)

Fig. 4: A nonfunctional ovule collected from the Himrod variety at full bloom. Note the central core of degenerating nucellus. (×125)

divided according to the number of nuclei, 2, 4, or 8 (Figs. 2 and 3). Until full bloom an ovule was classified as nonfunctional when it did not contain a developing gametophyte and the central core of nucellus was in some stage of degeneration (Fig. 4). This category probably included ovules in which early degeneration of megaspore mother cells, megaspores or immature embryo sacs had occurred. The endosperm category was subdivided according to the number of free-nuclear endosperm nuclei within each embroy sac. If an egg cell and a fused polar nucleus were found in the micropylar end of an embryo sac in a post-bloom collection the ovule was placed in the endosperm category 'zero endosperm' (Fig. 3). The nucellar degeneration category was similar to the nonfunctional one except this designation was only applied to post-bloom collections (Fig. 4). Perhaps the degenerating central core of nucellus found in these ovules was generally due to gametophytic breakdown. This category increased in frequency with time after full bloom and eventually included all ovules except those with endosperm development or unfertilized mature embryos sacs. A collapsed ovule type was an advanced stage of nucellar degeneration in which the nucellus and inner integument collapsed and separated from the outer integument.

In all 4 varieties female gametophyte development was of the *Polygonum* type: 3 mitotic divisions from the chalazal megaspore (Fig. 1) produced an 8-nucleate embryo sac with 4 nuclei at each pole. The antipodals degenerated prior to the differentiation of the egg apparatus, the migration to the center of the embryo sac and the fusion of the polar nuclei. The mature embryo sac actually contained only 4 nuclei: an egg, 2 synergids and a fusion polar nucleus (Fig. 3).

Table 1

Embryological conditions of ovules within blossoms collected at full bloom and two days after full bloom for four grape varieties (recorded as percentages within each ovule category)

Full bloom:

Variety	Embryo sacs			Nonfunctional	Number of ovules examined
	2- and 4-nucleate	8-nucleate[1])	degenerating		
Ontario	0	37.0	4.3	58.7	92
N. Y. 15302	6.7	66.2	13.5	13.5	99
Interlaken	10.9	38.2	21.8	29.1	55
Himrod	2.6	64.5	6.6	26.3	76

Two days after full bloom:

Variety	Number of endosperm nuclei			Nucellar degeneration	Number of ovules examined
	0	2 or 3	4 to 6		
Ontario	50.0	0	0	50.0	100
N. Y. 15302	54.5	8.1	0	37.4	99
Interlaken	39.8	2.1	0	58.1	93
Himrod	32.5	5.8	14.0	47.7	86

[1]) Includes mature sacs with only 4 visible nuclei, the egg, 2 synergids and fused polar nucleus.

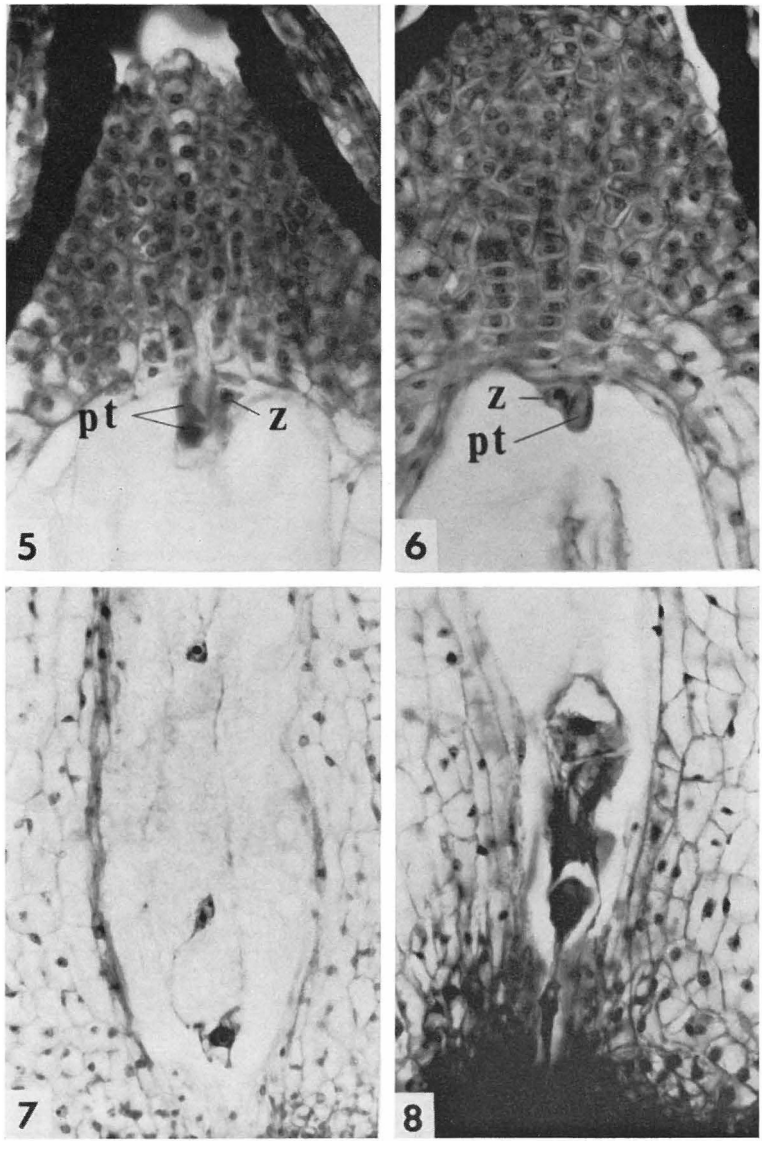

Fig. 5: A pollen tube (pt) and functional zygote (z) in an ovule of N. Y. 15302 grape collected 14 days after full bloom. (×400)

Fig. 6: A pollen tube (pt) and degenerating zygote (z) in an ovule of Himrod collected 8 days after full bloom. (×400)

Fig. 7: Three endosperm nuclei in an ovule of Himrod collected 2 days after full bloom. The 2 nuclei towards the top of the page are in the large micropylar cell and the third nucleus is in the chalazal endosperm cell. (×285)

Fig. 8: Degenerate endosperm cells in the chalazal end of an ovule of N. Y. 15302 collected 14 days after full bloom. (×285)

At full bloom more than one-third of the ovules of Ontario and Interlaken and two-thirds of the ovules of N. Y. 15302 and Himrod contained apparently functional 8-nucleate embryo sacs (Table 1). Ontario and Interlaken had a higher proportion of degenerating embryo sacs and nonfunctional ovules than did N. Y. 15302 or Himrod.

Endosperm development in all varieties was of the Helobial type: the first division of the primary endosperm nucleus resulted in a large micropylar cell and a small chalazal cell (Fig. 7). The micropylar nucleus produced 2 to 9 free nuclei by 8 days after full bloom. The chalazal endosperm cells produced 2 to 6 new endosperm cells by 14 days after full bloom; these cells were frequently in some stage of degeneration (Fig. 8). Cellular endosperm was first noted in the micropylar cell in the 25-day collection.

Endosperm development had begun in all varieties but Ontario by 2 days after full bloom (Table 1). By 8 days after full bloom 15.9 per cent (Ontario) to 39.1 per cent (N. Y. 15302) of the ovules contained endosperm (Table 2). Approximately one-half the ovules of all varieties were nonfunctional at 8 days. By 8 days those ovules which contained endosperm were consistently longer than ovules without endosperm. Average ovule lengths in mm, with and without endosperm, were 0.9 and 0.6 for Ontario, 1.1 and 0.7 for N. Y. 15302, and 1.1 and 0.8 for Himrod. The ovules of Interlaken, however, were uniformly 0.8 mm long. The differences were even more pronounced 14 days after full bloom: for all varieties ovules with endosperm were 1.4 to 1.8 mm long and those without endosperm were 0.7 to 1.0 mm long. Actual endosperm growth did not account for the size difference since each ovule, except those of N. Y. 15302, contained an average of 5 endosperm nuclei (Table 3). The increase in size of fertilized ovules resulted primarily from rapid growth of the nucellus and outer integument.

Table 2

Embryological conditions of ovules within adhering berries collected eight days after full bloom and ovules within shatter berries collected nine days after full bloom for four grape varieties (recorded as percentages within each ovule category)

Eight days after full bloom:

Variety	Number of endosperm nuclei				Total with endosperm	Nucellar degeneration	Number of ovules examined
	0	2—3	4—5	6—9			
Ontario	28.0	7.5	7.5	0.9	15.9	56.1	107
N. Y. 15302	11.5	16.1	16.1	6.9	39.1	49.4	87
Interlaken	29.5	12.6	7.4	4.2	24.2	46.3	95
Himrod	29.5	14.1	12.8	3.9	30.8	39.7	78

Shatter berries nine days after full bloom:

Variety	Number of endosperm nuclei		Nucellar degeneration	Collapsed	Number of ovules examined
	0	2 to 8			
Ontario	13.4	0	31.6	55.0	118
N. Y. 15302	7.1	0	12.4	80.9	113
Interlaken	2.5	2.5	19.3	75.6	119
Himrod	27.0	12.3	40.5	20.2	89

The shatter berries collected 9 days after full bloom of Interlaken and Himrod contained a small proportion of fertilized ovaries, 2.5 and 12.3 per cent respectively (Table 2). Shatter berries o fall varieties were characterized by a high proportion of collapsed ovules or ovules with nucellar degeneration. All ovules in the shatter berries were 0.8 mm long.

Both functional and degenerating zygotes (Figs. 5 and 6) were found at 14 days in the ovules which contained developing endosperm (Table 3). With Ontario and N. Y. 15302 less than 10 per cent of the ovules with endosperm contained degenerating zygotes, but with Himrod the proportion was 25.8 per cent and with Interlaken 50 per cent.

In a small sample of seeds examined 25 days after full bloom all the seeds of Ontario and N. Y. 15302 contained embryos with 2 to 7 cells and more than 500 endosperm cells. Of 9 Himrod seeds examined 5 contained 2- to 7-celled embryos, although 4 of these seeds contained degenerate free-nuclear endosperm or a reduced number of endosperm cells. The remaining 4 seeds did not have embryos and had degenerate free-nuclear endosperm. Seeds of Interlaken did not contain embryos and one-half the seeds contained degenerate free-nuclear endosperm and the other half had a reduced number of endosperm cells.

Table 3

Embryological condition of ovules within berries collected 14 days after full bloom for four grape varieties (recorded as percentages within each ovule category)

Variety	Number of endosperm nuclei							Total with endosperm[1])	Nucellar degeneration	Collapsed	Number of ovules examined
	0	2—4	5—9	10—14	15—19	20—29	30—40				
Ontario	23.9	12.5	19.3	0	0	0	0	31.8	31.4	10.2	88
N. Y. 15302	7.2	0	7.2	12.1	16.9	9.6	6.0	51.8	20.5	20.5	83
Interlaken	14.5	8.4	25.3	4.8	0	0	0	38.5	43.4	3.6	83
Himrod	26.8	25.2	18.4	0	0	0	0	43.6	29.6	0	71

[1]) Of the ovules with endosperm, 2 of 28 (7.1%), 4 of 43 (9.3%), 16 of 32 (50%) and 8 of 31 (25.8%) contained degenerating zygotes for the Ontario, N. Y. 15302, Interlaken, and Himrod varieties respectively.

Discussion

The sequence of events from embryo sac formation to the first division of the zygote and formation of cellular endosperm for Ontario and N. Y. 15302 were essentially similar to that described for other seeded grapes (3, 6, 7). Helobial endosperm development was noted in *Vitis* by KIM (3) and RAMIREZ (6) and confirmed here in all 4 varieties. The present findings agree with those of other workers that the first division of the zygote occurred between 2 and 4 weeks after full bloom (3, 4, 5, 7).

With the 3 seedless varieties in the present study normal 8-nucleate embryo sacs were found at full bloom in more than one-third of the ovules. In addition, fertilization, as measured by the production of endosperm, occurred in more than one-third of the ovules. These findings confirm observations with other steno-

spermocarpic varieties, Concord Seedless, Thompson Seedless, Sultanina Rose and Black Monukka, that normal embryo sacs were produced and that endosperm development began in many ovules (4, 5, 7). In contrast, in parthenocarpic varieties such as the Corinth group, few functional embryo sacs were produced and fertilization did not occur (5, 7).

Precocious endosperm development was observed in Himrod and unusually rapid endosperm development at 14 days was found in N. Y. 15302. NITSCH *et al.* (4) found that in Concord Seedless endosperm development started earlier and developed more rapidly than in the seeded Concord variety, but endosperm degeneration occurred about 3 weeks after full bloom.

Imperfect development of the zygote and endosperm contributed to the lack of seed development in Himrod and Interlaken. At 14 days half the zygotes of Interlaken had degenerated and at 25 days no embryos were found. Many of these seeds contained only degenerating free-nuclear endosperms. Endosperm at 25 days in Himrod had degenerated before becoming cellular in 6 of 9 seeds examined. Accompanying the limited endosperm and embryo development in Himrod and Interlaken was a sharp reduction in seed size.

STOUT (7) coined the term stenospermocarpy to desribe fruits that contained seeds which failed to develop completely because of embryo and/or endosperm abortion. The present study has shown that berries of Interlaken and Himrod fit this description. N. Y. 15302 had soft green and somewhat smaller seeds than Ontario, but up to 25 days after full bloom only slight deviation in ovule development was observed. It is reasonable to assume that embryo and/or endosperm abortion takes place in N. Y. 15302 after 25 days. It appears that the later embryo and/or endosperm breakdown occurs the larger the seed remnants at maturity. NITSCH *et al.* (4) reported in Concord Seedless that endosperm degeneration occurred about 3 weeks after full bloom and that the embryo did not develop beyond a few cells. STOUT (7) found in many ovules of Thompson Seedless, Sultanina Rose and Black Monukka that the embryo failed to develop completely but did not degenerate before the fruit was mature. Neither PEARSON (5) nor STOUT (7) mentioned when endosperm development ceased in Thompson Seedless, Sultanina Rose or Black Monukka, but they rarely found endosperm in the seed remnants in mature berries.

Summary

Female gametophyte and early embryo and endosperm development were examined in 3 seedless grape varieties, Himrod, Interlaken Seedless and N. Y. 15302, and in their seeded parent Ontario.

1. A high proportion of functional embryo sacs was found at full bloom in all varieties.
2. Fertilization occurred in all varieties.
3. Precocious initiation of endosperm was observed with Himrod and extremely rapid development of endosperm was found with N. Y. 15302.
4. Early degeneration of zygotes, failure of the zygotes to divide, and endosperm degeneration were observed in the seedless varieties with smallest seed remnants, Himrod and Interlaken Seedless.
5. Imperfect development of the zygote and endosperm resulted in seed abortion (stenospermy) and seedless fruit (stenospermocarpy) in Himrod and Interlaken Seedless varieties.

Acknowledgment

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Literature Cited

1. Barritt, B. H., 1969: Fruit set and ovule development in seedless grapes treated with the growth retardants Alar and CCC. Ph. D. Thesis Cornell Univ. 61 p.
2. Johansen, D. A., 1940: Plant microtechnique. McGraw-Hill Book Co., New York. 523 p.
3. Kim, K. S., 1967: A contribution to embryological studies on *Vitis (labrusca × vinifera)* Fredonia variety. Ph. D. Thesis. Rutgers, The State Univ. 123 p. (Diss. Abstr. 28, 2200-B).
4. Nitsch, J. P., Pratt, C., Nitsch, C. and Shaulis, N. J., 1960: Natural growth substances in Concord and Concord Seedless grapes in relation to berry development. Amer. J. Bot. 47, 566—576.
5. Pearson, H. M., 1932: Parthenocarpy and seed abortion in *Vitis vinifera*. Proc. Amer. Soc. Hort Sci. 29, 169—175.
6. Ramirez, O. D., 1968: Comparative embryogenesis of Erie, Concord and Golden Muscat grape varieties as related to the germinability of seeds. Ph. D. Thesis. Rutgers, The State Univ. 77 p. (Diss. Abstr. 29, 1230-B).
7. Stout, A. B., 1936: Seedlessness in grapes. N. Y. State Agricult. Exp. Sta. Tech. Bull. 238. 68 p.
8. Venning, F. D., 1954: Manual of advanced plant microtechnique. Wm. C. Brown Co., Dubuque, Iowa. 96 p.

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