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THE DEVELOPMENT AND ABORTION OF EMBRYO AND ENDOSPERM IN THE CROSS HORDEUM VULGARE X SECALE CEREALE

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INTRODUCTION

Post-fertilization breakdown in the reproductive processes is frequently the cause of incompatibility in interspecific and intergeneric crosses in plants.

Cooper and Brink (1940) report that sterility in the crosses *Nicotiana rustica* X *N. glutinosa*, *N. rustica* X *Petunia violacea* and *N. rustica* X *Lycopersicon esculentum* is due to a type of seed failure which they call somatoplastic sterility. Fertilization, although delayed, takes place, and the hybrid embryos grow normally until the collapse of the young seeds. The endosperm however is retarded in development; it loses ascendancy over the maternal tissues of the ovule, which proliferate and finally occlude the endosperm. Associated with these events is the non-differentiation of the vascular bundle to the ovule. Starvation of the endosperm results. Final seed collapse is initiated by the breakdown of the endosperm, which begins in the chalazal region and progresses towards the embryo.

Ledingham (1940) has investigated the development of the embryo and endosperm in the cross *Medicago falcata* X *M. sativa* and the reciprocal. He finds that "there is some evidence that if the development of the endosperm and embryo is too slow some part of the ovule or ovary develops at their expense until abortion occurs."

Autogenous endosperm breakdown is reported by several investigators. Kihara and Nishiyama (1932) found that in the cross _Avena strigosa_ X _A. fatua_ the endosperm grows very rapidly but abnormally. Mitotic divisions are disturbed, so that giant masses of chromatin are formed. Large irregular nuclei, unhealthy cytoplasm, and weak cell wall formation are also found. Seeds are formed but they do not germinate.

In the reciprocal cross, _A. fatua_ X _A. strigosa_, endosperm development is very slow and soon ceases. In some ovules it is regenerated later from a group of cells in the antipodal region, and these ovules may form fertile seeds.

Boyes and Thompson (1937) describe endosperm and embryo development in reciprocal crosses in _Triticum_ and _Secale_. The embryos develop normally. In the endosperm all conditions are found from a slight retardation in growth to extreme abnormalities and early abortion, depending on the width of the cross and the direction in which it is made.

Kostoff (1930) suggests that abortion of endosperm and embryo in _Nicotiana_ hybrids may be due to an immunity reaction. Antibodies are produced in the maternal tissues which attack the hybrid embryo and endosperm.

Laibach's theory (1929) which explains the non-germination of hybrid embryos in _Linum_ by unfavorable influences of the mother plant, is similar. When the embryos are dissected out it is found possible to raise them to maturity.

Preliminary work (Thompson, 1939) indicates that fertilization takes place in the cross Hordeum vulgare x Secale cereale, and that seed failure is due to a characteristic type of endosperm abortion. The development of the hybrid embryo and endosperm is traced in the present study.

ACKNOWLEDGMENTS

Acknowledgment is made to Mr. E. J. Britten who made some of the crosses and cytological preparations, to Dr. T. J. Arnason and Dr. W. P. Thompson who have given valuable supervision, and to Mr. N. Ferrier, who assisted with the photography. Financial assistance was provided by the National Research Council.

MATERIALS AND METHODS

Crosses were made in the field during the summers of 1939, 1940 and 1942, and in the greenhouse during a number of winters. Ovaries were collected at definite intervals. A series of self-pollinated barley was also collected. The varieties used were Regal barley and Prolific rye.

The material was fixed in Randolph's modified Navashin fluid or in formalin-aceto-alcohol, embedded by the tertiary butyl alcohol method and stained with Flemming's triple stain or Heidenhain's iron hematoxylin.

The sections were cut at from 12 to 16μ.

The drawings were made at table level with the aid of a camera lucida, and were reduced approximately one-half in reproduction. An 8x ocular was used for all figures. For Figs. 1 to 11 a 16 mm. objective was used, for Figs. 12 to 23 a 4 mm. objective. The tube length was 160 mm.

DESCRIPTION OF OBSERVATIONS

A. Development in Hordeum vulgare selfed

The mature barley ovule (Fig. 1) is anatropous. It has two integuments, each two cell layers in thickness. The outer integument does not reach to the micropyle, and at this stage is already somewhat flattened. The nucellus consists of an outer layer of regular, darkly staining cells, and an inner layer of vacuolate, lightly staining tissue, which is five or six cells thick at the chalazal end of the ovule and one layer thick at the micropylar end. A strand of vascular tissue runs up beside the ovule on the ventral side. In the embryo sac lie the egg nucleus, two synergids, the polar nuclei, closely appressed, and numerous large, vacuolate antipodal cells.

Fertilization takes place about 24 hours after pollination.

Post-fertilization development is as follows:

1 day after pollination: eight out of the 22 ovaries examined have been fertilized. In two the male nucleus was seen lying beside the egg; in the others the egg has been

fertilized and in two of these the zygote has divided once. The fertilized endosperm nucleus has divided two or three times; the nuclei lie free in the cytoplasm of the embryo sac. There has been a slight increase in the length of the ovule (Table I).

2 days: 22 out of the 25 ovaries examined have been fertilized. The embryo has from one to four cells; the majority are 2-celled. From 8 to 130 endosperm nuclei are present. The 2-celled embryos are accompanied by 30 to 40 endosperm nuclei, the 3- and 4-celled by 100 to 130. Fig. 2 is a drawing of a longitudinal section through a 2 day embryo sac.

3 days: the embryo consists of from five to twelve cells and averages .06 mm. in length. There are from 200 to 520 endosperm nuclei; they lie in a thin strand of cytoplasm near the periphery of the sac. (Fig. 3)

4 days: the embryo is pendant-shaped and has increased slightly in size. (Table II). The embryo sac is lengthening rapidly. Cell walls are forming in a pocket of endosperm tissue around the embryo; the peripheral layer is still non-cellular. (Fig 4). In some endosperms the cells immediately surrounding the embryo are highly vacuolate. The antipodals are flattened and the nuclei are reduced to dark staining,

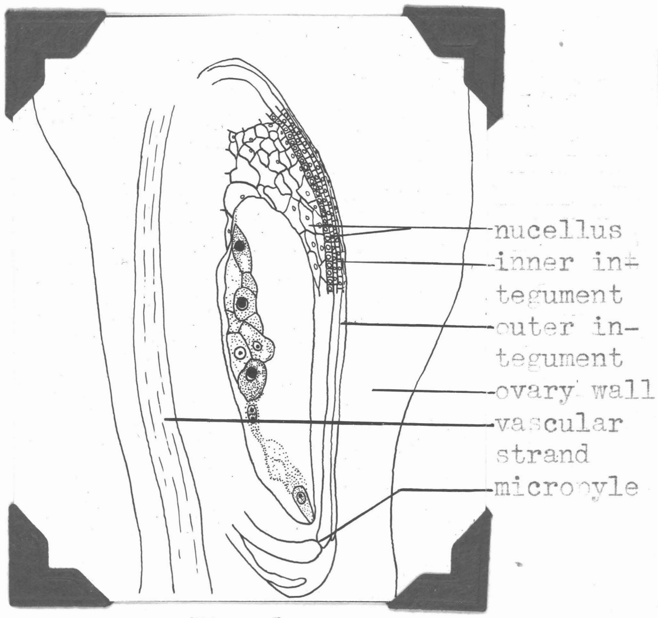

Fig. 1.

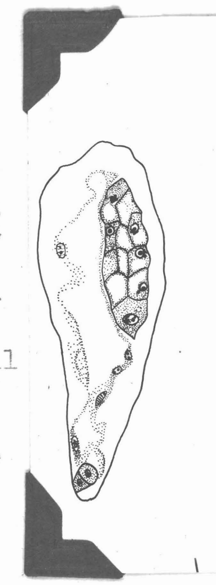

Fig. 2.

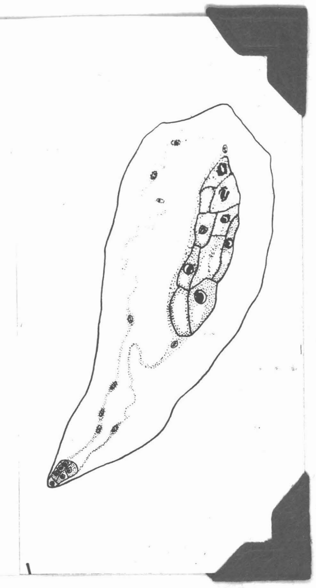

Fig. 3.

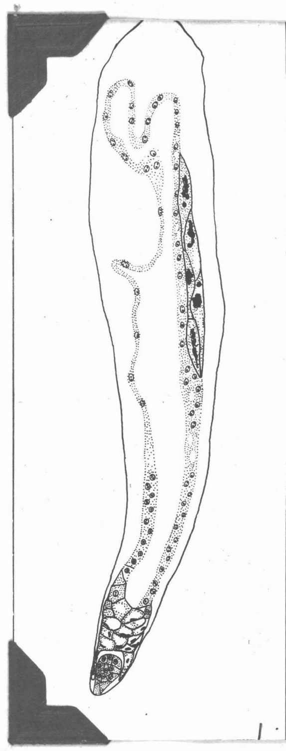

Fig. 4.

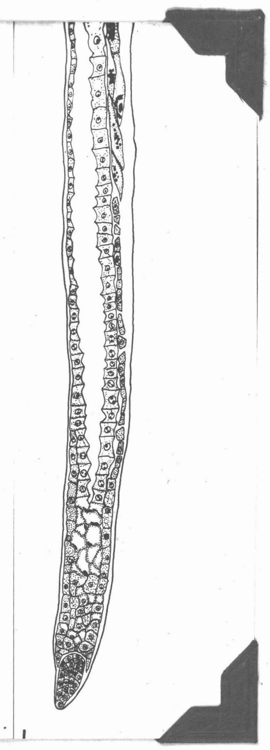

Fig. 5.

Fig. 1. Hordeum vulgare. Longitudinal section of a mature unfertilized ovary. The synergids do not appear in this section. x 68.

Figs. 2 - 5. Hordeum vulgare. Longitudinal sections of embryo sacs at 2, 3, 4 and 5 days after pollination. x 68.

misshapen masses. The inner layer of the nucellus is becoming crushed and disorganized.

5 days: the embryo is the same shape and has increased in length. The peripheral layer of endosperm is cellular. The cells in the pocket and extending up the outside of the peripheral layer on the ventral side are small and dense. (Fig. 5). In some endosperms long nuclei and streaks of chromatin material are present in the pocket. The inner layer of the nucellus is completely crushed.

6 days: the embryo has lengthened slightly. The endosperm is completely filled-in and cellular. The outer integument, which has been collapsing slowly, has finally disappeared.

7 days: development has been rapid throughout the ovule. The embryo has more than doubled in length and the coleoptile is beginning to grow around the stem apex. Starch grains are present in the endosperm, which has increased greatly in length and width. The antipodals are no longer visible.

8 to 15 days: the scutellum, root-tip and second foliage leaves become differentiated in the embryo. The starch grains in the endosperm enlarge and crush the cell nuclei. The nucellus and integuments are slowly flattened between the ovary wall and the growing endosperm.

B. Development in Hordeum vulgare x Secale cereale.

Fertilization takes place at approximately the same time as in barley. In material of 2 days and over, 63% of the ovaries have been fertilized. This is lower than the 83% fertilization found in barley in 2 to 5 day material. (It was not deemed accurate to include material over 5 days in this last computation, because unfertilized ovaries in the older stages are noticeably small in size and were often discarded.)

In Table I, the elongation of the embryo sac may be followed and compared with the normal development in barley. For three days the crossed ovule grows as rapidly as that of the pure species, but thereafter it falls behind.

In the embryo, retardation in growth is not noticeable until 4 days, when it is a pendant-shaped mass of 5 to 12 cells, averaging .063 mm. in length. It enlarges slowly until at 8 days, when all the embryo sacs have collapsed, it has reached an average length of .106 mm. Measurements of embryo lenth in barley x rye and in barley are given in Table II.

Differentiation of the growing point, coleoptile, scutellum, and other structures, which begins in barley at 7 days when the embryo is about .4 mm. long, never takes place in the hybrid. The longest hybrid embryo found measured .14 mm. in length. Many embryos maintain a healthy appearance after the ovules have collapsed (Figs. 10 and 11). In some 7 day material in which collapse is complete, cell division is still taking place in the embryo.

Development of the hybrid endosperm is from the beginning highly abnormal. In Table III, certain features of this development are outlined, together with data on barley selfed.

The first few nuclei formed are small, but irregular in shape. Some are slipper-shaped, some pointed sharply at both ends. By 2 days irregularities are more pronounced. Some nuclei reach a length 8 times that of barley endosperm nuclei. (Fig. 13) In shape some are long and pointed, some long and slipper-shaped (Fig. 13), some rectangular (Fig. 14), some twisted (Fig. 12). One or two large nucleoli, or very many small ones, are present in each nucleus. Sometimes the nucleolus is so large that it bulges out beyond the nuclear outline (Fig. 13). Several bean-shaped or flattened nucleoli were found.

The chromatin is in a coarser network than in a barley endosperm nucleus, and often appears granular. Since the cytoplasm of the embryo sac usually stains rather darkly, the endosperm nuclei show up as clear spaces in which chromatin granules and strands, and the nucleoli, are visible.

In several sacs pockets of very small nuclei are found. Fig. 16 shows a group of these, together with a large nucleolus, the remnant of a disintegrated nucleus.

It is often hard to distinguish the nuclear membrane, and it may be that its disappearance is the first stage in nuclear disintegration (Fig. 15). The chromatin dissolves also, leaving the nucleoli, which persist for some time after the rest of the nucleus is no longer visible.

Only one division figure was seen in the endosperms examined. (Fig. 17). It is of enormous size, extending through two sections besides the one which is photographed. Large masses of chromatin are lumped at the poles and the plate, and smaller fragments lie along the spindle fibres.

Cytoplasm is usually scant. A few sacs have pockets of darkly staining cytoplasm at the micropylar end or in the middle; most have only a thin peripheral strand in which the nuclei lie. No cell walls are formed in the endosperm.

The number of nuclei counted in typical sacs ranges between 0 and 35. However, two 3 day sacs were found with between 40 and 50 nuclei, and one 4 day sac with about 70. It was hard to determine whether fertilization had been with barley or with rye in these three. The endosperm nuclei were only slightly larger than those of pure barley and showed very few of the abnormalities typical of the hybrid nuclei.

Disintegrating nuclei are first seen at 3 days. More are visible in older material. By 6 days, many of the embryo sacs have collapsed, except for a small region around the embryo (Fig. 10). At 7 days, all are either partially or completely collapsed, and at

8 days, all are completely collapsed.

Some embryos begin to degenerate at 6 days. The cells become darkly staining, shrink, and may break apart. The embryo often becomes flattened and elongated because of the pressure of the collapsing ovary.

At 7 days, 80% show some signs of degeneration, and at 8 days, all are degenerating although some are healthier than others. By 12 days none is visible.

Changes in the nucellus and integument follow the same course as in barley. In the 7 day ovary, represented in Fig. 11, it may be seen that the inner integument and the outer layer of nucellar cells are all that remain of these tissues.

Figs. 17 to 23 are photomicrographs of portions of the ovary wall, integuments and nucellus in the selfed and crossed ovaries. These portions are from the dorsal side of the ovaries. At 1 day the nucellus and two integuments are visible in the selfed and the crossed ovaries. By 4 days the inner layer of the nucellus and the outer integument are crushed in both. In barley selfed at 8 days, integuments and nucellus are crushed beyond recognition. In barley crossed, the outer integument has disappeared, the outer layer of the nucellus is slightly flattened, and the inner layer is very thin.

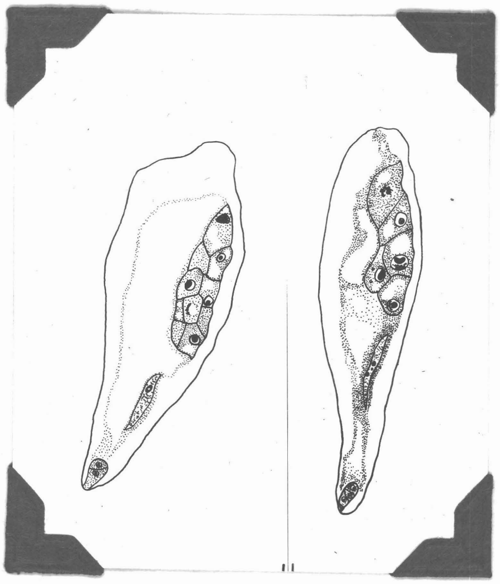

Fig. 6 Fig. 7

Fig. 8 Fig. 9

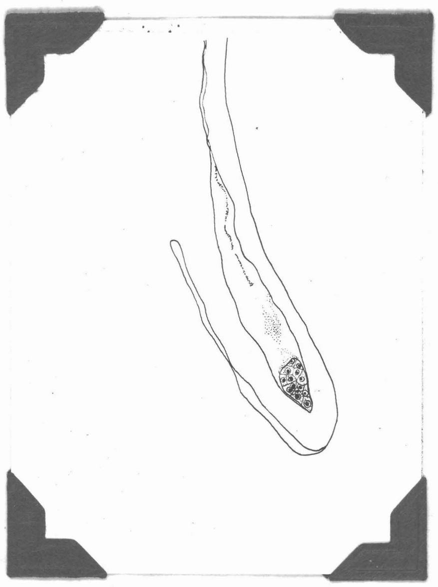

Fig. 10

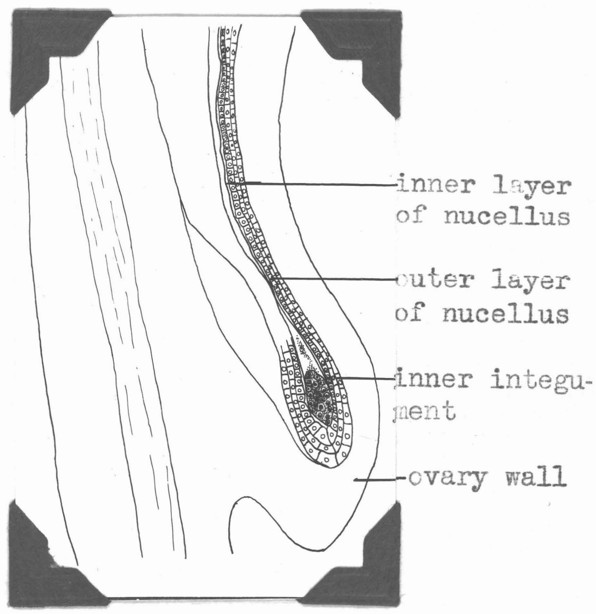

Fig. 11

Figs. 6 - 10. Hordeum vulgare x Secale cereale. Longitudinal sections of embryo sacs at 2, 3, 4, 5 and 6 days after pollination. x 68.

Fig. 11. Hordeum vulgare x Secale cereale. Longitudinal section of a collapsed ovary 7 days after pollination. x 68.

Fig. 12

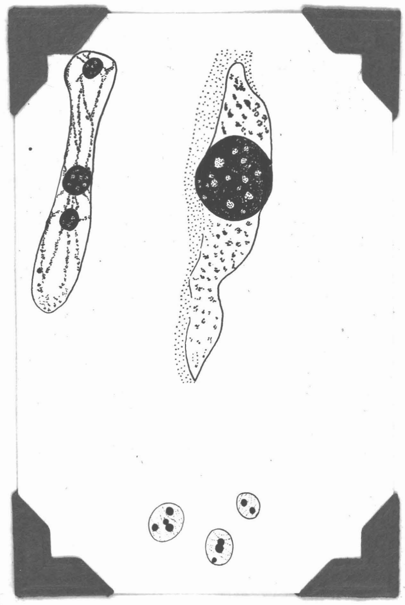

Fig. 13

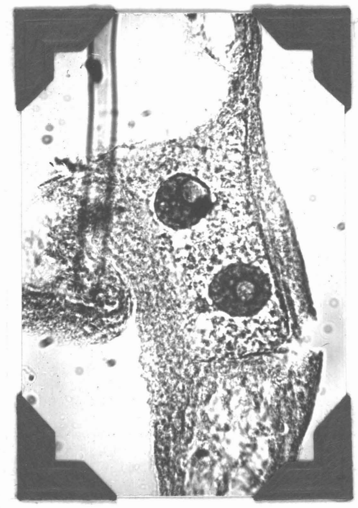

Fig. 14

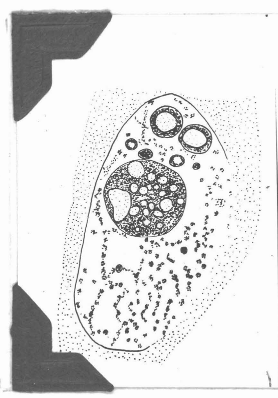

Fig. 15

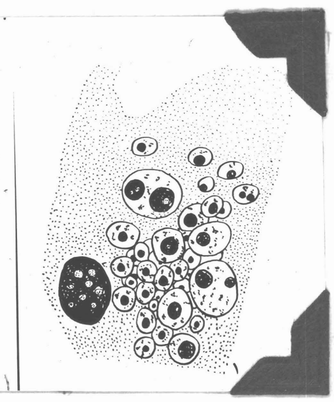

Fig. 16

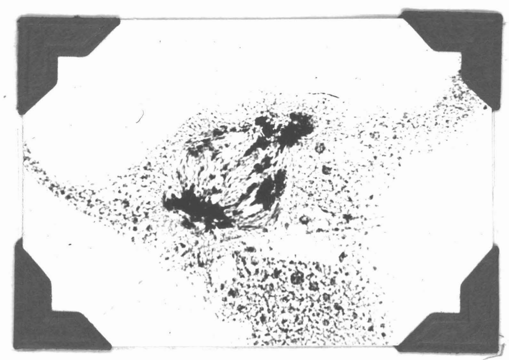

Fig. 17

Fig. 12 Hordeum x Secale. Photomicrograph of endosperm nucleus. x 300.

Fig. 13 Two Hordeum x Secale endosperm nuclei and three Hordeum selfed nuclei for comparison. x 300.

Fig. 14 Hordeum x Secale. Photomicrograph of endosperm nucleus. x 300.

Fig.15 Hordeum x Secale. Endosperm nucleus. x 300.

Fig. 16 Hordeum x Secale. Pocket of small nuclei. x 300.

Fig. 17 Hordeum x Secale. Photomicrograph of division figure in endosperm. x 300.

Figs. 18 - 20. Hordeum selfed.

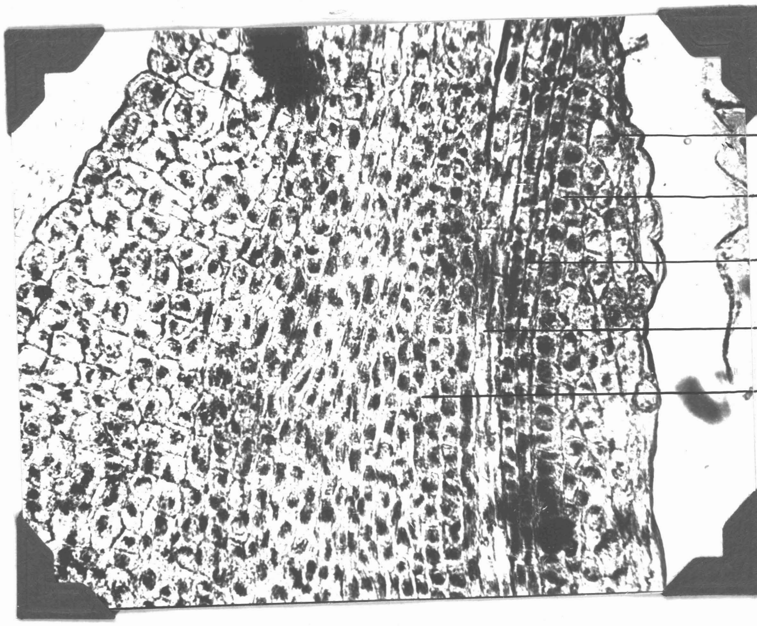

Fig. 18. Portion of ovary wall and ovule at 1 day. x 300.

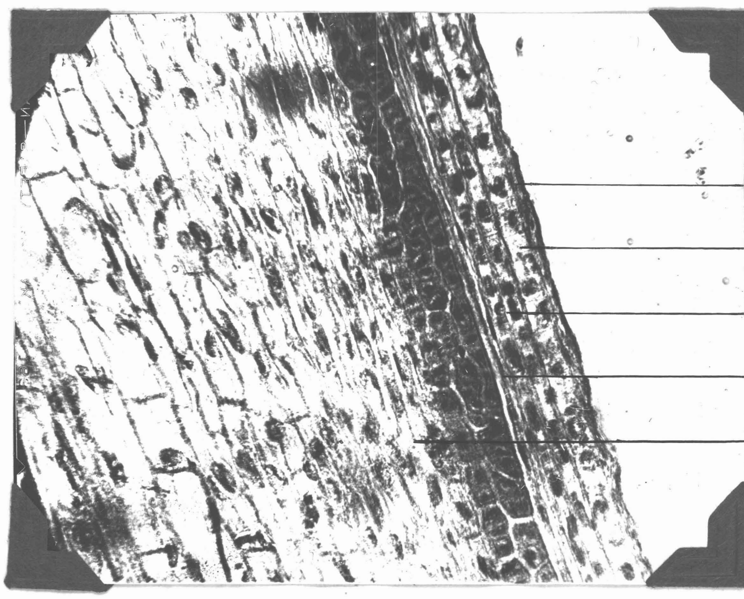

Fig. 19. Portion of ovary wall and ovule at 4 days. x 300.

Fig. 20. Portion of ovary wall and ovule at 8 days. x 300.

Figs. 21 - 23. <u>Hordeum</u> x <u>Secale</u>.

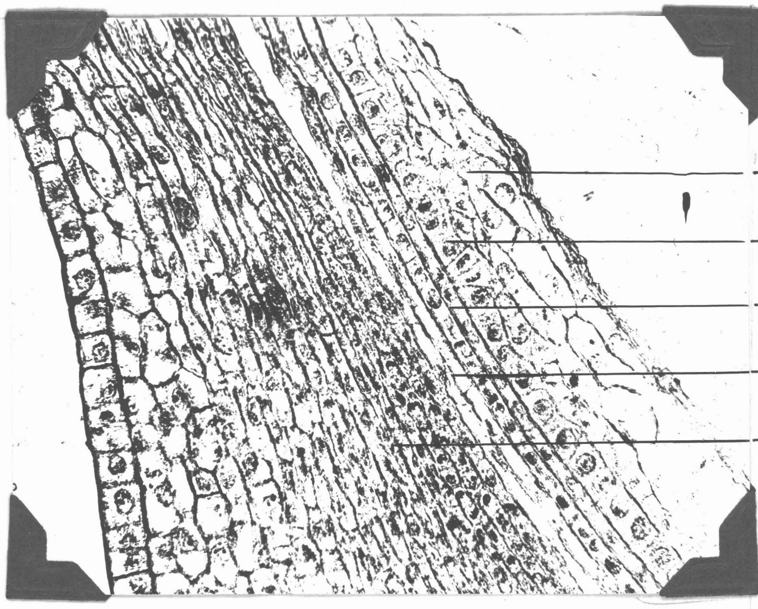

Fig. 21. Portion of ovary wall and ovule at 1 day. x 300.

inner layer of nucellus
outer layer of nucellus
inner integument
outer integument
ovary wall

Fig. 22. Portion of ovary wall and ovule at 4 days. x 300.

inner layer of nucellus
outer layer of nucellus
inner integument
outer integument
ovary wall

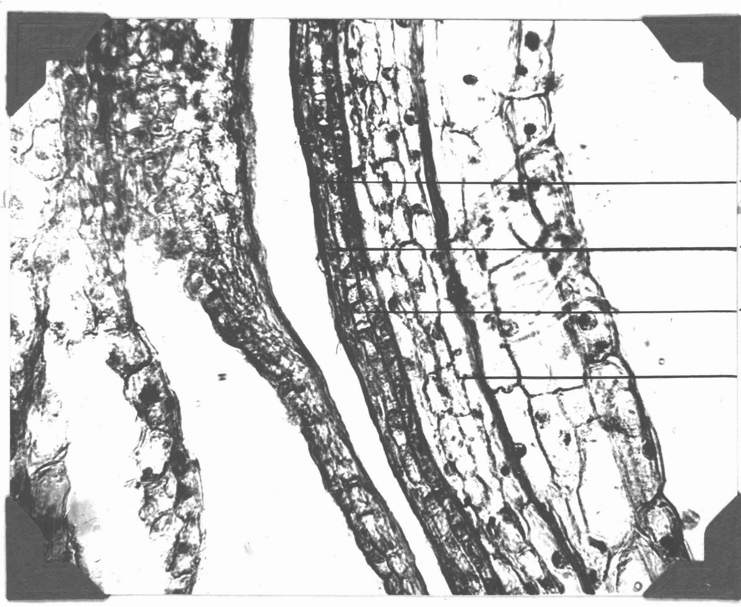

Fig. 23. Portion of ovary wall and ovule at 8 days. x 300.

inner layer of nucellus
outer layer of nucellus
inner integument
ovary wall

Table I

Length of Embryo Sac in Millimetres

Unfertilized	Range .3 - .6		Average .47	
No. of days after pollination	*Hordeum* selfed		*Hordeum* x *Secale*	
	Range	Average	Range	Average
1	.40 - .70	.52	.50 - .72	.62
2	.40 - .80	.63	.46 - .80	.67
3	.67 - 1.17	.95	.59 - 1.20	.92
4	1.10 - 1.66	1.35	.45 - 1.52	1.08
5	1.36 - 2.64	2.19	.67 - 1.68	1.14
6	1.90 - 3.45	2.80	1.09 - 1.84	1.51
7	4.40 - 5.12	4.74	.72 - 2.16	1.58
8	4.48 - 5.80	5.13	.96 - 2.08	1.59
10	4.80 - 5.44	5.23	---	---
12	---	---	1.12 - 2.08	1.54
15	5.6 - 5.92	5.76	---	---

Table II

Embryo Development, (Lengths are averages of all specimens).

No. of days after pollination	*Hordeum* selfed		*Hordeum* x *Secale*	
1	Egg + Sperm to 2-celled		Egg + sperm to 3-celled	
2	Zygote to 4-celled		Zygote to 5-celled	
3	5- to 12-celled	Length .060 mm.	2- to 7-celled	Length .066 mm.
4	Pendant-shaped	.072	5- to 12-celled	.063
5	" "	.085	Pendant-shaped	.068
6	" "	.117	" "	.078
7	Differentiation begins	.415	" "	.088
8	-----	.580	" "	.108
10	-----	.93	-----	---
12	-----	---	None visible	
15	-----	1.76		

Table III

Endosperm development.

No. of days after pollination	*Hordeum* selfed	*Hordeum* x *Secale*
1	4 - 10 nuclei	1 - 8 nuclei
2	8 - 100 "	2 - 16 "
3	200 - 520 "	1 - 35 "
4	Cell formation in pocket; peripheral layer of free nuclei.	0 - 12 "
5	Peripheral layer cellular; 2 - 4 cells thick.	0 - 20 "
6	Completely filled in and cellular.	0 - 20 " Some collapsed.
7	Starch formation	All partially or completely collapsed.
8	" "	All completely coll-apsed.

Table IV

No. of fertilized ovaries examined and measured.

No. of days after pollination	*Hordeum* selfed	*Hordeum* x *Secale*
1	8	10
2	21	22
3	10	17
4	14	30
5	12	16
6	18	13
7	7	21
8	5	18
10	4	—
12	—	9
15	2	—
Total	101	156

Discussion and Conclusions

It would appear from the evidence presented that somatoplastic sterility (Cooper and Brink, 1940) does not play a part in the failure of the Hordeum x Secale cross. No hyperplasia of the nucellus, integument, and ovary wall was observed; these structures followed the same course of development as in Hordeum vulgare selfed. Nor were any abnormalities observed in the vascular tissue.

If the immunity theory of Kostoff (1930) is adopted, the abnormal endosperm nuclei might be explained as resulting from the action of maternal antibodies, such as lysins and precipitins. In several interspecific crosses in Nicotiana, he found in the hybrid endosperms irregularities in cell division, deformation of the nucleus, and the destruction of the nuclear membrane. The dissolution of the membrane was preceded by a swelling of the nucleus. Some of the hybrid endosperm nuclei pictured by Kostoff resemble in appearance those of the Hordeum x Secale endosperm.

Kostoff's theory does not explain endosperm breakdown after selfing in Medicago (Brink and Cooper, 1939), and in rye (Landes, 1939). Here no foreign proteins would be introduced into the endosperm and embryo by the male gametes.

Laibach's work (1929) in raising young hybrid embryos in Linum by dissecting them out from the seed and thus removing them from the influence of the mother plant, lends support to the immunity theory. The hybrid embryo in the Hordeum x Secale cross is of such small size that it will probably be impossible to test the theory in this cross. The largest embryo found measured a little over one-tenth of a millimetre, whereas the Linum embryos were one millimetre and a half in length.

Probably the endosperm in the Hordeum x Secale cross aborts because of its unfavorable genetic constitution. The genes controlling endosperm development in the two parents do not work well when brought together in the hybrid. Those controlling the embryo, however, are apparently quite compatible; for embryo development is surprisingly normal when it is considered that only a small amount of nutriment can be supplied it by the aborting endosperm.

Summary.

When Hordeum vulgare, variety Regal, is pollinated by Secale cereale, variety Prolific, fertilization takes place in 63% of the ovaries. The cross is unsuccessful, however, because the endosperm aborts. All the ovaries are collapsed by 8 days. The embryo, although retarded slightly, develops normally until this time.

The basic cause of endosperm abortion is probably an incompatibility between the parental genes controlling its development. The incompatibility expresses itself in characteristically abnormal nuclei, slowness and irregularity in nuclear division, and absence of cell formation.

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