in fruit softening. According to Zauberman and Schiffmann-Nadel (10), softening of avocado fruit occurred in both immature and mature fruit when PE activity was minimal and PG activity was maximal. Softening generally occurred within 2 to 3 days after PE reached its minimal activity and PG its maximum activity. This could account for the delay in softening, assuming that CA conditions inhibit gradual increase in PG activity.

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Influence of Temperature on Seed Development of *Allium cepa* L.[1]

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Abstract. **Male-sterile onion lines, M2399A and P54-306A were hand pollinated with pollen from 2 male-fertile lines M611B and P52-371B, in 3 Biotron chambers with maxima temperatures of 24°, 35°, and 43°C, respectively, and a minimum temperature of 18°C. There was no significant effect of temperature on magagmetophyte development. Percent abortion of young seeds was 21, 11, and 66% at 24°, 35°, and 43°, respectively; 35° was more favorable for ovule, seed and ovary growth than 24° and 43°. The endosperm nuclei divided soon after fertilization and continued normally the first 5 days after pollination at all 3 temperatures. Subsequent growth of endosperm nuclei was retarded at 43° and only 1 or 2 seeds per ovary continued to grow at a normal rate. The first embryo division was observed 7 to 8, 5 to 6, and 6 to 7 days after pollination at 24°, 35°, and 43°, respectively.**

Franklin (3) reported that onion capsules seemed to be forming normally with developing seed but later contained only seed coats devoid of embryo and endosperm. High temperature may adversely affect onion seed production since ovary wall temperatures as high as 60°C have been measured (6).

We studied onion ovary, ovule, endosperm and embryo development at 3 controlled temperatures after hand pollination.

Materials and Methods

Onion plants (bulbs supplied by Crookham Company, Caldwell, Idaho) of 2 male-fertile lines, M611B and P52-371B, and 2 male-sterile lines M2399A and P54-306A, were transferred from the greenhouse to 3 chambers in the Biotron prior to anthesis. Three Biotron chambers simulating a diurnal cycle with temp maxima of 24°, 35°, and 43°C, and a minimum of 18° were used. Plants were held at each temp maxima for 4 hr (2). The light intensity was 21.6 klx with 12 hr light from 6AM to 6PM.

Table 1. Percent fertilized and aborted ovules as affected by temp and time after hand pollination.

Days after pollination	24°C % ovules fertilized	24°C % aborted	35°C % ovules fertilized	35°C % aborted	43°C % ovules fertilized	43°C % aborted
1	17[z]	0[y]	50[z]	0[y]	9[z]	0[y]
2	50	0	66	0	33	0
3	75	0	100	0	50	0
4	80	0	91	0	66	0
5	50	0	84	0	66	0
6	91	9	100	0	33	0
7	100	36	70	11	17	59
8	82	0	91	34	13	57
9	84	40	84	0	18	71
10	91	8	100	9	17	77
Avg	72.0	21[x]	83.6	13.5[x]	32.2	66[x]

[z]Avg of 5 ovaries.
[y]% fertilized ovules aborting.
[x]Avg of aborted ovules 7 through 10 days after pollination.

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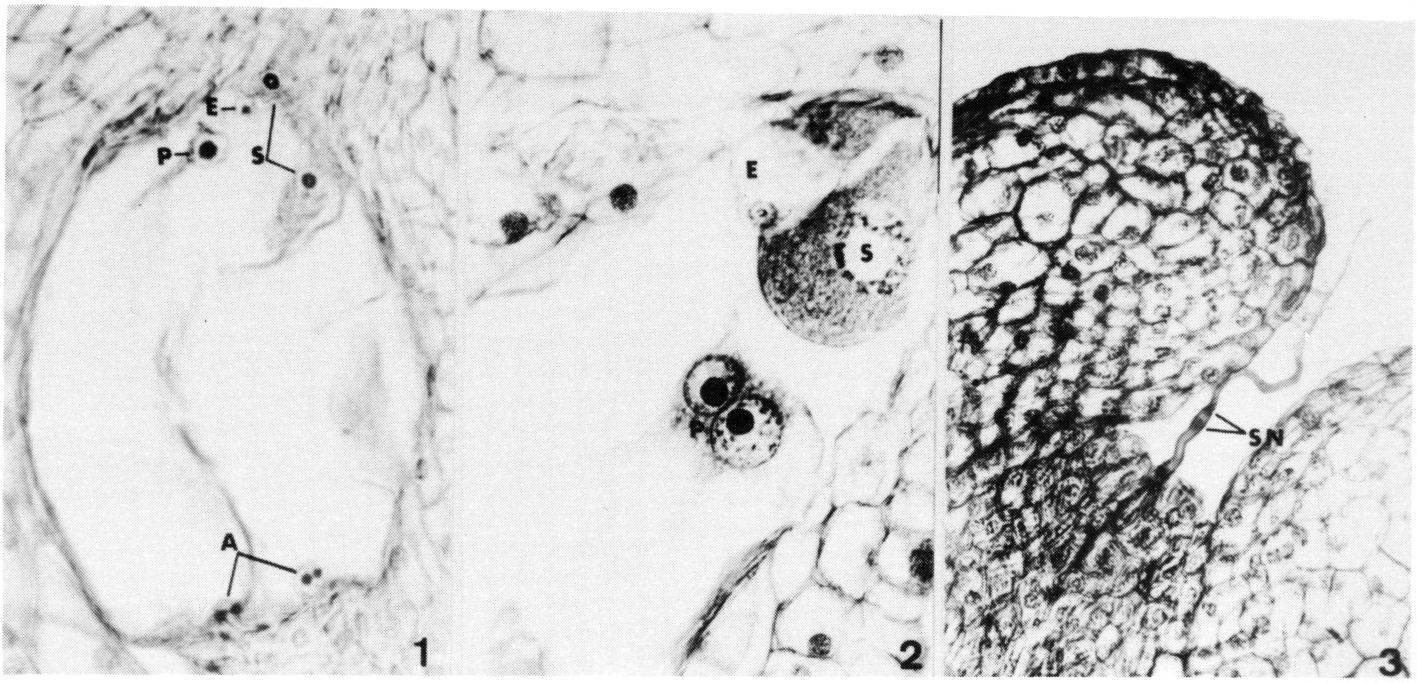

Fig. 1–3. Development of embryo sac and entrance of pollen tube into the ovule. Fig. 1. Embryo sac 2 days before anthesis showing 2 antipodals (A); egg cell (E); polar nucleus (P); and synergids (S) ×290. Fig. 2. Embryo sac 3 days after anthesis showing the egg cell (E); 2 polar nuclei (P); and synergid (S) ×100. Fig. 3. Ovule 1 day after pollination showing a pollen tube with 2 sperm nuclei (SN) entering the micropyle ×150.

Petals of pollinated flowers were marked with India ink to identify date of pollination. Flowers of the 2 male-sterile lines at anthesis were hand pollinated with pollen collected from the 2 male-fertile lines during the 4 hr period of maximum temp in each chamber. Flowers were collected 1 through 10 days after hand pollination, fixed in FAA (formalin-aceto-alcohol), dehydrated with tertiary butyl alcohol, infiltrated and embedded in Paraplast, sectioned at 10μ with a rotary microtome and stained with safranin, crystal violet and light green SF yellowish for anatomical studies.

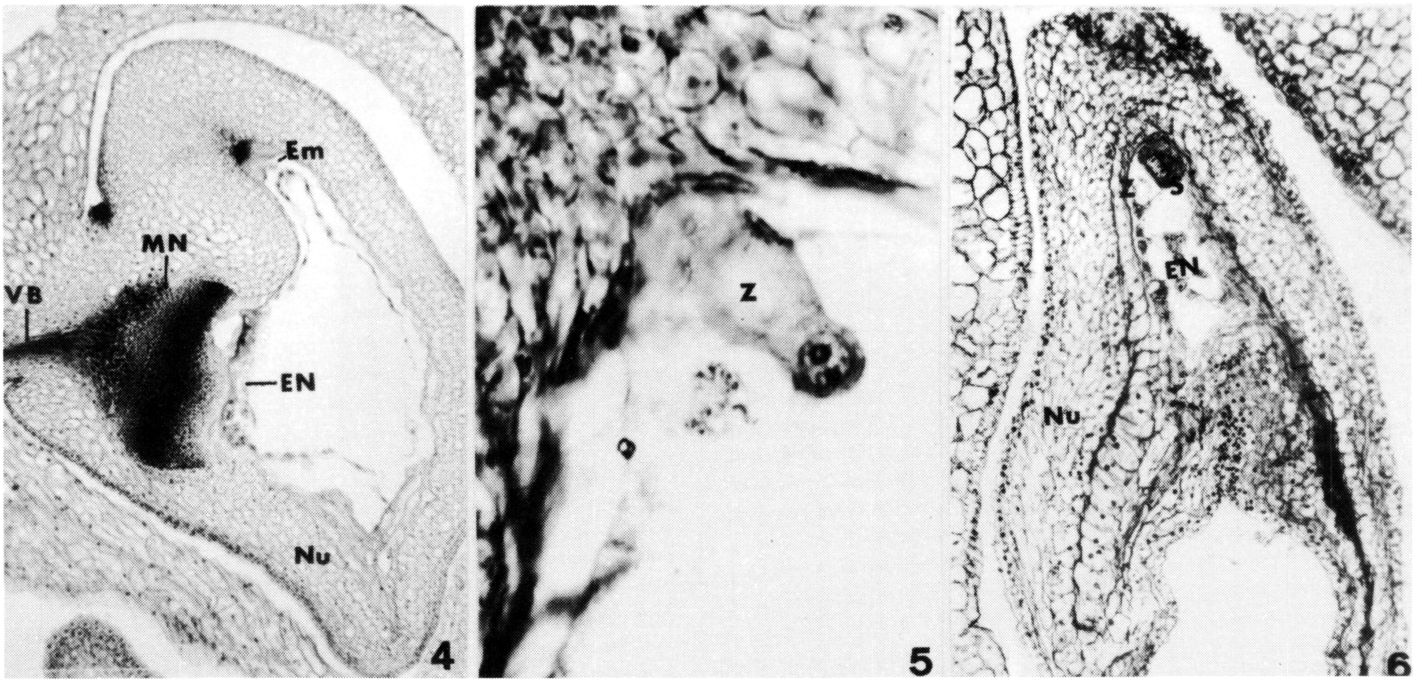

Fig. 4–6. Development of endosperm and embryo. Fig. 4. Ovule 2 weeks after pollination showing the relationship of nucellus (Nu) to the embryo sac. Note the embryo (Em), endosperm nuclei (EN), meristematic nucellus (MN) and vascular bundle (VB) ×65. Fig. 5. Part of ovule 5 days after pollination showing a zygote (Z) prior to first division at 35°C ×140. Fig. 6. Aborted ovule showing numerous endosperm nuclei (EN) with a vacuolate zygote (Z) 8 days after pollination at 43°C. Also note the nucellus (Nu) and synergid (S) ×54.

Table 2. Effect of temp on endosperm development after hand pollination.

Days after pollination	24°C No. of endosp. nuclei Range	24°C Mean[z]	35°C No. of endosp. nuclei Range	35°C Mean	43°C No. of endosp. nuclei Range	43°C Mean
1	2–4	3	2–6	3	2–8	4
2	2–18	9	2–28	14	5–40	15
3	42–70	52	17–67	57	7–85	45
4	35–78	59	67–138	93	17–102	67
5	57–80	67	38–125	78	3–70	43
6	4–75	61	70–107	85	2–57	40
7	15–139	84	48–250	135	5–150	52
8	39–236	174	48–626	231	5–90	47
9	18–390	230	167–998	354	4–229	73
10	103–444	301	211–770	392	8–201	55

[z]Avg of 5 ovaries.

Table 3. Embryo development affected by temp and time after hand pollination.

Days after pollination	24°C No. of embryo cells Range	24°C Mean[z]	35°C No. of embryo cells Range	35°C Mean	43°C No. of embryo cells Range	43°C Mean
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1–2	2	1	1
6	1	1	1–4	2	1–2	1
7	1–5	3	1–8	4	2–3	2
8	2–7	4	1–16	10	2–4	2
9	1–12	8	7–40	25	2–8	4
10	1–15	10	8–38	26	2–10	6

[z]Avg of 5 ovaries.

Results and Discussion

There was no significant difference in hybrid seed development from the 2 male-sterile lines; therefore, the results which follow were based on sampled flowers from line M2399A.

Megagametophyte development. The development of the megagametophyte has been described by Jones and Emsweller (4). In this investigation, it showed no differences at the 3 temp. The mature embryo sac, just before anthesis, was the typical elongate, 8-nucleate structure. The 3 antipodal cells were very small, and usually disappeared shortly after fertilization. The egg apparatus consisted of 2 densely stained synergid cells and the lighter-stained and considerably smaller egg cell. The volume of the 1 synergid was greater than the other which was apparent from serial sections for several samples. The 2 polar nuclei were closely appressed (Fig. 1, 2).

Pollination and fertilization. Using a fluorescence microscope to determine pollen tube growth, it was observed that pollen started to germinate ½ hr after pollination at the 3 temp (2). The 2 male gametes were apparent in the pollen tube entering the micropyle 12 hr after pollination (Fig. 3).

One day after pollination 50% of the ovules had been fertilized at 35°C compared to 17% and 9% at 24° and 43°, respectively (Table 1). Percent of ovules fertilized increased the first 4 days after pollination at 24° and 43°, and reached a maximum at 35° after 3 days. The average percent of fertilized ovules was 72.0 and 78.6 for 24° and 35°, respectively, compared to 46.6% at 43°C. There was no indication of abortion of unfertilized and fertilized ovules at any temp until 6 days after pollination when 9% abortion had occurred at 24° (Table 1).

Ovary and ovule growth. There was little difference in size between pollinated and unpollinated ovaries during the first 5 days except that ovary and ovule growth was faster at 43°C. At 43° the unfertilized ovules showed collapsed embryo sacs 7 days after pollination. A fully developed ovary ranged from 5.5 to 7.0 mm in diam. After 6 days ovary and ovule size was greater at 35° than at the other 2 temp. Ovary growth was less at 24° than at 35° but more than at 43° the 8th day after pollination. Ten days after pollination ovary diam was 1.5 mm greater at 35° than at 24° and 43°.

Endosperm development. After fusion of a sperm and the 2 polar nuclei more than 1 endosperm nucleus was observed 1 day after pollination at the 3 temp (Table 2). Temperature had no substantial effect on division of the endosperm nuclei the first 4 days after pollination; after 5 days there were more divisions at 35°C than at 24° and 43°. The fewer endosperm nuclei at 24° was associated with the slower growth rate of the embryo sac. At 43°, there were only 1 or 2 embryos per ovary with endosperm nuclei that continued to divide at the normal rate; the remaining were retarded and usually aborted. Free nuclear division of the endosperm continued for about 2 weeks after pollination after which nuclei became separated by cell walls. In the large central vacuole, endosperm nuclei were located at the periphery of the embryo sac wall (Fig. 4). At 35° the endosperm nuclei adjacent to the nucellus cells were larger and closer to the vascular bundle compared to other cells in the embryo sac. The nucellus possibly served as intermediary tissue for transferring nutrient materials for the growth of the endosperm. At 43°, endosperm nuclei were less closely associated with the nucelluar tissue, which possibly explains the higher abortion of ovules, and the shrinkage of embryo sac contents and vacuole also could account for more space between the endosperm nuclei and the embryo sac wall at the high temp.

Embryo development. The zygote underwent a period of rest (Fig. 5). At 35°C the zygote usually divided to form a 2-celled proembryo within 5 to 6 days; at 43° 6 to 7 days; and at 24° 7 to 8 days after pollination (Table 3). Possibly the unfavorable physiological conditions caused by the 24° and 43° temp interferred with mitosis and ultimately caused zygote and embryo abortion in the weaker seeds.

The first 5 days after pollination, seeds and unfertilized ovules possibly obtained nutrient elements through the vascular bundle in the chalaza. They grew at the same rate and showed no signs of abortion. At 43°C unfertilized ovules began to collapse 5 days after pollination (Fig. 6).

Endosperm, nucellus and integuments are auxillary to the embryo in maintaining growth of the embryo (1, 5). The results suggest that unfavorable temp could cause either an imbalance between growth of the embryo and the endosperm, or an early dissociation of the nucellus cells from the ovule resulting in failure of seed to develop.

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