Ultrastructural Changes in Peach Flower Buds During Rest[1]

Yeh Feng[2] W. F. Campbell and D. R. Walker
Utah State University, Logan

Abstract. Electron micrographs of 'Gleason Elberta' peach flower buds, *Prunus persica* (L.). Stobes, during rest indicated only moderate metabolic activity prior to December 20. In the December 27 collection, however, a single membrane-bound body (microbody or lysosome) and rough endoplasmic reticulum (RER) were present. Also, the Golgi bodies (dictyosomes) were nearly mature. Heterochromatin disappeared on January 3. By the end of February the number of mitochondria, Golgi bodies, and vesicles had increased markedly. During the first week of March, large nucleopores were observed in the nucleus. These data indicated that bud cells were changing at cold temperatures during winter. Organellar changes were compared to the predicted date for end of rest (13).

Numerous biochemical (2, 4, 5, 7, 8, 17) and physiological (3, 9, 11) changes that occur in plants during cold hardening have been observed, but they are not clearly understood. In addition, information is available on gross anatomical changes that occur in plant tissue during this time (1). Limited information is also available relative to the ultrastructural level of chloroplasts (10, 16) and other plant tissue (14, 18) exposed to low temperatures. Documentation of fruit flower bud ontogeny at the ultrastructural level, however, is lacking. Therefore, we used the electron microscope to follow ontogenetic changes of peach flower buds during rest.

Materials and Methods

'Gleason Elberta' peach, *Prunus persica* (L.). Stobes, flower buds were collected at weekly intervals from October 25, 1971 to March 29, 1972. On March 29 a cold field temperature (-8°C) killed all flower buds, precluding further sampling. The buds were collected from uniform trees growing at Utah State University's Howell Field Station, Ogden.

Twenty peach flower buds were selected on each sampling date. Whole buds, from which all bracts were removed, were fixed for 5 hrs at room temperature in a mixture of paraformaldehyde and glutaraldehyde (6) buffered to pH 7.2 with 0.2 M cacodylate buffer, rinsed twice with the same buffer, and kept overnight at room temperature. Secondary fixation was for 90 min with a 2% OsO_4 solution buffered to pH 7.2 in 0.2 M cacodylate buffer at 4° C. The tissues were then washed with 2 changes of the same buffer, with 15 min per washing.

All samples were dehydrated in an ethanol-propylene oxide series with each step lasting 15 min and embedded in Spurr's medium (15). The specimens were polymerized in plastic petri dishes and incubated for 24 hr at 45° C and 24 hr at 60° C.

Thin sections (500 to 700 Å) were cut from the parenchyma cells surrounding the ovary cavity using glass knives on a Sorvall Porter Blum MT-2 ultramicrotome. Light gold or silver sections were placed on acetone-cleaned, 3-mm, 200 mesh, uncoated copper grids. Sections were stained with saturated aqueous uranyl acetate at 60° C for 4 to 5 min (19) followed by lead citrate at 25° C for 4 min (12). The sections were examined with a Zeiss EM-9S-2A electron microscope and significant observations recorded photographically.

Results

Nucleus. The nucleus was the most conspicuous organelle in the cells of carpel tissue. Prior to January 3, it was spindle-shaped in the prophase (Fig. 1A), and essentially spherical in the interphase state (Fig. 1B). During interphase the cells contained more heterochromatin than euchromatin. The dense chromatin appeared as aggregates or patches in the nuclear interior.

The nuclear envelope that separates the nucleus from the cytoplasm was a tightly packed double membrane (Fig. 1A). No nucleopores were visible in the envelope of October 25, 1971, to February 29, 1972 samples (Fig. 4).

In samples taken from January 3, 1972, to March 7, 1972, the nucleus was irregularly shaped during interphase, with no heterochromatic patches in the nuclear interior (Fib. 1B). These observations indicated that the cells were producing more protein and RNA than previously.

In the February 29th specimens, the nuclear envelope membranes were loosely packed (Fig. 1C). In March samples, the nuclear envelope exhibited nucleopores (Fig. 1D, 4).

Endoplasmic reticulum. A few short cisternae of rough endoplasmic reticulum (RER) were found in cells of samples collected on October 25 (Fig. 1D, 4). Short, tubular, smooth endoplasmic reticulum (SER) was found near the cell membrane region in samples from October 25th through December 27 (Fig. 1E, 4). Long, sheet-like RER was observed from December 27 through March 7, (Fig. 1E, 4).

Samples collected on March 27th showed regional swellings of RER, forming several vesicles (Fig. 2A, B). The region surrounded by the vesicles usually contained cytoplasm that was less dense than elsewhere. The vesicles later tended to fuse to form a vacuole (Fig. 2C).

Golgi bodies. Prior to December 27, only a few Golgi bodies were observed scattered through the cytoplasm (Fig. 4). The flat cisternae extended in a parallel pattern and were closely packed. Vesicles formed only from the ends (Fig. 1A). Moreover, in December 27 and subsequent samples, the cisternae showed a convex surface-forming face and a concave surface- maturing face (Fig. 1F). On the concave surface, the cisternae pinches off vesicles. The larger of these vesicles contained granular inclusions, and are presumably lysosomes (Fig. 2D).

In February 22nd through March 7 specimens, the cisternae of the Golgi bodies were extended in a parallel pattern, and the 2 opposing surfaces differed. The cisternae near the maturing face were compressed so as to pinch off vesicles, while those on the forming face were often swollen (Fig. 2E).

The February 22nd samples had increasing numbers of Golgi bodies and Golgi vesicles. The vesicles either fused together in the cytoplasm to form one large vacuole (Fig. 2F) or dispersed toward the cell surface to fuse with the cell membrane (Fig. 2G).

The carpel size was somewhat increased in February 29 specimens, and substantially increased in the March 7 samples. This gives the impression that Golgi bodies may play an

[1]Received for publication February 5, 1974. A portion of a thesis submitted by the senior author in partial fulfillment of requirements for a M.S. degree. Published with the approval of the Director of the Utah Agricultural Experiment Station as Journal Article No. 1811.

[2]Present address: Department of Cell Biology, Kansas State University, Manhattan.

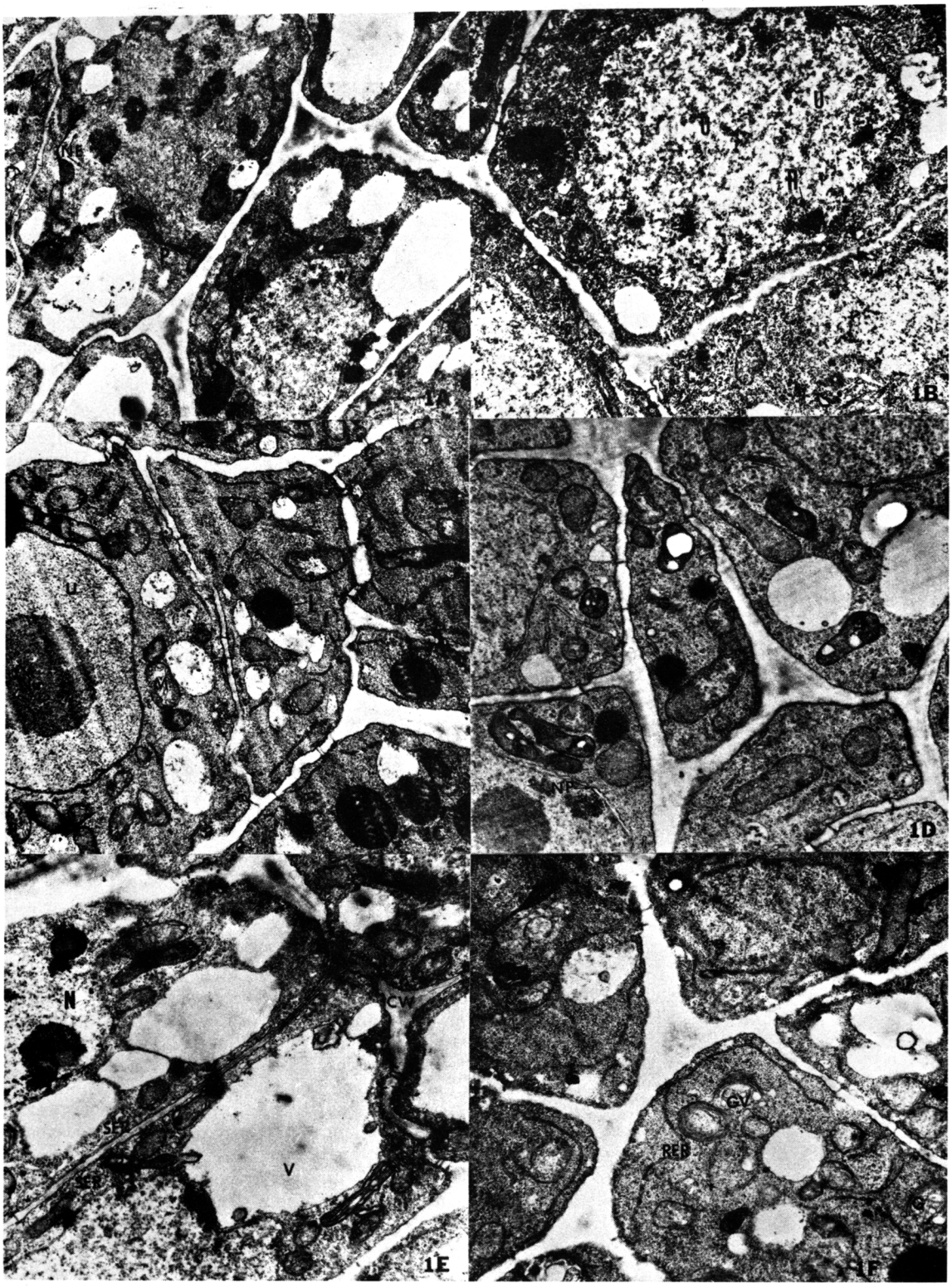

Fig.1. Ultrastructure of peach bud carpel cells. A. Cell in October 25 sample. Irregular-shaped nucleus. 8,000 X. B. Nucleus at interphase October 25, with patches of heterochromatin. 9,000 X. C. Cells at interphase October 29, with less heterochromatin in the nucleus and loosely packed nuclear envelope. 9,000 X. D. Cells from March 7 samples, note the presence of nucleopores. 9,000 X. E. Cells from October 25 showing SER near cell membrane region. 6,000 X. F. Cells at December 27 showing mature Golgi bodies and RER present. 9,000 X. A = Amyloplast, CW = Cell Wall, G = Golgi body, GV = Golgi Vesicle, H = Heterochromatin, L = Lipid body, M = Mitochondria, N = Nucleus, NE = Nuclear Envelope, NU = Nucleolus, NP = Nucleopore, P = Proplastid, RER = Rough Endoplasmic Reticulum, SER = Smooth Endoplasmic Reticulum, U = Euchromatin, V = Vacuole.

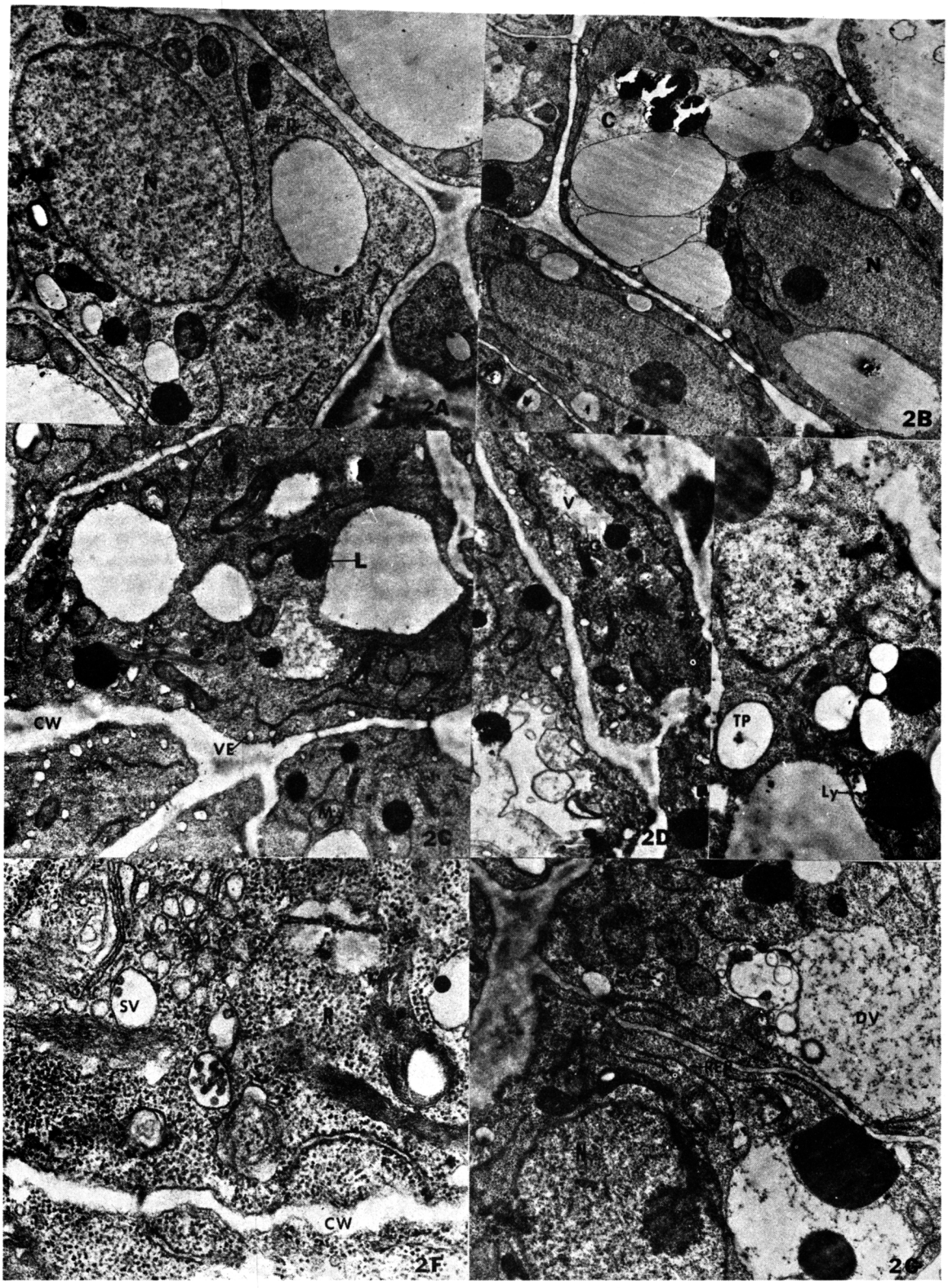

Fig. 2. Ultrastructure of peach bud carpel cells. A. Cells from March 7 samples. Note the regional swelling of RER. The RER was present near Golgi bodies. 8,000 X. B. Cells at March 7 showing vacuoles formed by swelling of RER. 8,000 X. C. Cells at February 22. Vesicles from Golgi bodies dispersed toward the cell surface and fused with the cell membrane. 8,000 X. D. Cells at February 22. Increased vesicles from Golgi bodies – fused to form vacuoles. 8,000 X. E. Cells at January 3. Lysosomes present with lipid droplets. 8,000 X. F. Cells at December 27. Numerous Golgi vesicles. 9,000 X. G. Cells at January 3. Digestive vacuoles and dispersed lysosomes are present in the cytoplasm. 9,000 X. C = Cisternae of RER, DV = Digestive Vacuole, Ly = Lysosome, R = Ribosomes, SV = Storage Vacuole, TP = Transparent Part, Ve = Vesicle.

Fig. 3. Ultrastructure of peach bud carpel cells. A. Cells at January 3. Membrane undulated. 14,000 X. B. Cells at February 22. Numerous mitochondria are shown in the cytoplasm. 9,000 X. C. Cells at February 29. Mitochondria were more numerous than on February 22. 8,000 X. D. Cells at March 7. Amyloplasts are present. 9,000 X. E. Cells at January 3. The presence of transparent parts in proplastids. 8,500 X. F. Cell at November 6. Lipid droplets present. 8,500 X. g= Mitochondria granule, RB = Residual body, S = Starch granule.

important role in cell growth by increasing the cell surface area. Also, these changes indicated an increased metabolic activity of cell wall formation during this time as evidenced by its secretory activity.

Lysosomes. It is hard to demonstrate the existence of lysosomes with electron microscopy because these particles have no characteristic shape or internal structure. Their size and shape may vary widely depending upon their state of activity as well as their internal inclusions. The primary lysosomal storage vacuoles originated from Golgi bodies (Fig. 2D) and differed in

size and granular inclusions from other small vesicles that were also pinches off from Golgi bodies.

Different sizes of digestive vacuoles were found. Typically, the vacuole was surrounded by a unit membrane and contained diluted cytoplasm, some irregularly shaped small vesicles and residues of digestion (Fig. 2G). After digestion was completed, the residues were excreted from the cell (Fig. 3C).

Lysosomes were first seen at the same time December 27 as the RER (Fig. 4), while the Golgi bodies were in their maturation state.

Mitochondria. During early development of the carpel cells, it was difficult to decide in which category, proplastid or mitochondria, a double-membrane bound organelle should be placed. The characteristically short, flat-like invaginations of the inner membrane, called cristae, ran transversely in the mitochondria, however, while sheet-like invaginations ran longitudinally in the proplastids. Also in the proplastids, connections between the invaginations and the bounding membrane were less clear and less frequently seen than in the mitochondria. Mature proplastids are commonly characterized by dense stroma and transparent parts (Fig. 3A).

There were at most 4 to 5 mitochondria per cell in October 25 through February 15 samples (Fig. 4). These were located around the nucleus and each had villus-like cristae projecting inward from the envelope into the matrix. The matrix contained a few mitochondrial granules and a few electron- dense particles, probably ribosomes (Fig. 3C).

In February 22nd samples, 7 to 8 mitochondria were observed per cell. One week later there were 10 to 12 mitochondria per cell (Fig. 3D, 4). The rapid increase in the number of mitochondria implied that more energy was needed for cell metabolic reactions by this time. However, only 4 to 5 mitochondria were present in a cell on March 7th.

Plastids. Among the various kinds of plastids, only irregular-shaped proplastids were observed in carpel cells before February 29 (Fig. 4).

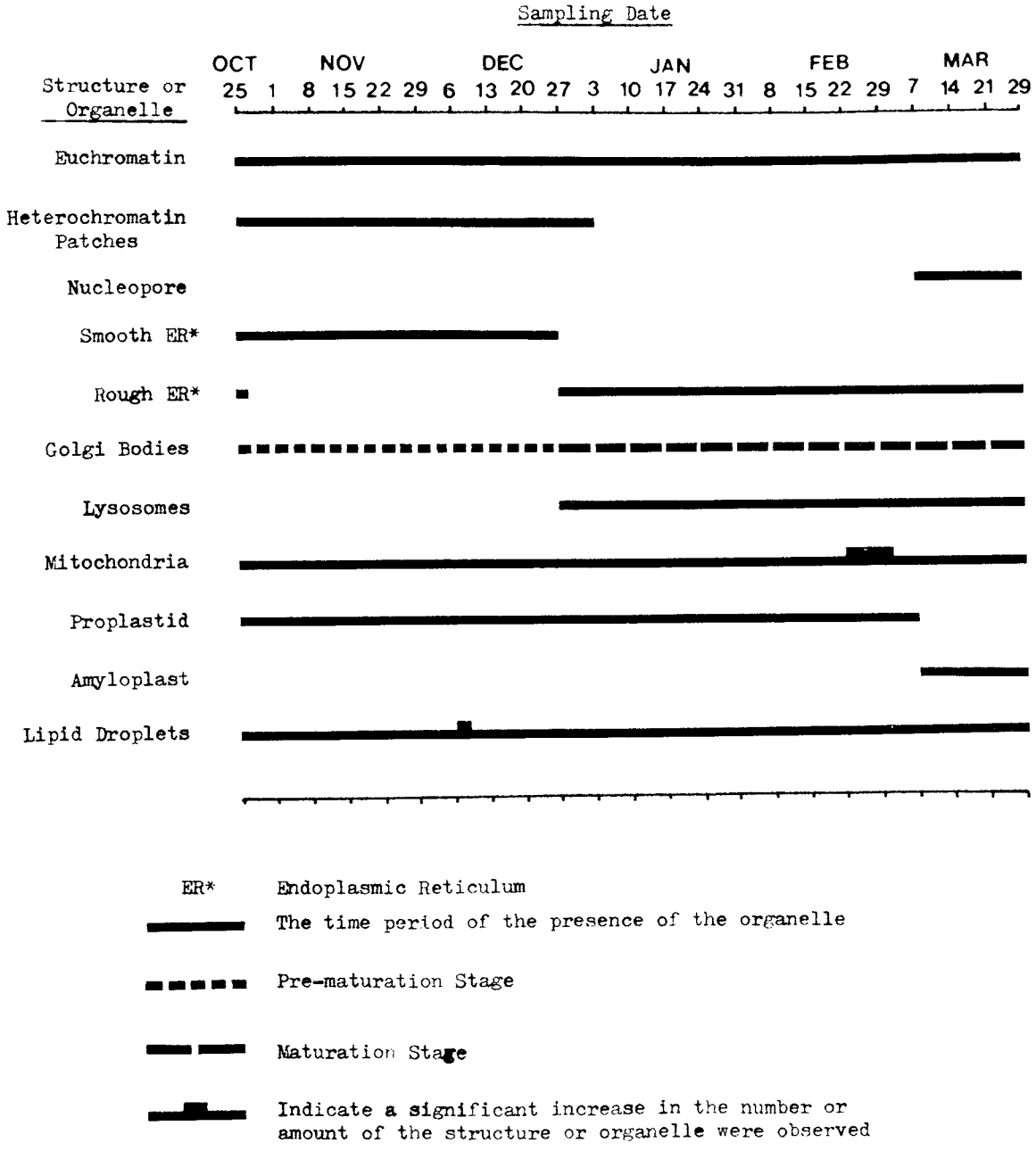

Fig. 4. A summary of seasonal changes in 'Gleason Elberta' peach flower bud cells in 1971-72 as viewed with the electron microscope.

In October 25 samples, proplastids looked similar to mitochondria (Fig. 3A). They can be distinguished with certainty only after the transparent parts become parallel to the envelope. This was first observed in November 22 specimens. As the temperature decreased, the transparent parts enlarged continuously. At this time, the proplastids were almost twice as large as they were in October (Fig. 3F).

In February 15 samples, the transparent parts were reduced in size as well as in number, so the proplastids were once again about the same size as mitochondria. More plastid granules were evident at this stage and the inner membrane system had started to increase in size (Fig. 2C).

In samples collected on March 7 the starch granules were formed in the plastid matrix. The innter membranes continued growing and tended to surround the starch granules. The plastids at this time were probably amyloplasts (Fig. 3E, F, 4).

Lipid. The lipid droplets reached a maximum number ca December 6 (Fig. 4). They occurred side by side along the plasmalemma or were aggregated together in the cytoplasm (Fig. 3F). The lipid droplets occupied about half of the volume of the cell. Lipid droplets were smaller and fewer in number in later samples, probably due to digestion by lysosome enzymes, as lysosomes were found very close, and sometimes attached, to the lipid droplets. The lipid droplets were presumably the source materials for membranes of new organelles as well as for the formation of plasmalemma.

Discussion

Based on the T_{50} (temperature required to kill 50% of the flower buds) 'Elberta' peach fruit bud data of Proebsting (11), a rapid loss of hardiness should occur ca March 14. In this study we observed nucleopores and amyloplasts on March 7 (Fig. 4). The nucleopores are areas through which fairly large molecules, such as RNA and ribosomal precusors, may pass from the nuclear interior to the cytoplasm and return. Their presence indicated the cells were metabolically active and may be associated with the rapid loss of hardiness. The time disparity may be due to seasonal or locational (Utah vs. Washington) variation.

El-Mansy and Walker (4, 5) demonstrated that, in peach flower buds, the total organic acids rose slightly after rest was completed, while malic and citric acids increased considerably. Mitochondria are the organelles that produce malic and citric acids through their respiratory function. We found that the number of mitochondria increased starting with the February 22 samples. The larger number of mitochondria could account for the higher rate of respiration and the increased malic and citric acids.

Sugars increased from 3- to 9-fold after rest, as compared with their concentrations during rest (4, 5). Sugar is the major source of energy for mitochondrial respiration and for cell wall formation by the excretion function of Golgi bodies as well as by SER. Thus, the increase in sugars can be correlated with the higher number of mitochondria, Golgi bodies, and Golgi vesicles in the February 22 samples. Also, the presence of starch granules in the amyloplasts of March 7 samples suggests that sugar could be in excess as early as that date.

The reason for the appearance in December 27 specimens of microbodies bounded by a single membrane, which we called lysosomes, is not clear at this time. They may represent a cellular way to store hydrolytic enzymes that are not currently needed or they may be involved in an, as yet, undetermined way with the plant's resting state. Moreover, the disappearance of heterochromatin may suggest that transcription had been completed as early as January 3.

The presence of SER and free ribosomes in pre-December 27 samples indicates cellular synthesis of polysaccharides and protein to be used within the cells. In the December 27 specimens, however, the SER shifted to RER. This indicates that the cells were preparing to synthesize protein that could be exported by the membrane-flow mechanism. These changes may be associated with the completion of rest. Using the chill unit model developed by Richardson et al. (13) for Utah temperatures, it was determined that rest for the 'Gleason Elberta' peach was completed on December 26 (Richardson – personal communication). Calculation of the completion of rest with the Richardson model was confirmed by later visual observations. We concluded that the changes associated with the completion of rest and the rapid loss of hardiness are evident at the cell level before they can be observed in gross morphology. This may also account for the disparity in time between Proebsting's data and the appearance of nucleopores.

Literature Cited

1. Bokazkiv, K. 1961. Anatomical characteristics of injuries to some woody species during the winter period. *Ukrain. Botan. Zhur.* 18:4.
2. Dowler, W. M., and F. D. King. 1967. Seasonal changes in starch and soluble sugar content of dormant peach tissue. *Hort. Abstr.* 4356.
3. Edgerton, L. J., 1954. Fluctuations in the cold hardiness of peach flower buds during rest period and dormancy. *Proc. Amer. Soc. Hort. Sci.* 64:175-180.
4. El-Mansy, H. I., and D. R. Walker. 1969. Seasonal fluctuations of amino acids, organic acids and simple sugars in 'Elberta' peach and Chinese apricot flower buds during and after rest. *Proc. Amer. Soc. Hort. Sci.* 94:184-192.
5. _____, and _____. 1969. Seasonal fluctuation of flavanones in Elberta peach buds during and after the termination of rest. *Proc. Amer. Soc. Hort. Sci.* 94:298-301.
6. Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixation of higher osmolarity for use in electron microscopy. *J. Cell. Biol.* 27:137A-138A.
7. Kelchie, D. O. 1966. Fatty acids in the bark of Halehaven peach as associated with hardiness. *Proc. Amer. Soc. Hort. Sci.* 88:204-207.
8. Lasheen, A. M., C. E. Chaplin, and R. N. Harmon, 1970. Biochemical comparison of fruit buds in five peach cultivars of varying degrees of cold hardiness. *J. Amer. Soc. Hort. Sci.* 95:177-181.
9. Meader, E. M., and M. A. Blake. 1943. Seasonal trend of fruit bud hardiness in peaches. *Proc. Amer. Soc. Hort. Sci.* 43:91-98.
10. Parker, J., and D. E. Philpott. 1961. An electron microscopic study of chloroplast condition in summer and winter in *Pinus strobus*. *Protoplasma* 53:575-583.
11. Proebsting, E. L. Jr. 1970. Relation of fall and winter temperatures to flower bud behavior and wood hardiness of decidous fruit trees. *HortScience* 5:422-424.
12. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17:208-212.
13. Richardson, E. A., S. D. Seeley, and D. R. Walker. 1974. A model for determining the completion of rest for peach trees. *HortScience* (In Press).
14. Sakai, A. and K. Otsuka. 1967. Survival of plant tissue at super- low temperature. V. An electron microscope study of ice in cortical cells cooled rapidly. *Plant Physiol.* 42:1680-1694.
15. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct.* Res. 26:31-43.
16. Taylor, A. O., and A. S. Craig. 1971. Plants under climatic stress. II. Low temperature, high light effects on chloroplast ultrastructure. *Plant Physiol.* 47:719-725.
17. Toman, F. R., and H. L. Mitchell. 1968. Soluble proteins of winter wheat crown tissues and their relationship to cold hardiness. *Phytochemistry* 7:365-373.
18. Wagenaar, E. B., M. K. Ross, and D. W. A. Roberts. 1970. Ultrastructural changes resulting from cold adaptation in cells of wheat and rye varieties. *J. Cell. Biol.* 47:218A.
19. Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4:475-478.