Seasonal Changes of Peach Flower Buds at the Ultrastructural Level

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SEASONAL CHANGES OF PEACH FLOWER BUDS
AT THE ULTRASTRUCTURAL LEVEL

by

Yeh Feng

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Yeh Feng

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Seasonal Changes of Peach Flower Buds at the Ultrastructural Level

by

Yeh Feng, Master of Science
Utah State University, 1974

Major Professor: Dr. W. F. Campbell
Department: Plant Science

This study was conducted to gain a better understanding of the seasonal changes of Gleason Elberta peach flower buds at the cellular level. Peach buds were obtained from the Howell Experiment Station in Ogden, Utah. Samples were collected at weekly intervals from October 25th, 1971 to March 29th, 1972. When a killing frost precluded further sampling, the buds were preserved by standard electron microscopic techniques. Thin sections (500 to 700 Å) of the cells around the ovarious cavity were examined with a Zeiss EM-9S-2A electron microscope.

From October 25th, 1971 to January 3rd, 1972, more heterochromatin than euchromatin was in the nucleus. After January 3rd, 1972, no heterochromatin patches were observed. Nucleopores were not observed until March 7th, 1972.

Proplastids looked very similar to mitochondria until November 22nd, 1971 when the alignment of the transparent part of proplastids
became apparent around the envelope. The number of lipid droplets increased and reached a maximum level near December 6th, 1971 then leveled off. A few short cisternae of rough endoplasmic reticulum (RER) were found only in the sample collected on October 25th. From October 25th through December 27th, short tubular smooth endoplasmic reticulum (SER) was observed near the cell membrane region and only a few Golgi bodies were found, scattered in the cytoplasm. They were in a pre-maturation stage. Starting from December 27th Golgi bodies were in a maturation stage, long sheet-like rough endoplasmic reticulum was noted and lysosomes, presumably pinched from Golgi bodies, were also observed.

On February 22nd, the number of mitochondria as well as Golgi bodies and Golgi vesicles had increased. The number of mitochondria was further increased on February 29th. On March 7th, amyloplasts presumably developed from proplastids were observed.

The results of this work revealed that polysaccharide metabolism was in progress from October 25th to December 27th as indicated by the presence of SER. The presence of lysosomes starting on December 27th suggested that the cell was undergoing intracellular digestion. The increased number of mitochondria and the Golgi bodies observed on February 22nd revealed an increased rate of respiration and the progression of cell wall metabolism respectively. The presence of nucleopores on March 7th would enhance exchange of information between the nucleus and the cytoplasm.
INTRODUCTION

Plant species vary considerably in their inherent capacity to resist freezing temperatures, and the resistance of individual plants changes dramatically during the year (Alden and Hermann, 1971). The ability of plants to develop cold resistance (cold hardiness) depends on the annual rhythm of physiological processes, the rhythm of climate, and on soil moisture conditions. The annual cycle of physiological processes consists of a period of development, rest and dormancy. The plant has a greater ability to resist freezing temperatures during the deep rest and dormancy periods. It loses this ability rather rapidly with the onset of warm weather and long photoperiods the following spring. After the plant has emerged from its dormancy, sudden freezing temperatures of a spring frost can cause severe damage, resulting in partial to complete loss of the fruit crop. Such freezing damage to fruit trees is a problem of major economic concern.

Many basic biochemical (Parker, 1963; Siminovitch, 1963; Biglov, 1964; Jablonskij, 1966; Ketchie, 1966; Dowler and King, 1967; Toman and Mitchell, 1968; Kuraishi, et al., 1968; Morton, 1969; El-Mansy and Walker, 1969a and 1969b; Lasheen, et al., 1970) and physiological (Meader and Blake, 1943; Chaplin, 1948; Edgerton, 1954; Hatch, 1967; Proebsting, 1970) changes that occur during cold hardening are well documented, though not clearly understood. In addition, data are available on the gross anatomical changes
that occur in plant tissue during this time (Bokazkiv, 1961; Fisk and Millington, 1962; Seeley, 1968). Few data are also available at the ultrastructural level on chloroplasts (Lindner, 1960; Parker and Philpott, 1961; Nishida, et al., 1966; Kislyuk, 1967) and other plant tissue (Shakhov and Golubkova, 1960; Sakai and Otsuka, 1967; Wagenaar, et al., 1970) exposed to low temperatures. Essentially no information is available, however, for fruit bud ontogeny at the ultrastructural level.

The objective of this study was to use the electron microscope and follow the ontogenetic changes of peach flower buds during rest, and to correlate the observed changes with the available literature.
REVIEW OF LITERATURE

Mitosis

Grif (1963) studied the effect of low temperature on mitosis in plants and found cell division to occur at or slightly above 0°C. When the temperature was slowly lowered to freezing and below, meristematic cells completed their mitotic cycles but did not initiate any new mitosis. In the case of a rapid temperature drop, cell division was completely suppressed so that mitosis appeared to stop at any given phase.

Ice Formation

Many semi-hardy or hardy economic plants are apparently killed at temperatures ranging from -15 to -45°C and at rates of cooling that commonly occur in nature. The rate at which the cooling and rewarming occurs, however, may be the clue to the plant's survival ability (Luyet, 1937). For example, Sakai and Otsuka (1967) studied the mechanism of survival of cortical cells of Morus bombycis Koidz. at super-low temperatures with rapid cooling and rewarming. Cortical cells remained alive and contained no ice cavities when taken rapidly to -60°C and held there as long as 16 hours before rapidly rewarming. By contrast, those cells at intermediate temperatures of -20 to -45°C were nearly all destroyed with many ice cavities being formed. They explained their results on the basis of the growth rate of intracellular ice crystals. Sakai (1966) and Sakai and Otsuka (1967) also reported that after
this rapid cooling which apparently removes all of the unbound water within the cell, plant tissue can then be slowly cooled to \(-196 \text{ C}\) without harmful effects.

Levitt's "rupture theory" (1956) stated that intracellular ice crystals grew to masses larger than the cells and caused the cells to contract and collapse. This contraction may be so severe that the opposite sides of the cell can be seen to come in contact with each other. Since the cells are firmly connected with each other, this results in a contraction of the tissues and of the organ as a whole; at the same time, the air from the intercellular spaces is squeezed out. Also, severe dehydration during the time of ice formation shrinks the entire cell including the cell wall and causes loosening of the tissues. More often the dehydrated cytoplasm contracts around the nucleus and the plasmatic strands break. Mechanical damage occurs when intercellular spaces are too small to accommodate all ice formation and cells are crushed, ruptured or separated by splitting of their walls along the middle lamellae.

Seeding with ice may take place through a wound or from cell to cell across pit fields when the withdrawal of water from unfrozen cells to the freezing cells cannot keep pace with decreasing temperature.

On thawing, the intercellular ice is converted to water, leaving the cell water-soaked. In the case of uninjured tissues, the cells rapidly reabsorb the water, but injured cells are unable to do so.
Cellular Membrane

Membrane destruction may be the primary cause of freezing damage in hardy plant cells. Electrolytic tests indicate a loss of selective permeability (Wilner, 1961). Heber (1967) has presented evidence that inactivation of phosphorylation may be one of the causes of freezing injury. This is apparently brought about by an alteration of the permeability properties of the chloroplast and mitochondrial membranes. The frozen membranes can no longer function as an osmometer. This leads to proton leakage through the membranes. Consequently, the formation of a pH gradient across the vesicle membranes is prevented (Mitchell, 1961; 1966). Weiser (1970b), however, does not fully accept the membrane destruction hypothesis.

Protoplasm

Protoplasmic streaming stopped abruptly in chilling sensitive plant cells when the temperature of the tissue dropped below 10 C, whereas streaming continued almost to 0 C in cells from chilling resistant plants (Lewis, 1956).

Das, Hildebrandt and Riker (1966) described cytoplasmic changes in single tobacco cells cooled from 26 C to -10 C. Both cytoplasmic streaming and motion of organelles slowed progressively and ceased movement at temperatures from 5 C to -7 C. Smaller organelles continued movement at lower temperatures than did larger organelles, which indicated a progressive increase in viscosity of the cytoplasm. Cytoplasmic strands became thinner and were replaced by numerous small vacuoles as the cytoplasm contracted on cooling. The small vacuoles disappeared as the cytoplasm was rewarmed and small
globular masses of cytoplasm joined end to end to reform cytoplasmic strands which then resumed normal streaming.

Samygin and Matveeva (1963) stated that injury by dehydration at low temperatures may result in removal of water that is essential to colloidal structures of the protoplasm. At the same time, non-uniform pressure of the extracellular ice caused invagination of cell walls and deformation of the plasmolyzed protoplasm. Injury to the protoplasm from mechanical stress, however, may be less significant than injury from dehydration in cells with small vacuoles and elastic walls (Samygin and Matveeva, 1963).

Tumanov (1967) discussed the importance of "gelling" to the plant in developing cold resistance. Gelling is promoted by aggregation and cohesion of protein molecules and occurs when the liquid phase of the protoplasm becomes trapped in the three-dimensional network of the solid phase. This protects the cell against mechanical deformation, dehydration and formation of intracellular ice by reducing mobility and orientation of water molecules. Also, gelling reduces the biochemical activity of the protoplasm and the harmful effects of concentrated solutes.

**Nucleus**

Siminovitch and Charter (1958) reported that the nuclei in black locust bark became more dense during the autumn and their prominence could serve as a guide to the degree of hardiness.

Some investigators had noted that the nucleus was larger in cold hardy tissue than in non-hardy tissue. This may be related to a higher degree of hydration (Parker, 1963).
Das, Hildebrandt and Riker (1966) observed that numerous small vacuoles formed in the nucleolar plasm of individual tobacco cells upon cooling to -10 C. The small vacuoles coalesced to a large vacuole which expanded and then sometimes closed within 8 to 32 seconds. Other vacuoles closed only on warming. They suggested that the nucleolus played a significant role in metabolic activities of cells because contraction of the nucleolar vacuole may release substances, mainly RNA, into the cytoplasm.

**Plasmodesmata**

Genkel, Oknina and Bakanova (1969) reported that deep dormancy was characterized by the absence of plasmodesmata. Thus, the protoplasm was isolated in plant cells during deep dormancy. Induced dormancy was evident by the presence of a few and growth by the presence of many plasmodesmata.

**Endoplasmic Reticulum and Ribosome**

Kimball (1971) working with hardy and nonhardy grass species observed that rough endoplasmic reticulum (RER) increased as temperature was lowered, indicating increased potential for protein production or a reduced rate of degradation for the RER. The latter alternative is the most likely one. The ribosomal component, however, did not vary with the different temperature treatment.

**Golgi Bodies**

Golgi bodies decreased in number as temperature was lowered, possibly signifying reduction in cell wall formation due to less cell division, enlargement and less secretory activity (Kimball, 1971).
Mitochondria

Richardson and Tappel (1962) found that the swelling of mitochondria was a physical phenomenon that resulted from osmotic changes and diffusion of solutes. The variation in physical behavior of mitochondrial membranes as a function of unsaturation and type of fatty acids in membrane phospholipids conceivably might have some significance relative to cold acclimation.

Semichatova et al., (1963) concluded that the disturbance of both respiration and phosphorylation was connected with the direct effect of temperature on the structural components of the mitochondria in the cells of pea seedlings.

Green and Young (1971) formulated the "conformational energy conservation hypothesis" to explain the behavior of the inner mitochondrial membrane in energy transductions. They mentioned that three distinct states of the tripartite repeating unit of the inner mitochondrial membrane was observed—nonenergized, energized and energized-twisted when the conditions (pH, temperature, etc.) were changed.

Lyons and Raison (1970) mentioned that the less flexible nature of the mitochondrial membranes from cold sensitive tissues, below the critical temperature for chilling injury, could alter the oxidative rate by reducing permeability to oxidizable substrates. This depressed rate of mitochondrial oxidation could lead to an accumulation of metabolic intermediates produced by extramitochondrial systems not adversely influenced by chilling temperatures and thus induce injury. Evidence that these events occur can be found in
tissue slices from banana fruit where it was shown that ethanol and acetaldehyde accumulated as a result of temperature treatments inducing chilling injury. In addition to accumulation of metabolic intermediates leading to cellular injury, reduction in the rate of ATP production could also induce injury. Steward and Guinn (1969) found that ATP levels decreased within 13 hours of chilling in two week old cotton seedlings at 5°C and suggested that below a certain level of available energy the tissue was unable to maintain the metabolic integrity of the cytoplasm necessary for survival. Either of these events, i.e., accumulation of metabolites or reduced ATP supply, resulting from depressed mitochondrial respiration at chilling temperatures could ultimately lead to injury.

**Chloroplast**

Reports of changes in chloroplasts as plants harden in response to low temperatures can be divided into two highly contradictory groups. Some workers have observed that chloroplasts retain their integrity, but migrate from a summer position near the cell wall to a crowded position in the cell interior in winter, with a concomitant loss in chlorophyll content. Other workers (Alden and Hermann, 1971) believe chloroplasts agglutinate, lose their integrity and merge with each other to become a continuous mass from which chloroplasts reform again as spring approaches.

Kislyuk (1967) studied the morphological and functional changes of chloroplasts after cooling of leaves of *Cucumis sativus* L. It was established that functional and morphological injuries occur in the
chloroplasts. Repression of photosynthetic capacity, decreased fluorescence of chlorophyll and changes in chloroplast structure were the earliest signs of cold injury. These signs developed simultaneously and became more evident as the period of cooling was prolonged. The most characteristic changes observed with the light microscope were: the chloroplasts became swollen and the part containing chlorophyll acquired the form of a cup and shifted to the periphery of the chloroplasts. The rest of the volume was filled with transparent colourless substances that faintly differed from the surrounding cytoplasm. The envelope of the chloroplasts became obvious. At this time they were very similar to the vacuolized chloroplasts that had been observed under various other conditions. After a more prolonged period of cooling, the chloroplasts stuck together.

Electron microscopic investigations have shown that changes in the chloroplast's structure begin with the swelling of the stroma (Kislyuk, 1967). Probably it is caused by the flow of water into the chloroplasts. In the chloroplasts of leaves subjected to cooling at 2°C for 25 hours in the light, the stroma increased considerably in volume. All of the lamellar system shifted to the periphery of the chloroplasts and became deformed as a result of the bending of the stroma lamellae. Thus, the transparent part of the swollen chloroplasts as seen with the light microscope was not a vacuole but a swollen stroma. In this state the chloroplasts almost always lost their photosynthetic ability and were characterized by a considerable repression of chlorophyll fluorescence. The fact that it was not a destruction but a shift of its elements could explain the good reversibility of these cold injuries.
The photosynthesis of leaves cooled to 20°C for 25 hours in light was 6% of that of untreated leaves (Kislyuk, 1967). However, after the chilled leaves were returned to room temperature for 40 hours photosynthesis returned to 83% of the control leaves. Light was particularly damaging during cooling, and its effect was believed to be connected with the sensitive, photo-oxidative constituents of the chloroplasts. Almost all of the chloroplasts, which retained any structure after cooling, regained their normal structure in 40 hours as was seen under the light microscope. During the same period the normal intensity of the chlorophyll fluorescence was restored. Thus, the pronounced morphological and functional changes in the chloroplasts appeared to be entirely reversible.

Taylor and Craig (1971) used the electron microscope to study the effects of low temperature (10°C) and high light intensities (170 W m⁻², 400-700 nm) on photosynthesis in C₄-pathway of Sorghum and Paspalum grasses and in the C₃-pathway of soybeans. The pattern of ultrastructural change was similar in these species. Starch grains in the chloroplasts were rapidly reduced in size when chilling stress was applied. At or before the time starch grains completely disappeared, the membranes of the individual stromal thylakoids closed together, reducing the intraspace between them while the chloroplast as a whole began to swell. Extensive granal stacking appeared to hold the thylakoids in position for some time causing initial swelling to occur in the zone of the peripheral reticulum when it was present. At more advanced stages of swelling, the thylakoid system unraveled while the thylakoid intraspaces dilated markedly. Initial thylakoid
intraspace contraction was tentatively ascribed to an increase in the transmembrane hydrogen ion gradient causing movement of cations and undissociated organic acids from the thylakoid intraspace to the stroma. At the initial stages of stress-induced ultrastructural change, a marked gradient in degree of chloroplast swelling was observed within and between cells, being most pronounced near the surface of the leaf directly exposed to light.

Heber (1967) presented evidence that uncoupling of photophosphorylation from the electron transport chain at subfreezing temperatures resulted from conformational changes in chloroplast membranes which, in turn, released protons and inactivated the light-induced increase in pH. A coupling factor removed from the membrane structure by ethylene diamine tetraacetic acid survived freezing and thawing and restored activity to unfrozen, but not to frozen membranes. Injury may result from altered properties of the membrane that arise from the dehydration that accompanies freezing.

In an electron microscope study, Parker and Philpott (1961) observed that winter-type chloroplasts appeared as distinct, intact entities often closely appressed to one another in the folds or sides of the cells. Further, the grana and lamellae were clearly visible in winter as well as in summer; the principal change from summer to winter appeared to be the loss of starch and plastoglobuli. It was thought that the larger size of the summer chloroplasts resulted from starch grain growth. In winter a network or reticulum occurred in all living cells, apparently emeshing mitochondria and chloroplasts, and in sieve cells extending through the plates.
MATERIALS AND METHODS

Sample Collecting

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

Fixation

Twenty peach flower buds were selected at each sampling date. Because of the small bud size, the entire bud less the bud scales was fixed prior to February 22nd, 1972. Only the carpel parts were fixed in buds collected after that date. The diagramatic longitudinal section of peach flower bud was shown on Figure 1.

Tissues were fixed for 5 hours at room temperature in Karnovsky's solution (Karnovsky, 1965) buffered to pH 7.2 with 0.2 M cacodylate buffer rinsed twice with the same buffer and kept overnight at room temperature. Tissues were fixed secondarily with 2% OsO₄ solution buffered to pH 7.2 in 0.2 M cacodylate buffer at 4 C for 90 minutes and washed with two changes of the same buffer for 15 minutes each.

All samples were dehydrated with an ethanol-propylene oxide series with changes of 15 minutes each and embedded in Spurr's medium (Spurr, 1969). Polymerization was carried out by placing the specimens in plastic petri dishes and incubating at 45 and 60 C for 24 hours each.
**Sectioning, Staining and Examining**

**1 μm sections for light microscopy**

The blocks were trimmed to 1 mm$^2$. For orientation purposes, 1 μm longitudinal sections were cut through the ovariary cavity with a glass knife on a Sorvall Porter-Blum MT-2 ultra-microtome.

Slides previously cleaned and stored in acidic ethanol were air-dried. Sections were floated on a drop of distilled water. A camel's hair brush was wetted and used to transfer the sections from the water trough of the knife to the slide. The slide was placed on a hot plate at 40°C. After drying for 15 minutes, Richardson's stain (Richardson, Jarett and Finke, 1960) was applied to the section and the slide was covered with a petri dish to minimize dust contamination. After 1 minute, excess stain was rinsed from the slide with a stream of demineralized water. The slide was dried again on the hot plate and then examined with a light microscope.

For staining the total lipids, the process used was as follows: sections were placed in 50% ethyl alcohol for 2 minutes, stained in a saturated and filtered solution of Sudan III in 70% ethyl alcohol for 12 minutes, differentiated in 50% ethyl alcohol for 1 minute, dried on a hot plate, and then examined at 25X with a Zeiss photomicroscope (Baker, 1947; Gomori, 1952).

**Thin sections for electron microscopy**

Blocks were trimmed to approximately 0.5 mm$^2$. Thin sections (500 to 700 A) from the parenchyma cells surrounding the ovariary
cavity were cut with a glass knife on a Sorvall Porter-Blum MT-2 ultra-microtome. 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 4-5 minutes (Watson, 1958), followed by lead citrate at 25 C for 4 minutes (Reynolds, 1963). 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 3rd, 1972, it was spindle-shaped in the prophase (Fig. 3) and essentially spherical in the interphase state (Fig. 4). There was more heterochromatin, the dense chromatin seen as aggregates or patches in the nuclear interior, than euchromatin in the interphase state.

The nuclear envelope separating the nucleus from the cytoplasm was a tightly packed double membrane (Fig. 4). There were no nucleoporos visible in the envelope from October 25th, 1971 to February 29th, 1972. This accounts for the high resistance of the membrane, even to small ions, and may also account for less energy supplied to the nucleus by the conversion of ATP to ADP through the ATPase that is present on the pores (Yasuzumi and Tsubo, 1966).

In samples taken from January 3rd, 1972 to March 7th, 1972, the shape of the nucleus was irregular in the interphase stage with no heterochromatic patches in the nuclear interior (Fig. 5). These observations indicated that the cells were producing more protein and RNA than they had been previously.

On February 29th, the nuclear envelope was loosely packed together (Fig. 5). On March 7th, 1972, the nuclear envelope exhibited nucleopores (Fig. 6). Such pores are areas in which fairly large molecules, such as m-RNA and ribosomal precursors, may pass from the
nuclear interior to the cytoplasm and back. Any change in the 
external environment could result in a change within the cell, 
causing the cell to adjust itself to the altered conditions 
(Ambrose and Easty, 1970).

Endoplasmic Reticulum

A few short cisternae of rough endoplasmic reticulum (RER) 
were found in cells from samples collected on October 25th, 1971 
(Fig. 19). Short tubular smooth endoplasmic reticulum (SER) was 
found near the cell membrane region from October 25th through 
December 27th, 1971 (Fig. 7). Ambrose and Easty (1970) mentioned 
that the presence of SER implied that polysaccharide metabolism 
was presumably occurring in this particular region of the cells. 

Long sheet like RER was observed from December 27th, 1971 
through March 7th, 1972 (Fig. 8). Before the shifting of SER to 
RER, ribosomes were distributed throughout the cell, which implies 
that the protein produced by these ribosomes was used by or incor­
porated within the cell itself. After the formation of long sheet-
like RER, the cell then has the ability to produce excess amounts 
of protein to be exported by the membrane flow mechanism.

Samples on March 7th, 1972 showed regional swelling of RER, 
forming several vesicles (Figs. 9 and 10). The region surrounded 
by these vesicles usually contained less dense cytoplasm. These 
vesicles later tended to fuse together to form a vacuole (Fig. 10).

Golgi Bodies

Prior to December 27th, 1971, only a few Golgi bodies were 
based scattered through the cytoplasm. The flat cisternae
extended in a parallel pattern and were closely packed. Vesicles were budded off from the ends (Fig. 3). Moreover, starting from December 27th, 1971, they showed face differences; a convex surface-forming face and a concave surface-maturing face (Fig. 8). On the concave surface, the cisternae pinched off vesicles. The larger vesicles contained granular inclusions which are presumably lysosomes (Fig. 13). 

From February 22nd, through March 7th, 1972, the cisternae of the Golgi bodies were extended in a parallel pattern and exhibited a difference between the two opposing surfaces. The cisternae near the maturing face were compressed so as to pinch off vesicles, while those on the forming face were often swollen (Fig. 12). 

On February 22nd, 1972, the number of Golgi bodies increased as did the number of Golgi vesicles. These vesicles either fused together in the cytoplasm to form one large vacuole (Fig. 12) or dispersed toward the cell surface to fuse with the cell membrane (Fig. 11). 

The presence of vesicles along the cell membrane in the February 22nd, 1972 samples were followed by a gradual increase in the carpel size on February 29th, 1972, and a substantial increase in the March 7th, 1972 samples. This gives the impression that Golgi bodies may play a very important role in cell growth by increasing the cell surface area. Also, this shows increased metabolic activity of cell wall formation during this time due to its secretory activity.
Lysosomes

It is hard to demonstrate the existence of lysosomes with an electron microscope, because these particles have no characteristic shape and internal structure. Depending upon their state of activity as well as their internal inclusions, the size and the shape of the lysosomes may vary widely. The primary lysosomal storage vacuoles originated from Golgi bodies (Fig. 13) and differed from the other small vesicles that were also pinched off from Golgi bodies by their size and granular inclusions. The primary lysosomal storage vacuoles contain digestive enzymes, including proteinase, nuclease, glycosidases, Carboxylic ester and thiolester hydrolases (Dingle, 1972).

The fact that storage granules were observed near proplastids, mitochondria and lipid droplets (Fig. 14) indicated that after formation, they wandered through the cytoplasmic matrix to digest the cytoplasmic organelles and the cytoplasmic substances to form digestive vacuoles (Clowes and Juniper, 1968). Different sizes of digestive vacuoles were observed. Typically one, like the one in Fig. 15, is surrounded by a unit membrane, containing diluted cytoplasm, some irregularly shaped small vesicles and residues of digestion. After digestion was completed, the residues were excreted out of the cell. This can be seen in the membrane undulation in Fig. 16.

The formation of lysosomes occurred at the same time as that of the RER. At that time, the Golgi bodies were in the maturation stage, which was on December 27th, 1971.
The presence of RER, Golgi bodies and lysosomes at the same time suggests that membrane flow was in an active progression and the protein synthesized was to be excreted, probably to intercellular space (Clowes and Juniper, 1968). Also the presence of lysosomes indicated that the biochemical properties of the cell were changing, enabling the cell to adapt metabolically to the changing environmental conditions (Ambrose and Easty, 1970).

**Mitochondria**

In the early developmental carpel cells, it was difficult to decide in which category, proplastid or mitochondria, a double membrane bound organelle should be placed. However, the distinct short flat-like invaginations of the inner membrane, called cristae, ran transversely in the mitochondria, while the sheet-like invaginations ran longitudinally in the proplastids. Also the connections of the invaginations to the bounding membrane were not so clearly or frequently seen in the proplastids. The characteristics of proplastids are their dense stroma and the presence of transparent parts (Fig. 19).

There were at most 4 to 5 mitochondria per cell from October 25th, 1971 through February 15th, 1972. They were located around the nucleus and had villus-like cristae projecting inward from the envelope into the matrix. Within the matrix there were a few mitochondrial granules and a few electron densed particles, probably ribosomes (Fig. 16).

On February 22nd, there were 7 to 8 mitochondria per cell observed. One week later (February 29th) there were 10 to 12
mitochondria per cell (Figs. 17 and 18). The rapid increase in the number of mitochondria implied that more energy was needed for cell metabolic reactions that might be occurring in the cell by this time. However, on March 7th only 4 to 5 mitochondria were present in a cell.

**Plastids**

Among the various kinds of plastids, only proplastids were observed in carpel cells before February 29th, 1972. Their shapes were irregular.

On October 25th, 1971, proplastids looked very similar to mitochondria (Fig. 19). It only becomes possible to distinguish them with certainty after the parallel alignment of the transparent parts to the envelope. These were not observed until November 22nd, 1971. As the temperature decreased the transparent parts enlarged continuously after formation. This made the proplastids almost twice as large as they were in October (Figs. 14 and 21).

On February 15th, 1972 the transparent parts were reduced in size as well as in number so as to make the proplastids about the same size as mitochondria. More plastid granules had developed in this stage and the inner membrane system started to increase in size (Fig. 10).

Samples collected on March 7th, 1972 showed that starch granules were formed in the plastid matrix. The inner membranes kept on growing and tended to surround these starch granules. The plastids in this stage are probably amyloplasts (Fig. 20).
Lipid

The lipid droplets reached a maximum level sometime around the week of December 6th, 1971. They were laid side by side along the plasmalemma or aggregated together in the cytoplasm (Fig. 22). The space occupied by the lipid droplets was about half of the volume of the cell.

The lipid droplets later became smaller and decreased in number, probably due to digestion by the enzymes in the lysosomes, as lysosomes were found very close to the lipid droplets, and sometimes they were attached together (Fig. 14). Obviously, the lipid droplets were the source materials for the construction of new organelles as well as for the formation of plasmalemma.

The lipid droplets were identified by staining of 1 μm sections with Suden III as shown in Fig. 2.
DISCUSSION

Proebsting (1970) had made an extensive study on cold hardiness of peach flower buds. He measured the $T_{50}$ temperatures for 14 consecutive years from 1954 to 1969. He noted that environmental temperatures did contribute significantly to the variation of hardiness. However, the results did not provide any good criteria to predict the hardiness directly from the variation of the environmental temperatures; nevertheless the pattern of the variation of $T_{50}$ temperatures was quite similar.

The first hardiness stage starts with a level of hardiness that is usually near -5 F ($T_{50}$) by late October or early November, and is quite constant throughout the rest period. The environmental temperatures during this period hardly affect the hardiness. The second stage starts with completion of the chilling requirement plus enough warm weather to initiate some bud development. If no cold weather intervenes, $T_{50}$ will increase gradually until, about a week before the first pink when there is a very rapid loss of hardiness into the tender bloom condition. During this period, $T_{50}$ is almost completely temperature (environment) dependent. The third stage starts with bloom. During the bloom period the buds lose their ability to harden and become completely tender.

One has to measure $T_{50}$ temperatures along with the observation on ultrastructural changes in order to correlate precisely the relationship between the two. In this work, only the ultrastructural change was investigated. The first pink of the buds was
observed on May 21st. Based upon Proebsting's analysis, the rapid loss of hardiness should occur near March 14th. The results of this work show the presence of nucleopores was observed on March 7th. The author had an impression that the presence of nucleopores might be an important step towards the rapid loss of hardiness.

El-Mansy and Walker (1969) did the biochemical analysis in peach flower buds. They concluded that "total organic acids rose slightly after rest, while malic and citric acids increased considerably" and "sugars increased from 3- to 9-fold during the after-rest period as compared with the rest period".

Mitochondria are the organelles that produce malic and citric acid through their respiratory function. It was found that starting on February 22nd the number of mitochondria increased. The increased number of mitochondria could account for the increased rate of respiration and the increased production of malic and citric acids.

Sugar is the major source material for performing respiration in mitochondria and for cell wall formation by the excretion function of Golgi bodies. So the increased amount of sugars can be correlated with the increased number of mitochondria, Golgi bodies and Golgi vesicles on February 22nd. Also, the presence of starch granules in amyloplast suggests that the excess amount of sugar could be in the cell starting on March 7th.
CONCLUSION

Peach flower bud cell had little activity prior to December 20th, 1971. By December 27th, 1971 lysosomes and RER were present, Golgi bodies were in a maturation stage. By January 3rd, 1972 heterochromatin had shifted to euchromatin. By February 29th, 1972 the number of mitochondria increased. By March 7th, 1972 nucleopores were observed. These data indicated that the cell was preparing for growth long before the temperature was favorable for growth.
LITERATURE CITED


APPENDIX
APPENDIX I

Table 1. Seasonal changes in the peach flower bud cells in 1971-72 as viewed with the electron microscope.

<table>
<thead>
<tr>
<th>Structure or Organelle</th>
<th>OCT</th>
<th>NOV</th>
<th>DEC</th>
<th>JAN</th>
<th>FEB</th>
<th>MAR</th>
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<td>Rough ER*</td>
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<td>Mitochondria</td>
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<td>Proplastid</td>
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<td>ER* Droplets</td>
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*ER* Endoplasmic Reticulum

The time period of the presence of the organelle

--- Pre-maturation Stage

--- Naturation Stage

--- Indicate a significant increase in the number or amount of the structure or organelle were observed
APPENDIX II

ABBREVIATIONS, LEGENDS AND FIGURES

Explanation of figures

Abbreviations are as follows:

A: Amyloplast  \hspace{1cm} \text{Nu: Nucleolus}
C: Cisternae of RER  \hspace{1cm} \text{NP: Nucleopore}
Ch: Chromosome  \hspace{1cm} \text{P: Proplastid}
CW: Cell Wall  \hspace{1cm} \text{R: Ribosome}
DV: Digestive Vacuole  \hspace{1cm} \text{RB: Residual Body}
G: Golgi body  \hspace{1cm} \text{RER: Rough Endoplasmic Reticulum}
g: Mitochondria granule  \hspace{1cm} \text{S: Starch granule}
GV: Golgi Vesicle  \hspace{1cm} \text{SER: Smooth Endoplasmic Reticulum}
H: Heterochromatin  \hspace{1cm} \text{SV: Storage Vacuole}
L: Lipid  \hspace{1cm} \text{TP: Transparent Part}
Ly: Lysosome  \hspace{1cm} \text{U: Euchromatin}
M: Mitochondria  \hspace{1cm} \text{V: Vacuole}
N: Nucleus  \hspace{1cm} \text{Ve: Vesicle}
NE: Nuclear Envelope
APPENDIX III

Figures 1-22
Figure 1. A diagramatic longitudinal section of pistil of peach flower bud
The Diagramatic Longitudinal Section of Pistil of Peach

Flower Bud
Figure 2. Samples taken on December 27th, 1972. 1 μm longitudinal section across the ovarious cavity. Stained with Richardson's stain and Sudan III. Note the lipid droplets, nucleus and cell wall. x 100
Figure 3. Sample taken on October 25th, 1971. Peach bud carpel cell undergoing cell division. Note the spindle shaped nucleus, the dissociation of nuclear envelope and the chromosome. The Golgi body was in an early developmental stage. x 36,000

Figure 4. Sample taken on October 25th, 1971. Interphase stage of the nucleus with patches of heterochromatin. x 12,000

Figure 5. Sample taken on February 29th, 1972. Interphase stage of the nucleus with less heterochromatin and loosely packed nuclear envelope. x 14,720

Figure 6. Sample taken on March 7th, 1972. Note the presence of nucleopore. x 32,000

Figure 7. Sample taken on October 25th, 1971. Note the presence of SER near the cell membrane region. x 28,000

Figure 8. Sample taken on December 27th, 1971. The maturation of Golgi bodies and the presence of RER. x 36,000
Figure 9. Sample taken on March 7th, 1972. Note regional swelling of RER. The RER was present near Golgi bodies. x 28,000

Figure 10. Sample taken on March 7th, 1972. The formation of vacuoles caused by regional swelling of RER. The inner membrane of the plastid was built up. x 14,350

Figure 11. Sample taken on February 22nd, 1972. Vesicles pinched off from Golgi bodies dispersed toward the cell surface to fuse with the cell membrane. x 17,400

Figure 12. Sample taken on February 22nd, 1972. Golgi bodies pinched off more vesicles than ever before. These vesicles fused together to form a vacuole. x 28,000

Figure 13. Sample taken on December 27th, 1971. The presence of lysosome which originated from the Golgi body. x 82,500

Figure 14. Sample taken on January 3rd, 1972. The presence of lysosome with lipid droplets. x 28,000

Figure 15. Sample taken on January 3rd, 1972. The dispersion of lysosomes in the cytoplasm. Also note the digestive vacuole. x 32,000
Figure 16. Sample taken on January 3rd, 1972. Membrane undulation and digestive vacuoles. x 28,000

Figure 17. Sample taken on February 29th, 1972. Mitochondria in the cytoplasm. x 7,200

Figure 18. Sample taken on February 22nd, 1972. Mitochondria in cytoplasm. The forming face of the Golgi body was swollen, the maturing face was compressed so as to pinch off vesicles and the cisternae extended in a parallel pattern. x 32,000

Figure 19. Sample taken on October 25th, 1971. Proplastids look similar to mitochondria. x 18,000

Figure 20. Sample taken on March 7th, 1972. Amyloplast present. x 17,600

Figure 21. Sample taken on January 3rd, 1972. The presence of transparent parts in proplastids. x 6,500

Figure 22. Sample taken on November 6th, 1971. The presence of lipid droplets. x 5,760
APPENDIX IV

The method of the preparation of fixation solution, embedding medium, and staining solution.

**Fixation Solution**

**Karnovsky's solution**

Two gm of paraformaldehyde powder are dissolved in 25 ml water by heating to 60-70 C and stirring. One to three drops of 1 N NaOH are added with stirring until the solution clears. A slightly milkiness may persist. The solution is cooled, 5 ml of 50 per cent glutaraldehyde are added, and the volume is made to 50 ml with 0.2 M cacodylate or phosphate buffer, pH 7.4-7.6. The final pH is 7.2. If cacodylate is used, 25 mg CaCl₂ anhydrous is added.

**Embedding Medium**

**Spurr's medium**

The composition is: ERL-4206 (vinyl cyclohexene dioxide) 10 gm, D.E.R. 736 (diglycidyl ether of polypropylene glycol) 6 gm, NSA (nonenyl succinic anhydride) 26 gm, and S-1 (dimethylaminoethanol or DMAE) 0.4 gm. Dispense the components, in turn by weight, into a single flask and mix thoroughly.

**Staining Solution**

**Richardson's stain**

Mix equal volumes of 1% azur II in distilled water and 1% methylene blue in 1% borax solution.
Reynold's lead citrate solution

Lead citrate is prepared by placing 1.33 gm Pb(NO₃)₂, 1.76 gm Na₃(C₆H₅O₇)₂H₂O and 30 ml distilled water in a 50 ml volumetric flask. The resultant suspension is shaken vigorously for 1 minute and allowed to stand with intermittent shaking in order to insure complete conversion of lead nitrate to lead citrate. After 30 minutes 8.0 ml 1 N NaOH is added, the suspension diluted to 50 ml with distilled water and mixed by inversion. Lead citrate dissolves and the staining solution is ready for use. The pH of the staining solution was routinely found to be 12.0. Faint turbidity, if present, is usually readily removed by centrifugation. The staining solution, stored in glass or polyethylene bottles, is stable for a period of up to 6 months. With "aged" staining solutions it is advisable to centrifuge before use.
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