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ULTRASTRUCTURAL EFFECTS OF IONIZING RADIATION ON PLANT CELLS

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Abstract

Ultrastructural effects of ionizing irradiation were investigated on the specific constituents of plant cells, primarily in fruits, with reference to mushroom cells and comparable data in the literature. In the cell wall the dissolution of the middle lamellae, probably due to radiation damage of pectin, and irregular thickenings were found. In the vacuole the quantity of inclusions changed, or unusual aggregations appeared, presumably in connection with altered phenolic biosynthesis. In chloroplasts the senescence was inhibited, and dedifferentiation occurred into agranal state. In amyloplasts starch hydrolysis was hindered. These plastidial effects are considered as visible signs of inhibition of synthesis of proteases, the LHCII, or amylolytic enzymes, respectively. Some lines of evidence suggest that the target for irradiation was outside of the plastids in all these cases. Starch resynthesis was observed in apple epidermis as a synergistic effect of $CaCl_2$ treatment and gamma irradiation. In this case presumably the sugar recycled from the vacuole into the plastids due to altered membrane permeabilities.

Key words: cell wall, vacuole, phenolics, plastids, senescence, starch grains, gamma irradiation, radiation effects, electron microscopy, ultrastructure.

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Introduction

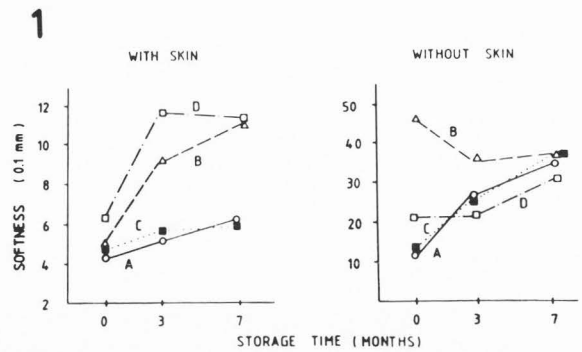
Plant cells are routinely irradiated by gamma rays in different foods (vegetables, fruits) for shelf life extension; such treatments, however, have been rarely accompanied by ultrastructural examination. On the other hand, basic research aimed at the subcellular effects of ionizing irradiation is carried out mostly on animal material. As a consequence, similar data on plant cells are limited. Collecting these data may be important, because the rapidly growing body of information in cell biology may serve as a basis for the interpretation or reinterpretation of earlier observations, while revealing new problems, further stimulating research on this topic.

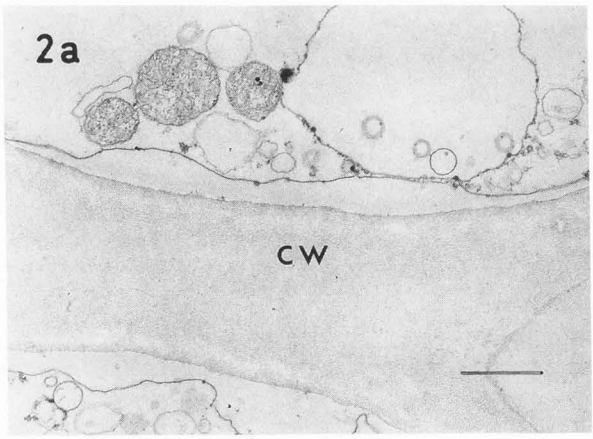
In this review we have concentrated on cell components specific to plants (cell wall, vacuole, plastids), and where possible, we have tried to relate the data to those from higher fungal cells.

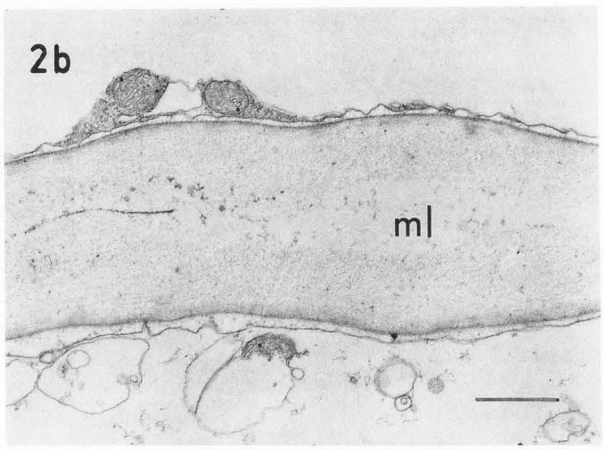
Alterations of the cell wall

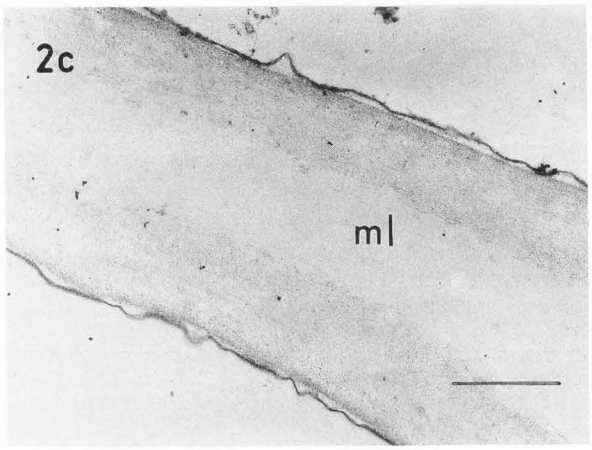
When fruits or vegetables are irradiated, four immediate responses have been found to occur: textural changes, decreases in ascorbic acid and sulphydryl content, production of ethylene and increase in respiration (Romani 1966). A textural change, i.e. a partial loss of firmness (Fig. 1) may be due to a decrease in turgidity caused by membrane permeability changes (for references see Romani 1966, Sommer and Mitchell 1986), as well as to alterations of the cell wall. These include random breaks in the glucosidic bonds of pectin (McArdle and Nehemias 1956, Skinner and Kertesz 1960, Somogyi and Romani 1964) and degradation of other components of the wall (Kertesz et al. 1964, Foa et al. 1980). Pectin molecules seem to be especially vulnerable to irradiation; decreases in their molecular weight were demonstrated at doses as low as 4 krad, while degradation of cellulose was found significant at a dose of 100 krad (Glegg and Kertesz 1956).

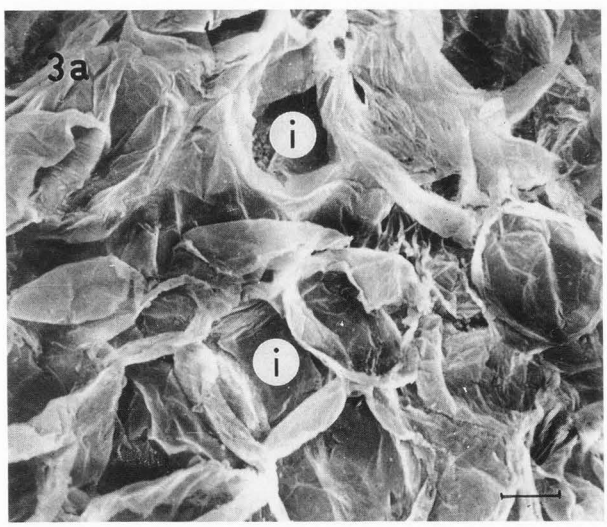
Changes demonstrated by TEM and SEM of cell walls (Kovács et al. 1988) may be the expressions of these molecular events. When the fruit flesh of irradiated, then stored Hardenpont pears was investigated by TEM, regions of the middle lamellae (normally rich in pectin) seemed to be dilated and "empty", as compared to both the fresh and











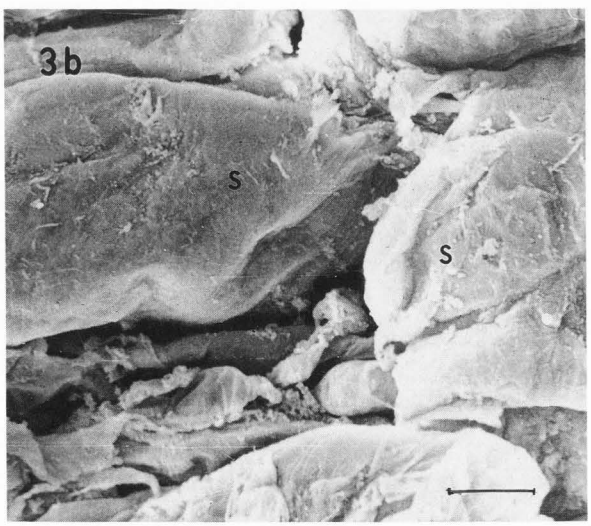
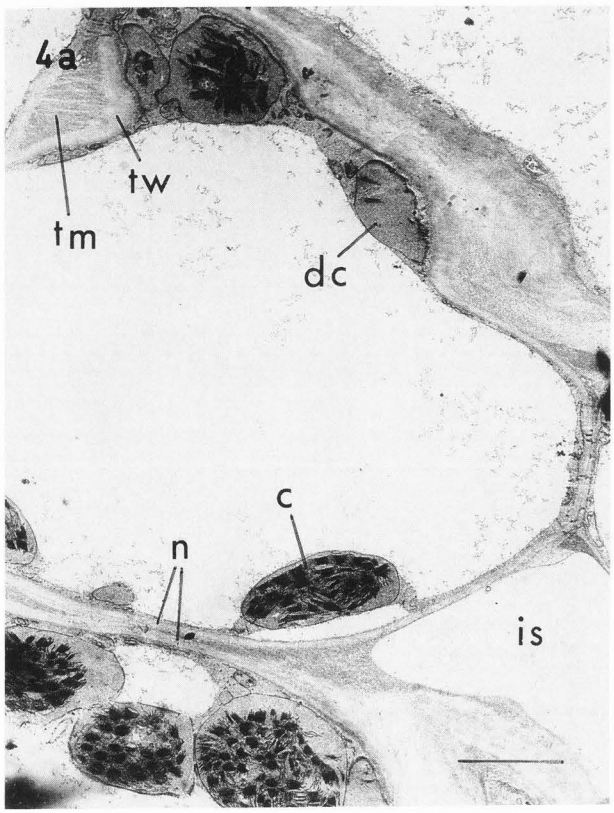


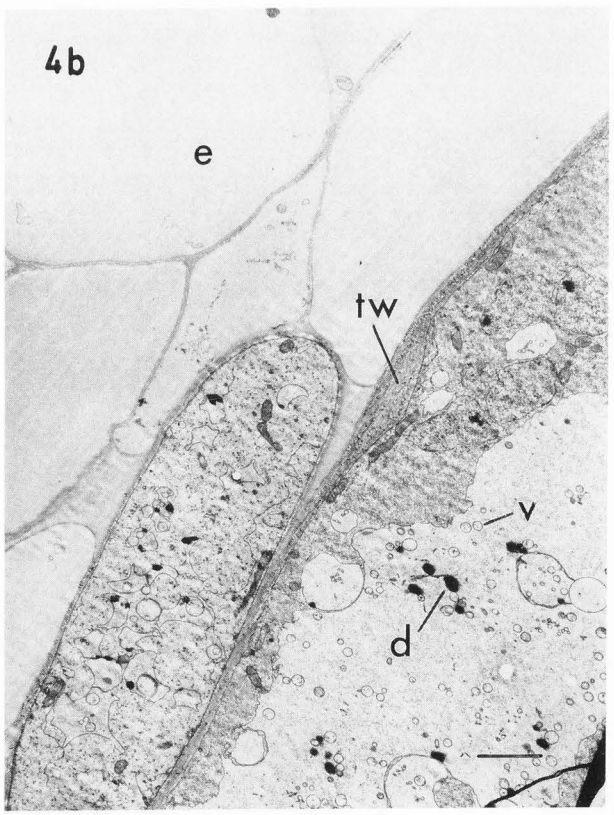
Fig. 1. Changes of firmness (expressed by penetrometric measurements of softness) in Gloster apple after different treatments, as a function of storage time. A= control, B= irradiated (1 kGy), C= calcium treated, D= calcium treatment combined with irradiation. (From Kovács et al. 1988).

Fig. 2. TEM of cell walls in the fruit flesh of Hardenpont pear. a= fresh control, b= stored (3 months) control, c= irradiated (1 kGy) then stored sample. cw= cell wall, ml= middle lamella. Here and in the other TEM micrographs, samples (unless specified otherwise) were fixed in glutaraldehyde and OsO_4, dehydrated in acetone, embedded in Spurr's resin, sections stained with uranyl acetate and lead citrate, then examined in a Tesla BS 500 electron microscope. Bars= 1 μm. (From Kovács et al. 1988).

Fig. 3. SEM of cells in the fruit flesh of Hardenpont pear. a= stored (3 months) control, b= irradiated (1 kGy) sample. i= cell interior, s= cell surface. Samples were fixed in glutaraldehyde and OsO_4, dehydrated in ethanol and amylacetate, then after critical point drying and gold coating examined in a JEOL JSM-50A scanning electron microscope. Bars= 50 μm. (From Kovács et al. 1988).

Fig. 4. Local thickenings of cell walls in Hordeum vulgare leaf mesophyll (a) and Agaricus bisporus stipe (b) after seed irradiation with fast neutrons (1130 rad), or irradiation with gamma rays (2.5 kGy), respectively. n= normal walls with normal middle lamella, tw= thickened wall, tm= thickened middle lamella, c= chloroplast, dc= defective chloroplast, is= intercellular space, d= dense body, v= vacuolar vesicle, e= empty cell. In Fig. 4a sample was fixed in $KMnO_4$, dehydrated in ethanol, embedded in Durcupan ACM. Bars= 3 μm. (4b from Keresztes and Kovács 1987).





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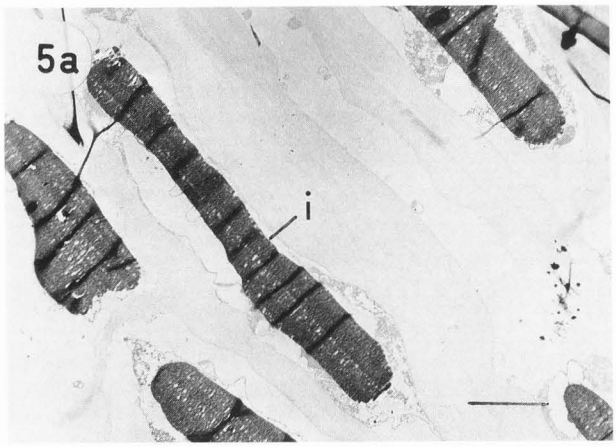
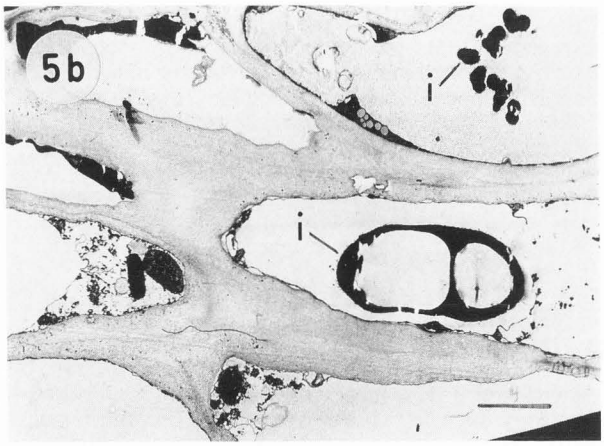
stored control (Fig. 2). When the same material was studied by SEM, in irradiated samples the adjacent cell walls seemed to be separated and the outer wall of most cells was seen, while adjacent cell walls in the control were connected, which resulted in cells being sectioned (Fig. 3). A similar degradation of middle lamellae was achieved both in apple and pear by polygalacturonase pretreatment of the tissue slices (Ben-Arie et al. 1979).

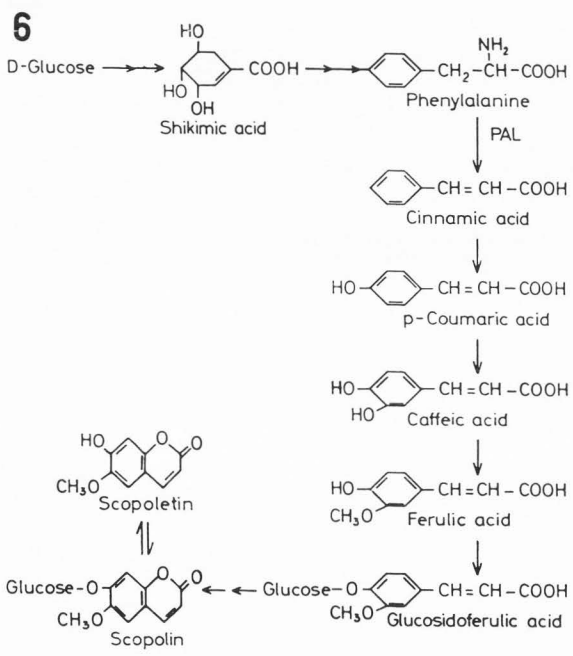
Another aspect of post-irradiation cell wall disturbances is thickening. In barley seedlings developed from seeds irradiated with fast neutrons (Kovács et al. 1979), unevenly thickened walls and middle lamellae appear in several groups of cells distributed randomly in the mesophyll (Fig. 4a). Similar local cell wall thickenings were encountered also in the stipe of gamma irradiated mushrooms (Keresztes and Kovács 1987, Fig. 4b). These thickenings may reflect an imbalance between cell wall synthesis and cell growth. In other cases, however, irradiation may stimulate cell growth, as giant cells were reported in Larix pollen (Eriksson et al. 1966) and Arachis suspension cell cultures (Verma and van Huystee 1971) after low or high doses, respectively. In our opinion it is possible that a disturbed division and subsequent increase in ploidy level are involved in such size increases.

Alterations in the vacuolar content

The vacuolar cell sap frequently contains phenolics. Some kinds of phenols form electron dense precipitate. Dense vacuolar precipitates







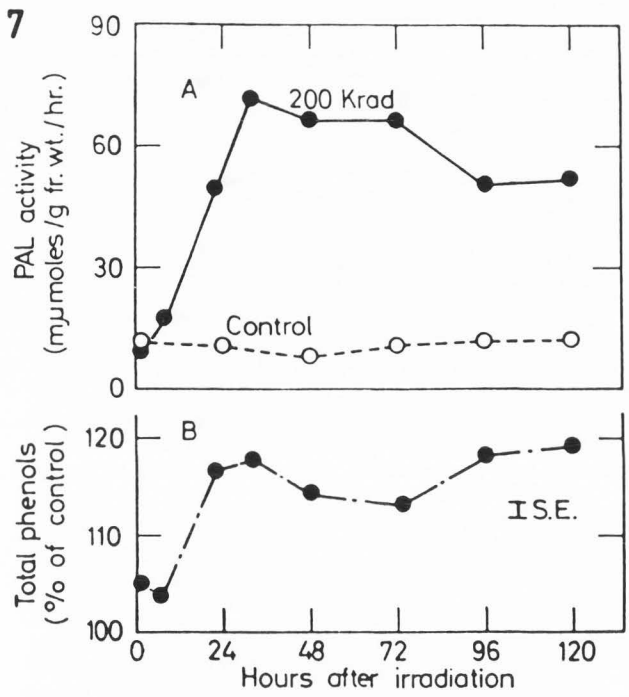
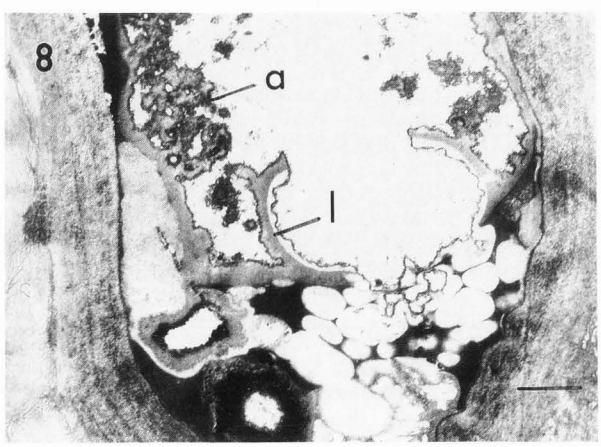


Fig. 5. Parts of the hypodermis from the stored (2 months) control (a) and gamma irradiated (1 kGy) sample (b) of Hardenpont pear. i= vacuolar inclusion. Bars= 5 μm.

Fig. 6. The pathway of phenolic biosynthesis showing scopolin and scopoletin formation. (From Riov et al. 1972).

Fig. 7. Effect of irradiation (200 krad) on PAL activity (A) and total phenols (B) of flavedo of Shamouti oranges. (From Riov et al. 1968).

Fig. 8. Epidermal cell from irradiated (1 kGy) Golden Delicious apple fruit. a= amorphous aggregate, l= layered aggregate. Bar = 1 μm.



usually occur in the peel of apple and pear fruits. Although these have not been directly identified, on the basis of phytochemical investigations by Williams (1960), Bain and Mercer (1963) inferred that these consisted of polyphenols.

In Hardenpont pear peel we found that the finely or coarsely granulated vacuolar precipitate aggregated into compact dense bodies during storing. After irradiation, however, smaller inclusions of variable shape and structure developed (Keresztes et al. 1989, Fig. 5). This may be a sign of a decreased formation of polyphenols, but not necessarily of phenolics in general.

Indeed, working with citrus fruit flavedo (the outer colored peel layer), irradiation proved to have a stimulatory effect on phenolic biosynthesis. Three compounds were found to accumulate markedly: scopolin, scopoletin and 6,7-dimethoxycoumarin (Riov et al. 1971, Riov et al. 1972, Fig. 6). This accumulation involved an increased amount of aromatic amino acids (Riov et al. 1972) and stimulated phenylalanine deamination, as measured by an increased activity of phenylalanine ammonia-lyase (PAL) (Riov et al. 1968, Fig. 7). The effect of irradiation on these events was mediated by ethylene production (Riov et al. 1970). This was in harmony with earlier reports on irradiated, wounded or infected plants producing more ethylene and exhibiting an increased PAL activity (for references see Riov et al. 1972). Still these findings are not easy to interpret if we consider that irradiation decreases total protein synthesis as measured by ^{14}C-leucine incorporation (Riov and Goren 1970). It seems that this biosynthetic pathway is somehow protected against radiation damage.

A practical consequence of these results is that a connection appears between irradiation induced peel damage (pitting) and the accumulation of phenolic compounds (Riov 1975). Other investigators related peel damage to an increased level of limonene, a volatile terpene, in different orange varieties (Belli-Donini et al. 1974, Belli-Donini and Baraldi 1975). Light microscopy and histochemistry showed, however, that the damaged cells were rich in phenolics and their pattern in the tissue was random, rather than coinciding with that of the oil cavities (Riov 1975).

In freshly harvested Gloster and Mutsu apple fruits epidermal and hypodermal cells contained dense vacuolar inclusions, which disappeared during storage, whether or not the fruits were irradiated (Kovács et al. 1986, 1988). In Golden Delicious apple epidermis, we observed the aggregation of a moderately electron dense material, often in bizarre forms, in or around the vacuole after gamma irradiation of 0.5 - 2 kGy dose (Fig. 8). Indeed, sometimes it is hard to tell, which side of the tonoplast the aggregation is attached to. Its appearance resembles that of the "material B" described by Bain and Mercer (1963) in superficial scald of Granny Smith apple; that material is believed to be an oxidation product of the normally occurring polyphenol in the vacuole. Authors could experimentally produce similar precipitations in the vacuoles by freezing and thawing the tissue or with chloroform vapour. All these data indicate that the precipitations are due to a non-specific stress reaction.

Probably another type of vacuolar inclusion appears in maize shoot apices after gamma irradiation. This seems to have affinity for RNA stains, rather than for protein stains by light microscopy (Graham and Generoso 1971). Electron microscopy revealed a quantitative difference between the treated and control groups, the dense lamellar inclusions were much larger after irradiation than normally (Graham 1972).

A well known function of plant vacuoles is autophagy and autolysis leading to partial or total digestion of the protoplasm (for references see Matile 1974). This activity was enhanced in Pleurotus hymenium, and in Agaricus gills and stipe after 2.5 kGy gamma irradiation (Keresztes et al. 1985, Keresztes and Kovács 1987). As seen in Fig. 4b, the vacuole contained smaller or larger vesicles and dense bodies, which may be a sign of autophagy, while empty cells in the vicinity show the end result of digestive processes. Ruptures of membranes and degradation of cytoplasm could also be seen in Golden Delicious apple fruit flesh after doses higher than 1 kGy (unpublished results).

Alterations of the plastids

Chloroplasts

Ionizing radiation inhibits chlorophyll synthesis; this was demonstrated in etiolated wheat and barley leaves (Gailey and Tolbert 1958, Sprey 1972), potato tubers (Mukhin and Sal'kova 1961) and Chlamydomonas y-2 strain (Kohn et al. 1967). On the other hand, exposure to doses of 30 to 50 krad delayed degreening of pineapple fruits (Upadhya and Brewbaker 1966) and bananas (Maxie et al. 1968). Investigation of irradiation effects on the structure, development and transformations of plastids may shed light on these phenomena.

In fruits with chloroplasts in the hypodermis at the time of harvest (e.g. Hardenpont pear) we found that gamma irradiation (1 kGy) altered chloroplast structure characteristically (Fig. 9). Plastids of the stored (2 months) control became senescent, as they contained several transparent plastoglobuli and few, weakly staining inner membranes. In the irradiated group, during storing, plastids retained a considerable part of their inner membranes in form of parallel, somewhat swollen thylakoids filled with an electron dense substance. In some plastids these thylakoids seemed to converge into a tubular complex. Grana stacks were entirely lacking. Accordingly, plastids were affected by irradiation in two ways: 1/ inhibition of senescence, 2/ dedifferentiation into the protochloroplast stage.

The mechanism, by which irradiation prevents senescence can be elucidated by the results of experiments with cycloheximide. Martin and Thimann (1972) found that this inhibitor of cytoplasmic protein synthesis delayed chloroplast senescence in detached oat leaves, due to the inhibition of synthesis of proteolytic enzymes that would normally enter the plastid and destroy its inner structure. The similarity of effects does not mean, however, that irradiation acts at

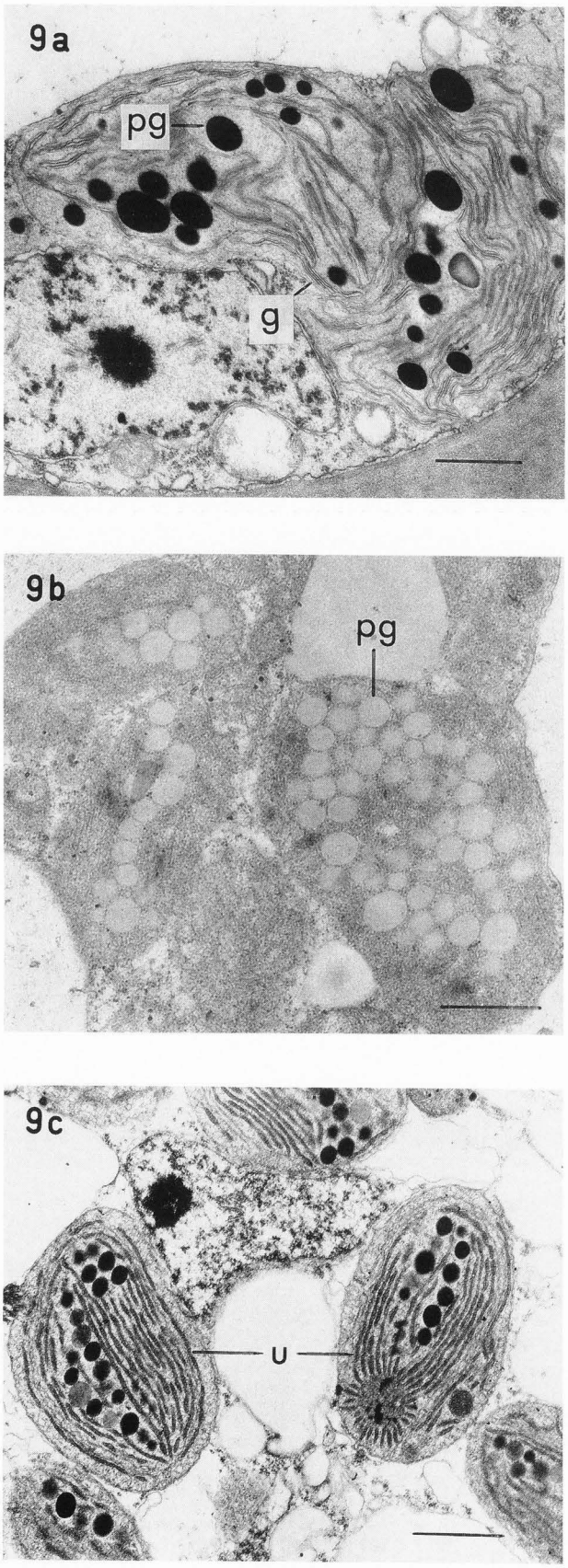


Fig. 9. Hypodermal plastids from the fresh control (a), stored (2 months) control (b) and irradiated (1 kGy) then stored sample (c) of Hardenpont pear fruit. g= granum, pg= plastoglobule, u= unstacked thylakoids. Bars= 1 µm.

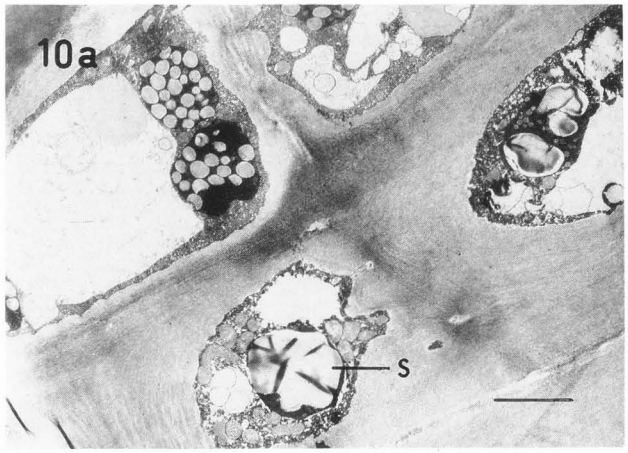
the same (transcriptional) level as cyclohexamide.

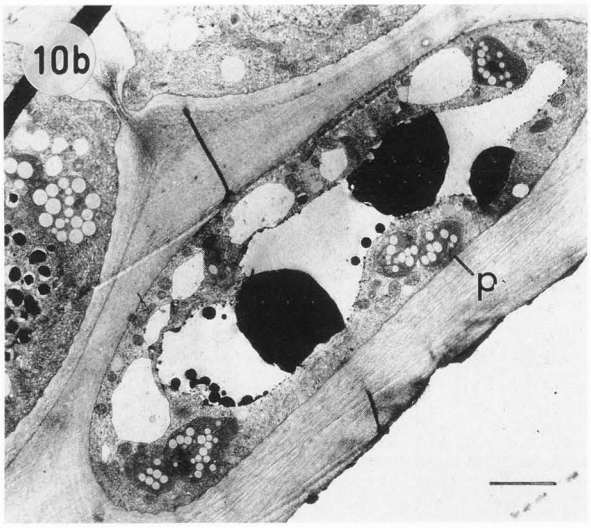
The developmental regression of chloroplasts can be assumed primarily from the destruction of grana. This is in line with the inhibitory effects of gamma or X-rays on granum development in etiolated bean and barley seedlings (Wrischer and Devidé 1967, Sprey 1972). Similar agranal plastids develop from proplastids, when seedlings are kept under intermittent light regime (Argyroudi-Akoyunoglou et al. 1971, Armond et al. 1976) and accordingly, these are called iml (intermittent light)-plastids or protochloroplasts. The component lacking in these plastids is the light harvesting pigment-protein complex (LHC II), which forms an antenna around photosystem II and also acts in membrane stacking, i.e., in granum formation (Argyroudi-Akoyunoglou and Akoyunoglou 1979, Akoyunoglou 1984, Zuber 1985, Thornber 1986). As LHC II is encoded by the nucleus (Cashmore 1984) and like the proteases, is synthetized in the cytoplasm, the target for irradiation is outside of the plastids. Similar protochloroplasts were found in the hypodermis of irradiated Golden Delicious apples (Kovács and Keresztes 1989).

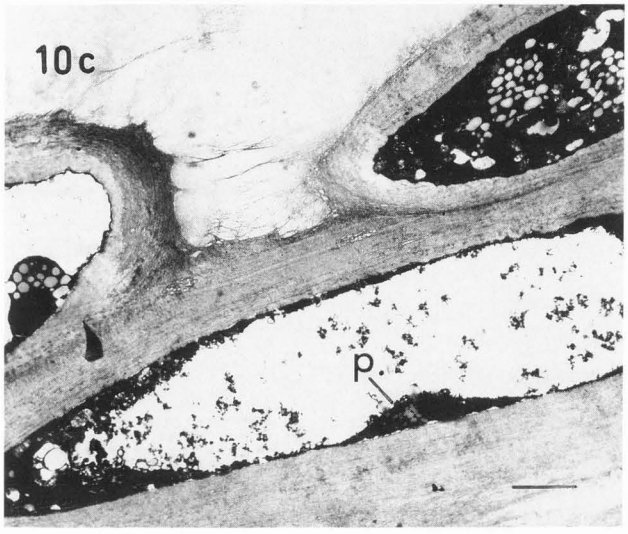
Apparently, when we irradiated hypodermal chloroplasts in fruits, we inhibited the synthesis not only of the proteolytic enzymes, but also of other proteins, including LHC II. This general inhibition of protein synthesis prevents plastid transformation from one type into the other, and this is why both greening and degreening could be inhibited by irradiation.

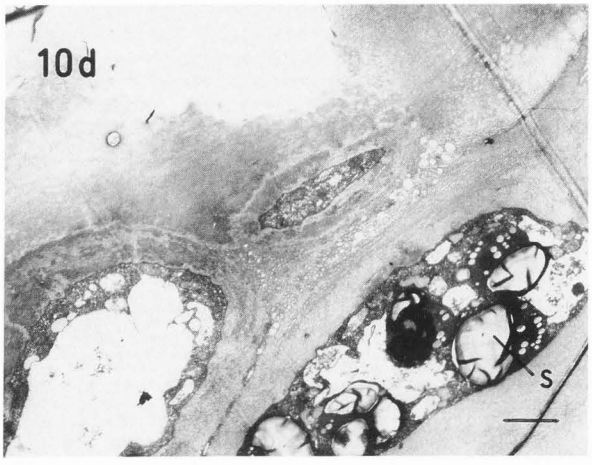
Amyloplasts

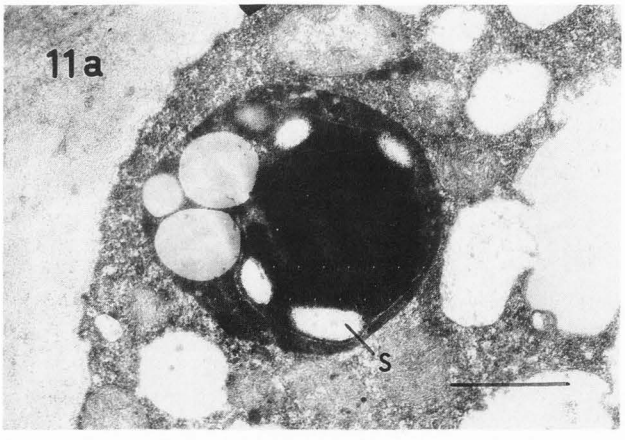
In fruits containing amyloplasts or chloroamyloplasts in their hypodermis at the time of harvest (e.g. Mutsu apple), gamma irradiation (1 kGy) preserved starch grains (Fig. 10). Starch was abundant in the hypodermis immediately after picking, but broke down after a few days. Cells became senescent by the end of storage. Irradiation, however, seemed to delay degradative processes notably including amylolysis, for at least 3 months. Persistence or even increase of starch content was found also in tomato microspores or in algae Brachiomonas submarina and Chlamydomonas reinhardtii after irradiation with fast neutrons, X or gamma rays (De Nettancourt and Eriksson 1968, Underbrink et al. 1969, Gruber et al. 1982). Preservation of starch grains is presumably due to the inhibition of synthesis of amylolytic enzymes. As the enzymes of starch metabolism are encoded by the nucleus and synthesized in the cytoplasm (Jacobsen and Knox 1973, Keresztes and Schróth 1984), the target for irradiation seems to be outside of the plastids again. De Nettancourt and Eriksson (1968) observed that irradiation was ineffective on starch hydrolysis in microspores if applied after the sixth day before anthesis (anthesis being the time of starch hydrolysis in their material), so we may assume that the transcription rather than











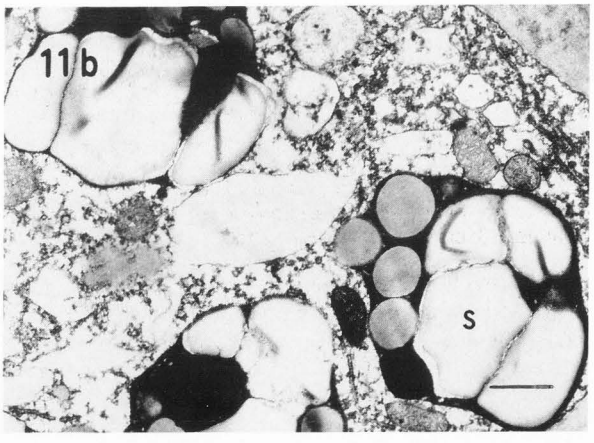


Fig. 10. Epidermal and hypodermal cells of Mutsu apple. a= fixed immediately after harvest, b= 4 days after harvest, c= stored for 3 months, d= irradiated (1 kGy) then stored for the same period. p= hypodermal plastid, s= starch grain. Bars= 3 μm.
Fig. 11. Epidermal plastids of Gloster apple after 1 kGy irradiation (a) and after $CaCl_2$ treatment + irradiation (b). s= starch. Bars= 1 μm.

translation was affected.

A more difficult problem is the irradiation induced synthesis of starch. We observed this in apple epidermis after the combination of gamma irradiation with $CaCl_2$ treatment (Fig. 11). Neither irradiation nor $CaCl_2$ was effective alone; the nature of this synergism is yet to be investigated. The appearance of newly synthesized starch is probably due to an increase in the substrate level, rather than in enzyme activities of the plastids, according to earlier in vitro experiments (Keresztes and Schróth 1979). In the apple fruit the substrate can be recycled from the vacuole into the plastids by the change of membrane permeability, which may be the effect of the combined treatment.

Embryonic plastids

It is worth mentioning that plastids may be also altered if plants are irradiated at the embryonic, rather than mature stage. With moderate seed irradiation (e.g. 500 rad), the chlorophyll content of the seedlings depended on the light conditions during cultivation. In the laboratory it was higher, whereas in the field it was lower than that of the control (Koepp 1978),which was considered as a sign of increased efficiency, reflected also by an increased yield (10-20%) of green mass (Degner et al. 1975). Irradiation of seeds with higher doses, however,may be mutagenic, resulting in an increased frequency of leaf variegation with decreased chlorophyll content and deficient plastids (Fig. 4a) in the pale parts (for references see Kirk and Tilney-Bassett 1978).

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Discussion with Reviewers

H.E. Gruber: You relate data on picked fruits to those on other cell systems. Could you briefly describe what normal cell functions can occur after the fruit has been picked?

Authors: Fleshy fruits undergo ripening under hormonal control (abscisic acid, ethylene), a process resembling normal senescence. Starch grains and to some extent pectins decay, sugars

accumulate. Chlorophyll content decreases, other pigments may increase. Respiration rises, exhibiting a climacteric maximum in several species. Prolonged storage leads to cell necrosis.

H.E. Gruber: In the various studies was irradiation applied immediately post-picking and irradiated and control tissues sampled at the same time?
Authors: Yes, we did so.

N. Rascio: The membranes of irradiated chloroplasts, besides the disappearance of thylakoid stacking, undergo also other modifications: aggregation in tubular complexes (probably related to the lipidic component changes), swelling, formation of electron dense intrathylakoidal substances. Are all these phenomena interpretable as due to effects of irradiation on the cytoplasmic compartment only?
Authors: We do not know. Not even the chemical nature of the dense intrathylakoidal substance is known. The hypothesis that it comprises membrane constituents (Salema et al. 1972, Damsz and Mikulska 1976), could not be confirmed by other authors (van Steveninck and van Steveninck 1980, Keresztes 1986).

N. Rascio: Why do you think that transcription was affected at starch hydrolysis? Do you know the length of time between transcription and translation, or the lifetime of the specific mRNAs either in the microspore or in the fruit?
Authors: These data are not known, but we think that if a two-step process can be inhibited in an early phase rather than in a late phase, then probably the first step is sensitive.

N. Rascio: Are you sure that the plastid marked with "dc" in Fig. 4a is really a deficient chloroplast? Could not be the absence of thylakoids due to a peripheral section plane?
Authors: If a barley leaf chloroplast at this developmental stage has such a large thylakoid-free section plane, it can not be normal. In addition, we have not seen cells of thickened walls without deficient chloroplast in this material.

N. Rascio: In this case two different plastid populations occur in the same cell, suggesting an irradiation effect on the plastid genome. Please comment.
Authors: Yes, we agree.

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