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EFFECT OF IONIZING IRRADIATION AND STORAGE ON MUSHROOM ULTRASTRUCTURE. I. THE GILLS OF *AGARICUS BISPORUS* (LGE.) IMBACH and *PLEUROTUS OSTREATUS* (JACQ.EX FR.) KUMMER

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Abstract

We have investigated the gills of both the control and gamma-irradiated groups of A.bisporus and P.ostreatus (2.5 kGy or 2.5 and 5.0 kGy doses, respectively) by scanning (SEM) and transmission (TEM) electron microscopy. The primary aim of our study was to see, how gamma-irradiation used for shelf-life extension inhibits spore production. We have found in both species that inhibition of spore production in irradiated specimens is caused by the destroying of basidia rather than by retarding normal spore development. In P.ostreatus the hymenium appears to be more sensitive to irradiation than in A.bisporus. In both species the subhymenium and trama seem less sensitive than the hymenium.

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Introduction

Staden (1964, 1965) was the first to report on a new method based on irradiation for the extension of storage life of mushrooms. Experiments have shown that irradiation inhibits the opening of the pileus and changes the sensory properties of the mushroom (Stoller 1968; Kovács et al., 1968, Kovács and Vas, 1970;1974a,b). An irradiation dose of 2.5 kGy strongly inhibited veil opening throughout the storage period (Wahid and Kovács, 1980). In the control samples the veil started to rupture on the first day, and on the second day all mushrooms were fully open. Spore formation was found to start while the caps were still closed. The beginning of opening coincided with the increase in spore count. After irradiation treatment not only does the pileus remain closed but the development of gills also stops, thereby inhibiting the formation and growth of spores as well (Kovács et al., 1981).

The question arises therefore whether the cessation of spore production is caused by lengthening of the juvenile stage of the cells, or due to the destructive processes, such as radiation damage.

In order to answer this question, we have performed scanning (SEM) and transmission (TEM) electron microscopic investigations on the gills.

Materials and Methods

Experimental materials and treatments

A.bisporus provided by Duna MgTSz (Budapest) and P.ostreatus provided by Borota MgTSz (Borota) were used for the experiments. From the freshly harvested young, closed A.bisporus carpophores, those of 3-5 cm diameter were selected for irradiation, while from P.ostreatus, 7-10 cm fresh fruit bodies were used.

Samples were irradiated at the Institute of Isotopes of the Hungarian

Academy of Sciences by ^{60}Co radiation source with a total activity of 3.7 pBq. A.bisporus was irradiated with 2.5 kGy, P.ostreatus with 2.5 and 5.0 kGy doses, the dose rate being 0.65 kGy x h^{-1}.

Carpophores were stored at 14-16 ^{o}C with a relative humidity of 90-95 % for 6 days.

SEM

Samples taken from the middle of the gills were fixed in cold 2 % glutaraldehyde dissolved in 0.14 M cacodylate buffer (pH 7.0) for 24 hours. After washing with the buffer, they were postfixed in cacodylate buffered 1 % OsO_4 for 2 hours, dehydrated in a series of ethanol and amyl acetate and dried through liquid CO_2 in a Dupont-Sorvall critical point drying apparatus. The samples were then coated with gold in a Zeiss HBA vacuum-evaporator and examined in a JEOL JSM-50A type scanning electron microscope at a 20kV accelerating voltage.

TEM

Samples were cut out from the middle of the gills (about halfway between the stipe and the brim of the pileus) and parts close to the edge of the gill were fixed.

The fixation and embedding procedure was based on the method used by Dieleman-van Zaayen and Igesz (1969) and on our earlier experiences with plant cells. Fixation was carried out in 6 % /v/v/ glutaraldehyde (in 0.035 M K-Na phosphate buffer, pH 7.2) for 2 hours at 4 ^{o}C. After thorough washing in the above buffer samples were postfixed in 1 % /w/v/ OsO_4 for 1.5 hours, dehydrated in an acetone series and embedded in Spurr's resin. Using flat molds, samples could be oriented so that the gill was always sectioned transversely. Sections were made with a Porter-Blum ultramicrotome equipped with an LKB glass knife, and after contrast-staining with uranyl acetate and lead citrate, were examined in a Tesla BS 500 electron microscope operated at 60kV.

Results

A.bisporus

SEM: In the fresh, closed control, developing basidia are seen, some of them with two protruding spores (Fig. 1 A). In the six day old control there are basidia with sterigmata which have lost their spores partly because of their maturity, or of preparation procedures (Fig. 1 B). In the six day old irradiated samples the basidia are deformed and the hymenium is discontinuous (Fig. 1 C).

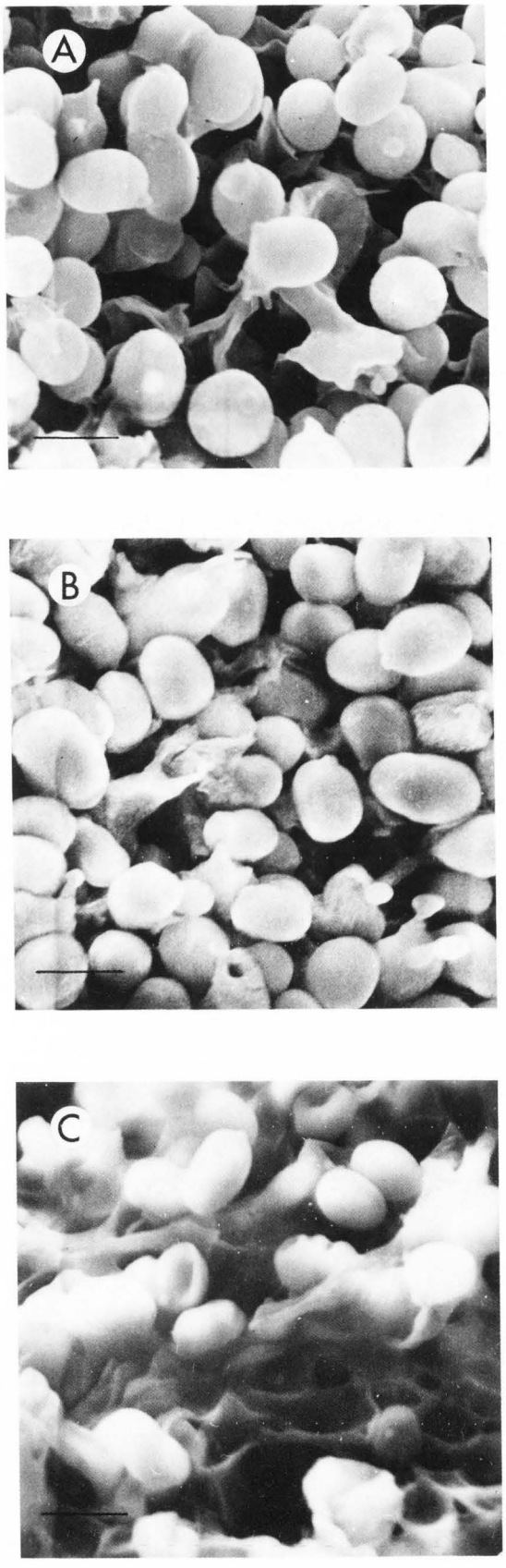
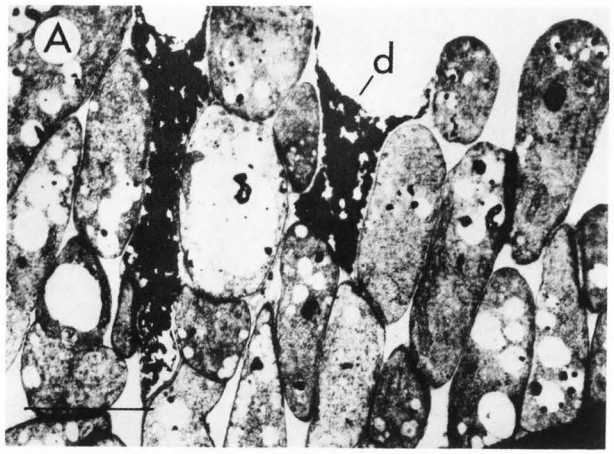
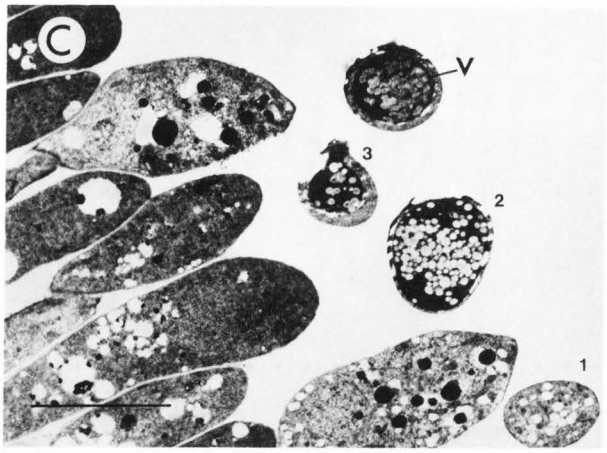
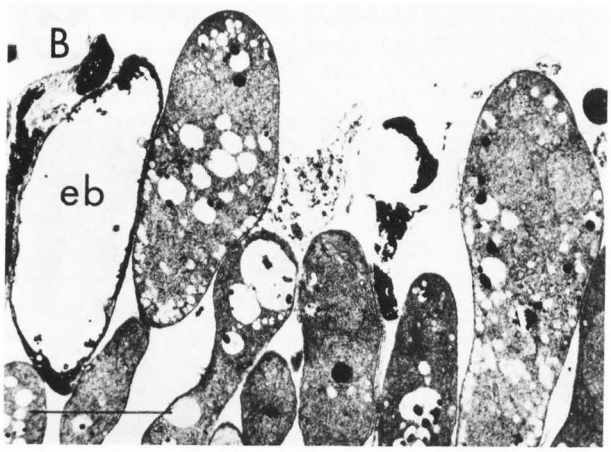


Fig. 1. SEM micrographs of the fresh control (A), stored control (B) and irradiated then stored (C) samples of A.bisporus. Bars equal 4 μm.







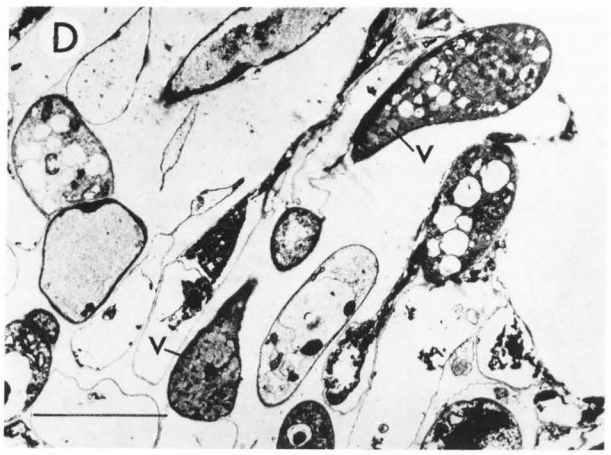


Fig. 2. TEM micrographs of the hymenium of A.bisporus. Bars equal 4 µm.
A: fresh, closed fruit body, d= dense cell
B: stored, open fruit body, eb=empty basidium
C: spore development on an open fruit body (from 1 to 3 the spores are in a developmental sequence), v=vesicle
D: irradiated then stored, closed fruit body, v=vesicle.

TEM: The hymenium of the fresh, closed carpophore consists of hypha cells elongated perpendicularly to the surface (Fig. 2 A). Basically two cell types build up this layer; convex cells with moderately electron dense plasm and the less frequent multiconcave cells with very dense plasm. We could not find forms intermediate between these two types. There occurred also cells with large vacuoles and rarely mature spores could be found on the surface. We identify the dominating cell type (i.e. the convex cells with moderately dense plasm) with developing basidia. They are connected with intermediate forms to the highly vacuolized cell type which is considered as the degenerative end stage of basidium development. The multiconcave dense cells may be cystidia (A. Keresztes, E. Kovács in preparation).

During six days of storage (and cap opening) the number of the vacuolized cells increased in the hymenium (Fig. 2 B). However, young developing basidia could be found even at this stage. The preparative procedures used permitted preservation of the inner structure of several spores (Fig. 2 C). Inside their thick walls vesicles dominate, probably containing storage lipids.

When storage was preceded by irradiation the number of vacuolized and necrotized cells increased dramatically (Fig. 2 D).

In some places the hymenium became discontinuous, or (mainly at the edge of the gill) separated from the hymenophoral part. Basidia (if not necrotized) attained an unusual structure characterized mainly by the accumulation of vesicles of medium density. Such masses of vesicles can be seen in spores (Fig. 2 C), which allows the supposition that certain cytoplasmic processes of spore formation may continue independently of the inhibition of nuclear processes in the basidium. Sometimes

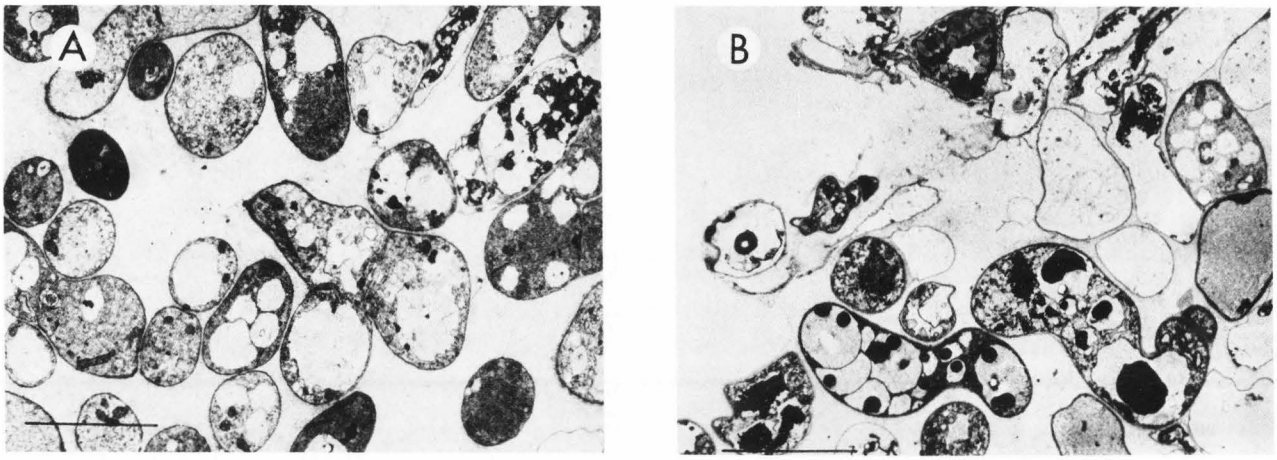


Fig. 3. TEM micrographs of the subhymenium and trama of the gill of A.bisporus. Hymenophoral cells in cross section, except the right upper corners of both micrographs, where parts of hymenial cells are in longitudinal section. Bars equal 4 μm. A: fresh, closed fruit body B: irradiated then stored fruit body.

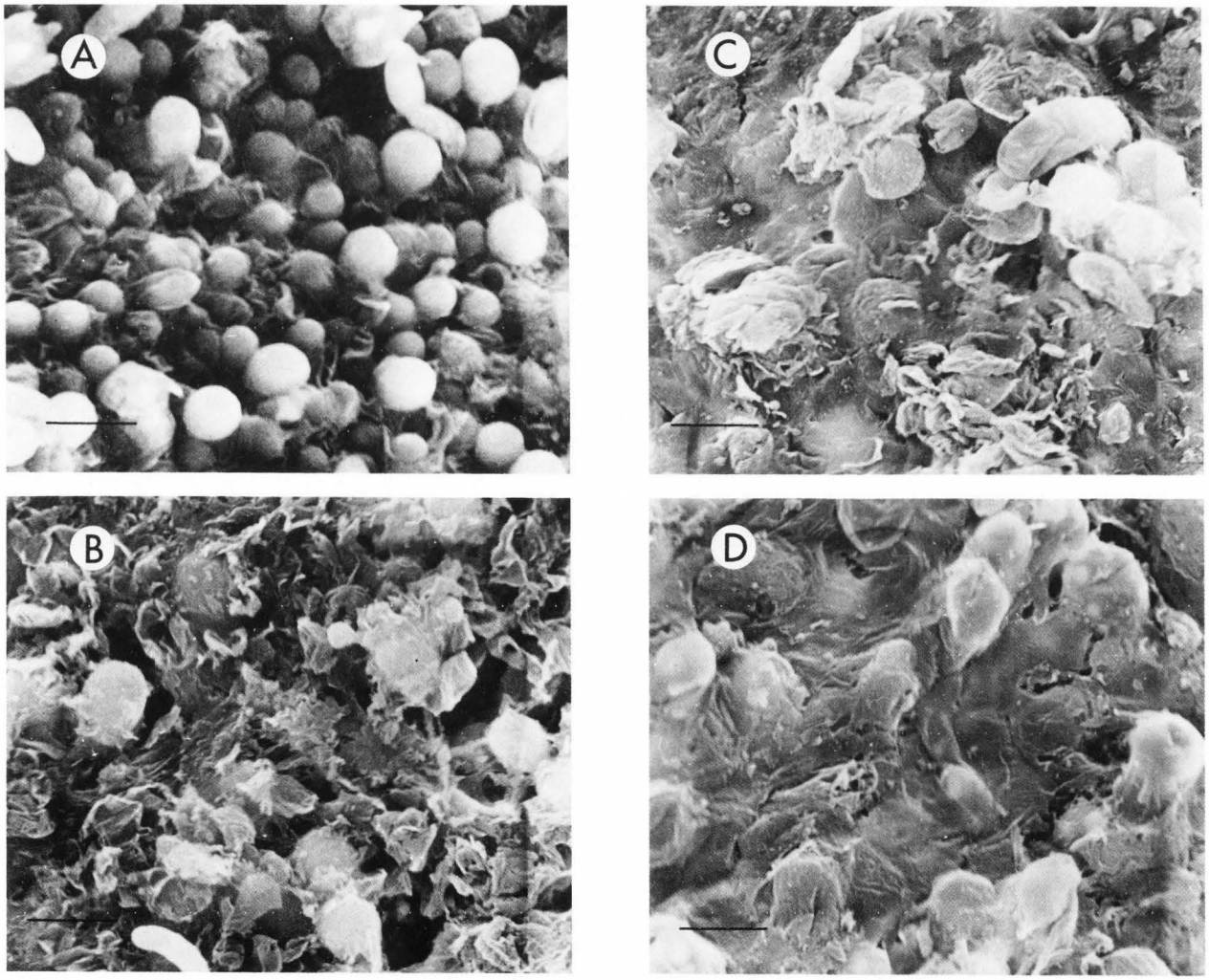


Fig. 4. SEM micrographs of the fresh control (A), stored control (B) and irradiated by 2.5 kGy in (C) or by 5.0 kGy in (D) samples of P.ostreatus. Bars equal 4 μm.

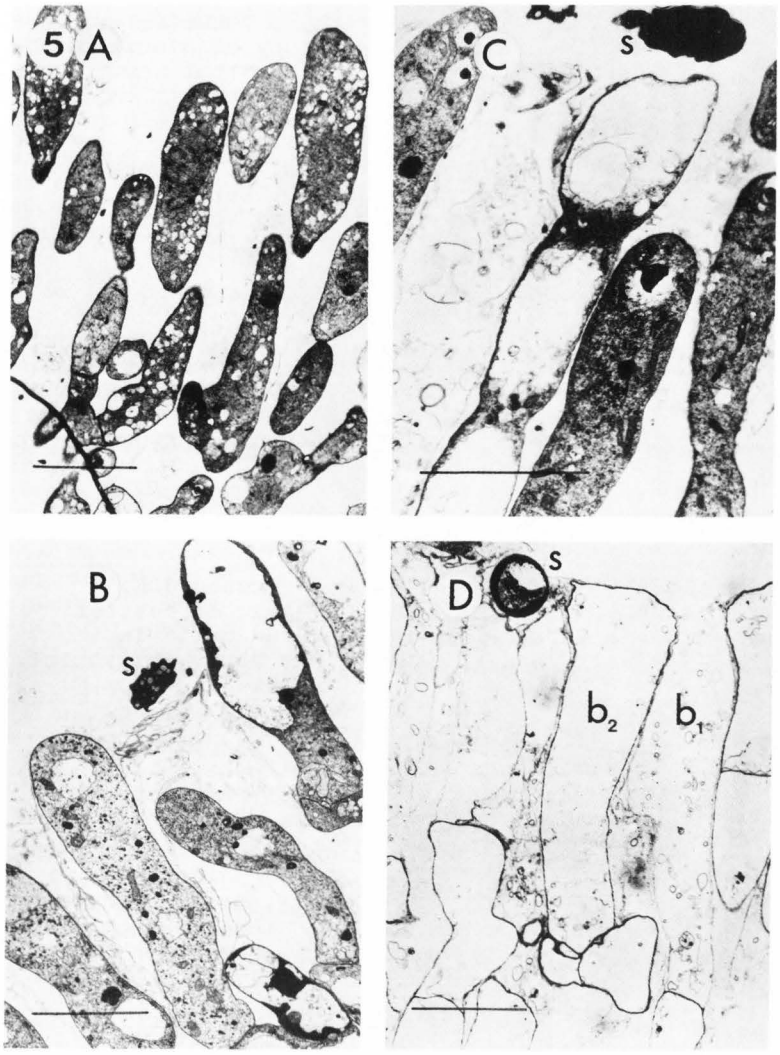


Fig. 5. TEM micrographs of the hymenium of P.ostreatus. s=spore. Bars=4 µm. A and B: fresh samples, C: stored sample, D: irradiated (2.5 kGy) then stored sample. Basidia necrotized before (b_1) or after (b_2) irradiation.

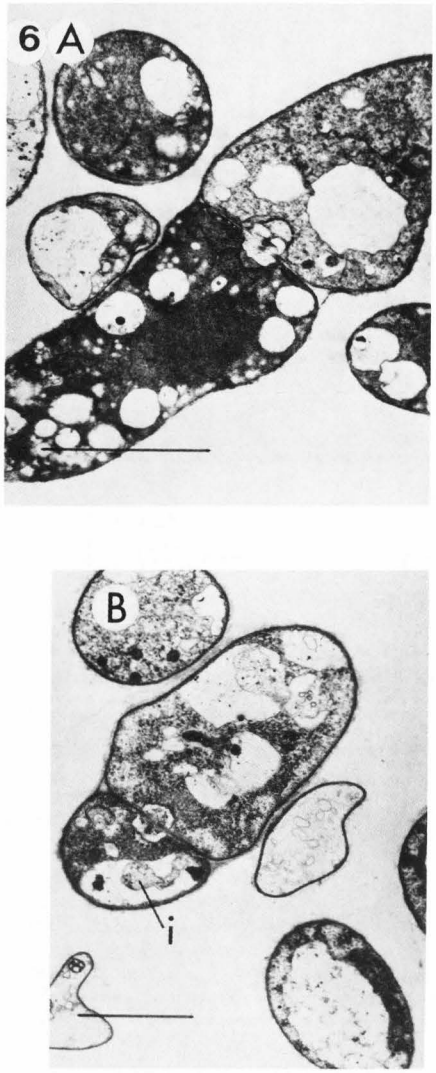


Fig. 6. TEM micrographs of the hymenophoral cells of P.ostreatus. Bars=2 µm. A: fresh sample, B: irradiated then stored sample, i=cytoplasmic intrusion.

apparently normal, mature spores were found in the sections, which may have formed before irradiation.

In the hymenophoral cells no significant changes were observed at the end of storage, so Fig. 3 A represents the ultrastructural features present in both fresh and stored controls. After irradiation (Fig. 3 B) many empty or even disintegrated cells occur in the subhymenium and trama. Also the plasma-containing cells show alterations; a relatively coarse cytoplasmic granulation as well as more frequent and larger dense bodies are present in the vacuoles.

P.ostreatus

SEM: In samples of fresh mushrooms basidia of different size are relatively close to each other (Fig. 4 A). In the stored control the surface is coated, the coat sparsely being broken through by basidia (Fig. 4 B). In the irradiated samples the basidia bulge out on the surface but do not seem to break through the coat. Basidia are less frequent than those of the control, their distribution is uneven, their shape sometimes being irregular (Figs. 4 C and D).

TEM: Hymenial cells of fresh P.ostreatus seem to be sparser than those of A.bisporus (Figs. 5 A and B). This is due to the presence of many necrotized and faint cell remnants among the living cells. The latter are all of the same cell type (basidia) as no idioblasts (e.g. cystidia) were found

among them. The fine structure of basidia, however, changed from sample to sample if they were taken from different freshly harvested carpophores. In one case, cells had moderately dense, finely granulated cytoplasm and numerous small vacuoles (Fig. 5 A). In another case, a more translucent cell content (possibly mictoplasm, see Angeli-Papa and Eyme, 1978) could be seen, with fewer vacuoles. In such sections there were also highly vacuolized basidia (Fig. 5 B). The reason for these differences may be that in P.ostreatus the age and developmental stage of fruiting bodies cannot be determined on a morphological basis. Therefore the carpophores used for sampling could be different in this respect.

As compared to the young samples of the fresh material, basidia in the stored material frequently had large vacuoles, sometimes with spores in their vicinity (Fig. 5 C). The spores seemed to be electron dense and flat in section, with a wavy outline (Figs. 5 B and C).

The effect of irradiation on cell structure was always pronounced (Fig.5 D). The hymenium consisted almost exclusively of empty cells, among which those necrotized after irradiation could be discerned on the basis of their denser wall from those necrotized earlier. The few plasma containing cells had a coarse granulation. Between some cells we observed unusual lateral fusion. The very few spores differed from those of the control.

The hymenophoral cells, although being mostly vacuolata did contain some cytoplasm and were less damaged than cells of the hymenium (Fig. 6). However, there were signs of destruction even in these cells, including coarse cytoplasmic granulation and intrusions into the vacuoles. These latter formations are regarded as the beginning of autophagy. Induced autophagy upon ionizing irradiation (X-rays or gamma rays) has been reported in other non-dividing cells or cell cultures (for references see Hamberg et al, 1976).

Conclusions

We have found in both species that ionizing radiation inhibits spore production by causing an abnormal development or necrosis of basidia rather than by the conservation of their juvenile stage.

In P.ostreatus the hymenium is more sensitive to irradiation than in A.bisporus. The hymenophoral cells in both species are less sensitive than the hymenium.

As the cellular responses of the gills cannot be generalized to other parts of the cap and to the stipe, we intend to investigate these parts of the carpophore after irradiation.

On the basis of these results, we regard electron microscopy as a suitable method to detect mushrooms that have received irradiation treatment.

Acknowledgements

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Discussion with Reviewers

D.A. Wood: Have the ultrastructural changes observed been correlated with any biochemical changes in the gill tissue?
Authors: According to our unpublished investigations the cytokinin level seems to decrease in the gills after gamma-irradiation.

D.A. Wood: Does storage temperature have any effect on the ultrastructural changes observed?
Authors: We have not investigated the temperature dependence.

T.Cayle: Is irradiation the only cause of the observed differences? I doubt it.
Authors: To our best knowledge irradiation was the only difference between the treated and the stored control groups.

E.A.Davis: Has irradiation been used by others in the early stages of mushroom growth to accelerate and shorten the growth time?
Authors: We do not know about such experiments but on the analogy of stimulation of plants we can imagine an accelerated growth of mycelia irradiated with low doses.