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IN VITRO DESTRUCTION OF THE EXINE OF RECENT PALYNOMORPHS II

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Abstract

We carried out the greater part of the experiments used first for the pollens of *Corylus avellana* on the pollen grains of *Taxus baccata*. The present results differ from those got on the pollen grains of *Corylus avellana*, namely well defined globular biopolymer units were not detectable on the experimentally degraded exine of *Taxus baccata*. In this way the present results support the statement of other authors obtained by chemical methods, there are differences in the chemical structures of the exines of the gymnosperms and angiosperms.
Key words: Taxus baccata, exine, experimental destruction.

Introduction

The first part of this series of publication (KEDVES, 1986) dealt with the most important results of the chemical composition of the sporopollenin reviewed. The present day state, and the problems of the researches of the molecular structure of the sporopollenin were also discussed. It is necessary to emphasize, in this place too, that the results got by different kinds of experiments gave different results. This is, in all probability a consequence of the complicated character of this field of investigations.

We cite the most important results of the earlier experiments on the pollen grains of *Corylus avellana* L., KEDVES (1986): "1. *Helix* enzyme with merkaptoethanol is suitable to decompose the sporopollenin of recent and fossil plant microfossils. 2. The partially decomposed wall, studied by the TEM method, may reveal the molecular structure of the sporopollenin. 3. Our results on recent *Corylus avellana* L. pollen grains suggest a globular structure of the biopolymers of the sporopollenin of this species." This paper, as the second part of this series of publications summarize the results achieved on the exines of the pollen grains of *Taxus baccata* L..

Material and Methods

Among the *Gymnospermatophyta* pollen grains an inaperturate type seemed to be the most suitable for the first experimental object. *Taxus baccata* L. from the *Taxopsida* was chosen, because this species have several peculiar characters, e.g.: the structure of the staminate inflorescence, lack of the cone, the characteristic arillus of the seed, and its alcaloids. In this way it may be presumed that there are differences in the chemical composition of the sporopollenin of the pollen grains of *Taxus baccata*, which will be appear in the molecular structure, too, in contrast to those demonstrated earlier.

Fresh pollen grains of *Taxus baccata* L. were collected 20 March, 1984 on Honvéd square, Szeged, by I. DÁVID. Only mature pollen grains, fallen out from the stamina were the subject of our experiments. The pollen material was placed into dark glass containers. The series of experiments are identical with series no 2. and 3. achieved earlier on the pollen grains of *Corylus avellana*. The so-called experimental series no 2, were complete on 15th May 1984, as follows:

T-5 — 20 mg. air dried pollen grains + 20 ml H_2O dest., temperature 30 °C, length of time: $2^h30'$.

T-5A — the same, only the length of time was 5^h.

T-7 — 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, temperature 30 °C, length of time: $2^h30'$.

T-7A — the same, only the length of time was 5^h.

T-9 — 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, + 20 µl merkapto-ethanol, temperature 30 °C, length of time: $2^h30'$.

T-9A — the same, only the length of time was 5^h.

T-11 — 20 mg. air dried pollen grains + 2 ml *Helix* enzyne 2% + 20 µl merkapto-ethanol + 20 mg. EDTA, temperature 30 °C, length of time: $2^h30'$.

T-11A — the same, the length of time was 5^h.

The second series of experiments (in the case of *Corylus avellana* the 3rd) were done on 26th September, 1984, as follows:

T-4b1 — 20 mg. air dried pollen grains + 1 ml merkapto-ethanol + 2 ml H_2O dest., temperature 30 °C, length of time: $2^h30'$.

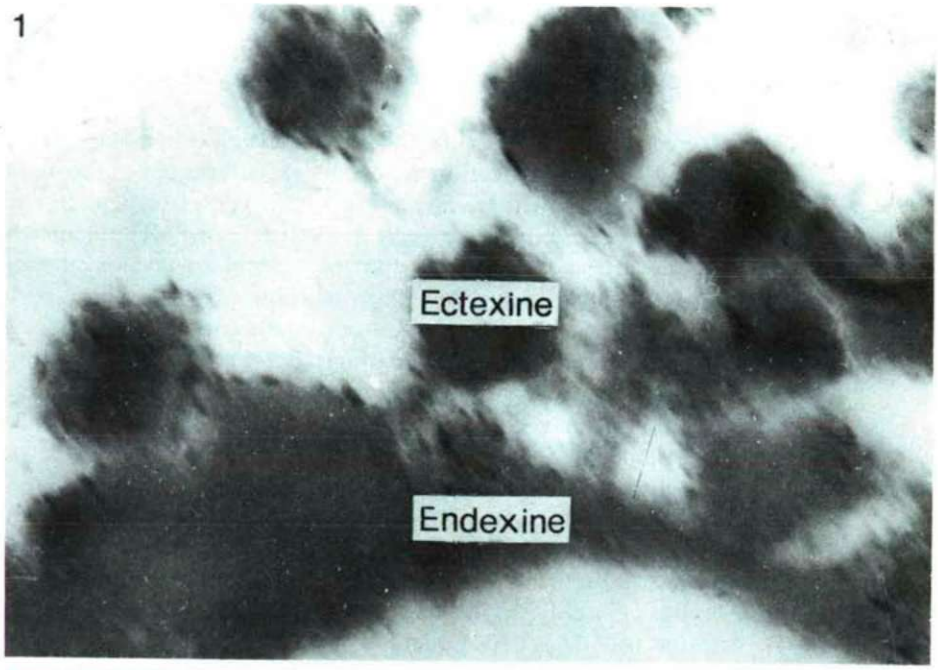
T-4b2 — the same, only the length of time was 5^h.

The procedures of the TEM method are identical with the earlier. So after the experiments the pollen material was washed with distilled water, fixed in OsO_4 (aqu. dil.) and embedded in Araldite (Durcupan, Fluka). Ultrathin sections were made on a Porter Blum ultramicrotome with glass knives. TEM pictures were taken in a TESLA BS-500 instrument, which has a resolution of 6 Å.

Plate I

Taxus baccata L.

1. Experiment T-5. Essentially the control infected microbiologically. Well is shown the degradation of the exine, the tiny particules may be microorganisms or the biopolymer units of the sporopollenin. The original lamellar ultrastructure of the endexine may not be discernible. x100000
2. Experiment T-5. General picture from the ultrastructure of the pollen wall. x5000
3. Experiment T-5A; the control of 5^h. The heterogeneous character of the microbial destruction of the exine is well shown on this photomicrograph. The lamellar ultrastructure of the endexine may hardly be recognized, and its electron affinity is stronger than that of the ectexine. x50000
4. Experiment T-5A. In opposition of the previous documentation the lamellar ultrastructure of the endexine is well preserved. Both principal layers of the exine are finely granular, these particules are presumably the globular biopolymer units of the sporopollenin. x100000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Ectexine
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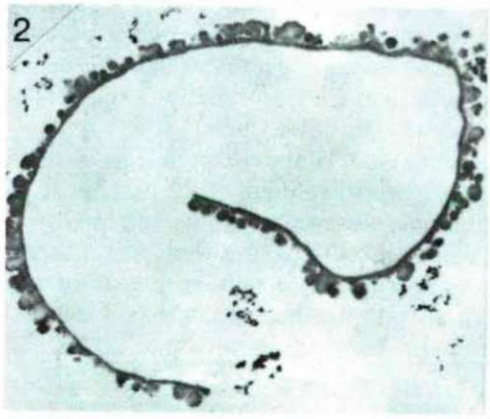
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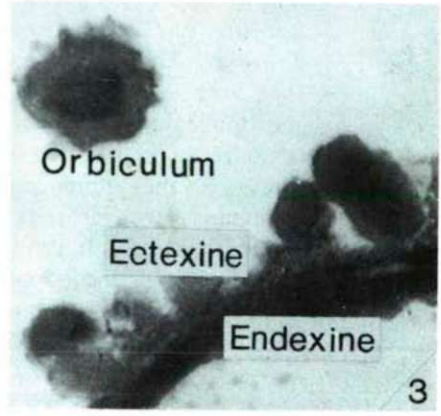

Orbiculum
Ectexine
Endexine
3


Ectexine
Endexine
4

Results

By the different experiments, the most important results may be summarized as follows:

T-5 — This is the control of $2^h30'$, its results are surprising (Plate I, figs. 1,2). The picture of low magnification gives a general aspect about the exine stratification of the pollen grain (Plate I, fig. 2). The picture, with high magnification represents a strongly degraded exine. The lamellar ultrastructure of the endexine may not be recognized on the ectexine there are particules with stronger electron affinity. During the experimental process this material was without doubt contamined, and the exine degraded in this way microbiologically. Concerning the ultrastructure of the non-degraded exine we refer to data published by AFZELIUS (1956) and GULLVAG (1966). The fine structure of the endexine is lamellar, the ectexine is composed of mostly isodiametric particules of various diameter.

T-5A — Essentially the same as previously but there were exines moderately degraded, namely in some cases the lamellar ultrastructure of the endexine was more or less discernible (Plate I, figs. 3,4).

T-7 — (Plate II, fig. 1) and T-7A — The two variants gave identical results. The ectexine was degraded moderately and uniformly; it contrast to this, the endexine dezorganized strongly. The characteristic lamellar ultrastructure of the endexine became homogeneous and partially destroyed. The electron affinity of the ectexine and the endexine at this experiment is identical. In spite of the strong degradation, well defined sporopollenin biopolymer units were not discernible.

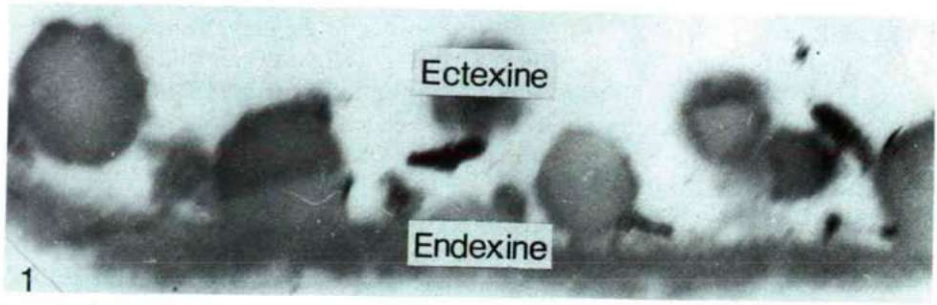
T-9 — (Plate II, fig. 2) and T-9A — The results of these experiments were the same, with the difference that the electron affinity of the homogeneous endexine is stronger in its total thickness, or only in its inner half part (Plate II, fig. 2). It is to be mentioned that the time factor is not considerable in these kind of experiments. It may be presumed that merkapto-ethanol in contrast to the previous results has a peculiar effect.

Plate II

Taxus baccata L.

1. Experiment T-7. The degradation of the exine is in general uniform, the lamellar ultrastructure of the endexine may not be discernible. x50000
2. Experiment T-9. Ultrastructure of a strongly degraded exine. The endexine is secondarily homogeneous, by its electron affinity two sub-layers may be distinguished. The electron affinity of the inner part is much more stronger than those of the outer part, this latter mentioned is identical in this respect with the exine. x50000

3,4. Experiment T-11A. The exine is moderately and uniformly degraded, the original, lamellar ultrastructure of the endexine is discernible in spite of its damaged character. x100000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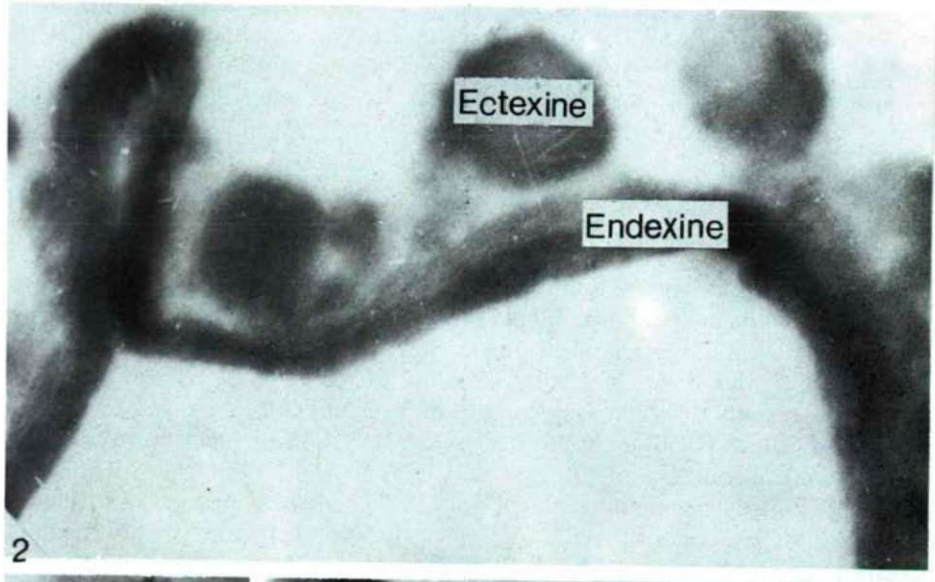
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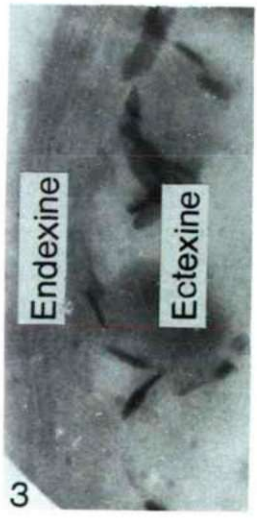
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Ectexine
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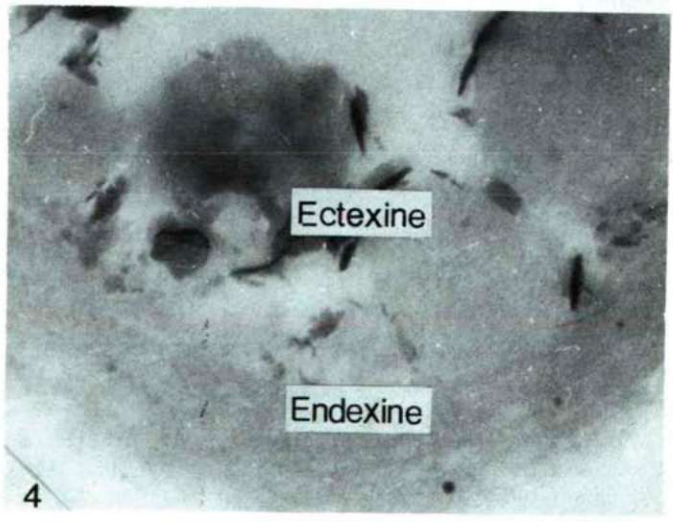

Ectexine
Endexine
4

T-11 and T-11A — (Plate II, figs. 3,4) — In contrast to the previous two experiments (T-7 and T-9) the exine degraded in a slight degree and uniformly. As regards the electron affinity of the different layers of the exine no differences were observable. The endexine was strongly degraded, but its original lamellar ultrastructure was observable on the biggest part of the ultrathin sections.

T-4b1 and T-4b2 — (Plate III, figs. 1-3) — This experiment produced a characteristic degradation. The endexine lost its lamellar ultrastructure, in some places, tiny granules occur (Plate III, fig. 3) which may be the globular biopolymer units of the sporopollenin. The electron affinity of the ectexine and the orbiculi are much more stronger than that of the endexine. Furthermore there are differences in the electron affinity of the wall of the orbiculi, a very narrow, inner layer has a stronger electron affinity than the thicker outer layer.

Discussion and conclusions

1. The experimental degradation methods, which resulted at the exine of *Corylus avellana* in well defined globular biopolymer units in the case of the *Taxus baccata* brought not a similar result. This relate that the chemical composition, and in consequence of this in the molecular structure of the exine of *Corylus* and *Taxus* there are essentially differences. In connection with this it is interesting to cite from the paper of UENO (1960) the following; p. 126/127: "The pigments were studied by SUITA (1948), KARRER and LEUMANN (1952) etc., and LUBLINER-MIANOWSKA (1955) investigated pigments in pollen grains of 67 species. According to him the pigments in pollen grains of conifers is not carotenoid, while that of entomophilous pollen of angiosperms is carotenoids." But BROOKS (1971) established as follows; p. 351: "The chemical study of various modern and fossil spore walls of gymnosperms, angiosperms, pteridosperms fungi and algae show a majority to be composed of sporopollenin.

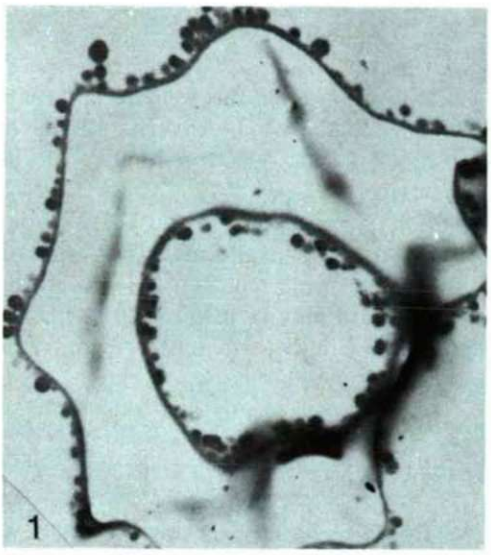
2. The effects of the degrading secrets used during the experiments are different. The *Helix* enzyme strongly degraded the endexine, but have not differentiate the electron affinity of the two principal layers of the exine.

Plate III

Taxus baccata L.

1—3. Experiments T-4b2

1. General picture from the exine ultrastructure of the pollen grain. x5000
2. Fine structure of the degraded orbiculum. x100000
3. Detail from the ultrastructure of the degraded exine. The electron affinity of the orbiculi and the ectexine is stronger than those of the endexine. In consequence of the degradation the lamellar ultrastructure of the endexine may not be discernible. x100000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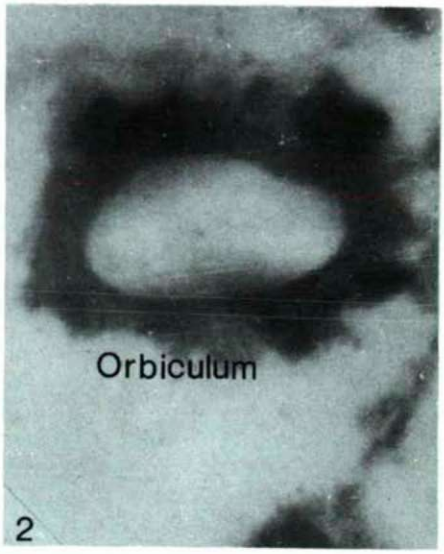
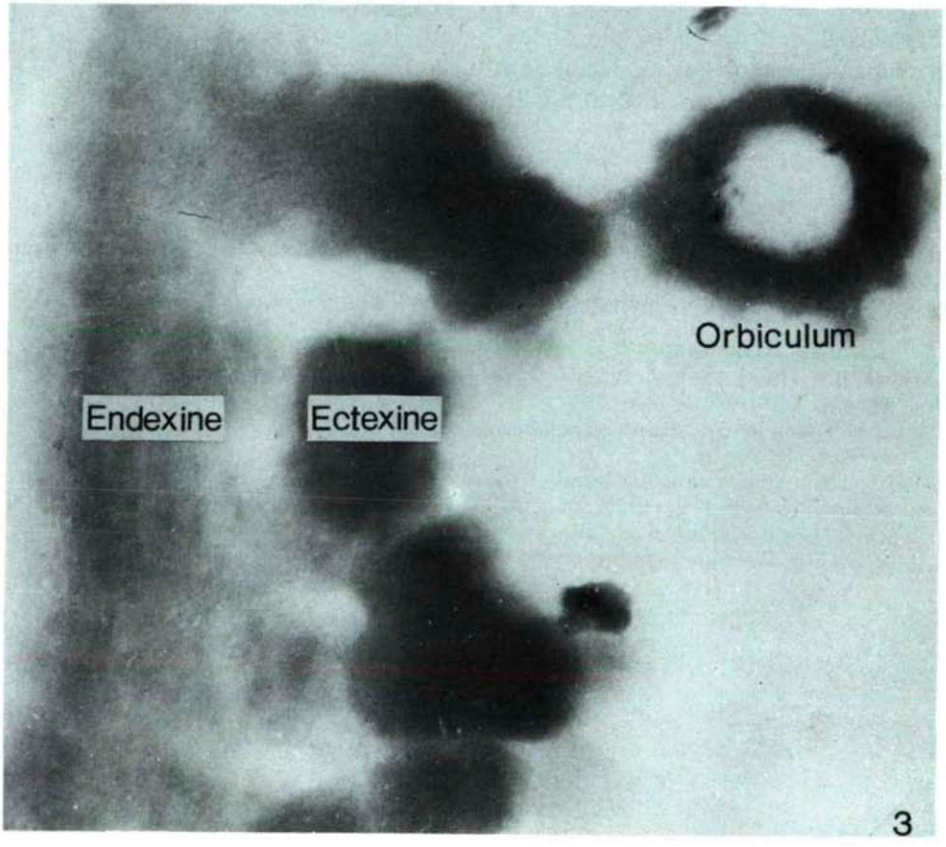

Orbiculum
2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3. The merkapto-ethanol cause such changes in the chemical structure which appear in the electron affinity of the two principal layers of the exine. The exclusive use of the merkapto-ethanol resulted that the electron affinity of the ectexine alternate stronger. The merkapto-ethanol, with *Helix* enzyme altered the electron affinity of the exine layers, but caused an opposing effect, so the electron affinity of the endexine became stronger than that of the ectexine.
4. The use of EDTA as a supplement of these experiments, on the basis of our present day knowledge moderate the effect of *Helix* enzyme and merkapto-ethanol.
5. The microbial degradation naturally was not projected, but it is warning concerning the control. On the other hand these data may be also useful in further researches. The experimental microbial degradation is in general another field of the researches of the exine.

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