1. HIGH TEMPERATURE EFFECTS ON THE SPORES OF EQUISETUM ARVENSE L.

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Abstract

Freshly collected spores of *Equisetum arvense* L. were examined by light microscopy after different exposures time of high temperature at 200 °C. Qualitative changes observed as follows: Elaters partially or completely separate from the spores. Perispore also folds out from the exospore. These changes and the alterations in maximum spore size are represented in diagrams. No difference is found between the quantitative changes at 200 °C with time, even after 300 hours.

Key words: Palynology, *Equisetum*, high temperature effect.

Introduction

During previous experimental investigations on the thermal alterations at high temperature of Recent pollen grains (KEDVES and KINCSEK, 1989, KEDVES et al., in press), we noted important changes in morphology, which reflect on taxonomy and phylogeny. Early results of similar experiments on spores, especially on the genus *Selaginella* (KEDVES, 1990), did not show equally important changes as a result of high temperature. Our research programme in this field includes all the most important groups of spores and pollen grains. Different concepts are involved, e.g. methods, taxonomy, phylogeny. As the present state of knowledge several problems of method are to be solved. The purpose of the present paper is partly to focus on methods and partly to study the pecularities of the *Equisetineae* in every respect. Morphological characteristics of the spores of *Equisetum* are dealt with in a previous paper (KEDVES, 1979). TEM data on the spore wall of the genus *Equisetum* were published by GULLVAG (1968), LUGARDON (1969), SAXENA (1980), and SEM data by KEDVES (1979). The biopolymer organisation of the sporoderm of *Equisetum* was studied by KEDVES and WINTER (1988).

Materials and Methods

The investigated material was collected by the senior author on 1. 4. 1989. Locality: left bank of the Tisza River. The spores were frozen at −20 °C after collection. For the experiments on high temperature effects 5 mg of spore material were used. Experiments were made as follows:

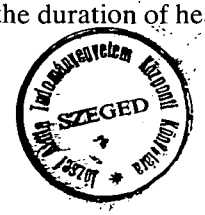
Number alloted to experiment	length of time	date
645	10'	1. 6. 1989
646	20'	1. 6. 1989
647	30'	1. 6. 1989
648	40'	1. 6. 1989
649	50'	1. 6. 1989
578	1h	3. 4. 1989
579	2h	3. 4. 1989
580	3h	3. 4. 1989
581	4h	3. 4. 1989
582	5h	3. 4. 1989
583	10h	8. 5. 1989
624	25h	8—9. 5. 1989
625	50h	8—10. 5. 1989
638	75h	1—4. 6. 1989
639	100h	1—5. 6. 1989
640	125h	1—6—6. 1989
650	150h	12—18. 6. 1989
761	200h	10—18. 12. 1989
762	250h	10—20. 12. 1989
763	300h	10—22. 12. 1989

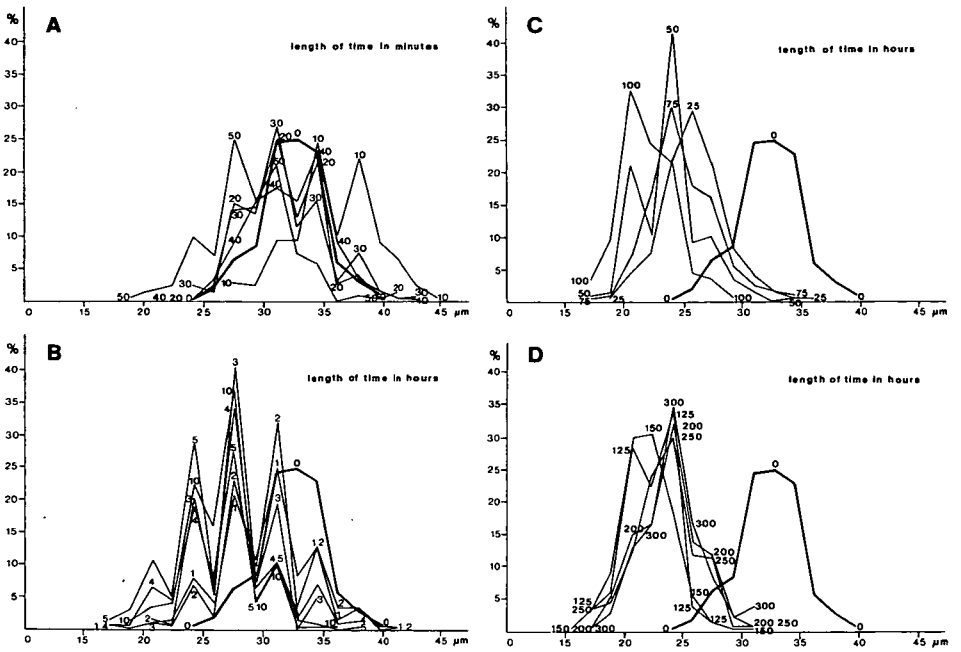
The slides for light microscopy were mounted in glycerin-jelly hydrated at 39,6%. 200 Specimens of each sample were investigated according to the following points of view: 1. Spore diameter. 2. Degree of degradation of the different wall layers, including elaters. 3. Thermal Alteration Index (TAI) in accordance with UTTING et al. (1989).

Results

1. Alterations in the diameter of spores (Text-fig. 1.1., A): After 10 min. at 200 °C the spore diameter increased, and two maxima appeared in the frequency distribution diagram. After 20 min. the spore diameter started to decrease. Indeed, after 20 min. heating the frequency distribution graph is nearly the same as that for fresh spores without heating. It is worth mentioning that the frequency distribution of the spores heated during 50 min. is the inverted graph of those heated for 10 min. The degradation of the different sporoderm layers is extremely peculiar (Text-fig. 1. 2., A). During these experiments an unexpectedly regular change has been registered. The highest quantity of complete sporoderms, i.e. exospore + perine + elaters, was observed in spores heated for 50 min. This quantity is higher than that found in spores that were not experimented upon. However, the frequency distribution of spores without elaters changes regularly in relation to the length of time, of heating. Perispore loss was not observed in these experiments.

2. Experiments during 1hr to 10hrs resulted in several maxima in the frequency distribution of the spore diameter (Text-fig. 1. 1., B). The decrease in spore size is more or less regular in relation to the duration of heating. In contrast (Text-fig. 1.



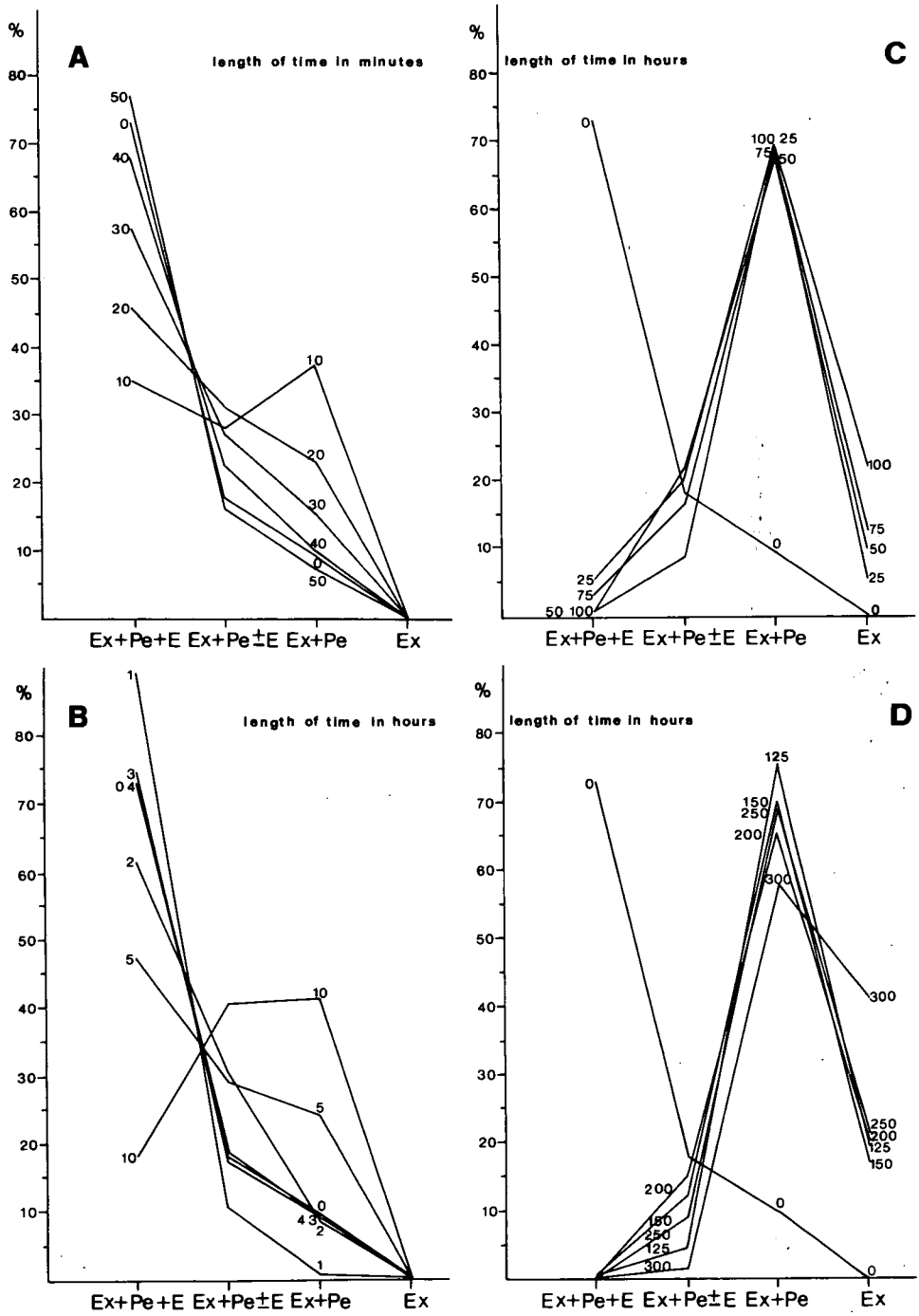


Text-fig. 1.1.
Variation-statistical diagrams of the spores investigated.

1., A), the frequency distribution graphs of spores subjected to experiments are quite different to those of fresh ones. The disappearance of the different sporoderm layers (Text-fig. 1.2., B) develops similarly (Text-fig. 1.2., A). Complete sporoderms were observed after heating for 1^{hr}. Heating during 3^{hrs} and 4^{hrs} brought nearly the same results as before heating. However, peculiar results were obtained after 2^{hrs}, and there the degradation of the sporoderm after heating for 5^{hrs}, and 10^{hrs} is remarkable. The frequency distribution graph of the latter is similar to that for 10^{min}.

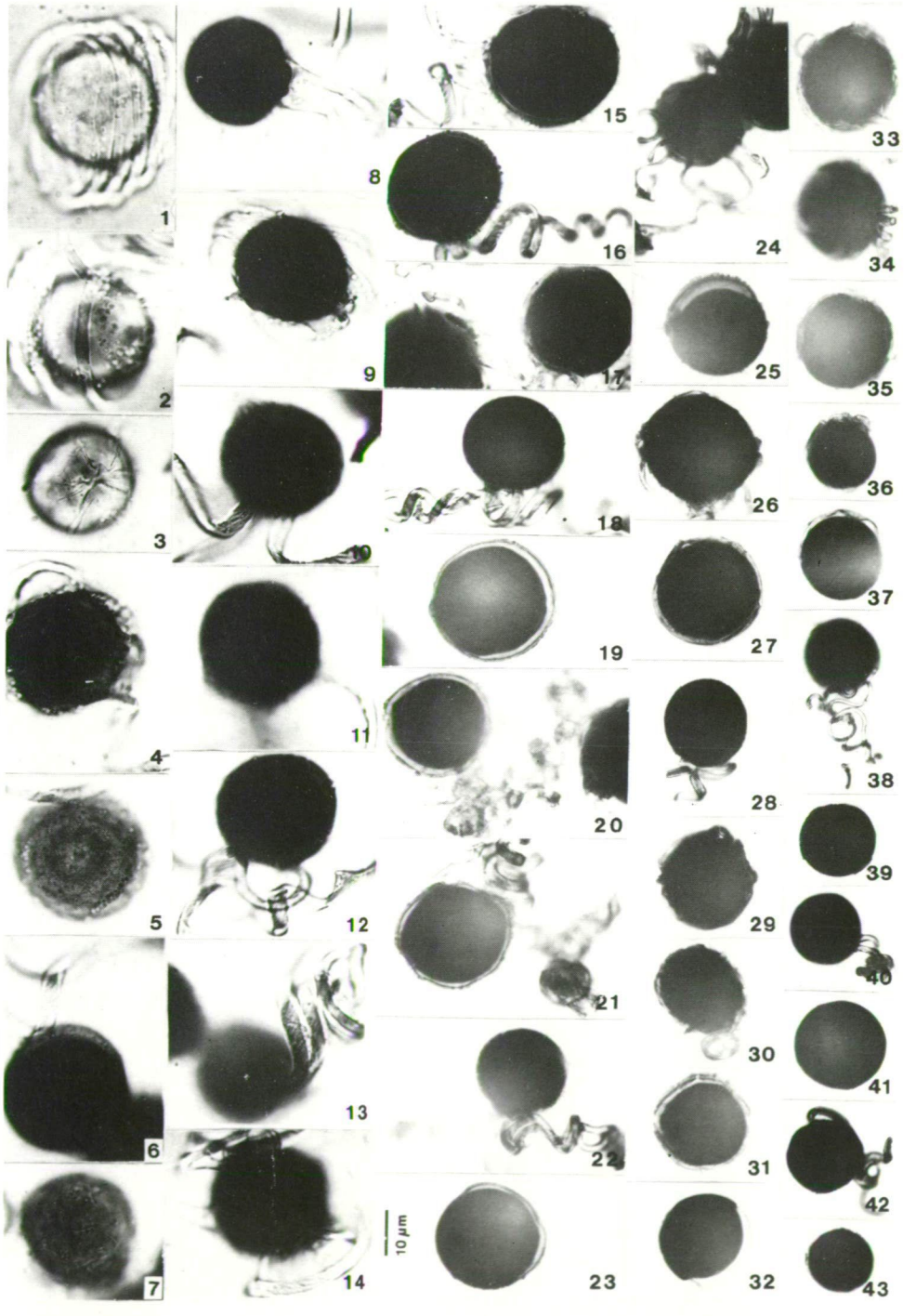
3. Heating the spores for 25^{hrs}, 50^{hrs}, 75^{hrs}, and 100 ^{hrs}, resulted in a more or less regular decrease in diameter. After 75^{hrs} the frequency distribution graph has two maxima, one of which corresponds to that of spores heated for 50^{hrs}. Changes in sporoderm degradation show about the same tendency. Very few spores bear elaters whereas most conserve perine. In these experiments the spores without perine appeared as a new form of degradation. The quantity of these spores changes regularly in accordance with the length of time of heating.

4. High temperature for 125^{hrs}, 150^{hrs}, 200^{hrs}, 250^{hrs}, and 300^{hrs}, did not result in a notable decrease in spore diameter. Regarding the detail, the two maxima in the frequency distribution graph at 125^{hrs}, and the two flat, more or less identical maxima at 150^{hrs} may be noted. In these experiments, the spores have practically all lost their elaters. However, the percentage of spores with perine (Text-fig. 1. 2., D)



Text-fig. 1.2.

Frequency distribution graph of the degradation process of the different sporoderm layers. E = elaters, Pe = perispore, Ex = exospore.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◀ Plate 1. 1.

1—43. *Equisetum arvense* L., Recent.
1—3. Spores without staning or heating.
4, 5. Experiment No 645, length of time 10 min.
6, 7. Experiment No 646, length of time 20 min.
8, 9. Experiment No 647, length of time 30 min.
10, 11. Experiment No 648, length of time 40 min.
12, 13. Experiment No 649, length of time 50 min.
14, 15. Experiment No 578, length of time 1 hr.
16, 17. Experiment No 579, length of time 2 hrs.
18, 19. Experiment No 580, length of time 3 hrs.
20, 21. Experiment No 581, length of time 4 hrs.
22, 23. Experiment No 582, length of time 5 hrs.
24, 25. Experiment No 623, length of time 10 hrs.
26, 27. Experiment No 624, length of time 25 hrs.
28, 29. Experiment No 625, length of time 50 hrs.
30, 31. Experiment No 638, length of time 75 hrs.
32, 33. Experiment No 639, length of time 100 hrs.
34, 35. Experiment No 640, length of time 125 hrs.
36, 37. Experiment No 650, length of time 150 hrs.
38, 39. Experiment No 761, length of time 200 hrs.
40, 41. Experiment No 762, length of time 250 hrs.
42, 43. Experiment No 763, length of time 300 hrs.

is nearly the same as previously (Text-fig. 1. 2., C), with the exception of spores heated for 300^{hrs}. Percentual changes in spores without perine are more or less regular except for spores heated during 300^{hrs}.

"Thermal Alteration Index" values are as follows. N. B. — S = spore wall, exospore and perine, E = elaters.

$0 (S = 1, E = 1), 10' (S = 1+, E = 1), 20' (S = 2, E = 1+), 30' (S = 2, E = 2-), 40' (S = 2, E = 2-), 50' (S = 2+, E = 2), 1^h (S = 1+, E = 1), 2^h (S = 2, E = 1), 3^h (S = 2+, E = 2), 4^h (S = 2+, E = 2), 5^h (S = 3-, E = 2+), 10^h (S = 3-, E = 2+), 25^h (S = 3-, E = 3-), 50^h (S = 3-, E = 3-), 75^h (S = 3, E = 3), 100^h (S = 3, E = 3), 125^h (S = 3, E = 3), 150^h (S = 4-, E = 3), 200^h (S = 4-, E = 3), 250^h (S = 4-, E = 3), 300^h (S = 4-, E = 4-).$

These data refer to the following: the colour of the spores changes gradually corresponding to the length of time of heating. As departures from gradual changes one notes the results after 50^{min}, 1^{hr}, and 2^{hrs}.

Discussion and Conclusions

New results are as follows.

1. Taking into consideration the irregularities in the results of the different experiments the following can be presumed: The diagenesis of the chemistry of the spore-pollen wall was not completely interrupted by freezing at −20 °C, and the experiments were not all made at the same time. Another thing is there may also have been differences in the maturity of spore samples in spite of the careful collection of the experimental material.

2. In a previous paper (KEDVES et al., 1990), dealing with inaperturate gymnosperm pollen grains the separation of non-experimental and experimental frequency distribution graphs has been noted. With *Taxus baccata* this is between 50—100 hrs with *Juniperus virginiana* at 125—150 hrs. As was pointed out previously with spores of *Equisetum arvense* heated for 300 hrs this has not happened. Probably, this phenomenon is a consequence of the relatively thick wall of *Equisetum* spores. Taking into consideration the tendencies of the frequency distribution graphs it may be assumed that after a certain diameter/wall thickness ratio the separation of the non-experimental and experimental frequency distribution graphs occurs. This problem needs further investigation.

3. The two maxima occurring occasionally in the frequency distribution of the spores of *Equisetum arvense* after some experiments probably relase to the "sexual dimorphism" of the homosporous spores of this genus, cf. PIÉRART (1974).

4. Spores without elaters or without perispore do occur in fossil material. In this way the altered *Equisetum* spore can be similar and/or identical with some algal cysts.

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