8. EXPERIMENTAL INVESTIGATIONS ON THE POLLEN GRAINS OF MALVA SYLVESTRIS L. AND HIBISCUS SYRIACUS L. I.

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Abstract

The allergenic pollen grains of *Malva sylvestris* L. and as comparative material, that of *Hibiscus syriacus* L., were partially degraded with 2-aminoethanol during 30 minutes, 1, 5, 10 and 24 hours and investigated by the LM method. Dissolution of the mucilage drops and alterations in the general aspect of the pollen morphology, diameter, length of spinae and the size of the apertures were investigated. In general pollen grains of both species are resistant to the experimental processes employed. Important alterations in the general aspect of the pollen grains were observed after 10 hours and in particular, 24 hours of treatment.

Key words: Experimental Palynology, recent, Malvaceae, LM method.

Introduction

The characteristic pollen grains of the family, Malvaceae have been the subject of several studies: following THANIKAIMONI (1972, 1973) the first palynological data from the genus *Malva* were published by FRITZSCHE (1832) and MOHL (1835). For the genus *Hibiscus* the bibliographical data of FRITZSCHE (1832) and HASSALL (1842) may be mentioned based on the Bibliographical index of THANIKAIMONI (1972, 1973). Several bibliographical data and LM morphology of the Malvaceae pollen grains were published by ERDTMAN (1952) also. As important papers in this subject the following are selected: LANG (1937), ERDTMAN and VISHNU-MITTRE (1958), ERDTMAN (1959), SAAD (1960), ERDTMAN, BERGLUND and PRAGLOWSKI (1961), FREYTAG (1964), GULLVÄG (1964), SOWUNMI (1973), UENO (1978), GOETZ (1982), CHRISTENSEN (1986A), CULHANE and BLACKMORE (1988) and LA SERNA RAMOS and DOMINGUEZ SANTANA (1991). In the monograph of CHESTER and RAINE (2001) there are important informations on the Malvaceae pollen grains too.

This object of the investigations is contribution to the ongoing studies of pollen degradation in various taxa.

Materials and Methods

The investigated material was collected in Szeged on the 22.09.2000, by Miss K. PRISKIN, and Miss Zs. IMRE 30 stamens were used for the experiments. Experimental parameters inwolved were: Temperature (30 °C), length of time (30 minutes, 1, 5, 10 and 24 hours). The pollen grains after washing were mounted in glycerine jelly hydrated for 39.6% (LOBREAU, 1966). The following characters were measured: diameter of the pollen grains, length of the spinae and the size of the apertures.

General problems

Following KHAN (1992) the Malvaceae originated in the southern Gondwana continent and underwed speciation at two centres, Neotropical and Australian-Oceania. The evolutionary trend of the Malvaceae pollen grains is from ancestral tricolporate pollen type with small spines to a large polytreme type with long spines (CHRISTENSEN 1986b). NAIR (1958) established, that the pollen grains of *Malva parviflora* LINN. collected from Kashmir are ornamented with two types of spines (those with pointed apices, and those with blunt apices). He pointed out, that it is difficult to explain the dimorphism, but as a possible explanation is that, this is a consequence of some meiotic disturbance. SRIVASTAVA (1982) compiled an evolutionary trend of spines of the different taxa of the Malvaceae, as follows: blunt tips to pointed tips. Unbranched to branched. HANKS and DRYXELL (1979) investigated 9 characters of some Malvaceae pollen grains. MATEU, GÜEMES and SALVADOR (1988) used the following characters during its investigations on the pollen grains of Malvaceae from Valencia: diameter, characters of the spinae diameter of the apertures. Interesting schemas of the spinae morphology were published. LA SERNA RAMOS and DOMINGUEZ SANTANA p. 106 (1991) who used the pollen biometry method of different populations of the species of the genus *Lavathera* L. in the Canary Islands. New morphological data from the pollen grains of *Hibiscus syriacus* L. were published by MAR TRIGO, GARCIA and CABEZUDO (1994).

TAKAHASHI and KOICHI (1988) investigated with the LM, SEM and TEM methods the ontogenetic development of the spinous exine of *H. syriacus* with special reference to the formation of the spines. The mature pollen grain is polycolporate, 160-170 μm in diameter, with supratectal spines 10-25 μm long.

KNOX and HESLOP-HARRISON (1969) investigated the cytochemical localization of enzymes in the wall of the pollen grains of several species, among which were malvaceous species, *Malvaviscus arboreus* and *Hibiscus rosa-sinensis*. DIAZ DE LA GUARDIA et al. (1994) described osmiophyl granules on the surface and the holes of the infratectal layer of the pollen grain of *Lavatera oblongifolia* BOISS.

We believe that the above mentioned and incomplete review concerning this subject, support the importance of the investigations of the pollen grains of the Malvaceae. In this place we have not discussed the experimental and the electronmicroscopic results, because such investigations are in progress.

Results

1. *Malva sylvestris* L.

1. Fresh pollen grains (Plate 8.1., figs.1-3)

Amb circular, polyaperturate tectum ornamented with spines. Mucilage drops are well shown. Diameter of the investigated pollen grains: 75.0-100.0 μm, maximum: 82.5-87.5 μm. Length of the spinae: 5.0 - 10.0 μm, maximum: 7.5 μm. Aperture size: 5.0-10.0 μm, maximum: 7.5 μm.

Pollen grains partially degraded with 2-aminoethanol

2.1. Degradation for 30 minutes (Plate 8.1., figs. 4-6)

No important alterations in the general morphology of the pollen grains except the partial dissolution of the mucilage drops. Diameter: 80.0-100.0 μm, maximum: 85.0-92.5 μm. Length of the spinae: 6.25-10.0 μm, maximum: 7.5 μm. Aperture size: 5.0-10.0 μm, maximum: 7.5 μm.

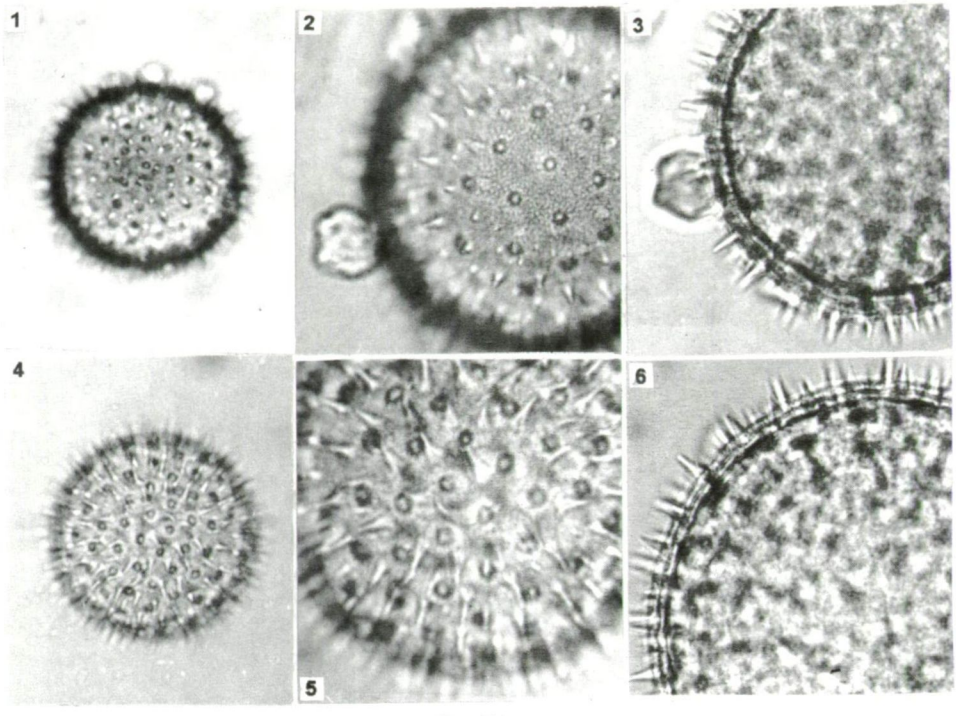

Plate 8.1.

1-6. *Malva sylvestris* L.
1-3. Fresh pollen grains
4-6. Pollen grains partially degraded with 2-aminoethanol for 30 minutes. Magnifications: 1, 4. 330x., 2,3,5,6. 660x.

2.2. Degradation for 1 hour (Plate 8.2., figs. 1-3)

The general aspect of the pollen grains is identical to the fresh ones, but the mucilage drops dissolved. Diameter: 77.5 - 105.0 µm, maximum: 85.0 - 95.0 µm. Length of spinae: 5.0 - 12.5 µm, maximum: 7.5 µm. Aperture size: 5.0 - 12.5 µm, maximum: 7.5 µm.

2.3. Degradation for 5 hours (Plate 8.2., figs. 4-6)

The qualitative morphological characteristic features are identical to the previous experiment. Diameter: 80.0 - 125.0 µm, maximum: 97.5 - 110.0 µm. Length of spinae: 5.0 - 11.5 µm, maximum: 7.5 µm. Aperture size: 5.0 - 10.0 µm, maximum: 7.5 µm.

Plate 8.2.

1-12. *Malva sylvestris* L.
1-3. Pollen grains partially degraded with 2-aminoethanol for 1 hour.
4-6. Pollen grains partially degraded with 2-aminoethanol for 5 hours.
7-9. Pollen grains partially degraded with 2-aminoethanol for 10 hours.
10-12. Pollen grains partially degraded with 2-aminoethanol for 24 hours. Magnifications: 1, 4, 7, 10. 330x, 2, 3, 5, 6, 8, 9, 11, 12. 660x.

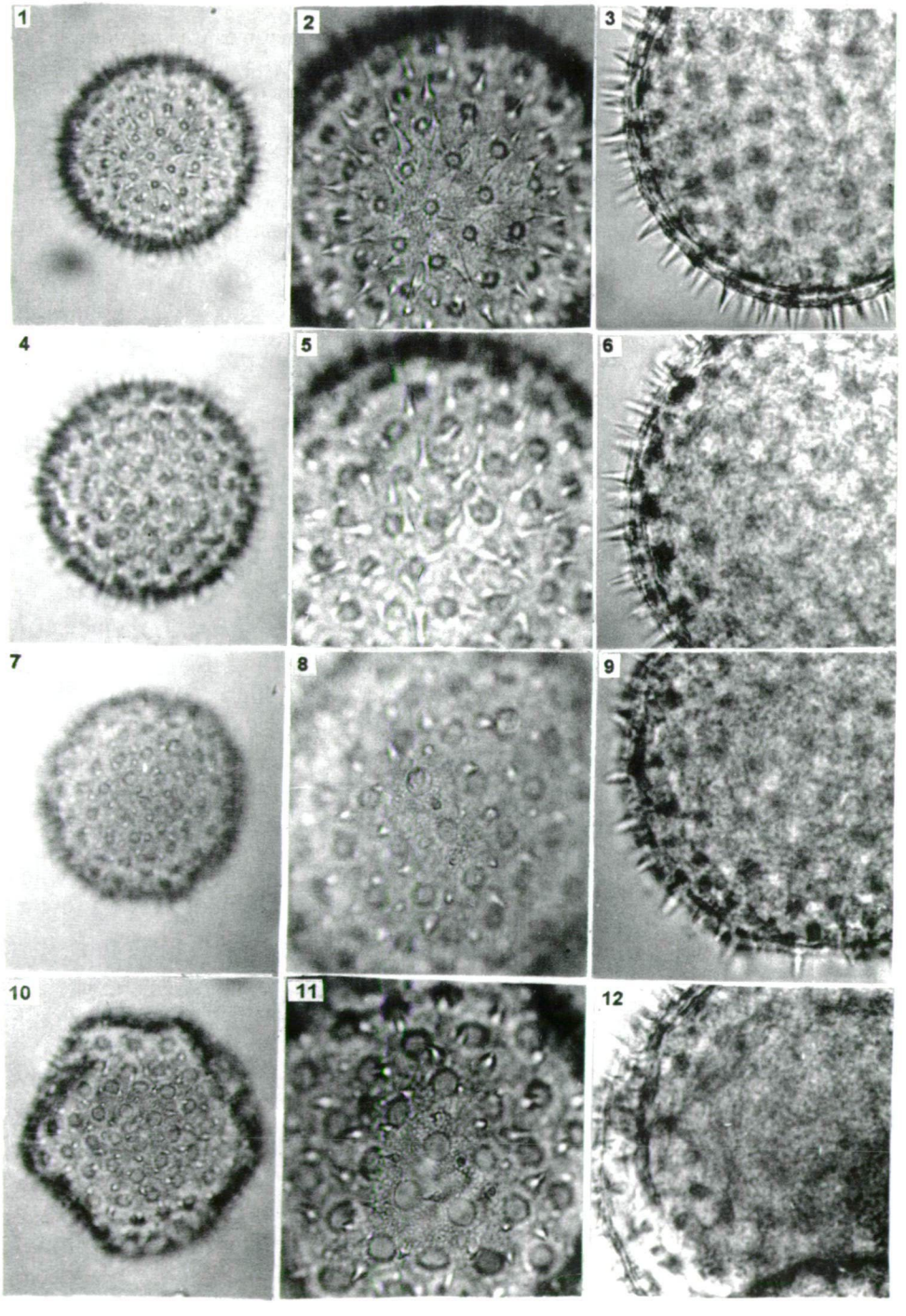

Plate 8.2.

2.4. Degradation for 10 hours (Plate 8.2., figs. 7-9)

The globular form of the pollen grains altered slightly, sometimes it is not completely globular. Diameter: 77.5 - 130.0 µm, maximum: 85.0 - 107.5 µm. Length of spinae: 5.0 - 8.75 µm, maximum: 7.5 µm. Aperture size: 5.0 - 8.75 µm, maximum: 6.25 µm.

2.5. Degradation for 24 hours (Plate 8.2., figs. 10-12)

Remarkable deformation of the originally globular pollen grain was observed, illustrated in picture 10, of Plate 8.2. Diameter: 90.0 - 130.0 µm, maximum: 112.0 - 122.5 µm. Length of spinae: 5.0 - 10.0 µm, maximum: 7.5 µm. Aperture size: 5.0 - 10.0 µm, maximum: 7.5 µm, but the number of the apertures of 10.0 µm large are also frequent.

2. *Hibiscus syriacus* L.

1. Fresh pollen grains (Plate 8.3., figs. 1-3)

Polyaperturate, globular pollen grains ornamented with large spines. Numerous characteristic mucilage drops were observed. Diameter: 105.0 - 170.0 µm, maximum: 132.5 - 142.5 µm. Length of spinae: 12.5 - 27.5 µm, maximum: 15.0 - 17.5 µm. Aperture size: 14.0 - 22.5 µm, maximum: 12.5 - 15.0 µm.

2. Partially degraded pollen grains with 2-aminoethanol

2.1. Degradation for 30 minutes (Plate 8.3., figs. 4-6)

The general aspect of the pollen grain is similar to the fresh ones, except the mucilage drops were partially dissolved. Diameter: 97.5 - 170.0 µm, maximum: 117.5 - 125.0 µm. Length of spinae: 10.0 - 25.0 µm maximum: 13.0 - 17.5 µm. Aperture size: 7.5 - 22.5 µm, maximum: 12.5 - 15.0 µm.

2.2. Degradation for 1 hour (Plate 8.3., figs. 7-9)

Amb circular, alterations were not observed by the LM method. It is worth of mentioning that the mucilage drops are still present and more common than at the previous experiment. Diameter: 110.0 - 147.5 µm, maximum: 125.0 - 132.5 µm. Length of spinae: 12.5 - 22.5 µm, maximum: 15.0 µm. Aperture size: 10.0 - 20.0 µm, maximum: 12.5 - 15.0 µm.

2.3. Degradation for 5 hours (Plate 8.4., figs. 1-3)

The general aspect of the pollen grains have not altered during this experiment, but the mucilage drops are mostly dissolved. Diameter: 117.5 - 157.5 µm, maximum: 125.0 - 142.5 µm. Length of spinae: 12.5 - 25.0 µm, maximum: 17.5 µm. Aperture size: 10.0 - 22.5 µm, maximum: 12.5 - 15.0 µm.

2.4. Degradation for 10 hours (Plate 8.4., figs. 4-6)

Deformations started at this experiment. The ectexine wrinkled, the amb is not always circular, sometimes slightly angular. Mucilage drops dissolved completely. Diameter: 100.0 - 150.0 µm, maximum: 125.0 - 132.5 µm. Length of spinae: 10.0 - 25.0 µm, maximum: 15.0 - 17.5 µm. Aperture size: 7.5 - 22.5 µm, maximum: 10.0 - 15.0 µm.

2.5. Degradation for 24 hours (Plate 8.4., figs. 7-9)

Important deformations were observed after this experiment. The amb is polyangular. On the surface protrusions appeared sometimes similar to the dissolved mucilage drops. Diameter: 107.5 - 152.5 µm, maximum: 120.0 - 132.5 µm. Length of spinae: 12.5 - 22.5 µm, maximum: 15.0 - 17.5 µm. Aperture size: 7.5 - 20.0 µm, maximum: 10.0 µm.

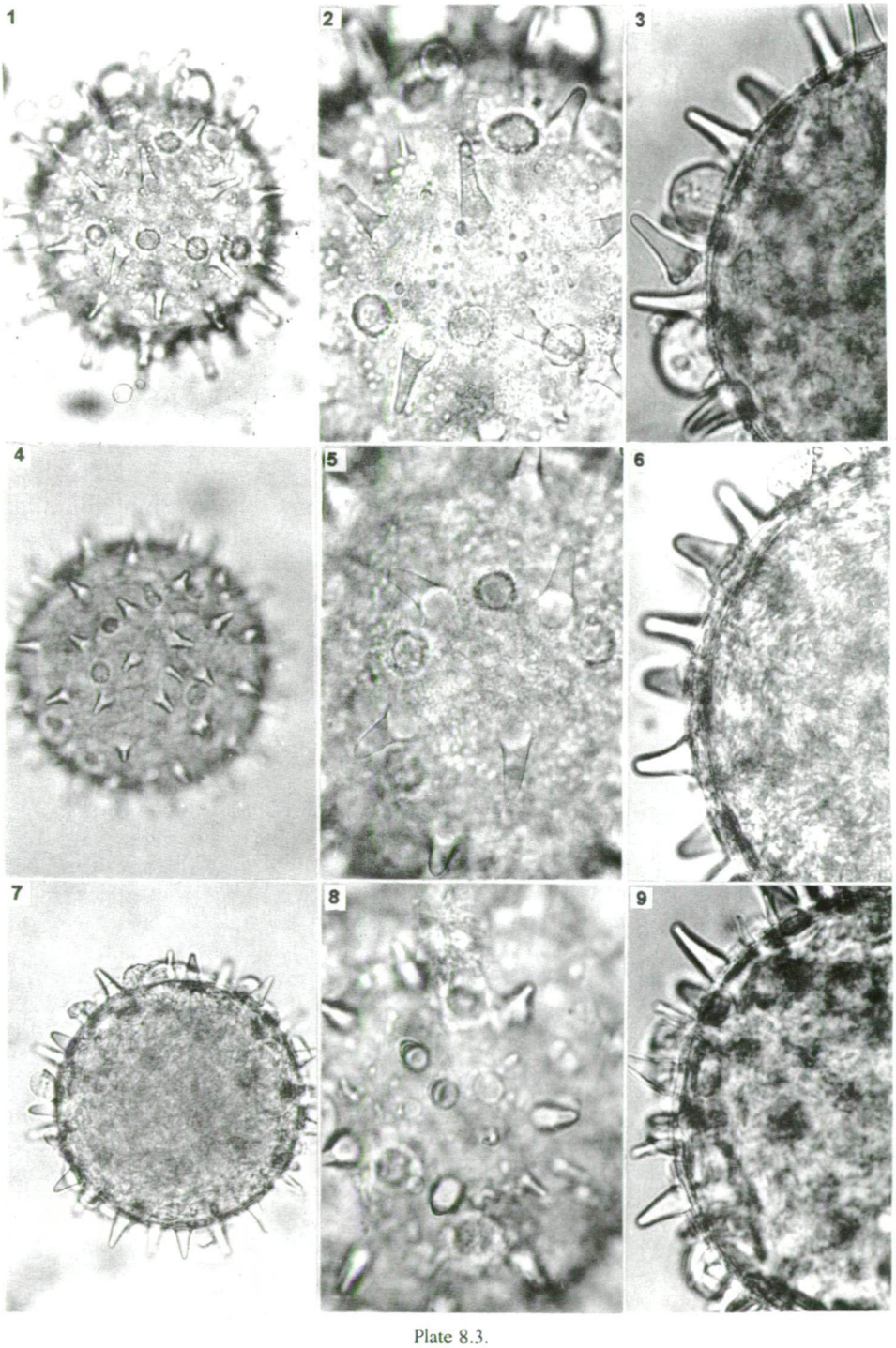

Plate 8.3.

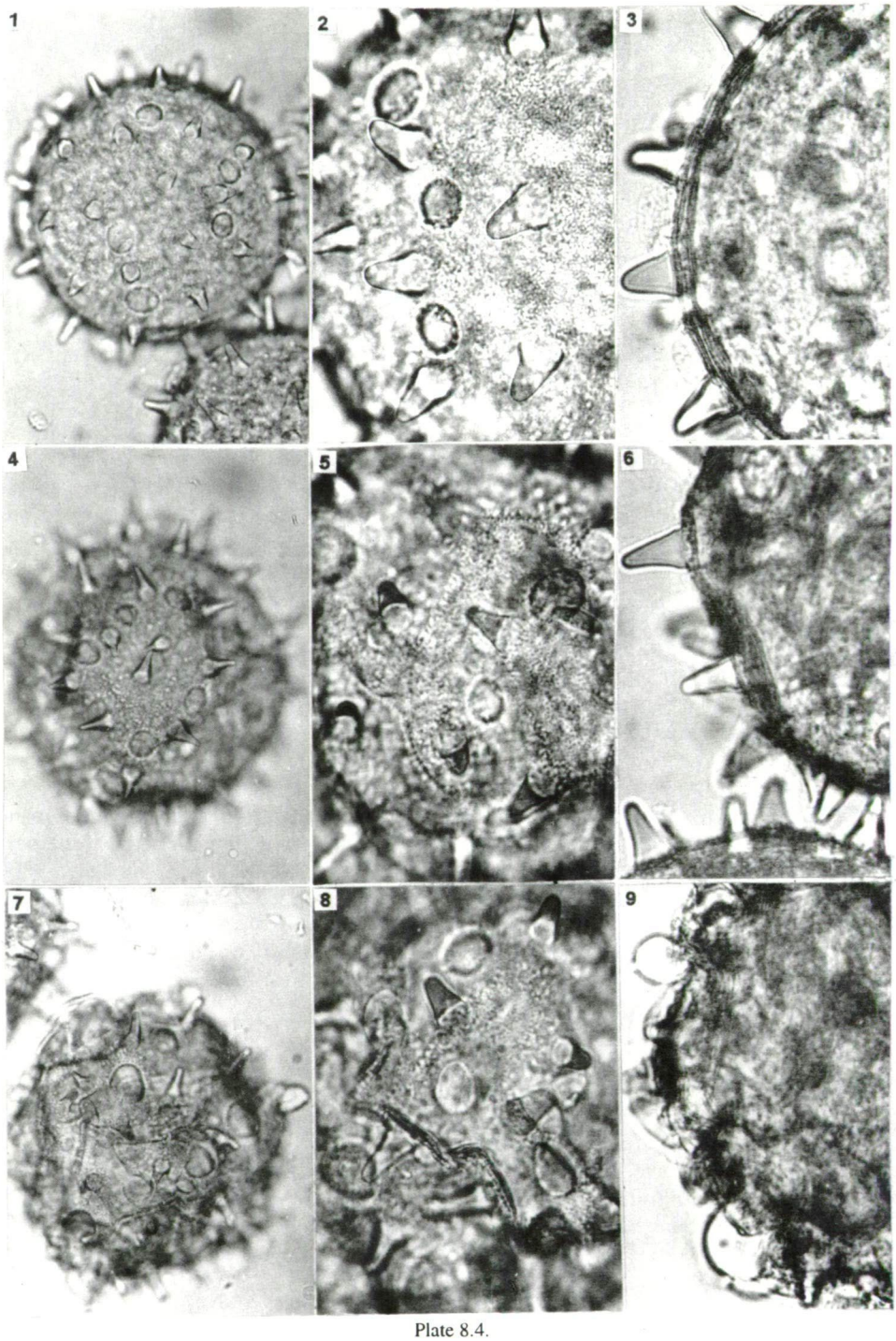

Plate 8.4.

Plate 8.3.

1-9. *Hibiscus syriacus* L.
1-3. Fresh pollen grains.
4-6. Pollen grains partially degraded with 2-aminoethanol for 30 minutes.
7-9. Pollen grains partially degraded with 2-aminoethanol for 1 hour. Magnifications: 1, 4, 7, 330x, 2, 3, 5, 6, 8, 9. 660x.

Plate 8.4.

1-9. *Hibiscus syriacus* L.
1-3. Pollen grains partially degraded with 2-aminoethanol for 5 hours.
4-6. Pollen grains partially degraded with 2-aminoethanol for 10 hours.
7-9. Pollen grains partially degraded with 2-aminoethanol for 24 hours. Magnifications: 1, 4, 7, 330x, 2, 3, 5, 6, 8, 9. 660x.

Discussion and Conclusions

1. The sporopollenin of the ectexine of both species investigated are relatively resistant to other species investigated previously for example some species of the genus *Quercus*, and *Elaeagnus angustifolia*. Relatively long degradation is necessary to observe important morphological alterations at these pollen grains.

2. Regarding the dissolution of the characteristic mucilage drops of these pollen grains based on our present day data the mucilages of *H. syriacus* are more resistant than that of *M. sylvestris*. The importance of these drops in the allergenic character of the pollen grains is in question.

3. Regarding the quantitative data of *M. sylvestris* there are no important differences in the measured characteristic features. Probably the maximum of the diameter is a bit larger after partial degradation for 24 hours. Worth of mentioning is that the maximum of the length of the spinae is 7.7 μm. Small differences are at the size of the apertures. 10 μm large aperture was observed after 24 hours of degradation.

4. At the pollen grains of *H. syriacus* some differences were established in the quantitative data of the partially degraded pollen grains. Concerning the diameter of the pollen grains it is worth of mentioning, that TAKAHASHI and KOICHI (1988) published 160-170 μm, and for the fresh pollen grains from Hungary (Szeged) our data are: 105.0 - 170.0 μm.

5. SEM investigations of these partially degraded pollen grains and the TEM study of partially degraded pollen grains of *M. sylvestris* by C60 fullerene/benzol solution will be published before long.

6. Later, in possession of the EM data we will have the opportunity to establish without doubt the character of the apertures, which may be polyporate, polyforate or (brevi)polycolporate with short colpi. After the establishment of the polycolporate condition by TAKAHASHI and KOICHI (1988) for the pollen grains of *H. syriacus* this will be investigated again.

7. Finally we will take into consideration the findings of ERDTMAN, BERGLIUND and PRAGLOWSKI (1961): Pollen dimorphism seems to occur at least in certain specimens of *M. pusilla* (some grains have long pointed spines, other grains shorter with blunt processes (p. 40).

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