ULTRASTRUCTURAL STATES OF PRESERVATION IN CLARKIA ANGIOSPERM LEAF TISSUES: IMPLICATIONS ON MODES OF FOSSILIZATION

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Ultrastructural analyses of Miocene leaf tissues from the Clarkia flora indicate the preservation of various protoplasmic features such as chloroplasts, mitochondria, and nuclei. Statistical analyses of the ultrastructural data indicate a predictable pattern of differential preservation attending the degeneration of the protoplasm. Of the 2,300 randomly sampled cells from the type locality (P-33), 26% contain mitochondria, 90.1% contain chloroplasts, and 4.3% contain nuclei. Comparable analyses of tissues from site P-40 indicate that 34.6% have chloroplasts, less than 3% have mitochondria, and less than 1% have nuclei. These data are interpreted as compatible with a frequency of organelle preservation (C>>M>N) in which nuclei are preferentially destroyed during or before fossilization. Simulated fossilization involving the compression of living leaf tissues and studies of senescent and dehydrated tissues are used to evaluate the probable effects of these factors on the sequence of events leading to the observed ultrastructure of the Clarkia fossils. From these studies we conclude that 63% of the sampled tissues appear to have entered the depositional environment as the result of leaf abscission and 15% were detached from their branches as a result of trauma; 89% of the tissues examined show evidence of compression wall failure, while 17% show concertina cell wall distortion similar to that seen in dehydration of living tissues.

The opportunity to study fossil cell contents with the electron microscope and thereby draw inferences as to the frequency and extent of protoplasmic preservation in the fossil record has been rare. In part, this is due to the vagaries in preservation and sampling of fossil floras that preclude the consistent application of electron microscopy and the gathering of adequate sample sizes for requisite statistical analyses. The abundant angiosperm leaf compressions recently found in the St. Maries River area of northern Idaho (cf. Smiley, Gray and Huggins 1975; Smiley and Rember 1979; Huggins, this volume) provide an opportunity to study fossil protoplasm in a detailed and systematic manner hitherto unparalleled. This paper presents a survey of the ultrastructural states of preservation found in a variety of fossil plant tissues, collected in the Miocene strata near the town of Clarkia, Idaho, as well as a preliminary investigation of the physical and biochemical processes that appear to have affected protoplasmic survival in different environments of geologic preservation. Representative angiosperm leaf fossils were collected from two sites, and their ultrastructural states of preservation were determined by means of transmission electron microscopy. Intra- and intertaxonomic variability of preservation was determined within and between these two sites on the basis of the statistical examination of randomly selected cells from which the frequency of organellar preservation was determined. To determine the extent to which various physical factors affected the integrity of protoplasmic features, modern taxa referable to the fossils studied (*Betula, Hydrangea, Platanus*, and *Quercus*) were subjected to dehydration and compression and then examined ultrastructurally. In addition, leaves of the modern forms that had undergone abscission or senescence were studied. Data derived from both the fossil and modern

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plants studied will be placed within the context of protoplasmic degeneration seen to attend fossilization, necrosis, and senescence.

MATERIALS AND METHODS

Fossil tissues of *Betula, Hydrangea, Platanus*, and *Quercus* were collected from freshly exposed strata at sites P-33 and P-40 of the Clarkia flora. Site P-33 is interpreted by Smiley and Rember (1979) to be derived from quiet water, offshore lacustrine sedimentation, while Site P-40 in the Emerald Creek embayment is interpreted to be due to nearshore sedimentation (cf. Smiley and Rember, this volume). The vertical sequence of sediments exposed at P-33 shows a temporal change from the base of the outcrop (alluvial sands) to the top (probable flood plain deposits) indicative of a progressive infilling of the Miocene Clarkia Lake. Thus the precise environment of deposition associated with any particular horizontal suite of fossils must be largely inferred from their relative position within the column. The Clarkia fossils are considered to be Lower Miocene in age, on the basis of paleofloristic and potassium/argon dating (cf. Smiley and Rember, 1979, this volume).

Fossils were handled at a minimum, as described by Niklas (1979, 1981), and reference samples were taken for cuticular and chemical analyses, and for light and transmission electron microscopy (TEM). Tissues were placed either directly in a fixative (2%, vol/vol glutaraldehyde; 1% wt/vol tannic acid; 50 mM cacodylate buffer at pH 7.2, cf. Niklas et al. 1978; Niklas and Brown 1981) or stored in nitrogen for subsequent fixation and TEM analysis. Referable modern tissue samples were fixed with glutaraldehyde and with tannic acid to determine fixation-induced variability in ultrastructure.

For purposes of statistical analyses, 100 cells from random areas of random TEM sections taken from every specimen were photographed. The number of specimens for each taxon collected is given in Table 1. Tracheids and vessel members were excluded from the random selection of cells, since these cell types lack protoplasmic structure and maturity and would skew the data. Transmission electron micrographs were printed at standard magnifications (10,000 and 16,000X), and their identifiable contents tabulated.

The percentage of cells having an ultrastructural feature (e.g. chloroplasts, nuclei, etc.) for any taxon from a specific locality is given by x/100k x 100%, where x is the number of cells having the feature, and k is the number of fossil specimens examined. The total number of sampled cells for a taxon is given by 100k or N. The variance, σ, or standard deviation of cells having a feature for any specific taxon is given by:

$$\sigma = \frac{[(p_1 - \pi)^2 + (p_2 - \pi)^2 + \ldots (p_k - \pi)^2]^{1/2}}{k}, \text{ Equation 1}$$

where p is the proportion of cells having the feature for a fossil specimen and π is the proportion of cells having the feature for the taxon. Equation 1 must be used to compute the intrataxonomic variance in ultrastructural preservation instead of a more generally used standard deviation formula, $s = (\xi x^2/N)^{1/2}$, since the data are in the form of a "presence vs. absence" tabulation (cf. Croxton 1953). The Kolmoganov-Smirnov one-sample test was used to compute the cumulative distribution of cells possessing various permutations of organelles within the between fossil localities. Subcellular features such as a nucleus, chloroplast, or mitochondrion, which were present in >50 of the random cells, were entered as a "typical" protoplasmic feature of the taxon–noted by a "+" in Table 1. If the feature was seen in <50, then a "–" was tabulated. Some of the raw data upon which the + and − entries were made are given in Table 2.

Observed sequences in the differential preservation of various organelles were quantified by means of an index of fidelity to the presumed living state. Each organelle and cell walls/cuticles,

TABLE 1. DIFFERENTIAL PRESERVATION OF ULTRASTRUCTURAL FEATURES IN CLARKIA FOSSIL LEAF TISSUES

	Betula (9)[1]	*Hydrangea* (2)	*Platanus* (6)	*Quercus* (6)
Site P-33				
ER, golgi	0[2] -	0 -	0 -	0 -
Nuclei	5 ± 0.8 -	1 ± 0.6 -	5 ± 0.9 -	2 ± 0.5 -
Mitochondria	10 ± 1.9 -	52 ± 6.6 +	42 ± 6.2 -	26 ± 6.5 -
Chloroplasts	83 ± 2.6 +	85 ± 3.3 +	96 ± 9.2 +	98 ± 9.5 +
Starch grains	76 ± 5.2 +	82 ± 9.2 +	59 ± 1.3 +	73 ± 6.2 +
Cell wall	100 +	100 +	100 +	100 +
Cuticle	100 +	100 +	100 +	100 +
Biotic effects	10 ± 0.7 -	5 ± 2.3 -	8 ± 5.5 -	10 ± 5.2 ±
FI-value	10	15	10	9
	Betula (0)	*Hydrangea* (2)	*Platanus* (4)	*Quercus* (8)
Site P-40				
ER, golgi	0 -	0 -	0 -	0 -
Nuclei	0 -	0 -	0 -	1 ± 0.3 -
Mitochondria	3 ± 1.2 -	0 -	1 ± 3.6 -	3 ± 0.3 -
Chloroplasts	42 ±20 -	39 ± 9.3 -	39 ±20 -	23 ± 6.9 -
Starch grains	0 -	0 -	0 -	0 -
Cell wall	69 ± 9.3 +	53 ± 7.2 +	59 ±5.2 +	72 ±6.2 +
Cuticle	100 +	100 +	100 +	100 +
Biotic effects	50 ± 7.2 ±	48 ± 5.2 -	40 ± 3.1 -	36 ± 5.2 -
FI-value	2	3	3	3

[1] Number of specimens.

[2] Percent of cells possessing the feature.

typical for a living cell, were assigned an arbitrary value in inverse order of the frequency of their preservation. ER and golgi = 7, nucleus = 6, mitochondria = 5, chloroplasts = 4, starch grains = 3, cell wall with cellulose microfibrils = 2, cuticle = 1. The fidelity index, FI, of a tissue was computed as the total of the values for each component, C_i, which was present in 50% of the 100 random cells, i.e. $FI = \sum_{1}^{7} C_i$. In addition, biotic degradation due to fungi and/bacteria was denoted by FI-1. The fidelity index provides a rough quantitative expression of ultrastructural preservation, as well as assigning a tissue to a generalized numerical sequence indicative of its protoplasmic deterioration.

RESULTS

Ultrastructural States of Preservation

The macroscopic and ultrastructural appearance of *Betula, Hydrangea, Platanus*, and *Quercus*, as well as some other genera from P-33 and P-40, have been reported elsewhere (Niklas 1981, 1982, 1983; Niklas and Brown 1981). Details of *Betula* (Fig. 1), *Quercus* (Fig. 2), *Platanus* (Fig. 3), and *Hydrangea* (Fig. 4) are reviewed here for purposes of comparison with their modern counterparts. Quantitative expressions of organelle frequencies are given in Table 1.

Betula. Gultaraldehyde fixed tissues of *Betula* from site P-33 lack golgi bodies and endoplasmic reticula, occasionally possess nuclei and mitochondria, and show chloroplasts with excellent infrastructure revealing the grana fretwork membrane system (Fig. 1). The cell walls of most

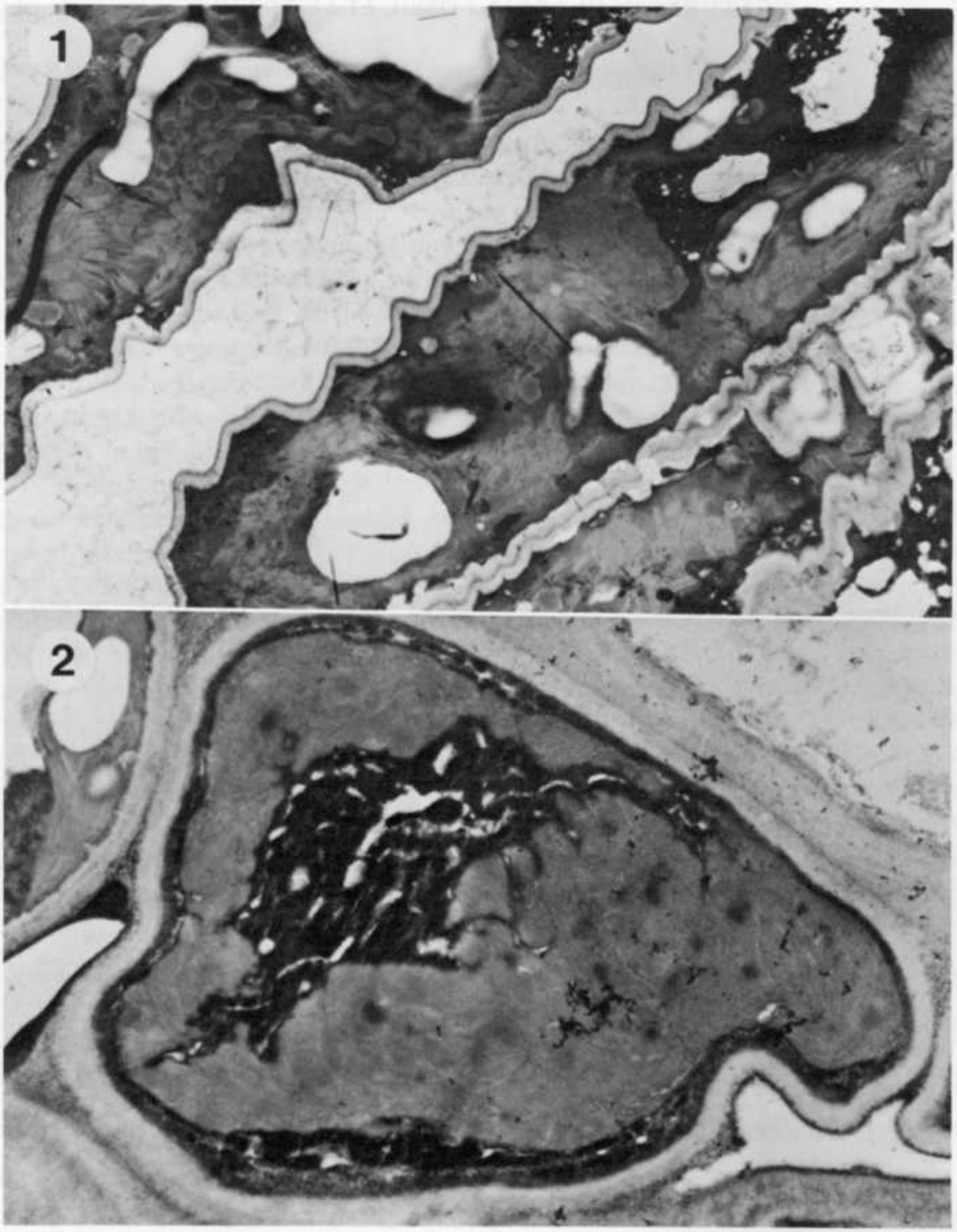


Figure 1. Transmission electron micrograph (TEM) of fossil *Betula* palisade cells showing concertina folding of cell walls and protoplasmic remnants. X 7,600.

Figure 2. TEM of fossil *Quercus* spongy mesophyll cells revealing occluded cell lumin. Portions of chloroplasts are seen to be contiguous with amorphous, electron dense regions. X 11,000.

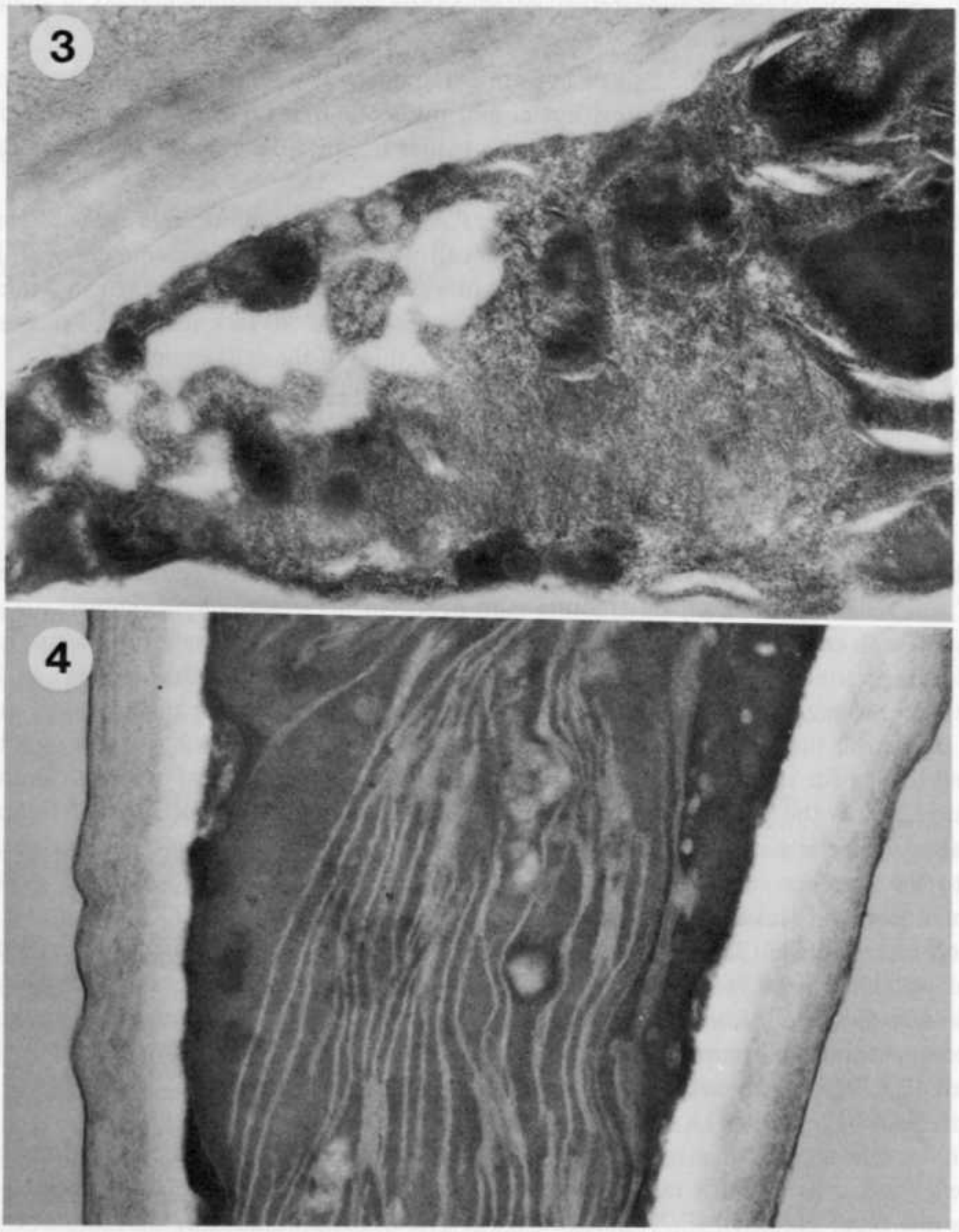


Figure 3. TEM of fossil *Platanus* palisade cell showing multi-layered cell wall (upper left) and heterogenously preserved protoplasm. X 32,000.

Figure 4. TEM of fossil *Hydrangea* floral bract showing "negative staining" property of chloroplast infrastructure and slightly crinulate cell wall. X 25,000.

mesophyll tissues are well preserved. *Betula* tissues from Site P-40 lack nuclei, in addition to Golgi apparati, and rarely possess demonstrable mitochondria. Chloroplasts are present in some tissue samples but are not abundant.

Quercus. Tissues from P-33 fixed in both glutaraldehyde and tannic acid show chloroplast infrastructure and occasionally possess nuclei and mitochondria. Chloroplasts are present in the majority of the cells examined (Fig. 2). *Quercus* tissues from P-40 are poorly preserved; the most common organelle is the chloroplast.

Platanus. Tissues from leaves identified as *Platanus* fixed in tannic acid show moderate to good preservation from Site P-33. Evidence for cell wall compression is common, with lacunae occluded with electron-dense cytoplasmic remnants (Fig. 3). Some cell lacunae show nuclei and mitochondria, and a majority have chloroplasts. Tissues from P-40 lack nuclei and possess infrequent mitochondria; chloroplasts are present in about a third of the cells examined.

Hydrangea. Glutaraldehyde-fixed tissues of *Hydrangea* bracts from P-33 possess plastids, which appear as if in negative contrast (Fig. 4), as well as mitochondria and occasional nuclei. The only organelle detected in P-40 tissues is the chloroplast.

Statistical Analyses of Ultrastructural Preservation

The percentages of randomly sampled cells (100 cells per specimen) containing various organelles, of cell walls with microfibril structure, and of specimens with *bona fide* cuticles are given in Table 1 for each of the four taxa studied from Sites P-33 and P-40. Some of the actual numbers of cells having various permutations of nuclei, chloroplasts, and mitochondria are given in Table 2. Variation of the percentage of cells possessing any of the protoplasmic or ergastic features examined is given in the form of standard deviation values. A chi-square, 2x2 table for each taxon is presented to test for the significance of differences between the number of cells with and without nuclei in regard to the presence of chloroplasts and mitochondria (cf. Table 2). In all but one case (*Hydrangea*), there is a significant difference between groups of cells with and without nuclei with regard to the presence of chloroplasts ($p<.25$ to $<.001$). Only two cells from P-33 showed evidence of nuclei and lacked chloroplasts, while 1,994 cells out of the 2,300 sampled had chloroplasts and lacked nuclei (213 cells lacked both nuclei and chloroplasts). Two cells had mitochondria and no chloroplasts, 600 cells had both, while 1,481 had chloroplasts and no evidence of mitochondria (Table 2). Statistical examination of Site P-40 cells (data not shown) indicates an even more pronounced trend for a preferential preservation of chloroplasts: 8 of all the cells examined (2,300) have nuclei, while all cells showing some ultrastructural features have chloroplasts (cf. Niklas 1982, 1983).

To test the significance of the data with regard to the relative number of chloroplasts per living cell, fossil cells for each taxon were stereologically examined and the chloroplast-to-nucleus ratio for the fossil specimen (C/N) computed. An overestimate of the C/N ratio in modern angiosperm palisade parenchyma was used ($C/N \cong 250$). On the basis of chi-square tests (Table 3), the probability of finding the fossil C/N ratios observed by chance is less than 0.15 to 0.001. A similar computation for mitochondria-to-nucleus (M/N) ratios was not performed owing to the lack of reliable M/N ratios in modern angiosperm tissues.

Based upon the observed differential preservation of protoplasmic structure, where chloroplasts are the most likely organelle to be found and ER/Golgi bodies are the least likely to be preserved, an index of fidelity (F1-value) to the living condition of each leaf was computed (cf. Table 1 and 2). The maximum possible score for any specimen is 28. The maximum observed score is 15, i.e. *Hydrangea* (Table 1). The lowest scores were tabulated for fossils from the P-40 site. Intrataxonomic comparisons of F1-values for specimens from the different geologic settings of P-33 and P-40 in the same flora indicate that preservational differences are for the most part not due to specific anatomical or chemotaxonomic variations. For example, the F1-values for

TABLE 2. CHI-SQUARE, 2x2 TABLE OF SITE P-33 FOSSIL LEAF CELLS POSSESSING CHLOROPLASTS, MITOCHONDRIA, AND NUCLEI

	Betula nuclei			*Hydrangea* nuclei			*Platanus* nuclei			*Quercus* nuclei		
	+	-		+	-		+	-		+	-	
Chloroplasts												
+	45	45		2	102		30	223		12	144	
-	0	153	p<.005	0	30	p<.70*	0	24	p<.25	2	10	p<.01
Mitochondria												
+	45	45		2	102		30	223		12	144	
-	0	810	p<.001	0	96	p<.20*	0	347	p<.01	0	444	p<.001
	mitochondria			mitochondria			mitochondria			mitochondria		
	+	-		+	-		+	-		+	-	
Chloroplasts												
+	90	657		104	66		252	324		154	434	
-	0	153	p<.005	0	30	p<.05	0	24	p<.01	2	10	p<.02

*Computed by the exact method of variance analysis.

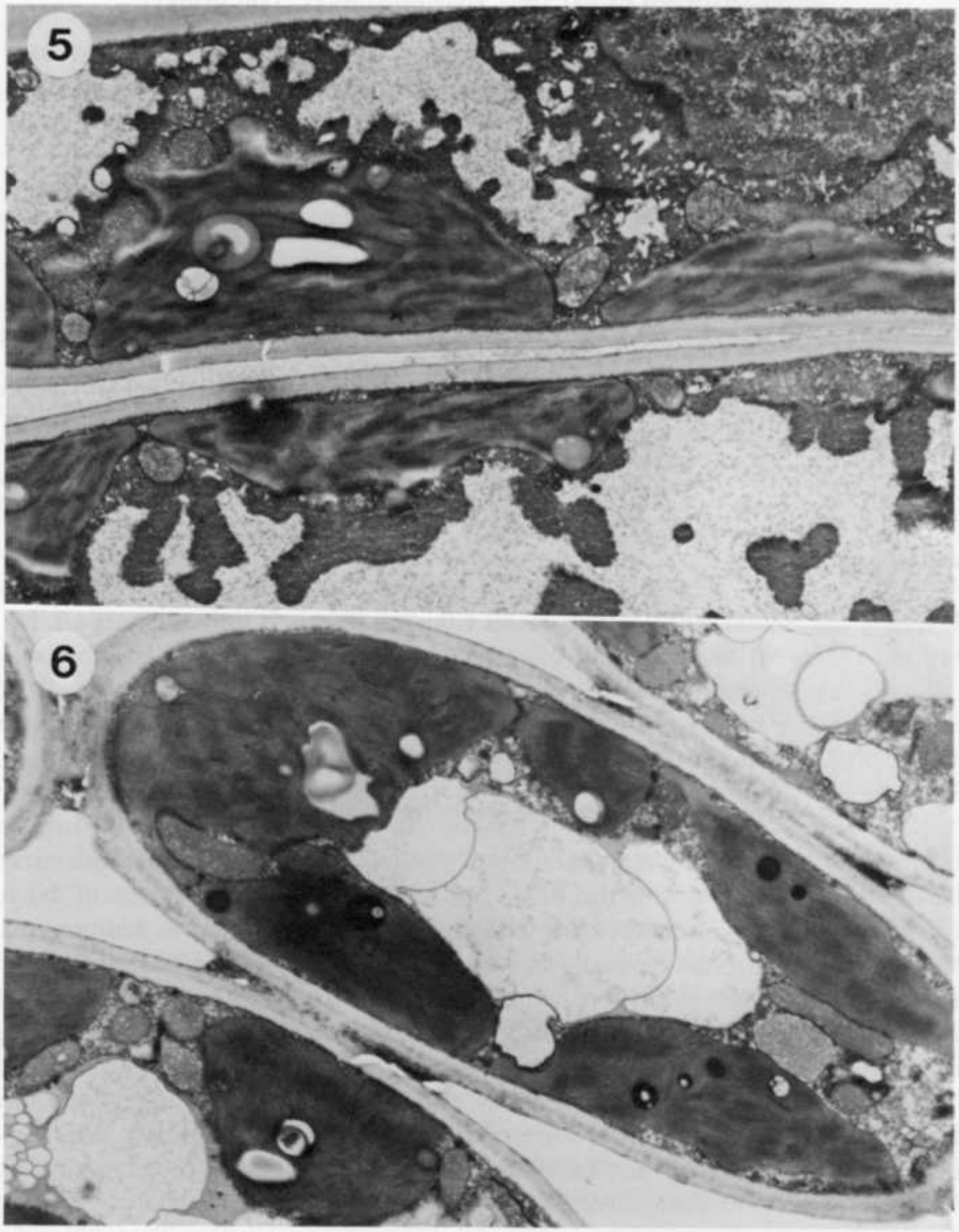
Betula, Hydrangea, Platanus, and *Quercus* fossils from the Clarkia P-33 locality are 10, 15, 10 and 9, respectively (Table 1)—indicating an intertaxonomic variability of preservation within this locality. Representative fossils of these same taxa from the P-40 locality have virtually identical and low F1-values, i.e. F1=3. (The F1-value of *Betula* is 2 and is due to evidence of excessive biotic degradation—50%.)

Ultrastructural Effects of Dehydration and Compression

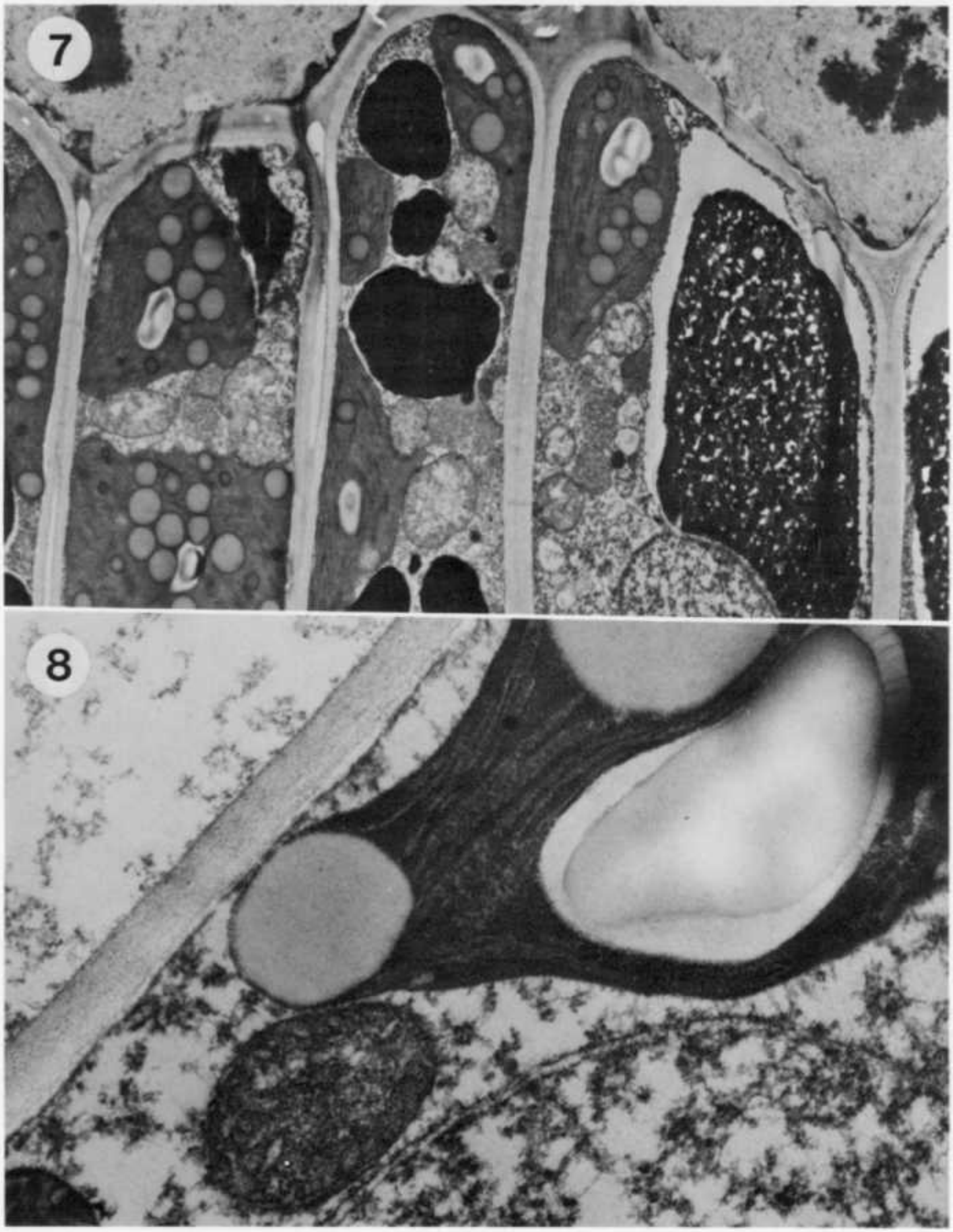
To provide a basis for the interpretation of fossil angiosperm leaf ultrastructure, referable modern leaves were examined with the TEM, and subjected to various regimes of dehydration, hydration, and/or compression before their ultrastructure was determined. In addition, the effects of tannic acid fixation were determined, since we had speculated on the ultrastructural fidelity of leaves submerged in an aqueous environment supercharged with this and other naturally occurring phenolics (cf. Niklas et al. 1978; Niklas and Brown 1981). The ultrastructure of unstressed mesophyll cells is well documented in the literature and will not be repreated here; however, in all of the genera we examined, such characteristic protoplasmic features as Golgi apparatus, ER, chloroplasts, mitochondria, and nuclei were evident (Figs. 5-8). As expected, dehydration and compression treatments severely altered the characteristic appearance of the mesophyll tissue and its cellular ultrastructure components.

Dehydration by air-drying results in a random or profuse concertina-like folding of palisade mesophyll cells along their longitudinal axes, while mesophyll cells appear to invaginate and collapse (Figs. 9-12). Attending these deformations, various protoplasmic alterations were observed that are consistent from one genus to another: (1) the loss of observable Golgi apparatus and well-defined ER, (2) the collapse or rupture of the tonoplast membrane(s), (3) vesiculation and/or rupture of the outer chloroplast membrane with a concomitant loss of a well-defined stromagrana orientation parallel to the plastid's major axis, (4) a decrease in the fidelity of mitochondrial cristae, and (5) the loss of the plasmalemma. In addition, demonstrable nuclei were rare or wholly lacking in many of the preparations (e.g. *Betula* and *Quercus*).

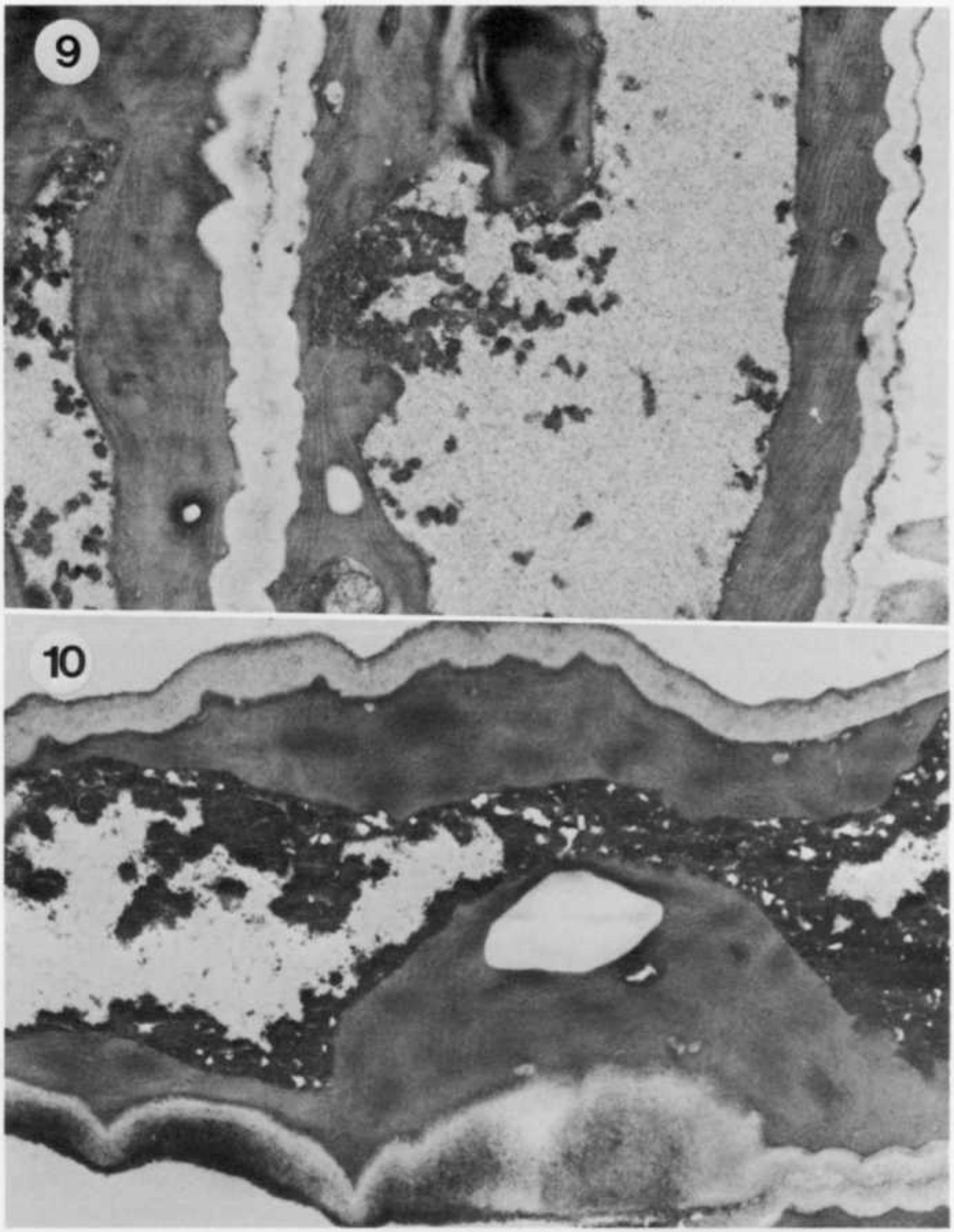
In contradistinction to the cell wall deformations induced by dehydration, compression of leaves (buried in a fine-grain sand and mud mixture) resulted in a general vertical collapse of mesophyll cell walls without the formation of concertina-like foldings characteristic of dehydration. Subsequent dehydration of compressed leaves resulted in the aforementioned ultrastructural



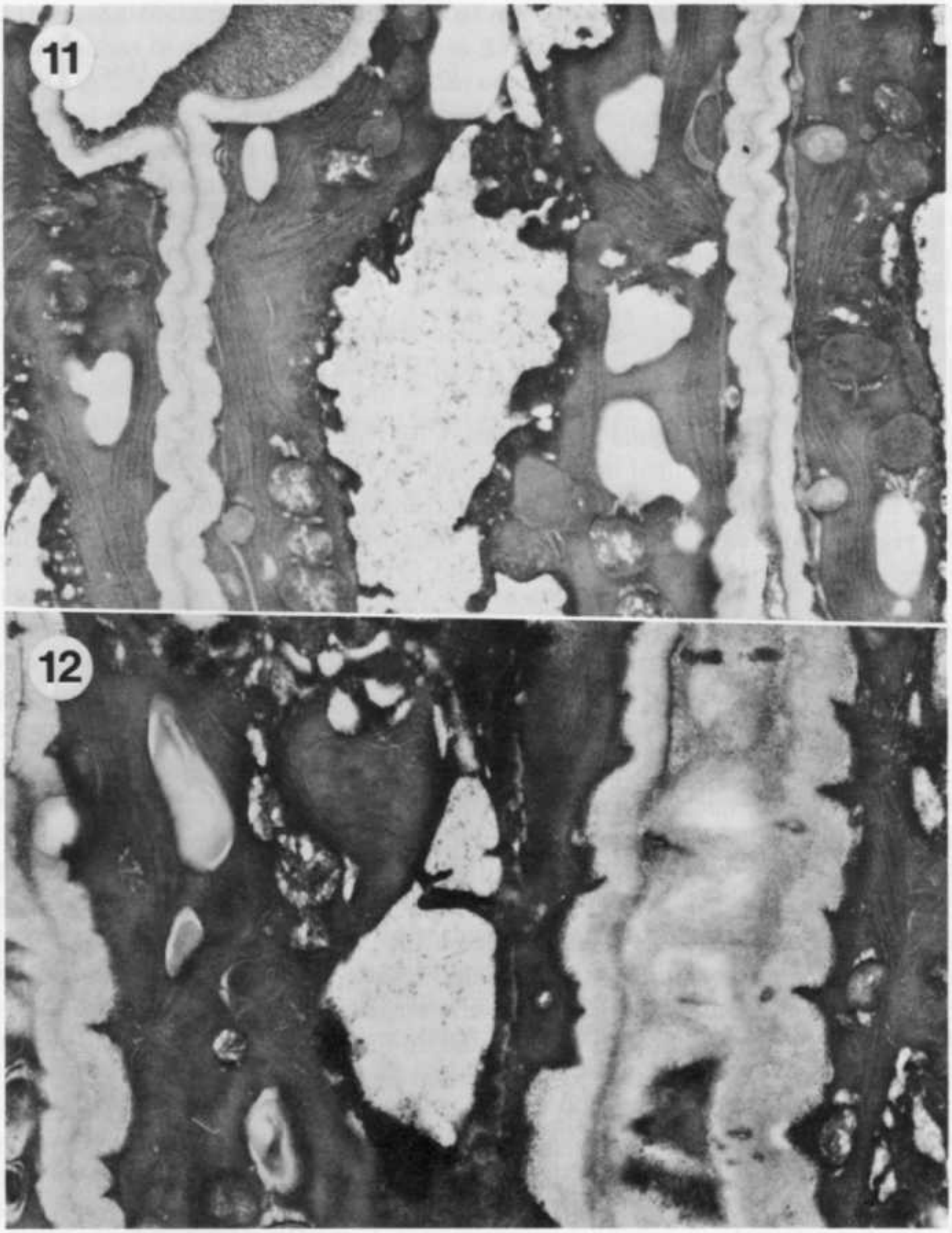
Figures 5 and 6. TEM of extant *Quercus* palisade cells prepared without tannic acid (TA) fixation (Fig. 5) and with TA fixation (Fig. 6). Cell walls are characteristically undistorted in both treatments; however, grana fretwork membrane systems are more pronounced in TA-prepared material. Figure 5 at X 19,000; Fig. 6 at X 11,000.



Figures 7 and 8. TEM of extant *Betula* palisade cells, without TA fixation (Fig. 7) and with TA fixation (Fig. 8). Figure 7 at X 8,000; Fig. 8 at X 42,000.



Figures 9 and 10. TEM of air-dried extant *Quercus* palisade cells prepared without TA fixation (Fig. 9) and with TA fixation (Fig. 10). Dehydration results in a concertina-like distortion of the cell walls in both treatments. Tannic acid fixation enhances the contrast seen between protoplasmic features. Figure 9 at X 14,000; Fig. 10 at X 14,000.



Figures 11 and 12. TEM of air-dried extant *Betula* palisade cells without TA fixation (Fig. 11) and with TA fixation (Fig. 12). Figure 11 at X 14,000; Fig. 12 at X 16,000.

TABLE 3. CHI-SQUARE TABLES OF "OBSERVED" VS. "EXPECTED" CHLOROPLAST/NUCLEUS RATIOS IN FOSSIL LEAF TISSUES

	f[a]	fc[b]	f-fc	$(f-fc)^2$	$(f-fc)^2/fc$	
Clarkia site P-33	750	250	500	2.5×10^4	1×10^3	$p < .001$
Clarkia site P-40	275	250	25	625	2.5	$p < .15$

[a] f = observed C/N ratio.

[b] fc = expected C/N ratio.

alterations observed in non-compressed, dehydrated mesophyll cells. Subsequent rehydration of air-dried and compressed tissues resulted in a partial re-establishment of the original cell wall configuration.

In at least two genera (*Betula* and *Quercus*), TA fixation of both fresh (Figs. 6, 8) and dried leaves (Figs. 10, 12) resulted in the appearance of "negative contrast" membrane systems, particularly in the infrastructure of chloroplasts. In general, TA fixation resulted in what appears to be the preferential preservation of chloroplast ultrastructure at the expense of the fidelity of other organelles (compare Figs. 9 and 11 with 10 and 12).

DISCUSSION

Ultrastructural examinations of angiosperm leaf fossils, presented here and elsewhere (cf. Niklas et al. 1978; Niklas and Brown 1981), indicate that protoplasmic features may be preserved provided that the physical and biochemical factors attending fossilization are sufficiently mild (Figs. 1-4). Nuclei, nucleoli, chloroplasts, pyrenoids, and even such delicate structures as chromosomes have been described from a wide range of plants and from various geologic periods (Baschnagel 1966; Baxter 1950; Bracken-Hanes and Vaughn 1978; Bradley 1946, 1962; Darrach 1938; Dwivedi 1959; Eisenack 1967; Florin 1936; Gould 1971; Mamay 1957; Millay and Eggert 1974; Schopf 1968, 1974; Stevens 1912; Vishnu-Mittre 1967; see, however, Niklas 1982, 1983). The interpretation of fossil protoplasm is difficult, however, due to: (a) possible pre-depositional alterations of the living state due to necrosis and/or senescence, and (b) post-depositional changes (= diagenesis) attending sediment consolidation and lithification. The present study has attempted to document the intra- and intertaxonomic variation in the ultrastructural preservation of four angiosperm leaf taxa (*Betula, Hydrangea, Platanus*, and *Quercus*) from two depositional sites of the St. Maries River fossil area of northern Idaho, near the town of Clarkia. The interpretation of these fossils must, however, be placed within the context of the ultrastructure of comparable modern genera, both in their pristine living condition and in states of dehydration and compaction designed to simulate some of the traumatized conditions possibly induced by fossilization (Figs. 5-12). By comparing the ultrastructure of fossil and modern taxa from different depositional environments and under conditions of physiologic stress, respectively, we hope that a more precise quantitative expression of ultrastructural states of preservation may be attained, and inferences concerning the pre- and postdepositional changes in protoplasm may be useful in reconstructing the conditions all ending in fossilization.

Of the 2,300 randomly sampled cells from tissues of *Betula, Hydrangea, Platanus*, and *Quercus* from site P-33 (cf. Smiley and Rember 1979), 90.1% contain remnants of chloroplasts (C), 26% contain mitochondria (M), and 4.3% have demonstrable nuclei (N) (Table 1). Similarly, a comparable study of tissues from site P-40 indicates that C = 34.6% M < 3%, and N < 1% (Table 1). Statistical analyses of the data from both sites indicate that a well-defined pattern exists in the frequency of preservation of various organelles such that C>M>N regardless of the presumed

differences in the environments of deposition and of the taxon selected (cf. Table 2). The significance of the raw data, when tested against the numerical abundance of chloroplasts over nuclei in the cell populations of leaves (C/N was taken as 250), indicates that the statistical probability of these data being the result of chance is less than one in one thousand ($p<.001$; Table 3). The data and the inferred pattern of protoplasm degeneration are consistent with studies based upon other angiosperm leaf tissues of comparable age (e.g. Succor Creek Flora; Niklas 1981, 1982, 1983).

The apparent susceptibility of nuclei to degeneration during fossilization, as opposed to the stability of chloroplasts, is not consistent with some studies of modern plant material subjected to physical or biological stress. From a survey of the literature, the death of a cell appears to follow in most cases a repeatable pattern of protoplasm degeneration, regardless of a variety of causes (Butler and Simon 1971; Israel and Ross 1967; Hoefert 1980; Hurkman 1979; Toyama 1980, and references therein). The fidelity index (F1) provides a method whereby both fossil and modern patterns of degeneration may be placed in a common framework. The first detectable changes in a modern plant cell undergoing necrosis are a decrease in the number of ribosomes and a loss of ER and Golgi body membranes. This is comparable to fossil tissues with an F1-value of 21, and is consistent with the lack of ER and Golgi in all of the fossil cells examined. Chloroplast breakdown is the next most obvious event in cellular necrosis. The stroma disappear, thylakoids swell and disintegrate, and the number and size of osmophilic globuli (= plastoglobuli) increase. While mitochondria are in most cases present until terminal necrosis, they may show a reduction in size, a swelling of their cristae, and a reduction in their number concomitant to chloroplast breakdown. The tonoplast ruptures before most organelles degenerate (F1 = 18). The plasmalemma and nucleus are relatively stable during necrosis (F1 = 11); death of the cell is marked by the lysis of the plasmalemma and the vesiculation and disappearance of the nucleus (F1 = 4). Except for the initial disappearance of ER, Golgi bodies, and the tonoplast, the pattern of modern cell degeneration is basically the reverse of what may be inferred to be the pattern induced by fossilization (cf. Table 2). As will be pointed out later, in some cases chloroplasts have been observed to be the only surviving organelle just before death of the cell (cf. Hurkman 1979).

An appropriate null hypothesis to explain the apparent disparity between these neo- and paleobotanical observations is that the reconstruction of protoplasmic degeneration is biased and ultimately unrepresentative of the phenomenon of fossilization. Given the proposition that all organelles in a cell undergoing fossilization have the same rate of alteration, then inherent differences in the way organelles manifest alteration ultrastructurally would lead to differences in their apparent survival, i.e. organelles with the most diagnostic infrastructure will be diagnosed as present in highly altered cells. The chloroplast, by virtue of its complex grana fretwork system of membranes, may be much more distinctive, even in the altered state, than a nucleus or mitochondrion. This combined with the numerical superiority of chloroplasts over nuclei could account for the low frequency of observed nuclei in our fossil material. A number of observations, however, mitigate this null hypothesis: (a) studies of various modern plant tissues in which nuclei preferentially degenerate (e.g. development and maturation of sieve cells, cf. Evert et al. 1973; sieve elements, cf. Hoefert 1980) provide the ultrastructural context in which to identify even highly altered nuclei; (b) our own studies, based upon dehydrated and/or compacted tissues, indicate that nuclei, while highly altered, are demonstrable even after severe osmotic stress and mechanical trauma; and (c) such techniques as stereological analysis (designed to compute volumetric and frequency distributions) when applied to the fossil material have failed to show a "hidden" population of nuclei in the sample set (data not shown). It is clear, however, from our manipulation of the living material, that chloroplasts are the most distinctive organelle even when the protoplasm is highly altered.

While the inferred degeneration pattern of organelles in the fossils studied cannot be fully explained, information is accumulating as to potential mechanisms. The factors attending

fossilization may either be different from those effecting changes in modern cells undergoing necrosis, or the factors may be the same but result in different ultrastructural patterns, due to as yet unknown diagenetic processes. Considering the diversity of exogenous and endogenous factors studied, it is difficult to identify one factor that is unique to the fossilization process. Similarly, the pattern of degeneration observed in the Clarkia fossils is the same for two different environments of deposition, as well as for leaves preserved in pyroclastic (ashfall) deposits of the Succor Creek (Niklas, 1983), indicating that the extent of tissue hydration during fossilization is not as critical as was previously thought. Our preliminary observations do, however, indicate that dehydration and the possibility of "auto-fixation" by naturally occurring plant phenolics before fossilization may play a critical role in both the extent of preservation and in the degenerative pattern seen in organelles. Luzzati and Husson (1962) have shown that at 80% dehydration, the phospholipid bimolecular leaflets of some membranes rearrange into hexagonal arrays that provide few sites for reactions with fixatives such as osmium tetroxide or tannic acid. A "negative staining" results, very similar to the condition seen in many fossil cells (Figs. 10, 12; cf. Niklas et al. 1978), as well as dehydrated modern leaf tissues that have been subsequently fixed with tannic acid. In this regard, we have observed that those taxa characterized by high, naturally occurring concentrations of tannic acids are those often found in the best state of preservation. The negative staining seen in many of these fossils may indicate that a period of dehydration (in which the molecular nature of cellular membranes becomes reorganized) was followed by a period of fixation due to tannic acids or other plant phenolics. The unit membranes of mitochondria and nuclei remain intact even after 95% of their lipids have been removed. In contrast, chloroplast membranes, because of their unusual chemistry (≅80% mono- and digalactosyl diglycerides) are less stable. However, after dehydration and subsequent tannic acid fixation, chloroplast membranes appear to be more stable than those of mitochondria or nuclei. Thus, the differential preservation of the chloroplast over other organelles in some fossils could have resulted from: (a) fully mature leaves undergoing senescence with attending decrease in water content, or dehydration due to premature removal from trees by some mechanical agent; (b) phospholipid-rich bimolecular leaflets of membranes reorganizing into hexagonal arrays that provided few sites for tannic acid fixation, while galactolipid-rich membranes resisted such a rearrangement; (c) subsequent rehydration of leaf tissue in tannic acid rich waters or, in leaves preserved in dry environments, rupture of tonoplasts (containing high concentrations of plant phenolics) resulting in (d) the infiltration of cells by a "natural fixative." Such a simple scenario could explain the diversity of depositional environments in which excellent ultrastructural preservation has been reported, various ultrastructural features suggestive of dehydration (e.g. concertina-like distortion of fossil cell walls, Fig. 2; differential staining of organelles, Fig. 4), and the apparent stability of chloroplasts over nuclei and mitochondria.

Differences in the sequence of chloroplast degeneration due to such factors as senescence, dehydration, and compression are sufficiently pronounced and consistent as to provide some information on the predepositional status of fossil leaves (Table 4). Naturally senescent or detached/aging leaves initially show similarities in the degeneration of chloroplasts. These include the appearance of osmophilic globuli, reorientation of the thylakoidal system, and a distention of the grana-intergrana lamellae (Table 4). However, in detached aging leaves, the chloroplast envelope ruptures before the breakdown of the lamellae, swelling of the grana-intergrana lamellae is not pronounced, and the thylakoidal system of membranes degenerates without vesiculation or the formation of numerous osmophilic globuli. Both types of senescence result in a characteristic negative staining of membranes, much like tissues fixed with tannic acid, perhaps indicative of dehydration-induced reorganization of the unit membranes. Rapid dehydration of leaves results in the formation of irregularly shaped chloroplasts, small numerous osmiophilic globuli, and in advanced plasmolysis, the appearance of myelin-like membrane configurations (Table 4). A nonplastid character of rapid dehydration, previously noted, is a concertina-like failure of cell walls.

TABLE 4. DIAGNOSTIC FEATURES OF ALTERED MESOPHYLL ULTRASTRUCTURE

	SENESCENCE		
	Naturally Senescing		*Detached and Aging*
10 days	a. chloroplasts elliptical b. stroma-grana parallel to major axis c. stroma have ribosomes and small plastoglobuli	3-4 days	a. chloroplasts elliptical b. lamellae swollen c. chloroplast membrane swollen and distended
21 days	a. stroma-grana disoriented and swollen b. contain large plastoglobuli c. stroma compact to granular	5 days	a. chloroplast envelope swollen b. lamellar structure intact c. stroma flocculent d. with large plastoglobuli
or	a. chloroplasts spherical b. stroma granular with c. grana swollen and stacked; appearing as vesicles d. intergrana lamellae not recognizable	or	a. chloroplast envelope rupture b. stroma-grana system still evident c. very large plastoglobuli d. parts of the lamellar system and outer membrane appear digested e. negative staining of membranes
>21 days	a. stroma flocculent b. no thylakoidal system c. contains numerous vesicles d. very large plastoglobuli e. negative staining of membranes		
	TRAUMA		
	Dehydration		*Compression*
1-2 days	a. chloroplasts elliptical b. lamellae swollen c. small plastoglobuli		a. chloroplasts irregular b. stroma-grana disorganized, undulating c. small and few plastoglobuli d. myelin-like stroma
3-4 days	a. chloroplasts irregular b. lamellar structure intact c. stroma granular d. small, numerous plastoglobuli		
>10 days	a. chloroplast membrane discontinuous or ruptured b. stroma-grana still evident; or myelin-like stroma c. negative-staining of membranes		

Compression results in irregular chloroplast outlines, an undulated stroma-grana, and myelin-like membranes within the chloroplast (Table 4). Cell walls usually rupture or fail in the axis normal to compression resulting in elliptical wall outlines.

Many of the observed ultrastructural features typically associated with senescence, dehydration, and/or compression have been observed in fossil protoplasm. Negative staining, irregular chloroplast outlines, and myelin-like membrane systems are common in fossils and are indicative of dehydration and/or compression. Similarly, the concertina-like failure of cell walls in some

specimens indicates that these ultrastructural features were probably the result of pre-burial dehydration. As noted earlier (cf. Results), rehydration does not totally remove the cell wall failure pattern, perhaps because of a plastic deformation component in failure.

From the foregoing ultrastructural observations, it is possible to identify fossil leaves that had undergone dehydration, senescence (either naturally or due to premature leaf fall), and/or compression. The bulk of the Clarkia leaves examined (63%) appear to have been the result of natural leaf fall due to leaf abscission, and 15% appear to have been detached due to mechanisms other than leaf abscission. Roughly 89% of all the leaves examined showed some evidence of compression cell wall failure, while 17% show concertina failure patterns.

The ultrastructural examination of the Clarkia fossils reveals a wealth of information on vagaries in the preservation of protoplasm, correlative to pre- and postdepositional factors, which collectively define the Clarkia environment of deposition. Perhaps the most surprising conclusion drawn from the statistically well-defined frequency of organelle preservation is the relative stability of chloroplasts over other organelles, which is counterintuitive to neobotanical patterns of protoplasmic degeneration (Butler and Simon 1971). However, in a few cases, the chloroplast has been reported to be the only organelle surviving terminal necrosis (cf. Hurkman 1979). Further research on fossil tissues and on patterns of simulated fossilization must follow if paleo- and neobotanical observations on ultrastructure are to be reconciled.

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