Taphonomic constraints on preservation of cuticles in compression fossils : fungi induced ultrastructural changes in cuticular membranes

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A comparative investigation has been made of the ultrastructure of the cuticular membrane recovered from healthy and fungal-infected leaves of *Thinnfeldia indica* Feistmantel, a fossil taxon to understand the nature of changes brought about in the cuticular membrane by the fungi. In general, the structural configuration of both the cuticular membranes is similar. In the infected leaf, precursors of cutin accretions are irregularly present at the sub-cuticular surface. These accretions are interpreted as possible results of breakdown of the cutin due to the secretion of an enzyme by the fungi infecting the leaf. It thus seems that the fungi, besides edaphic factors, do play a role in the break-down of the cutin and thus constrain the preservation of the cuticular membranes.

Key-words — Cuticular membrane, Ultrastructure, Taphonomy, *Thinnfeldia*.

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सारांश

संपीडनाश्मों की उपचर्मों के परिरक्षण में जैवसादिकीय अवरोध : उपचर्मीय झिल्लीयों में कवकों द्वारा व्युत्पादित परासंरचनात्मक परिवर्तन

ऊषा बाजपेयी

उपचर्मीय झिल्ली में कवकों द्वारा किये गये परिवर्तनों को जानने के लिए *थिन्फेल्डिआ इन्डिका* फाइस्टमॅन्टेल नामक एक अश्मित वर्गक की स्वस्थ एवं संक्रामित पत्तियों से प्राप्त उपचर्मीय झिल्ली की परासंरचना का तुलनात्मक अन्वेषण किया गया है। सामान्यतः दोनों ही उपचर्मीय झिल्लीयों में संरचनात्मक सदृशता एक जैसी ही है। ऐसा देखा गया है कि संक्रामित पत्ती में क्यूटिन संवर्धन करने वाले पूर्वग उप-उपचर्मीय सतह पर अनियमित रूप से विद्यमान रहते हैं। ये पूर्वग सम्भवतः पत्ती को संक्रामित करने वाले कवकों द्वारा स्रावित एन्जाइमों के कारण क्यूटिन के टूट जाने से बनते हैं। अतएव ऐसा प्रतीत होता है कि क्यूटिन के ह्रास में, मृदीय प्रभावों के अलावा, कवकों की प्रमुख भूमिका है और इसीलिए उपचर्मीय झिल्ली परिरक्षित नहीं हो पाती।

THE remarkable resistance of cutin, the major component of the plant cuticles, to most inorganic and organic chemicals under normal conditions has enabled the plant cuticles to persist through the aeons, right from Late Devonian (375 million years approximately) to Recent. The stability of the 'cutin' is due to the presence of 'cutan', a highly aliphatic non-saponifiable biomacromolecule, that has a high fossilisation potential (Nip *et al.*, 1986). However, not all the fossil leaves have a cuticle preserved, and in many a case the preservation of the cuticle is highly unsatisfactory. Tegelaar *et al.* (1991) have expressed the opinion that cutan is not necessarily present in the cuticle of all the species, and this may be a reason for potential bias in the preservation of the cuticular membranes of leaf fossils. The plant surface mostly carries a microflora which grows in the environment provided by the leaf surface, i.e., 'phyllosphere' (Last, 1955). The phyllosphere is colonised by bacteria and fungi which penetrate the plant tissue either through natural openings in the cuticle, i.e., stomata, or through openings caused by wounds. Many fungi penetrate directly through the cuticle by release of enzyme cutinase which weak-

ens the thick cutin layer and helps the entry of the hyphae. Though the process of fungal colonisation of the leaf tissue is understood to some extent, yet no study, more so on the fossil leaves, has been made to know if any changes are brought about in the ultrastructure of the cuticular membranes by the fungal infection. An attempt has been made here to document differences in the ultrastructure of healthy and fungi-infected cuticular membranes of *Thinnfeldia indica* Feistmantel 1876.

MATERIAL AND METHOD

The cuticular membranes were recovered by acid-alkali processing of specimens of *Thinnfeldia indica* Feistmantel, a presumed pteridospermous leaf collected from the Early Cretaceous Sivaganga Formation exposed in a water-well near Naicolam, Tiruchirapalli District, Tamil Nadu (Maheshwari, 1986). Epiphyllous microthyriaceous fungal remains on these leaves have been earlier reported and studied under the light microscope (Bajpai & Maheshwari, 1988). Ultrastructure of the fungi-infected cuticular membrane has been studied by Maheshwari and Bajpai (1996). Here we document the ultrastructure of the cuticular membranes recovered from a healthy (i.e., uninfected) leaf and compare it with that of the infected leaf to understand the taphonomic changes in the cuticular membrane.

For processing the cuticular membranes for ultrastructural studies, the method described in Maheshwari and Bajpai (1997) was followed in general. A variety of staining techniques was employed to attain good contrast. A large number of sections of the cuticular membrane have been cut and were picked on uncoated Copper grids for examination under the Transmission Electron Microscope.

OBSERVATIONS

Transmission electron micrographs of the ultrathin sections of an uninfected leaf of *Thinnfeldia indica* reveal that the leaf, at the air-leaf interface, is covered with remnants of the epicuticular wax (Pl. 1, fig. 6). Beneath the epicuticular wax is the cuticular membrane proper which is amorphous in composition and is structurally homogeneous (Pl. 1, figs 6, 7). The thickness of the cuticular membrane is not uniform all over. This cuticle conforms to Type-6 of Holloway's classification of the cuticle types in the leaves of modern plants. The inner face of the cuticular membrane that is in contact with the epidermis develops cuticular pegs or anticlinal flanges (Pl. 1, figs 6, 7). These pegs/flanges are pointed at the tips and have wide bases. The cuticular flanges are located between the walls of adjacent epidermal cells (Cutter, 1978). Cuticular flanges have also been reported in another fossil leaf, *Ticoa harrisii* from the Early Cretaceous of Argentina (Archangelsky, Taylor & Kurmann, 1986). Several ovoid gaps are seen in the matrix of the cuticular membrane which are the result of not very highly satisfactory impregnation of the embedding medium.

The cuticular membrane of *T. indica* leaf that is infected with epiphyllous fungi is also covered by remnants of the epicuticular wax at the leaf-air interface (Pl. 1, fig. 5). The matrix of the cuticular membrane is amorphous (Pl. 1, figs 1, 2); at certain places electron dense spots are seen which in all probability are particles of the stain uranyl acetate (Pl. 1, fig. 4). The inner region of the cuticular membrane appears to have been formed of aggregations of irregular bodies (Pl. 1, fig. 3), which have the same density as the other region (Pl. 1, fig. 4). This region is termed here as the 'disturbed zone'. These irregular bodies were possibly formed by the break down of cutin at the sub-cuticular surface due to the action of some enzyme (?cutinase) released by the

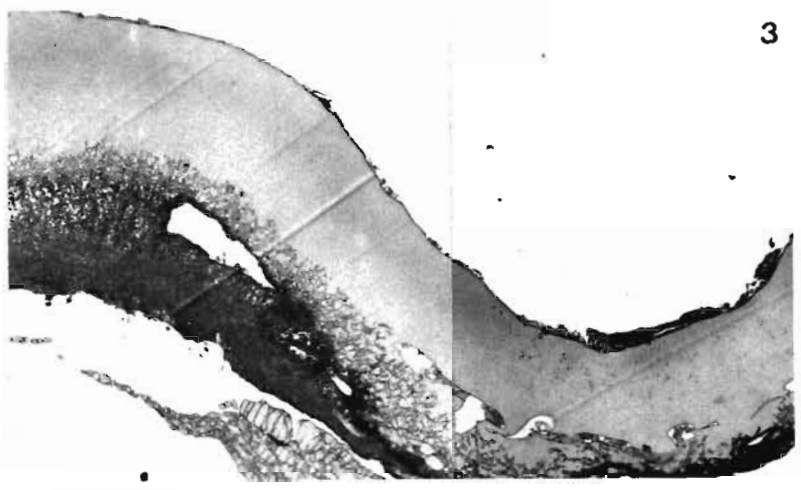
PLATE 1

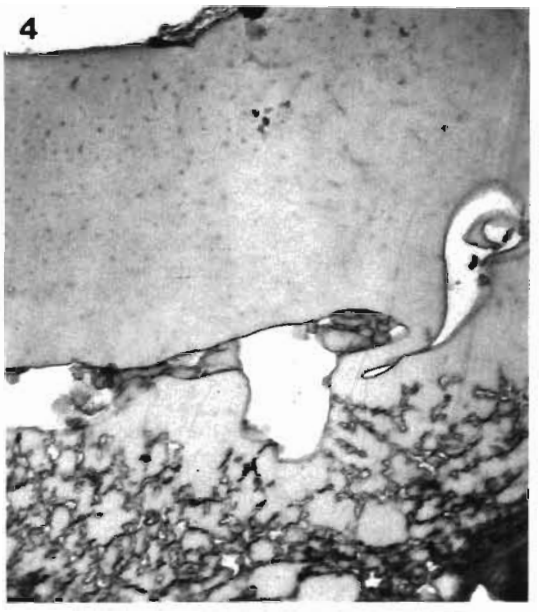
1-3. *Thinnfeldia indica* Feistmantel, micrographs of cross-section of an infected cuticular membrane showing 'disturbed' sub-cuticular layer. 1, x 4400; 2, x 7100; 3, x 7100.

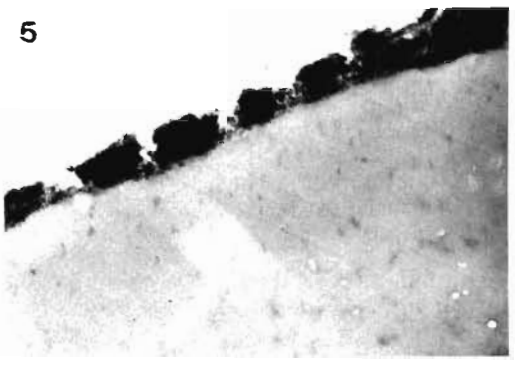
4. A part of the same enlarged further to show the amorphous matrix and aggregations of irregular bodies at the sub-cuticular level, x 21000.

5. Epicuticular wax on the infected cuticular membrane, x 21000.

6-7. Micrograph of the cuticular membrane of an uninfected leaf showing the amorphous matrix and cuticular pegs, x 8800.







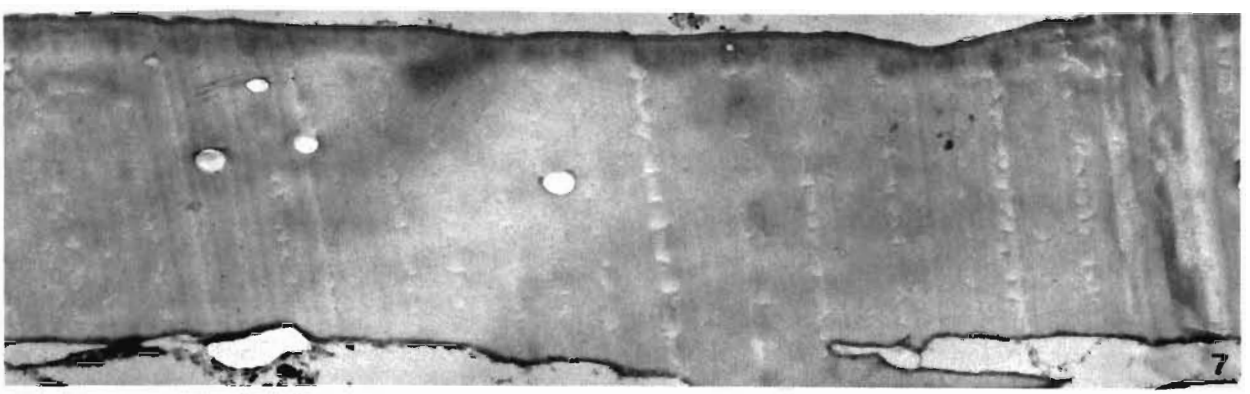


PLATE 1

infecting fungi. Certain other structural deformities of the amorphous matrix are also seen in the disturbed zone as discussed by Maheshwari and Bajpai (1996).

REMARKS

As a result of the available information on the fine structure of uninfected and infected cuticular membranes of *Thinnfeldia indica,* it has been classified under the Holloway's Type-6. Both the cuticular membranes described here possess an amorphous homogeneous region of significant thickness. The well-developed anticlinal flanges indicate a machinery to minimise water loss from the leaf surface, but whether this information supports the views that the climate of the region during the Early Cretaceous was warm tropical is open to question. From the palaeogeographical maps of that period it is evident that India lay in the sub-temperate zone, south of the palaeoequator.

Degradation of the epicuticular waxes may be due to burial and diagenesis. The consistency in the fine structure of both the normal and infected cuticular membranes demonstrates that diagenetic factor did not disturb the structural identity but the fungal infection did result in degradation of the cuticular membrane on the inner surface. Fungi are known to secrete cutin-hydrolysing enzymes, such as, cutinase (Kolattukudy, 1980).

The most common plant fossils are compression fossils of leaves that have lost their carbonified crust. Such fossils frequently occur in argillaceous shales, and in extremely rare cases in arenaceous sediments. The carbonified crust consists of an almost unaltered cuticle and badly crushed unidentifiable remnants of other tissues of the leaf. How and when the cuticle is lost from the compression fossils is yet not very clearly known. It is possible that the cuticle along with other tissues of the leaf is lost during transport due to abrasion, or more likely it is lost due to microbial activities during diagenesis.

A host of phytopathogens is known to grow under natural conditions on both living and dead plants. In the fossil record, fungal plant pathogens have a long history. In the Gondwana deposits, epiphyllous fungi and bacteria have been reported on certain Permian and younger leaves. It has been found that such leaves invariably do not have a sufficiently well-preserved cuticle. In such cases the initial breakdown of the cuticle by cutinase is probably followed by a bacterial attack as has recently been observed in a *Glossopteris* leaf from a grey shale associated with a Late Permian coal (Bajpai & Tewari, 1990). It would thus seem that degradation of the cuticle in compression fossils is induced by fungi , and in later stages is taken over by bacteria.

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