Primo contributo dell'Università di Firenze allo studio delle realtà ambientali dell'America Latina

Primera contribucion de la Universidad de Florencia al estudio de las realidades ambientales de America Latina

Atti

Ed. L. Brighigna

Firenze, 11 ottobre 1991

Why did Columbus order a defensive wall on the site on wich La Isabela
d from the near village, as early as January 1494?

e only sure that La Isabela had to be abandoned because of the fights and
ns among the colonists, after realizing that no gold mines were available,
· cause can be traced back to epidemic fevers due to the high concentration
n a relatively small settlement. More than a thousand men, with horses,
d hens were living on the tiny promontory, surrounded by 3 or 4 thousands
atives became gradually hostile, since spanish settlers urged them to hard
o which they were no used. Many Taino, after trying to escape, choose to
own relatives and themselves. When Columbus arrived in the island, it was
populated (there were nearly a million inhabitants), according to the
l sources; in three decades, only a few survivors certified the first genocide
y the colonist of the New World.

ssary therefore to trace back not only the life and problems of the european
but also the tragedy of Taino people and culture. It's a work to be
ned with the helps of anthropo-ethnological data, compared and integrated
orical sources not always in agreement.

centenary of the discovery of America seemed to offer a good change to
study of an historical period relevant also for Italy. Columbus was not the
ian in the expedition: Bernardo da Pisa, Michele da Cuneo, Columbus
Bartolomew, and son, Diego and perhaps many others crossed the ocean,
of a Mediterranean sea and of an Europe without frontiers. Unfortunately,
could not be carried out because local authorities in Florence choose not
heir support to the research.

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REPRODUCTIVE ASPECTS IN TILLANDSIA (BROMELIACEAE): ULTRASTRUCTURE OF THE TAPETUM IN TILLANDSIA ALBIDIA MEZ ET PURPUS.

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RIASSUNTO

Tillandsia albida Mez et Purpus vive esclusivamente in una limitata stazione presso Meztitlan in Messico. Alla luce di un'alta sterilità del polline prodotto dalla pianta, che può essere una delle cause della difficoltà di questa epifita ad allargare il proprio areale, abbiamo intrapreso uno studio ultrastrutturale del tappeto dell'antera, tessuto importante nella maturazione delle microspore. Sono state identificate tre fasi funzionali successive nello sviluppo del tappeto: la prima comprende differenziamento e maturazione, nella seconda una secrezione eccrina di sostanze idrofile che interessa le pareti precede una secrezione di tipo granulocrino rivolta alla digestione del callosio che circonda le tedradi, la terza consiste nella progressiva degenerazione e conseguente secrezione olocrina nel loculo. Il Tappeto di *T. albida* risulta essere di tipo secretorio e si sviluppa rapidamente; inoltre il precoce annullamento dei collegamenti simplastici porta a una perdita della sincronia dello sviluppo delle cellule del tappeto. L'asincronia e le fasi secretorie anticipate rispetto allo sviluppo delle microspore sono considerate una causa della non vitalità dei granuli pollinici.

SUMMARY

Tillandsia albida Mez et Purpus subsists exclusively in a limited station near Meztitlan, Mexico. The pollen produced by the plant have a high rate of infertility; this may be one of the reason that the ephiphyte has difficulty in expanding its area of distribution. In view of this, we have undertaken an ultrastructural study of the tapetum of the anther, a tissue that is important for the maturation of the microspore. Three consecutive functional phases have been identified in the development of the tapetum: the first comprises differentation and maturation; in the second, eccrine secretion of a hydrophilic substance that affects the walls precedes a granulocrine secretion whose function is to digest the callose that surrounds the tetrads; the third

consist of the progressive degradation of the tapetum and its consequent holocrine secretion into the loculus. The tapetum of *T. albida* is secretory and develops rapidly; furthermore, the premature distruption of the symplastic connections leads to a loss of syncrony in the development of the cells of the tapetum. This developmental asynchrony and shift in the secretory phases, which occur early with respect to the development of the microspore, are considered a cause of non-viability of the pollen granules.

Tillandsia is, a significant genus of the Bromeliaceae (Benzing, 1980; Francini Corti, 1981) a large family of herbaceous monocotyledons, almost exclusively distributed in the intertropical range of the American continent. The only exception is *Pitcairnia feliciana* Harms et Mildred which grows in West Africa.

Owing to its epiphytic choice the taxon is the most evolutionarily specialized of the Family. This is due to the high morphological and fuctional specialization of the absorbing trichome, a foliar apparatus which is the peculiar characteristic of all Bromeliacee, and to the absence of absorbing roots.

As of today 414 species of Tillandsia have been identified.

The large number of species and the wide diffusion of many of them, as well as their vast range, testify to a remarkable efficiency in the gamic reproduction of the taxon. Nevertheless, some Tillandsias are not very widely diffused and can only be found in a restricted habitat.

For these, it is reasonable to think that something in their reproduction mechanisms does not work properly. This is the case of *Tillandsia albida* Mez & Purpus, a rare Mexican species which lives only one restricted "station": the Barranca of Meztitlan near the homonymous Mexican town. Here the species survives thanks to a very active vegetative propagation.

In an attempt to explain the reason for its restricted habitat, we addressed ourselves to the study of the reproductive organs, taking into consideration that in the literature there is no information at all about the anther tapetum in the Bromeliads. In this paper, we report our ultrastructural observations done on the anther tapetum, a tissue which is very important during the maturation of the male sporogenous tissue.

In the anther, the tapetum is the tissue which divides sporogenous cells, the inner most ones in each of the pollen sacs, from the outer tissues which primarily carry out a mechanical role.

Tapetum is always present in terrestrial plants (Pacini 1985). In the spermatophyta we can distinguish two main kinds of tapetum (Davis, 1966): glandular (or secretory) tapetum typical of almost all of the dicotyledons, and amoeboid (or

invasive) tapetum, present in almost half of the monocotyledons.

Fig. 1 - *Tillandsia albida* Mez et Purpus

The first one is formed by an uninterrupted layer (stratum) of cells, frequently binucleate, which never lose their cytoplasmic individuality in spite of walls disgregation.

It is charecterized by an early loss of direct contact with sporogenous cells, therefore delimiting a cavity, the loculus, in which the sporogenous cells immersed in locular fluid are contained.

In the second kind of tapetum, cells, by means of amoeboid movements, send evaginations among the meiocites, and in most cases this leads to cytoplasms fusion into periplasmodial tapetum. This last, according to Chiarugi (1927), can be either a false periplasmodium, whose nuclei are already degenerating to cytoplasm fusion, or real periplasmodium whose nuclei keep functioning in the syncytiun for a long time.

Both kinds of tapetum give the same contribution to the nourishment, growth an vitality of the microspore (Hesslop-Harrison and others, 1969; Lombardo an Carraro 1976a, 1976b; Pacini and Jumiper 1979).

In the secretory tapetum, the cells maintain their initial position. They are polarize and comunicate with the microspores through the locular fluid.

In the amoeboid, we see a direct contact of the amoeboid cytoplasmic mass wit the microspores.

An hypothetical phylogenesis of the tapetum on morphological bases and on th basis of the nutritional relationship with the microspores, seem to indicate

tendency of the tissue to reach a greater contact between its cells and the pollen grains, using three inter-connected modes: 1) intrusion of cells among the spores, 2) loss of cellular walls, 3) reduction of the anther.
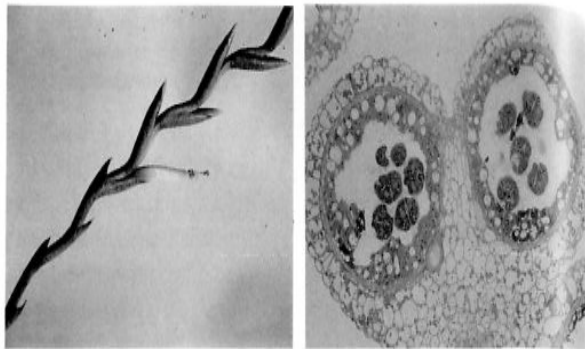

Fig. 2 - The inflorescence (helicoid cime) of *T. albida*

Fig. 3 - Detail of a cross section of *T. albida* anther before the deiscence. The gland tapetal cells which cover the pollen sacs (locule) are intensively stained. Inside the sacs the fertile cells are at the stage of tetrads. Stained with toulidine bleu.

MATERIAL AND METHODS - The anthers used in our research were obtained from *Tillandsia albida* Mez et Purpus plants gathered in Mexico in 1986, during a scientific expedition financed by the Deparment of Plant Biology of the University of Florence, and still living in the Botanical Garden of the above mentioned University. Tanks to the acropetal development of the flowers, the simultaneous collection of buds at different heights permitted the testing of stamens at various stages of maturity.

Small pieces of anthers, about 2mm in length, were fixed in 2,5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7,4, postfixed in 2% OsO4 in the same buffer, successively dehydrated and embedded in epoxy resin

KEY TO LABELING: (C) Callus; (d) dictiosome; (I S) intercellular space; (L) locule; (m) mitochondrion; (M T) mechanic tissue; (m l) middle lamellae; (n) nucleo; (P) plastid; (Pk) pollenkit; (RER) rough entoplasmic reticulum; (S C) sporogenous cells; (T C) tapetal cell; (V) vacuole; (W) wall.

Fig. 4 - The tapetum at the time when the sporogenic tissue is still undifferentiated. The tapetal cells may be distinguished from the esternal ones which are smaller and flat, by the isodiametric shape. The sporogenous cells reveal organelles at an early stage of development. Noteworty in the tapetal cells are: the fragmented vacuome (note the presence of fusion figures), the prominently lobate nucleus and the plasmodesmata that connect the cytoplasms. The sporogenous cells are already wrapped in a thin layer of callose of low electrondensity. (x 3,900).
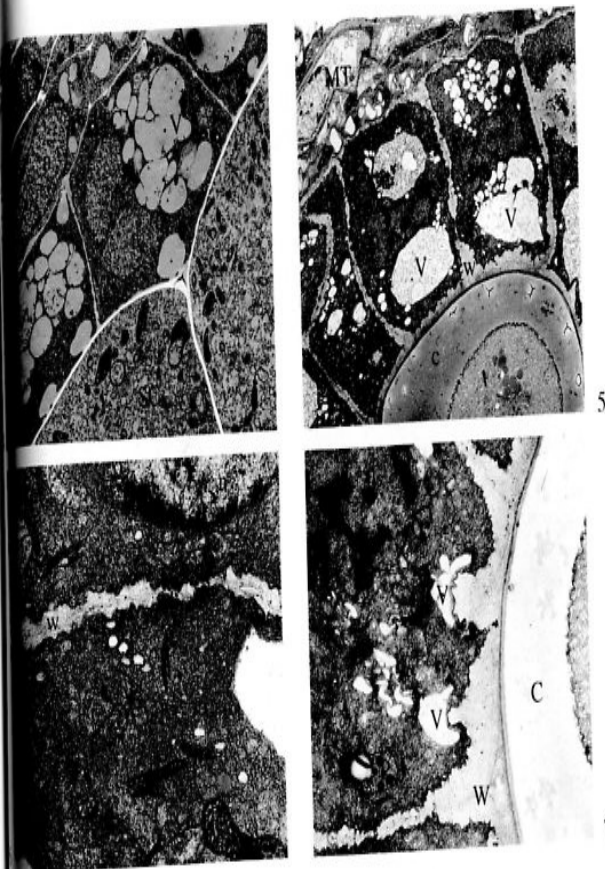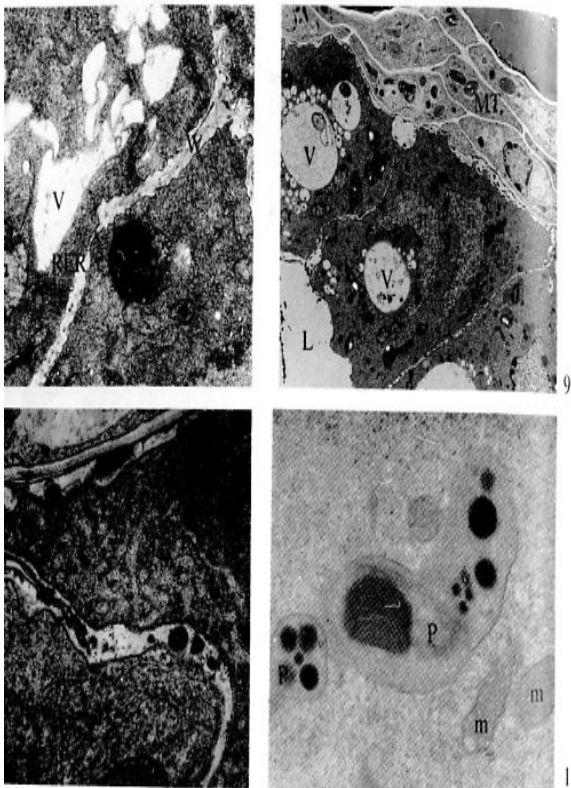

Fig. 5 - The tapetum during the meiotic prophase of the mother cells of the microspores. The tapetal cells have assumed an elongated shape and, except in areas in which plasmodesmata are present, feature prominent parietal swellings. Their vacuome are larger and have diverse contents, while the cytoplasm is very dense. In the lower cell, two nuclei are seen. The sporogenous cell is wrapped in a thick layer of callose. (x 2,300).

Fig. 6 - The tapetum during the meiotic prophase of the mother cells of the microspores. Detail of a tapetal cell. The radial wall tract has a very irregular profile and is crossed by plasmodesmata. Clear golgian vesicles are moving towards the wall. (x 14,300).

Fig. 7 - The tapetum during the meiotic prophase of the mother cells of the microspores. Detal of a tapetal cell. Medium-sized vacuoles are in contiguity with the more prominent parietal thickening on the side of the mother cells, which are wrapped in the callose sheath. Note the dense cytoplasm, the highly-scalloped shape of the plasma membrane, and the fusion figures that characterize the vacuoles (x 7,400).

- The tapetum during the meiotic prophase of the mother cells of the microspores. Detail of :tal cell. The RER profiles more numerous near the wall, and the cytoplasm is very ondense. (x 10,700).

- The tapetum during the tetrad stage. The digestion of the wall is already advenced, as iced by the intracellular electrondense aggregates. The nuclei are lobate, while the vacuome dominantly composed of large, spherical elements. Note in the cytoplasm a circular zone ') occupied by dense collectin of electrondense particles. (x 2,500).

D - The tapetum during the tetrad stage. Detail of a tapetal cell. Residual segments of the : lamella, whose profiles are marked by the accumulations of electrondense materials, persist n the radial intercellular space, together with higly electrondense gates. (arrows) (x 11,300).

1 - The tapetum during the tetrad stage. The detail shows a plastid containing a compact f grouped membranes and black globules. (x 22,200).

(Luft, 1961). Ultrathin sections obtained using a REICHERT OM U3 ultramicrotome were contrasted with uranile acetate (Gibbons and Grimstone, 1960) and lately with lead citrate (Reynolds, 1963).

Observations were made using a PHILIPS EM300 at 80 KV transmission electron microscope.

RESULTS - The first picture (fig. n. 1) shows a *Tillandsia albida*. As we can see the base is dried and already dead but what remains is in excellent condition and does not show any traces of roots, this is a characteristic of almost all "atmospheric" Tillandsias.

The second picture (fig. n. 2) shows a *T. albida* inflorescence with a helicoid cime. The lowest flower is already open, the others have still to bloom. The next picture (fig. n. 3) shows two pollen sacs of an anther of *T. albida*, observed with an optical microscope and coloured with toluidine blue at 0,1%. We can anticipate that the tapetum of the *T. albida* is of the glandular type. In the picture fertile cells are still assembled in tetrads inside the two loculi. The first picture obtained with an electron microscope (fig. n. 4) shows how the tapetum appears at the moment in which *the fertile cells slightly differentiated (shown by the thin and non-homogeneous matrix, by the small mitochondria and by the intensely contrasted amoeboid plastids) have already formed a first thin parietal layer of callose that appears electrontrasparent.*

The tapetum cells have a rather isodiametric shape and very thin walls. The radial ones are crossed by a lot of plasmodesmata (ultrathin pores) which maintain the connection between their cytoplasms. The cytoplasm is electrondense due to the great number of ribosomes; mitochondria are small and elongated. The plastids are also small containing a few starch granules, and the system of inner membranes is restricted to small vesicles. The nucleus is large and lobate. The vacuome is well developed but fragmented in numerous elements. In the upper-right corner, the outer mechanic tissue cells are flat and elongated in periclinal direction. The following picture (fig. n. 5) shows the tapetum cells *when they are at the same stage of maturation as the mother cells of the microspores characterized by a very thick callose sheath.* The first thing we notice is that the tapetum cells are elongated in a radial direction.

The greatest phenomenon concerning these cells is connected with the irregular swelling of the walls which remain thin only where plasmodesmata are present. The wall swellings are more remarkable in the lower part, or to be more exact, near the sporogenous tissue. The cytoplasm is now richer in ribosomes which increase its

electrondensity especially in the peripheral band. The nucleus has divided as we can see in one of the two central cells and this is confirmed by optical microscope observations. The nucleoli are large, porous and emit swarms of ribosomes. The vacuome tends to collect into two larger elements and shows electrondense contents. The dictyosomes (fig. n. 6) bud large clear vesicles which successively migrate to the periphery and enter into contact with the cell walls. In the lower-left side is a plastid showing vesicles.

The following pictures show two aspects, which soon become apparent in these elongated cells. Here (fig. n. 7) we can see little vacuoles entering into contact with the more marked wall swellings; we can easily see an electrondense fibrillar component contrasting with the electrontransparent matrix.

In the following one (fig. n. 8) the presence of longer or shorter RER profiles is evident near all walls. This is a generalized aspect.

From these first pictures at the electron microscope we can conclude that after having reached the elongated shape, the tapetum cells start an activity of proteic (enzymatic) synthesis attacking their own walls, as will be shown in the following pictures.

This is evidenced by the preferential positioning of the RER and by the spatial contiguity of the vacuoles with the parietal swellings, while the early activity of the dictyosomes must be considered only destined toward the wall growth. Therefore, in this primary phase the tapetal cells show a secretory activity of eccrine type.

This secretory process is more evident in the following pictures. The figure n. 9 shows the alteration taking place in the tapetal cells. Because of the total disappearance of the inner tangential walls, a locular cavity is formed. *Inside the loculus now there are tetrads sheathed by callose.* Even the other walls (radial and outer tangential) have lost electrondensity and are similar to electrontransparent clefts between the cytoplasms. In their interior, there are small masses of different shapes but all strongly electrondense, as we can clearly see in the figure n. 10. Here, the clear transverse sinuous band is the image of one of these clefts. On the right side there are three small masses of different sizes and electrondensity; on the left side, a residual line of the middle lamella shows on both sides accumulation of electrondense materials.

Obviously the dissolution of the walls brings about the disappearance of the cytoplasmic bridges and it is evidenced by the loss of synchrony during the development of the tapetal cells. This phenomenon is more evident with the optical microscope. Now the cytoplasm is less electrondense compared to the previous
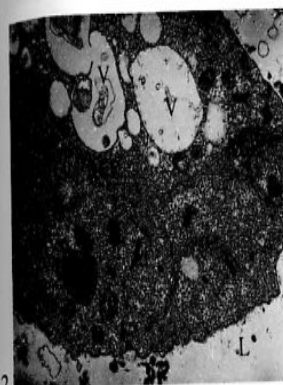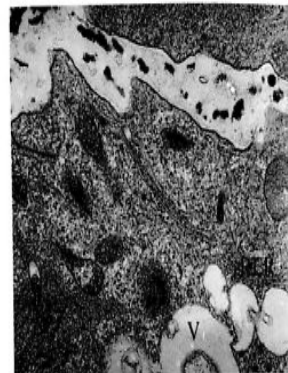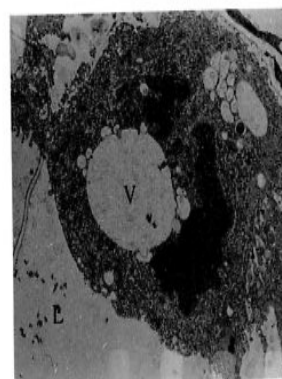

Fig. 12 - The tapetum during the tetrad stage. Detail of a tapetal cell. Noteworty in the cytoplasm the presence of RER long parallel profiles assembled in bundles. The dictyosomes are abundant and compact. (x 7,900).

Fig. 13 - The tapetum during the tetrad stage. Detail of a tapetal cell showing a RER profile which makes contact with the plasmamembrane through one of its dilated edges. Now the dictyosomes are budding very small vesicles. (x 12,000).

Fig. 14 The tapetum during the young microspore stage. Detail of a tapetal cell which demonstrates the marked sinuousness of the intercellular space. The ER network, whose elements are dilated and laden with contents of medium electrondensity, also stands out. (x 6,200).

Fig. 15 - The tapetum during the young microspores stage. Detail of a tapetal cell. It completely lacks the inner tangential wall, and the plasmamembrane profile reveals several point of granulocrine secretion. (x 3,000).

phase and thus it is possible to observe clearly the mitochondria. In the plastids appear small black globules and only one compact group of membranes (fig.n. 11). Microtubules are found along the plasma membranes in various positions.

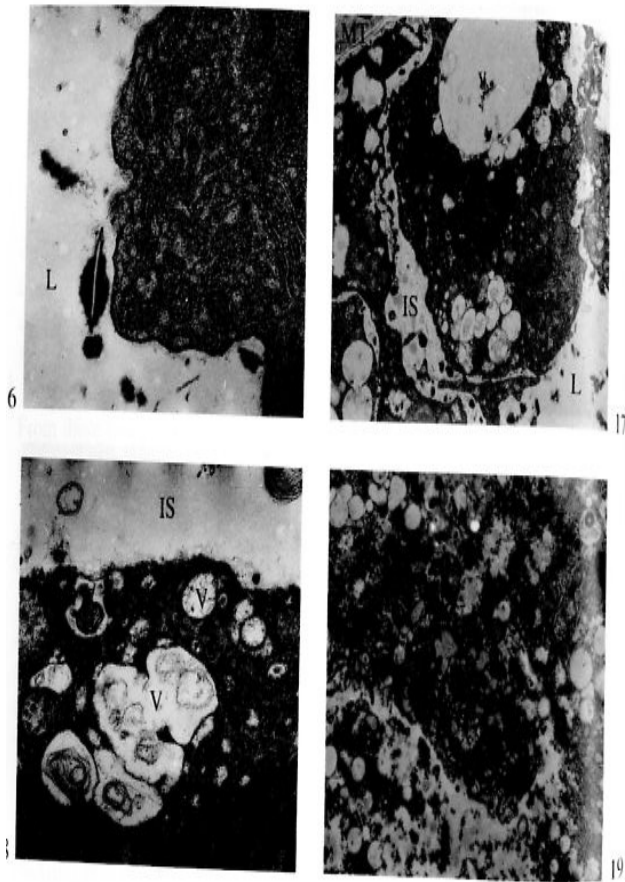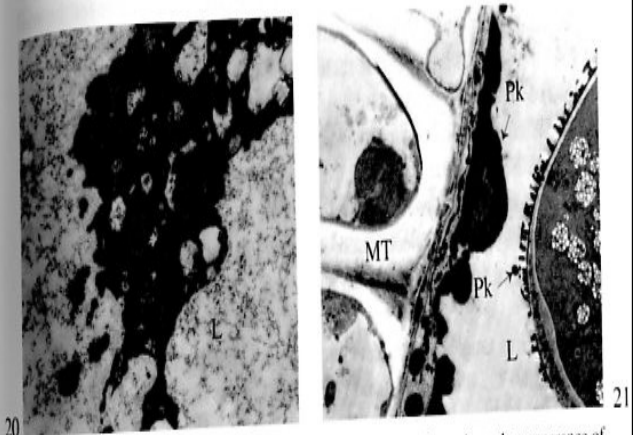The enzymatic phase, involving the walls, is completed.

Fig. 16 - The tapetum during the microspores stage. Detail of a tapetal cell in which sings of secretory activity are present. (x 21,000)

Fig. 17 - The tapetum at the microspore stage. The cytoplasm contained among the close ER network is marked osmiophilic. The appearance of small, osmiophilic bodies (arrow) which make contact with the plasmamembrane is significant. (x 4,600).

Fig. 18 - The tapetum at the microspore stage. Detail showing the presence of small dark bodies (orbicula) and similar osmiophilic bodies in the vacuoles located near the plasmamembrane. Within the same vacuoles, membranous tangles are present. (x 14,100).

Fig. 19 - The tapetum during the young gametophyte stage. Sings of cytoplasmic disorganization and lysis are evident. The organelles are less distinct from the cytoplasm because of the progressive loss of their membranes. Only the mitochondria are still identifiable for a longer period. A disordered clump of tubules and vesicles represents the ER residue. (x 6,000).

Fig. 20 - Tapetum during the young gametophyte stage. The cytoplasms have the appearance of irregularly-shaped masses in which no distinct compartment can be recognized. More strongly osmiopilic materials are present. A floccular component appears in the locule. (x 13,200).

Fig. 21 - Detail, of a pollen sac during the young gametophyte stage. Note the ovoid or spherical osmiophilic globes (pollenkit) of variable size that are present both on the tapetal residua and on the exine. (x 2,500).

Before other changes will occur in the sporogenous tissue (the loculus still contains tetrads) other alterations will be manifested in the cytoplasm of the tapetal cells, as we can see in this image (fig. n. 12). A wide diffusion of bundles of long RER profiles appear and at the same time the cytoplasmic matrix has rarefaction areas; the still numerous dictyosomes have changed their morphology; instead of budding vesicles of clear content they secrete very small vesicles. The most peripheral bundles of RER profiles can make contact with the plasma membrane as we can see in the following figure (fig.n. 13). In this picture we also notice numerous masses of elecrondense material and abundant fibrils in the electrontransparent cleft (formerly the wall) at the lower side and figures of fusion involving the vacuoles. Later on, in the cytoplasmic tapetal masses which, deprived of wall constriction, press on the plasma membranes giving them the more sinuous profiles (fig. n. 14), the extensive endoplasmic network, now swollen, can be clearly seen. In the

following figure (fig. n. 15) this phenomenon is fully demonstrated. Near the loculus, ER elements come into contact with the plasma membrane and they open showing a granulocrine secretion clearly visible in the following image (fig.n. 16). Going back to the previous picture (fig. n. 15), we also notice a reduction of osmiophilia of the plastidial matrix. Figures of fusion still involve the vacuome. In summary, we can say that the eccrine secretive phase, responsible for the wall degradation, is followed by a different synthesis phase (bundles of RER profiles), which anticipates a granulocrine secretion on the part of extensive ER network. The appearance of a large number of small electrondense bodies, prevalently located along the radial plasma membranes, characterizes a following phase of the tapetal cells, as we can see in tha next figure (fig. n. 17). *The phase starts simultaneously with the end of the tetrad stage, which involves the dissolution of the callose layer.* Small electrondense bodies, like the ones described above, are also present in small peripherical vacuoles (fig. n. 18), which can also contain membraneous tangles.

Membraneous tangles which resemble plastids being digested by vacuoles (this phenomenon has been noted and published for the *Tillandsia caput-medusae* pollen) can be seen. In this phase, the entire electrondense membranous complex inside the plastids is dissolved. The same is observed for the plastoglobules. We can see the electrondense cytoplasm divided into small portions of different shapes among the lightest strands of the very extensive ER network. A brief consideration of the phenomena deducible from the aspects seen in the latest figures, lead us to identify the small electrondense bodies placed along the plasmalemma as orbiculi because of their analogy to images and situations found in literature (Echlin, 1971; Heslop-Harrison and Dickinson, 1969). Their production seems connected to the enzymatic activity of the vacuole with respect to the plastids. The maturation process of the tapetum ends with the appearance of chaos within the cytoplasmatic masses (fig. n. 19), where a confused heap of tubules and little vesicles is all that remains of the ER network and of the Golgi apparatus. The plastids are no longer seen. The mitochondria (which are more persistent) become small roundish organelles, delimited by only a membrane and containing few cristae; more or less accentuated rarefaction areas open within their matrix. In the following picture (fig. n. 20) we can see how the tapetum cells have transformed: in spongy aggregates of more or less strongly osmiophyllic masses. Around them, in the locular fluid, a flocculent material appears. *In the loculus the microspores have already formed their most external sculptured wall (exine).* The last picture (fig. n. 21) refers to the final destiny of the tapetum. The tissue is now

reduced to irregular black heaps adhering to the mechanical tissue and sometimes to the exine of the pollen grains.

The first consideration that we can draw from our observations is that in *Tillandsia albida*, the tapetum is glandular and has a rapid devalopment (with reference to the life of the sporogenous tissue), compared to the cases found in literature. This is to say that *Tillandsia* seems to place itself among those monocotyledon genera characterized by an inferior stage of evolution (Pacini and other, 1985; and Chiarugi, 1927) with respect to the functionality of the examined tissue. On the other hand, since glandular tapetum is the most diffused kind among plants living in xeric enviroments (Pacini and others, 1985), this characteristic of *Tillandsia albida* may not be correlated with its evolutive stage, but instead with the choice of environment. In fact, we can speculate that the liquid, gathered in the loculus and covered by the glandular tapetum, may have an insulating function related to the fertile cells.

The morphological variations observed in the tapetum devolopment have permitted us to identify three functional phases.

The first, which can be defined as differentation and maturation, inclused the attainment by the cells of an elongated shape in a radial direction and the preparation of a rich proteinic (enzymatic) supply sufficient for a following phase of secretory activity. This phase ends approximately when the microspore mother cells appear encircled by a thick sheath of callose.

The second phase is characterized by aspects connected with the secretion of hydrophilic substances: more enzymes destined for the digestion of the walls (eccrine secretion) and other substances introduced into the loculus (granulocrine secretion). It starts exactly when the loculus is formed and ends as soon as the callose sheath around the tetrads (whose microspores have completed their exine endogenous layer) disappears.

The last stage includes a progressive cellular degeneration and a generalized lysis of the cells which is simultaneous with the first phase of maturation of free pollen grains.

During the first stage the tapetum cells are joined together by cytoplasmatic strands (plasmodesmata) which insure the synchrony of their differentiation. This is true even when the walls undergo the swelling process. This modification, derived either from the contribution of new material, through the considerable dictyosome activity, or, at least in part, from the diminished compactness of the amorphous matrix, as suggested by the untidy disposition of the fibrillar electrondense component, does not involve those radial wall tracts which are crossed by group

of contiguous plasmodesmata.

Plasmodesmata are also initially present between the tapetum and the sporogenous cells, but, as cells deposit callose, the plasmodesmata are interrupted. Similar behaviour has been recorded by Pacini and Juniper (1979) in the secretory tapetum of *Olea europea*.

The irregular and lobate shape of the nucleus and its division testify to the secretory nature of the cells and the necessity of rapid and efficent nucleus-cytoplasm inter-relationships which are realized through an increase of the exchange surface. The porosity of the nucleolar mass, which is large, and the swarms of electrondense granulules they emit are connected to the large increase in the number of ribosomes needed for the marked protein synthesis required by the cells.

The spatial contiguity of some of the vacuoles with the more marked wall swellings, can be explained as a indication of enzymatic activity already in act in connection with the walls.

The second functional stage is characterized by an early loss of synchrony. As a matter of fact, we observe different degrees of development in the cells and in their cytoplasmatic osmiomphilia, probably due to the disappearance of the symplastic connections. The digestion of the substances of which the walls were composed is evidenced by the electrontransparency of the spaces between the radial plasmamembranes and by the fact that the inner tangential plasmamembrane delimits the locular cavity. The enzymatic attack on the walls, indicated by the presence in the peripheric cytoplasm of RER cisterns parallel to the plasma membranes and of a large quantity of free ribosomes, causes the appearance of the various electrondense materials distributed in the intercellular spaces. Without excluding a variety in the kinds of substances involved, careful observation of the different masses has lead us to consider most of them as subsequent stages of aggregation of material of parietal origin (see diagram A). This because the electrondense detritus deposited on middle lamella fragments and the porous granular aggrgations, prevail in the first stage, while later, the quantity of compact masses increases, some of them still diametrically divided by a middle lamella residue.

The degradation of the walls assures a glycosic contribution to the fertile cells. But since Echlin (1971) denies the possibility that molecules bigger than a triose can penetrate the callose filter and Southworth (1971) points out the incorporation of glucose in the callose wall which acts as a barrier, only later can the cytoplasm of the fertile calls benefit from the greater part of the products of parietal hydrolysis of the tapetum.

Therefore, initially the locular fluid should be enriched by the glycosic contribution. The presence of microtubules near the plasma membranes is probably connected to the disappearance of the walls and therefore to the necessity of an intra-cytoplasmic cytoskeletal alternative. However, this becomes less effective when the cytoplasmic masses take up very irregular countours.

A contibution of the microtubules to the maintenance of the shape of the tapetal cell has been reported by various authors (Steer, 1977; Pacini and Juniper, 1979; Tiwari and Gunning, 1986a, 1986b; Pacini and Keijzer, 1989).

The fact that radial plasma membranes of the contiguous cells do not meet can be explained by the persistence on their outer side of residual wall components which are not sufficiently electron-opaque.

The appearance of bundles of extended RER profiles and the modified apperance of the dictyosomes are the first signals of the different secretory activity in which the tapetal cells will be involved. This aspect of the RER is a clear sign of a proteinic synthesis extended troughout the whole cytoplasm since the bundles are widely distributed. This synthesis takes place before the appearance of the ER network (fig. 11) and the successive chromatic modification (darkening) of the inter-connected cytoplasm (fig. 14): therefore, it must be connected to the auto-lithic mechanism which possibly gives even the dictyosomes a part in the distribution of enzymes. The granulocrine activity, evidenced by the vesicles which come into contact with the plasma membranes and subsequently open pouring out their content, appears at the same time as the auto-lithic phenomenon and is connected *with the degradation process of the callosic walls of the tetrads*. At the end of this process the microspores are free.

Regarding the callase synthesis we do not find untrastructural proof in the literature though its production by the tapetum has been demonstrated by Mepham and Lane (1969) but only the observation of the significant presence of RER (Pacini and Juniper, 1979) is reported.

The disappearance of the starch reserves and the high number of the mitochondria complete the picture of this intense cellular activity.

The third functional stage of the tapetum is initially characterized by the appearance of electrondense bodies located along the plasma membrane. On the basis of their morphology and location we identify them as orbicules (or sporopolleninic bodies) (Echlin, 1971; Heslop-Harrison and Dickinson, 1969). This appearance is observed simultaneously with the reduction of the osmiophilia of the plastid matrix and their corpuscles and with the presence, in the peripheral cytoplasm, of vacuoles also containing electrondense small bodies.

Concerning the orbicules formation, Brooks and Shaw (1968-1971) have pointed out that the carotenoids of plastidial origin are precursors of the sporopollenin.

The orbicules formation occurs in *Tillandsia* while the sporogenous tissue concludes the tetrads stage, as reported in *Lilium* by Heslop-Harrison and Dickinson (1969). These authors, moreover, attribute the role of precursors of the sporopollenin to the chloroplast carotenoids of the tissues outside the tapetum and not to the tapetum cloroplasts.

The function of the orbicules seem to be to favour the dispersion of the pollen grains avoiding their adhesionto the internal surface of the anther. It should be said however that another possible purpose of the lipids and of the carotenoids of the tapetum plastids is a contribution to the formation of the pollenkitt (Hesse, 1980; Echlin, 1969).

After the formation of the orbicules and in concurrence with the separation of the pollen grains from the anther, the degenerative process of the tapetum appears generalized and irreversible. The mitochondria, wich have lost all of their cristae, are the most persistent type of organelle maintaining for a rather long time a delimiting membrane, an aspect which has also been reported by Lombardo and Carraro (1976a) in *Antirrhinum maius;* the plastids instead are rapidly absorbed by the cytoplasmatic mass.

This cytoplasm degeneration represents a secretion that must be defined as holocrine. With the generalized osmiophilia of the cellular materials, a fine flocculent electrondense content represent the last secretory stage of hydrophilous material dispersed in the loculus.

When the pollen grains reach complete maturity (the young gametophyte stage), electrondense residual tapetal heaps of different shapes and sizes are adherent to the mechanic tissue and randomly distributed among the isolated grains, more or less in contact with the exine.

This material represents the lipophilic residue of the tapetum which, as is well known, is the main component of the pollenkitt. In conclusion, the tapetum of *Tillandsia albida,* in spite of peculiar ultrastructural aspects (such as the early loss of synchrony in the functionality of its cells) basically develops in a similar way as other secretory tapeta. It is important to point out the rapidity with which its function procedes as compared to the sporogenetic process. In plants from extreme environments, as is the examined species, a rapid development of the biological process is most likely a necessity for survival.

The tapetum has been indicated as the site of a possible cause of sterility by Chiarugi (1927) in *Achillea clavensae* (Asteraceae) in which the occasional lack of

degeneration of this tissue (here of a periplasmodial type) causes the digestion of the pollen granes in a pollen sac or in a whole flower.

The precocious cellular asynchrony that we obseved in *Tillandsia albidia* and the secretory phase that precedes the development of the fertile cells, can be considered the causes of a different kind of tapetal disfunction which explains the high percentage (80%) of *non-vital* pollen grains that we have observed.

This condition is surely a determinant in the difficulty of the species to extend its habitat.
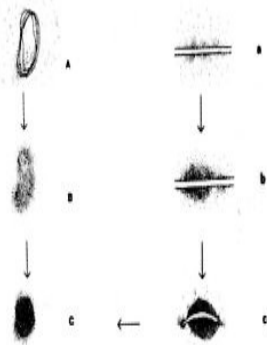
D. 1 - Pathway of the formation of elecrodense aggregates of parietal origin.

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The phyllosphere is a biological entity that is defined (Last, 1955; Ruinen, 1956) by the relationships that exist among the leaves, their exudates, and the epiphtic microflora that grows at their expense. It is the epigeal equivalent of the "rhizosphere" of the root apparatus. This is particularly true as regards the zone called the "rhizoplane".

In this zone, the microflora is in contact with the roots. For this reason, Kerling (1988) proposed the designation "phylloplane" to describe both this entity and the nature of the relationships between the leaves and the epiphytic microflora.

The factors that affect the settlement and characteristics of the micro flora are: the microorganisms that, although telluric in origin, are air-borne; the epiphytic microorganisms that are intimately associated with the leaf; the quantity and nature of the exudates produced by the leaf; the foliar structure and the conditions of the microclimate (i.e., temperature and humidity).

The microclimatic conditions, such as temperature, humidity, and ventilation, in which the leaf exists assume importance for the outcome of the exchanges of energy between the leaf and the environment. Furthermore, they are particularly influential factors in the development of the microflora that populates the phyllosphere. In general, the foliar microclimate does not quite correspond with the environmental climate. Even in the same leaf, it can vary in temperature and humidity according to location (for example, central as opposed to marginal).

The chemical conditions of the phyllosphere are influenced by the foliar exudates. The nature and quantity of the substances released by the leaves are not insignificant, even though they vary considerably from plant to plant. Even in each individual plant, they vary, depending on such factors and its nutritional state, and climate. Substances released by the leaves include mineral compounds; organic compounds, including sugars; pectic substances; organic acids; alkaloids and phenolic compounds; gibberellic acid; and vitamins.

A rich microflora settles on this chemical substrate. It is influenced by the microecological conditions of the soil and on the degree of contamination of the air. Depending on the climatic conditions, this microflora can develop with relative ease.

Following the development of this microflora, a number of interactions are established at the foliar level.

One may easily guess the effect of such a microbial coating on the principal functions of the leaves, they include assimilation respiration and transpiration. Indeed there is no group of microbes, photosynthetic organism included that is not represented, even with appreciable variations from one plant to another, according

to the position and age of the leaves, and location. Clearly, an appreciation of the phyllospheric microbial complex and its ecologic niche contributes important elements to a comprehension of the relationships between microorganisms and plants.

THE PHYLLOSPHERIC MICROFLORA

Leaf surface are colonized both by a random microflora that is conveyed from the environment and by epiphytic microorganisms that are intrinsically associated with the leaves by the utilization of foliar exudates as nutrients.

The most salient characteristics of the phyllospheric microflora are predominance of chromogenic bacteria and fungi; presence of Gram-negative bacteria; absence or scarcity of actinomycetes; and the frequency of bacterial antibiotic producers which are predominatily active on phytopathogenic fungi. The microbial colonization of the leaf occurs by degrees. The first inhabitants of the phyllosphere are the bacteria that originate principally from the buds. These are followed by fungi and yeasts. Among the plants with perennial leaves, the phyllospheric population grows with age. Variations are registered according to the season, achieving maximal density in Autumn.

In certain cases, the bacterial and blastomycetic population of the leaf attains a density greater than that found in the soil. Its estimated weight can represent up to 0.1% of that of the leaves (Di Menna, 1959; Stout, 1960). In general, the foliar microbial load of plants in tropical countries achieves values higher than those of temperate zone (Ruinen, 1951).

The abundance of pigmented forms confers upon the epiphytic microbial population its distinctive nature. Chromogenic bacteria comprise more than 90% of the foliar microbial population. Specifically, these include species of *Flavobacterium, Pseudomonas, Xanthomonas*, and *Achromobacter*. Similary, the yeast population is composed of pigmented species. Often, 90% of the population is composed of red and black pigmented species, such as *Rhodotorula* and *Aureobasidium pullulans*.

There are, however, exceptions. Unpigmented bacteria dominate during periods of active growth of the leaf, and yeasts of the genus *Cryptococcus* can, in certain cases, be more numerous than pigmented species (Di Menna, 1959; Ruinen, 1966). Among the fungi, species of the genera *Cladosporium, Alternaria, Epicoccum*, and *Aureobasidum* are particularly widespread.

The predominance of pigmented microbial species reflects a selective adaptation against the effect of light, which eliminates those species that lack the protection

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LITTLE-KNOWN GROUP OF NITROGEN FIXERS IN THE̶ PHYLLOSPHERE OF NEOTROPICAL EPIPHYTES

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RIASSUNTO

Viene presentata una breve revisione dei più recenti dati bibliografici sui rapporti tra̶ le piante e i microrganismi della fillosfera. Una particolare attenzione è rivolta alla̶ fissazione dell' Azoto, una delle attività più importanti nella fillosfera, e ai batter̶ coinvolti in questo processo. I risultati di due consecutivi indagini sull' azotofissazione̶ nella fillosfera di diverse specie di *Tillandsia*, piante epifitiche che vivono i̶ ambienti tropicali, vengono qui riportati e discussi; in particolare il profilo dell̶ microflora azotofissatrice che colonizza le foglie di *Tillandsia* ed il suo ruolo nel̶ nutrizione azotata di queste piante. Le ricerche suggeriscono che c'é una connessio̶ fra la distribuzione della popolazione microbica delle foglie e lo sviluppo morfologi̶ dei loro tricomi assorbenti in differenti specie di *Tillandsia*.

SUMMARY

A short review of the most recent data on the relationships between plants a̶ microorganisms at phyllospheric level is presented. Particular attention is paid̶ nitrogen fixation, one of the most important activities in the phyllosphere, and to̶ bacteria involved in this process.
The results of two consecutive investigations on nitrogen fixation in the phyllosph̶ of several species of *Tillandsia*, which are epiphytic plants that live in trop̶ environments, are reported and discussed. The profile of the nitrogen-fi̶ microflora that colonizes the *Tillandsia* leaves and its role in the nitroge̶ nutrition of these plants are discussed.
The investigations suggests that there is a connection between the distribution o̶ microbial population of the leaf and the morphological development of the abs̶ trichomes in different species of *Tillandsia*.

THE PHYLLOSPHERE

The surface of the leaf constitutes a distinct ecological environment re̶ the "phyllosphere". It has aroused the interest of many biologists, since it̶ to the welfare of the plant by providing a niche for its microflora, whi̶ bacteria, yeasts, and fungi.