Leaf surface of two understorey shrubs *Rudgea decipiens* Müll. Arg. and *Rudgea macrophylla* Benth. (Rubiaceae)

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ABSTRACT

(Leaf surface of two understorey shrubs - *Rudgea decipiens* Müll. Arg. and *Rudgea macrophylla* Benth. (Rubiaceae)). Micromorphological characteristics of leaf surface of two species of *Rudgea* are presented. The study was based on material from the understorey of Atlantic Forest. Special attention is paid to the occurence of smooth and striated cuticle, to paracytic stomata (some of which exhibit an unusual pattern in the arrangement of the surrounding epidermal cells) and to papillae, which are irregularly distributed on the abaxial leaf surface. Correlations between the leaf surface features and environmental factors are discussed.

Key Words: shade leaves; *Rudgea*; Rubiaceae

RESUMO

(Superfície foliar de *Rudgea decipiens* Müll. Arg. e *Rudgea macrophylla* Benth. (Rubiaceae). São apresentados dados relativos à micromorfologia da superfície foliar de duas espécies de *Rudgea*. O estudo foi realizado em indivíduos que ocorrem no interior da Floresta Pluvial Tropical (Mata Atlântica). Destaca-se a ocorrência de cutícula lisa ou estriada, cera epicuticular sem ornamentação, estômatos paracíticos e paralelocíticos, sendo alguns diferentes do tipo padrão, e papilas distribuídas somente na face abaxial. A correlação entre as características da superfície foliar e fatores ambientais é discutida.

Palavras-chaves: folhas de sombra; *Rudgea*; Rubiaceae.

INTRODUCTION

The National Park of Floresta da Tijuca is one of the last reserves of Atlantic Forest (Tropical Rain Forest) in the city of Rio de Janeiro, Brazil. Due to human activities this ecosystem has been reduced to approximately 10% of its original area in the country as a hole. Considering this decimation, and the limited nature of the existing data about this forest, it is important that a greater attempt should be made to investigate this flora. This present paper represents the second study on leaf surface structure in Rubiaceae from the Floresta da Tijuca, one of the most important families among those occuring in the Atlantic Forest (Robbrecht 1988).

The study of the surface structure of leaves is extremely important due to its recognition as a dynamic covering which represents the first barrier between the ambiance celullar and the variable environmental conditions (Juniper & Jeffrey 1983).

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There are few scientific investigations on leaf surfaces in Brazil based on scanning electron microscopy. Among them, we can include the work of Salatino *et al.* (1986), on leaf surfaces of woody species from cerrado, and the study of Vieira & Machado (1992), which discusses aspects of leaf surfaces from ecotypes of *Bauhinia radiata* (Leguminosae). We can also mention Fontenelle et al. (1994), who studied species of Myrtaceae, and Vieira & Gomes (1995), who have provided informations about the surface structure of shade leaves from four species of the genus *Psychotria* (Rubiaceae) from the National Park of Floresta da Tijuca.

In the present work the authors examine micromorphological aspects of the leaf surfaces of *Rudgea decipiens* and *Rudgea macrophylla*, in order not only to provide valuable structural data for taxonomic purposes, but also to correlate some of this aspects with these plants environment.

MATERIAL AND METHODS

The specimens, *Rudgea decipiens* and *Rudgea macrophylla*, grow in the National Park of Floresta da Tijuca, Rio de Janeiro. *Rudgea decipiens* was collected from the forest reserve of the Rio de Janeiro Botanical Garden, while *R. macrophylla* was found next to the second kilometer of Dona Castorina road which winds throught the Floresta da Tijuca.

In order to investigate the leaf epidermis of the species with the scanning electron microscope (SEM), the following procedure was employed: in the field, leaves from the fifth node were select, cut and fixed in alcohol 70°GL. Then, in the laboratory the leaves were dehydrated in increasing ethanol solutions (Cutler 1979). After this preparation, portions with 1 cm^2 were sectioned from the middle of the lamina and separated in two batches. One batch was immersed in chloroform and boiled twice, for five minutes each time, in order to remove superficial wax cover, according to the process set out buy Martin & Juniper (1970). The other batch was untreated. Fragments from the two batches were mounted on stubs with double-sided adhesive tape, in coated with gold. The scanning electron microscope study of the specimens was carried out with a Jeol 25-S-II instrument, operating at accelerating voltages of 12,5 to 25 K e V.

Leaf epidermis samples were isolated using the Jeffrey's method (Johansen 1940) and observed with a light microscope to analyse the density and the distribution of stomata and pappilae. The samples were placed on a slide and immersed in a drop of glycerin of 50% underneath a coverslip and observed at 400X. Light microscope was used to confirm some anatomical traits.

RESULTS AND DISCUSSION

Rudgea decipiens and *Rudgea macrophylla* are understorey shrubs of the Tropical Rain Forest. Due to this fact, the leaves of *R. decipiens* and *R. macrophylla* have a dark green colour, which acoording to Boardman (1977) and Hart (1988), is due to a greater proportion of type a and b chlorophyll in the chloroplasts of such plants subject to reduce illumination. One distinguishing characteristic of the plants is the size of their leaves: *R. decipiens* is 2 to 5 centimeters long, while *R. macrophylla* measures 20 to 40 centimeters in length.

An analysis of the leaf surface reveals straight anticlinal walls on the two surfaces of the plants in study, while these walls are markdly tricker on the adaxial surface os *Rudgea decipiens* (figures 1 and 5), The non-underlating nature of the anticlinal walls represents an exception to generalizations by Isanogle (1944) and Hughes (1959) that the leaves of plants growing in shade areas, in general, present undulated anticlinal walls with densities proportional to the level of such shade.

In addition to the shape of anticlinal walls on the two surfaces, the environment may influence the cuticular ornamentation. Bergen (1904) and Dunn et al (1965) reported smooth cuticles in leaves growing in shaded environments and striated cuticles in leaves subject to direct sunlight. In the species under study, *R. decipiens* exhibits striations on the adaxial surface. These striations have an irregular and random distribution from cell to cell and confer a wrinkled look to the cuticle (figures 1 and 5). In contrast, the cuticle of the abaxial surface of *R. decipiens* is smooth, as are both surfaces of *R. macrophylla* (figurs 2,3 and 4). However, on the abaxial surface of *R. macrophylla*, there may occur striations on the cuticle of the epidermal cells adjacent to the stomatal apparatus (figures 9, 10 and 11). These figures show that the striations are parallel and confined to the periclinal walls, thus differing from the pattern observed in *R. decipiens*.

The present study also reveals no differences in relation to the epicuticular wax. No ornamentation was detected on either leaf surface of both species of Rudgea. The pattern of was deposition observed is in a continuous manner over the leaf surface. Barthlott & Wollemweber (1981) believe that this latter pattern seems to be most frequent situation in the vascular plants.

Martin (1964) and Juniper & Jeffrey (1983) admit that in leaves with high incidence o fungal flora, the fungi secrete enzymes that are able to dissolve the epicuticular wax. Very large quantities of fungi are encountered on the leaf surfaces of the two species of *Rudgea*, principally on the adaxial surface of *R. macrophylla* (figures 2 and 4). The environment in which these species grow, provides ideal conditions for fungal to flourish. The relative humidity of the Floresta da Tijuca is 82% (Mattos et al. 1970) and the average annual precipitation is more than 2000mm (Vieira 1994).

The stomata, which are distributed only on the abaxial surfaces, are predominantely of the paracytic and parallelocytic type, following the classification of Payne (1970). However, in *R. macrophylla* we observed a type os stomatal apparatus that differs from the rubiaceous type. This different type is characterized by a change in the shape of the stomatal apparatus (figures 10 and 11). Similar structures were described as abnormal stomata for some species of Rubiaceae by Pant & Mehra (1965).

Coutinho (1962) emphasizes that hypostomatism is the norm in species of the tropical rain forests. This author believes that this phenomenon may constitute a protection against the obstruction and obliteration of the stomatal pores by small mosses, liverworts, fungi and other components of the epiphyllous flora that constantly cover the adaxial surface of the species of the rain forest. This is evident in figures 2 and 4 where part of the epiphyllous flora was removed from the adaxial surface of *R. macrophylla*.

The abaxial epidermis of the species studied is characterized by the presence of papillae (figures 12,13 and 14). These epidermal appendages have surfaces ornamented with small wrinkled forms in *R. decipiens* or are smooth-walled in *R. macrophylla*. The papillae can contribute to the protection against the loss of water by the stomata (Napp-Zinn 1988) and improve the reception of light stimuli, important to plants submitted to shade (Bone et al. 1985). Therefore, due to the fact that *R. decipiens* and *R. macrophylla* recive reduced light and that their environments provides a great avaiability of water, one may conclude that the papillae act as converting lenses in terms of light stimuli for the mesophyll, thus meeting the needs of photosynthesis.

Micromorphological features of leaf epidermis have been recognized as helpful tools for taxonomic purposes (Metcalfe & Chalk 1979, Barthlott & Wolleweber 1981, Fontenelle et al 1994). Our observations related here revealed two characters that may be of taxonomic value (Table 1):

Table 1: Micromorphological differences between leaf surface of *R. decipiens* and *R. macrophylla*.

Micromorphological characteristics	*R. decipiens*	*R. macrophylla*
Sttriated cuticle on the adaxial surface	+	-
Pappilae with ornamented surface	+	-

ACKNOWLEDGEMENTS

The authors are indebted to Heitor Monteiro Duarte, for his help taking the photos. The authors would like to thank Conselho Nacional de Pesquisa (CNPq), wich provided the research training grant (N° 110828/92-5), awarded to the first author, Fundação Universitária José bonifácio (FUJB), and also Laboratório de Microscopia Eletrônica do Instituto de Biofísica (UFRJ).

REFERENCES

AHMAD, I & WAINWRIGHT, S.J. 1976. Ecotype differences in leaf surface properties of Agrostis stolonifera from salt marsh, spray zone and inland habitats. *New phytol.* 76:361-366.

BAKER, E.A. & HUNT, G.M. 1986. Erosion of waxes from leaf surfaces by simulated rain. *New. Phytol.* 102:161-173.

BARTHLOTT, W. & WOLLENWEBER, E. 1981. *Zur Feinstruktur, chemie und taxonomischen signifikans epicuticularer wachse und ahnlicher skrete. Tropische und subtropische Planzenwelt.* Akademie der wiss. u. d. Literatur. 32:1-67.

BERGEN, J.Y. 1904. Transpiration of sun leaves and shade leaves of Olea europea and broad-leaved evergreens. *Bot. Gaz.* 38: 285-296.

BOARDMAN, N.K. 1977. Comparative photosynthesis of sun and shade leaves. *Ann. Rev. Pl. Physiol.* 28:355-377.

BONE, R. A., LEE, D. W. & NORMAN, J. M. 1985. Epidermal cells functioning as lenses in leaves of tropical rain-forest shade plants. *App. Opt.* 24: 1408-1412..

COUTINHO, L. M. 1962. Contribuição ao conhecimento da ecologia da mata pluvial tropical. *B. Fac. Fil. Ciên. Letr. U.S.P. Bot.*, São Paulo, 18: 219pp.

CUTLER, D. F. 1979. The scanning electron microscope in recent systematic plant anatomy. In *Anatomy of the Dicotyledons* (C.R. Metcalfe & L. Chalk eds.). Clarendon Press, Oxford, vol. 1, p. 9-11.

DUNN, D. B., SHARMA, G. K. & CAMPBELL, C. C. 1965. Stomatal patterns of dicotyledons and monocotyledons. *Am. Mild. Nat.* 74: 185-195.

FONTENELLE, G. B., GOMES, D. M. S. & MACHADO, R. D. 1994. Foliar anatomy and micromorphology of eleven species of *Eugenia* L. (Myrtaceae). *Bot. J. Linn. Soc.* 115:111-133.

HALLAM, N. D. 1070. Growth and regenerative of waxes on the leaves of *Eucalyptus. Planta* 93: 257-268.

HART, J. W. 1988. Light and plant growth. Unwin Hyman. London.

HUGHES, A. P. 1959 Effects of the controlled shading upon the development of leaf structure in two deciduous tree species. *Ecology* 25: 404-413.

ISANOGLE, I. E. 1944. Effects of controled shading upon the development of leaf structure in two deciduos trees species. Ecology, 25:404-413.

JOHANSEN, D. 1940. *Plant Microtechnique.* Clarendon Press, Oxford.

JUNIPER, B. E. & JEFFREY, C. E. 1983. *Plant surfaces.* Edward Arnold. London.

MARTIN, J. T. 1964. Role of the cuticle in plant defense against plant desease. *An. Rev. Phytopath.* 2: 81-100.

MARTIN, J. T. & JUNIPER, B. E. 1970. *The cuticles of plants.* Edward Arnold. London.

MATTOS, C. C. L. V., MATTOS, M. D. L. V. & LAROCHE, R. C. 1970. Aspectos do clima e da flora do Parque Nacional da Tijuca. *Bras. Fl., Rio de Janeiro*, 7 (25): 7-12.

METCALFE, C. R. & CHALK, L. 1979. *Anatomy of the Dicotyledons*. Clarendon Press Oxford.

NAPP-ZINN, K. 1988. *Anatomie der Blattes.* Gerbrude Borntraeger, Berlin.

PANT, D. D. & MEHRA, B. 1965. Ontogeny of stomata; unrecognized patterns in the Dicotyledoneae. *Am. J. Bot.* 57: 140-147.

PAYNE, W. W. 1970. Heliocytic and allelocytic stomata; unrecognized patterns in the Dicotyledoneae. *Am. J. Bot.* 57:140-147.

ROBBRECHT, E. 1988. *Tropical wood Rubiaceae. Opera Botanica* Belgium, v.1, 269pp.

SADDI, N. 1988. Micromorphological evidence in the genus *Kielmeyera* Martius (Guttiferae). *Rev. Bras. Biol.* 48(4): 697-720.

SALATINO, A., MONTENEGRO, G. & SALATINO, M. L. F. 1986. Microscopia eletrônica de varredura de superfícies foliares de espécies lenhosas do cerrado. *Revta. Bras. Bot.* 9: 117-124.

SKOSS, J. D. 1955. Structure and composition of plant cuticle in relation to environmental factors and permeability. *Bot. Gaz.* 117(1): 55-72.

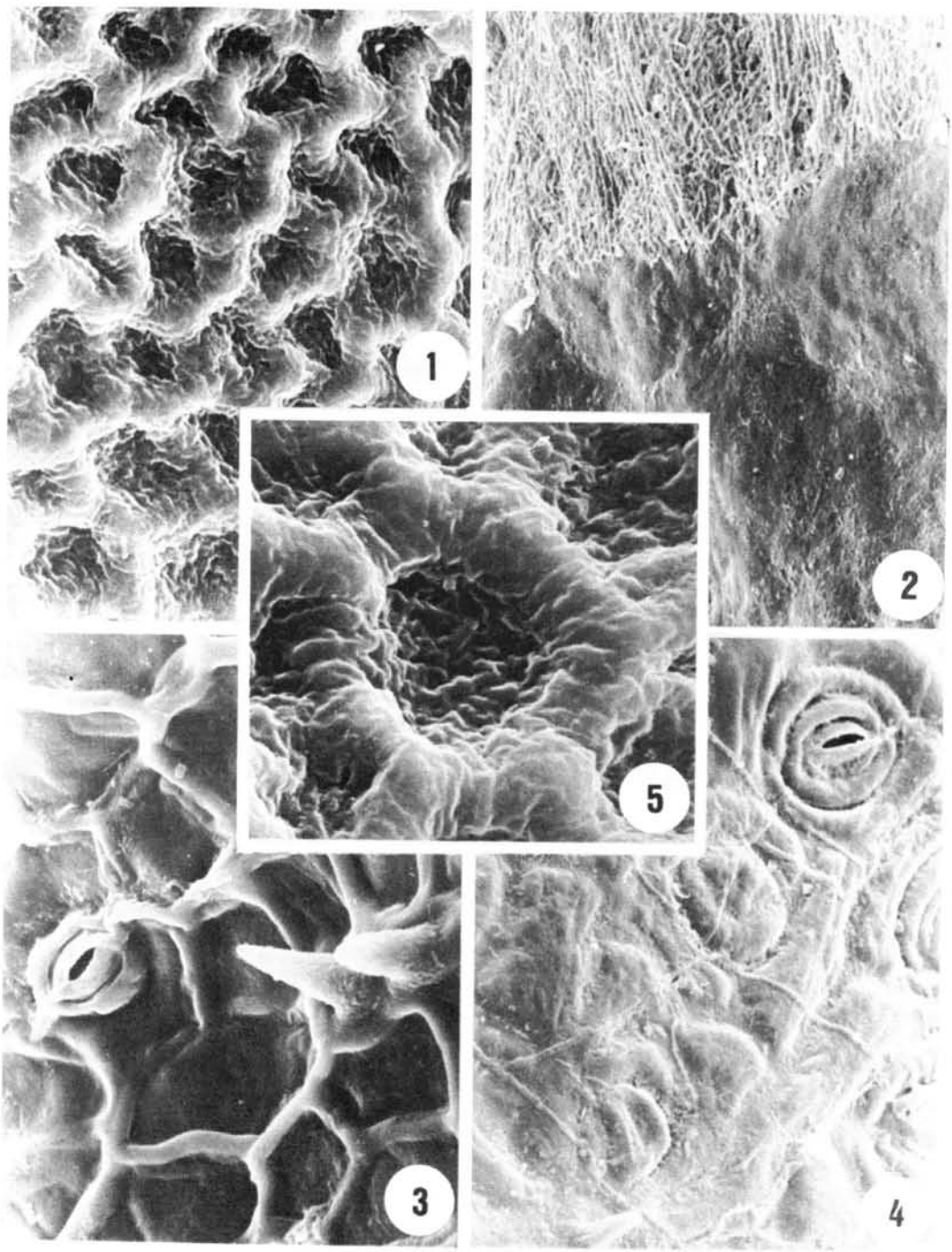
STACE, C. A. 1965. Cuticular studies as an aid to plant taxonomy. *Bull. Br. Mus. Nat. Hist.* (Bot). 4:3-78.

VIEIRA, R. C. & GOMES, D. M. S. 1995. Superfície da lâmina foliar de Psychotria nuda (Cham. & Schltdl.) Wawra, *P. leiocarpa* Cham. & Schltdl., *P. stenocalyx* Müll. Arg. e *P. tenuinervis* Müll. Arg. (Rubiaceae). *Acta bot. bras.* 9(2): 263-270.

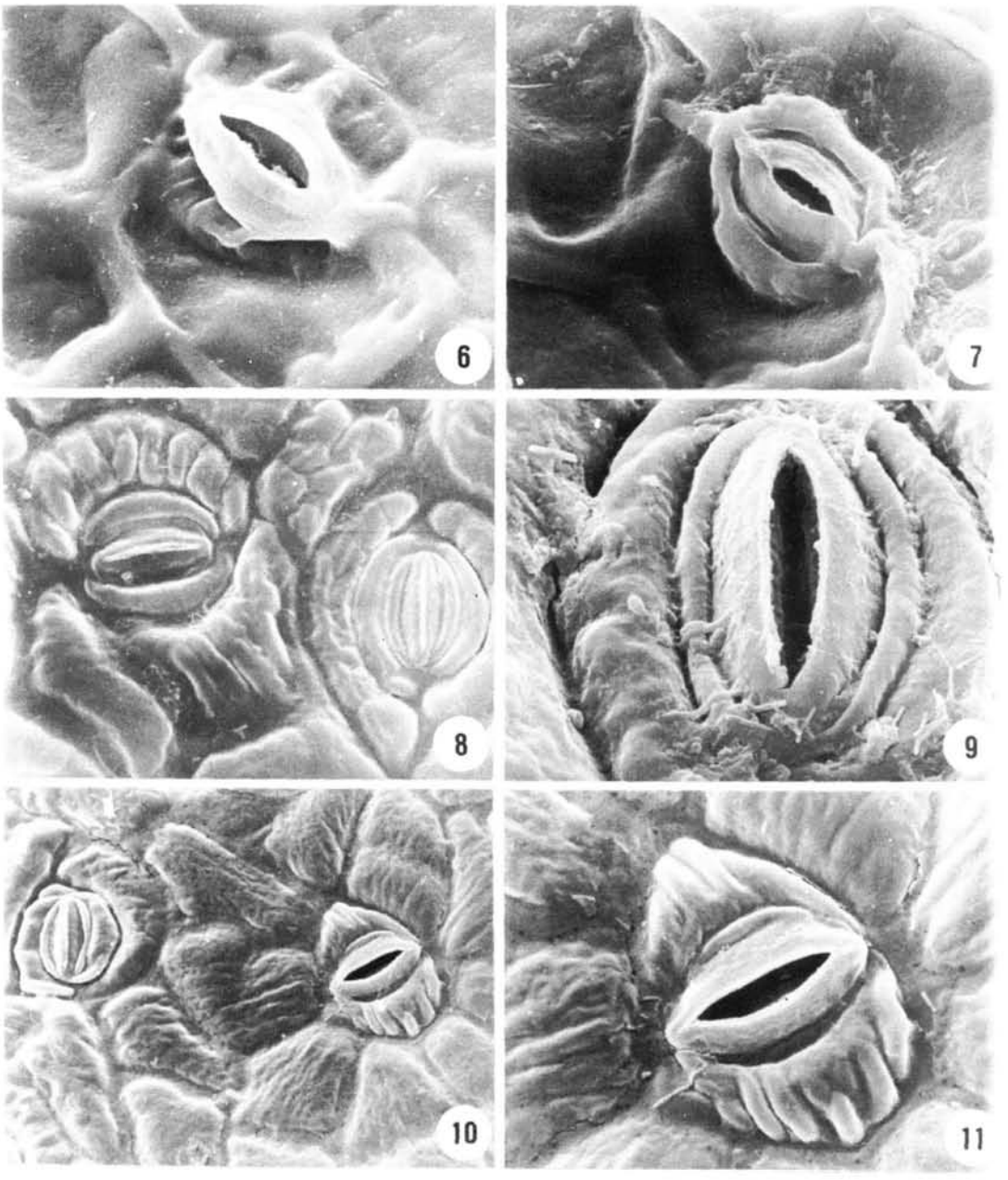
VIEIRA, R. C. 1994. Considerações sobre o clima e o solo da Floresta da Tijuca e de Búzios. *Cad. Geoc.* 12:45-50.

VIEIRA, R. C. & MACHADO, R. D. 1992. Superfície foliar de *Bauhinia radiata* Vell. em dos ambientes. *Hoehnea* 19(1/2):111-116.

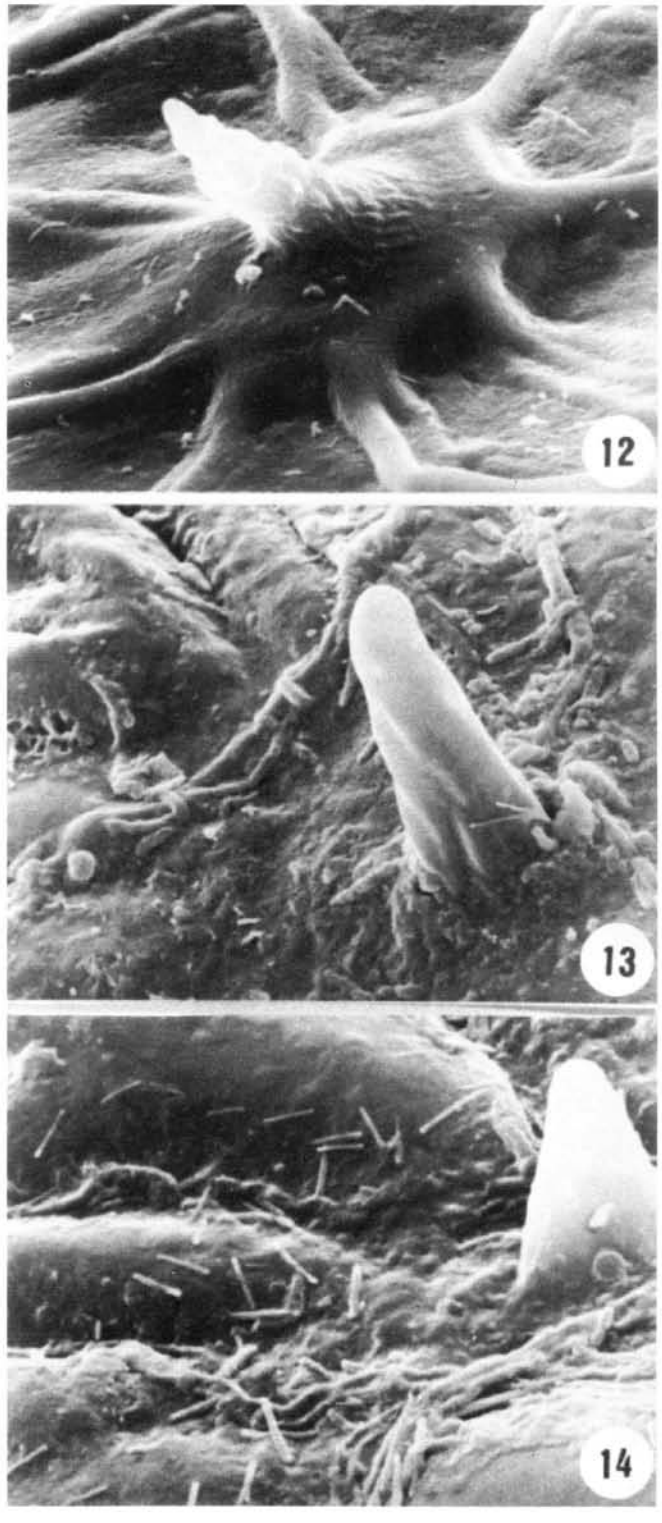
WILKINSON, H. P. 1979. The plant surface. In *Anatomy of the Dicotyledons.* (C. R. Metcalfe & L. Chalk, eds). Clarendon Press, Oxford, v.1, p.97-165.



Figures 1-5. Scanning electron micrographs of leaf surfaces from species of *Rudgea*. General view. 1. Adaxial surface of *R. decipiens* showing striated cuticle (600X). 2. An adaxial surface view of *R. macrophylla* where can be seen the smooth cuticle and epiphyllous flora (600X). 3-4. Abaxial surfaces of *R. decipiens* and *R. macrophylla* (600X). 5. Detail of the adaxial of *R. decipiens* illustrating the wrinkled look given by the striations (1200X).



Figures 6-11. Scanning electron micrographs of abaxial surfaces from species of *Rudgea*. Stomata. 6-7. Paracytic stoma from *R. decipiens* (1250X). 8-9. Stomata from *R. macrophylla* showing the striated and smooth cuticle of subsidiary cells (800X; 2600X). 10-11. Variations on stomata apparatus from *R. macrophylla*. Note the arrangement of the surrounding epidermal cells, radially disposed around the stoma, whose subsidiary cells differ from the standard type (610X; 1300X).



Figures 12-14. Scanning electron micrographs of abaxial surfaces from species of *Rudgea*. Papillae. 12. A cone-like papilla with ornamented cuticle from *R. decipiens* (1200X). 13-14. Smooth-walled papillae from *R. macrophylla* (2600X).