Observations on the Structure and Function of Hydathodes in Blechnum lehmannii

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The fronds of many ferns in the Polypodiaceae (Ogura, 1972) and Cyatheaceae (Weiler, cited in Lippmann, 1925) possess swollen vein endings associated with specialized adaxial epidermal cells. Their structure is similar in all ferns (Gardiner, 1883; Potonié, 1892; Poirault, 1893; Goebel, 1930; Guttenburg, 1934), including *Blechnum lehmannii* Hieron. (*Figs. 1–3*). The depressed epidermis (*Fig. 3*) suggested the term "Wassergrübchen" for the vein endings to European anatomists of the nineteenth century (e.g., Potonié, 1892). Vein endings on developing fronds secrete water (*Fig. 1*) when conditions reduce or stop transpiration (Mettenius, 1856; Haberlandt, 1894a; Goebel, 1926, 1930). In some species, the secreted water contains salts in solution which form "chalk scales" after the water has evaporated (Mettenius, 1856; Potonié, 1892; Lippmann, 1925).

Most sources report that water secretion is dependent on the metabolic activity of the specialized epidermal cells adaxial to the vein ending (*Fig. 3*, e); it is thus regarded as an active, or glandular, process (Gardiner, 1883; Haberlandt, 1894a, 1914; Lepeshkin, 1906; Stocking, 1956; Fahn, 1979). This conclusion is reflected in various references to the vein endings as water glands, salt glands, or chalk glands. There is, however, very little evidence to support any conclusions on the mechanism of secretion. All work on the subject was performed in the last century, and the results obtained are questionable due to the bias in experimental design which prevented testing of alternative hypotheses and to the possibility of artifacts in technique (see comments in Spanjer, 1898; Haberlandt, 1898; Lepeshkin, 1923). Moreover, the results are contradictory, since they have been interpreted as supporting either an active (Gardiner, 1883; Haberlandt, 1894a, 1914) or a passive (Spanjer, 1898) mechanism of secretion. Finally, all experiments were performed on a single species, *Polypodium aureum* L., and need not represent the situation in other ferns.

Because the mechanism of secretion from vein terminations is unknown, they will be referred to in this paper by the term "hydathode" which, as originally defined by Haberlandt (1894b), did not presuppose a mechanism of secretion. Haberlandt used his term to refer to structures on above-ground portions of the plant, particularly foliage leaves, which function in water transfer to and from the plant surface.

Previous work on the mechanism of hydathode secretion in ferns neglected important aspects of the problem. Despite the fact that root pressure could play a significant role in the secretion process, the relationship of root pressure to secretion in intact plants was not studied. In fact, there is no well documented account of root pressure in ferns. In addition, investigators could not analyze secretion from a structural standpoint because anatomical studies were confined to the mature, non-secreting hydathodes; the structure of the secreting hydathodes on developing leaves was unknown. In this study, the relationship of root pressure to secretion as well as developmental changes in hydathode structure were investigated in *B.*

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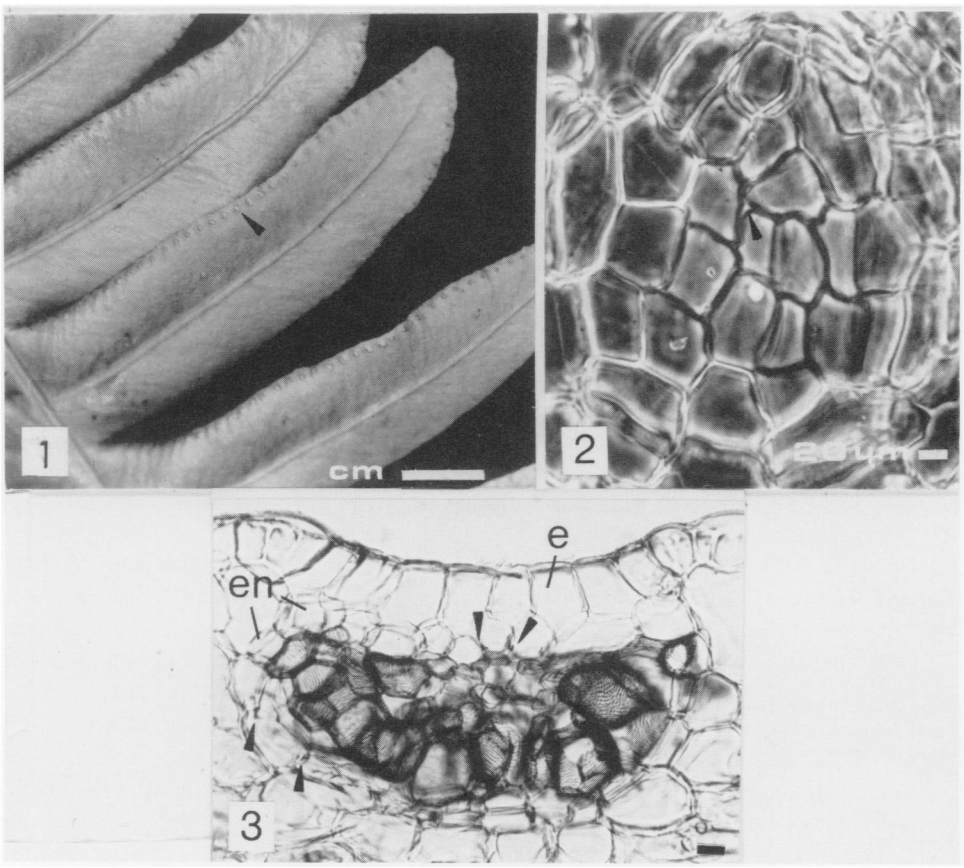


FIG. 1. Hydathodes secreting water on a developing frond; arrow indicates a droplet of water above a hydathode. FIG. 2. Epidermal peel of hydathode photographed with dark field illumination. There is no intercellular space in the epidermal layer; the separation of cells marked by the arrow is an artifact of preparation due to the weak adherence between epidermal cells. FIG. 3. Transverse section of a hydathode on a mature frond made parallel to the leaf margin and treated with I-KI/H_2SO_4. e = epidermal cells of hydathode, en = endodermis; arrows indicate Casparian strip.

lehmannii in its native locality, the Central American cloud forest. Observations suggest that one mechanism of secretion from fern hydathodes is the passive transmission of xylem sap from vein endings to the plant surface through the apoplast of the hydathode under a pressure gradient induced by root pressure.

MATERIALS AND METHODS

Plants growing in the cloud forests near Xalapa in the state of Veracruz, Mexico; provided material for both experimental and anatomical work. Individual plants consist of a single, erect rhizome with short internodes.

Root pressure was measured with a bubble manometer attached to the cut stipe (*Fig. 4A*) and a thermometer. The manometer was made with surgical tubing (ca.

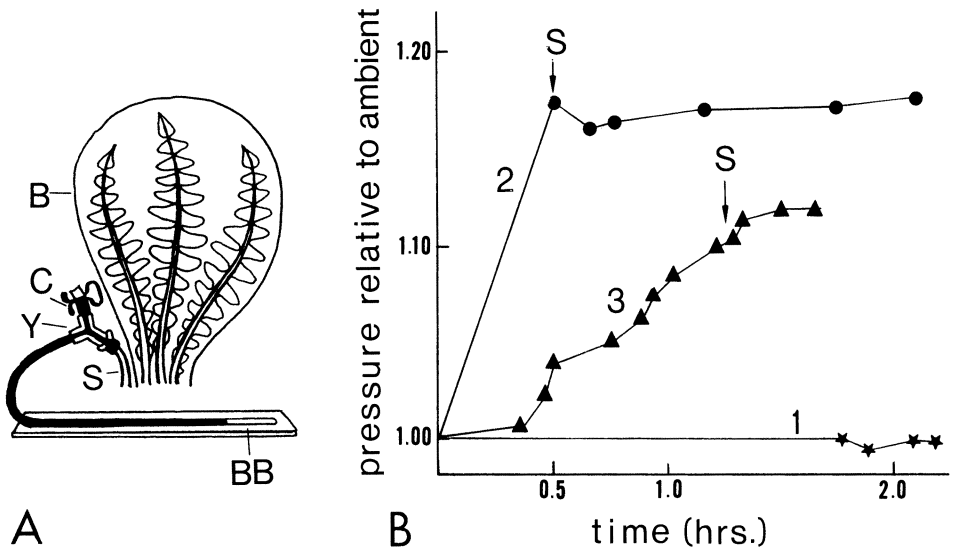
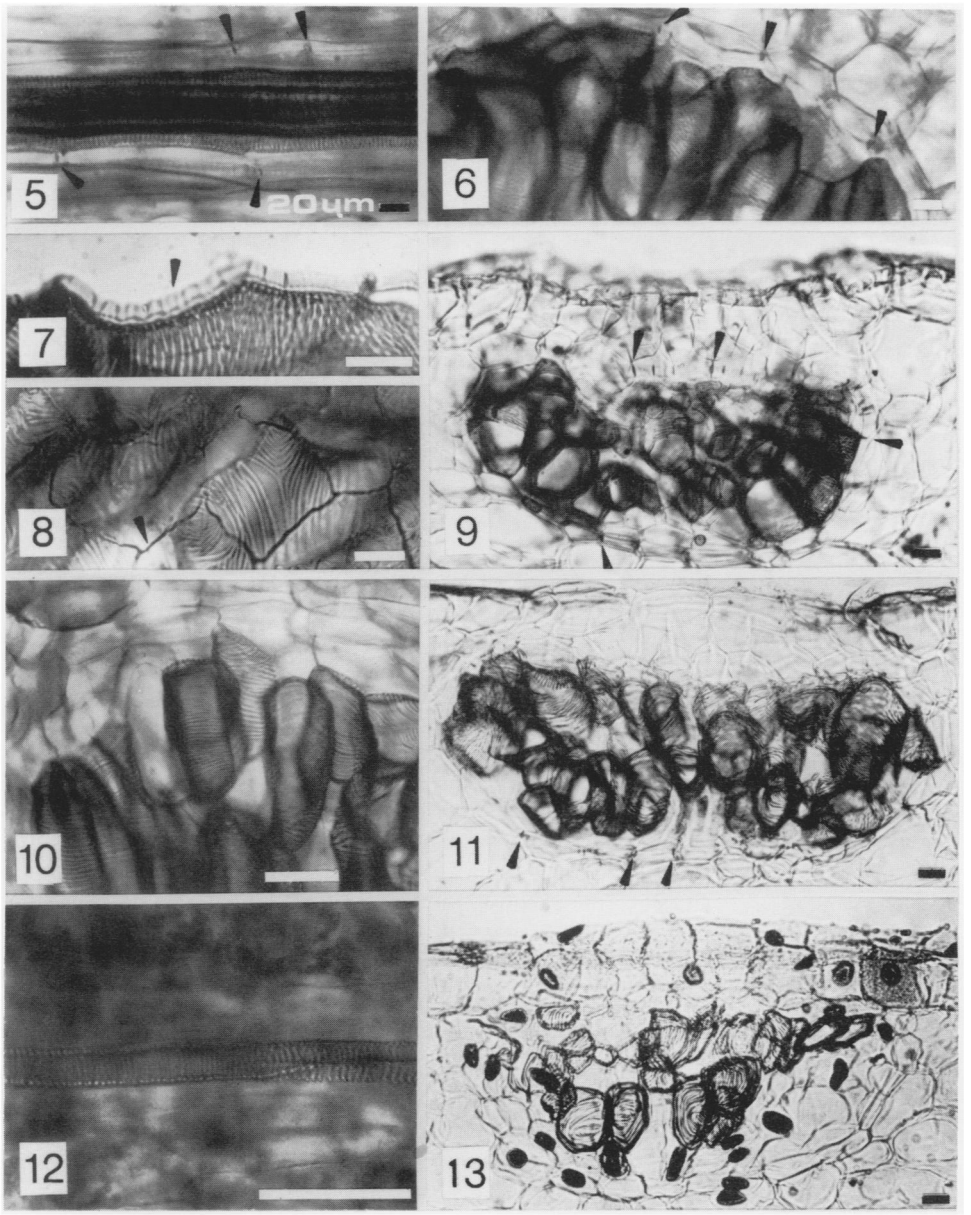


FIG. 4A. Manometer attachment for measuring root pressure. B = clear plastic bag enclosing the entire crown of an individual plant, S = cut stipe to which the manometer is attached, C = clamp, Y = forked connector, BB = bubble at sealed end of the manometer tube. FIG. 4B. Root pressure vs. time for plants 1, 2, 3. S = onset of hydathode secretion.

0.8 mm inside diameter) partially filled with acid fuchsin and sealed at one end so as to leave a bubble at the sealed end (*Fig. 4A*, BB). Bubble length was assumed to be proportional to bubble volume. At the beginning of the experiment, bubble length at ambient pressure and air temperature was determined by releasing the clamp (*Fig. 4A*, C). After the clamp was closed, subsequent readings of bubble length and air temperature were made, and exudation pressure relative to ambient was calculated according to Gay-Lussac's law (Weast & Astle, 1981). Clamping itself did not affect bubble length. Care was taken when attaching the manometer so that a continuous column of liquid was present from stipe to bubble, and the attachment was gently secured with twist-ties to help guard against leakage.

The relationship between root pressure and hydathode secretion was studied in three plants growing along a shaded stream in the cloud forest on a clear day. The entire above-ground portion of each individual plant was enclosed in a clear plastic bag and root pressure was monitored with a manometer attached to a single cut frond for each plant (*Fig. 4A*). Observations of xylem pressure and hydathode secretion were then made.

Anatomical differences between the secreting and non-secreting hydathodes on these three plants and others were studied from hand sections and clearings from both fresh and material fixed in FAA. Clearings were made by treating the leaf with alcoholic sodium hydroxide followed by lactic acid. Observations were made on preparations left unstained or stained in toluidine blue. The distribution of lignin was determined from hand sections stained in phloroglucinol and concentrated HCl. Suberized and cutinized cell walls were identified using the I–KI/H_2SO_4 test.



FIGS. 5–13. Development of Casparian strip. All preparations were treated in I–KI/H_2SO_4. FIGS. 5–8, 10, and 12 are from cleared preparations; FIGS. 9, 11, and 13 are transverse sections made parallel to the leaf margin. Arrows = Casparian strip. FIGS. 5–9. Non-secreting hydathodes on mature frond. FIG. 5. Vein subtending a hydathode and surrounded by endodermis with fully developed Casparian strip. FIG. 6. Hydathode viewed from the adaxial epidermis and focussed on the Casparian strip bordering the edge of the vein ending. FIG. 7. Higher magnification of the Casparian strip as viewed in

OBSERVATIONS

Secretion.—Secretion was observed on developing, unenclosed fronds in foggy and rainy weather in the cloud forest and when individual crowns were enclosed in plastic bags. In bagged plants, secretion was initiated on successive pinnae in an acropetal direction so that the last hydathodes to begin secreting on developing fronds were those on the most distal pinnae. Based on qualitative observations, distal hydathodes appeared to have a greater rate of secretion than more proximal hydathodes, which on developing fronds sometimes showed no secretion. Observations of fronds at different stages of development as judged by relative size, color, and texture suggested that the permanent loss of secreting capability by hydathodes occurs acropetally. No salt deposits of any kind were observed to accumulate above the hydathodes.

Root pressure.—Root pressure was determined by examining the bleeding behavior of cut stipes (*Fig. 4A*, S) on plants growing in conditions which were assumed to severely reduce or stop transpiration. Such conditions were either natural, as on rainy or foggy days, or induced by enclosing the entire above-ground portion of an individual plant in a clear plastic bag (*Fig. 4A*, B). Root pressure was assumed to be present when the attached stipes showed continual bleeding after being cut. Initially, some of this bleeding was due to mucilage flow as judged by the viscous nature of the exudate. However, after continual blotting over a period of several minutes, the viscosity of the bleeding fluid decreased considerably, and with the aid of a hand lens, xylem sap could be seen issuing from the stele. Plants in which transpiration was not inhibited did not show this bleeding behavior from cut stipes; here the only exudation noted was a small amount of mucilaginous material.

Although the secretion of mucilage from the cut end of the stipe was a potential problem for root pressure measurements, the results indicate that the pressure produced by the secretion is insignificant. Certain plants (e.g., plant 1, *Fig. 4B*) did not show root pressure even when transpiration was reduced by enclosure of the crown in a plastic bag. The absence of root pressure in these plants was apparently due to insufficient soil moisture. Despite the fact that the stipes of these plants showed mucilage secretion when cut, no significant increase in pressure above ambient was noted from attached manometers.

The relationship between root pressure and hydathode secretion studied in the three bagged plants (labelled 1, 2, 3) is summarized in the graph of *Figure 4B*. In plants 2 and 3, the buildup of root pressure in the xylem correlated positively with the observation of water secretion from hydathodes of developing fronds. In both plants, the measured pressures were sufficient to support a column of water taller than the height of the plant (1.8 m for plant 2, 1.2 m for plant 3). Plant 1 developed

Fig. 6 but with cellulosic walls dissolved by long exposure to sulphuric acid. The Casparian strip abuts directly on the tracheids of the vein ending. FIG. 8. Casparian strip adaxial to the vein ending in surface view with cellulose dissolved. FIG. 9. Hydathode with depressed epidermis and uniform development of Casparian strip around the vein ending. FIGS. 10–13. Secreting hydathodes on developing frond. FIG. 10. Hydathode viewed as in *Fig. 6* but with no Casparian strip. FIG. 11. Slightly protuberant hydathode with Casparian strip only developed adaxially. FIG. 12. Vein subtending a hydathode and without a Casparian strip. FIG. 13. Protuberant hydathode with no Casparian strip; dark, round structures are probably phenolic vacuoles.

no root pressure, and no secretion was observed from its hydathodes.

Variation in root pressure in the three plants is correlated with position relative to the stream flowing under the bank where they were growing. Plant 2, which showed the most dramatic rise in pressure, was growing roughly 0.3 m above the stream level. Plant 1, which showed no root pressure, was 2.5 m above the stream. Plant 3 was intermediate in pressure and bank position. Possibly the explanation for this correlation is decreasing soil moisture with increasing height above the stream; greater soil moisture would favor the generation of higher root pressures.

Hydathode development.—The ability of hydathodes to secrete is correlated with specific features of their development. Hydathode cells reach their mature size and are capable of secreting water long before the cells of the surrounding leaf tissue have finished expanding. Hydathodes capable of secreting protrude above the surrounding epidermal cells (*Fig. 13*). Only after the leaf cells reach their full size and the leaf achieves its mature thickness do hydathodes become the depressed "Wassergrübchen" consistently described by early anatomists (*Figs. 3* and *9*). In the recessed stage, hydathodes were observed to be incapable of water secretion.

The structural change that appears to be most closely related to secretory function is the progressive suberization of the hydathode endodermis. The Casparian strip surrounding the hydathode (*Figs. 3* and *9*) is the distal extremity of a network which surrounds the entire mature vascular system of the plant. The development of this continuous Casparian strip is acropetal, and lags considerably behind the maturation of protoxylem and hydathode cells (*Figs. 10, 12,* and *13*). Thus, hydathodes in developing tissues do not possess a suberized endodermis.

The acropetal extension of the Casparian strip to the hydathode is first evident in the endodermis abaxial to the vein ending (*Fig. 11*, arrows). The vein subtending the hydathode is completely surrounded by a Casparian strip (*Fig. 5*). At this time, the Casparian strip around the vein ending is like a mitten with the abaxially turned palm cut out. Development of the Casparian strip proceeds with the first steps represented by thin bands of suberin extending from the well developed strip below the vein. Eventually, the abaxial endodermis of the vein ending possesses a Casparian strip as well developed as anywhere else in the frond (*Fig. 9*).

The degree of Casparian strip development at the hydathode correlates closely with the capability of the hydathode to secrete water. Examination of developing fronds from plants 2 and 3, which possessed distal secreting hydathodes and proximal non-secreting hydathodes, indicated that secretion only occurred from hydathodes where the Casparian strip was either absent or incompletely developed. In one frond, for example, distal secreting hydathodes which from qualitative observation showed the highest secretion rate possessed no Casparian strip (*Figs. 10* and *13*). Hydathodes on pinnae from the middle part of the frond, which appeared to have a lower rate of secretion, possessed an incompletely developed Casparian strip adaxial to the vein ending (*Fig. 11*). Hydathodes at the base of the frond showed no secretion and were found to be completely surrounded by a thick Casparian strip (*Figs. 6–9*). In addition, examination of fronds at different stages of Casparian strip development indicated that the acropetal progression of complete hydathode suberization correlates with the acropetal loss of secretion capability in the hydathodes.

DISCUSSION

The correlation of root pressure with secretion from hydathodes on the one hand, and the correlation between Casparian strip development and the cessation of hydathode secretion on the other, suggest a simple explanation for the occurrence of water secretion from hydathodes in *B. lehmannii*. Root pressure is directly responsible for secretion by supplying a pressure gradient sufficient to drive the passive movement of xylem sap from the vein ending of the hydathode, through the apoplast of the sub-epidermal and epidermal cells, and through the thin cuticle to the outside of the plant. When the Casparian strip is completely formed around the vein ending of the hydathode, the apoplast is sealed and secretion from the hydathode is blocked. Preliminary results from *Polypodium* and *Nephrolepis* species indicate that this hypothesis can also apply to hydathodes in these genera.

Root pressure is known to be directly responsible for secretion from the epidermal-pore hydathodes of angiosperms (Fahn, 1979). Although pores are not present in the epidermis of fern hydathodes, root pressure can move water to the plant surface through the anticlinal walls of the epidermal cells. According to Meidner (1977), root pressure causes the secretion of water from the epidermal cell walls of *Gladiolus* leaves which lack epidermal-pore hydathodes, and the same explanation may account for Goebel's observation (1930) of secretion from the blade margins of ferns without hydathodes.

The results presented in this paper do not preclude the possibility that water secretion is partially or wholly active, and more research is required to decide the question. Hydathode epidermal cells could play a role in the modification of the solute content of the secreted xylem sap. Thus, they would be analogous to glandular cells found associated with epidermal-pore hydathodes of the angiosperms (Dieffenbach, et al., 1980). If the epidermal cells are capable of secreting salts, there may also be a metabolically dependent osmotic mechanism which contributes to water secretion, at least for those fern species that characteristically secrete large amounts of salts and form "scales" (see Lepeshkin, 1906). Regardless of the specific mechanism of secretion, however, the correlation of root pressure with hydathode secretion suggests the functional significance of the fern hydathode is to be sought in its relationship with root pressure.

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LITERATURE CITED

DIEFFENBACH, H., U. LÜTTGE, and M. G. PITMAN. 1980. Release of guttation fluid from passive hydathodes of intact barley plants. II. The effects of abscisic acid and cytokinins. Ann. Bot. 45:703–712.

FAHN, A. 1979. Secretory Tissues in Plants. Academic Press, London.

GARDINER, W. 1883. On the physiological significance of water glands and nectaries. Proc. Camb. Phil. Soc. 5:35–50.

GOEBEL, K. 1926. Morphologische und biologische Stunden. IX. Beiträge zur Kenntnis der Verwandschaftsverhältnisse einiger Javanischer Farne. Ann. Jard. Bot. Buitenz. 26:107–160.

————. 1930. Archegoniatenstudien. XX. Farne mit punktierten Blättern. Flora 124:410–422.

GUTTENBERG, H. 1934. Studien an Pflanzen der Sunda-Inseln. Ann. Jard. Bot. Buitenz. 34:1–62.

HABERLANDT, G. 1894a. Ueber Bau und Function der Hydathoden. Ber. Deutsch. Bot. Ges. 12:367–378.

————. 1894b. Anatomisch-physiologische Untersuchungen über das tropische Laubblatt. II. Über wassersecernirende und -absorbirende Organe. Sitzungsber. Akad. Wiss. Wien., Math-Nat. Kl. 103:489–583.

————. 1898. Bemerkungen zur Abhandlung von Otto Spanjer "Untersuchungen über die Wasserapparate der Gefässpflanzen." Bot. Zeit. 61:177–181.

————. 1914. Physiological Plant Anatomy. (Translated from the 4th German edition by Montague Drummond.) Macmillan, London.

LEPESHKIN, W. W. 1906. Zur Kenntniss des Mechanismus der aktiven Wasserausscheidung der Pflanzen. Beih. Bot. Zentralbl. 19:409–452.

————. 1923. Über active und passive Wasserdrusen und Wasserspalten. Ber. Deutsch. Bot. Ges. 41:298–300.

LIPPMANN, E. 1925. Ueber das Vorkommen der verschiedenen Arten der Guttation und einige physiologische und ökologische Beziehungen. Bot. Arch. 11:361–464.

MEIDNER, H. 1977. Sap exudation via the epidermis of leaves. J. Exp. Bot. 28:1408–1416.

METTENIUS, G. H. 1856. Filices Horti Botanici Lipsiensis. Leopold Voss, Leipzig.

OGURA, Y. 1972. Comparative Anatomy of Vegetative Organs of the Pteridophytes. Gebrüder Borntraeger, Berlin.

POIRAULT, G. M. 1893. Recherches anatomiques sur les cryptogames vasculaires. Ann. Sci. Nat. Bot., VII, 8:113–256.

POTONIÉ, H. 1892. Über die den Wasserspalten physiologisch entsprechenden Organe bei fossilen und recenten Farnarten. Sitzungsber. Ges. Naturf. Freunde Berlin 1892:117–124.

SPANJER, O. 1898. Untersuchungen über die Wasserapparate der Gefässpflanzen. Bot. Zeit. 61:6–81.

STOCKING, R. C. 1956. Guttation and bleeding. Pp. 489-502 *in*: W. Ruhland, ed. Encyclopedia of Plant Physiology, vol. 3. Springer-Verlag, Berlin.

WEAST, R. C. and M. J. ASTLE, eds. 1981. CRC Handbook of Chemistry and Physics. CRC Press, Inc., Boca Raton, Florida.