

V.—THE ANATOMY OF CERTAIN PLANTS FROM THE BELGIAN CONGO,
WITH SPECIAL REFERENCE TO MYRMECOPHYTISM

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INTRODUCTION

In examining a dried specimen of the myrmecophytic *Vitex Staudtii* Guerke collected by Lang and Chapin at Medje in the Belgian Congo, I was impressed by the close correlation between the distribution of the lateral cavities (supposed excavations) and the phyllotaxy of the plant. Through the courtesy of my colleague, Professor Wheeler, to whom the material had been sent by the collectors, I secured the opportunity of sectioning nodes and internodes of portions of the specimen and of studying their anatomical structure. So many features of unusual interest were encountered that it seemed advisable to study in detail the anatomy of other myrmecophytes from tropical Africa. All of the available material of ant-inhabited species of *Sarcocephalus*, *Barteria*, *Plectronia*, and *Cuviera* secured by Dr. Bequaert and Messrs. Lang and Chapin was very kindly turned over for my use. The myrmecodomatia of these genera proved to be fully as interesting as those of *Vitex*. Indeed, in so far as the anatomy of the host plants is concerned, the African myrmecophytes are even more remarkable than the much discussed *Acaciæ*, *Cecropiæ*, and *Myrmecodiæ* and are specially significant in any general consideration of myrmecophily.

MINUTE ANATOMY OF AFRICAN MYRMECOPHYTES

Vitex Staudtii Guerke¹

The taxonomy and general gross morphology of this verbenaceous liana are described by Dr. Bequaert on pages 447-452. The plant exhibits a typical decussate phyllotaxy. In other words, pairs of opposite leaves alternate with each other at right angles, resulting in four vertical rows or orthostichies of leaves. As is frequently the case in plants having this type of phyllotaxy, the stems are four-sided and quadrangular, each side corresponding to one of the orthostichies of leaves. At each node there are two circular apertures which are located at approximately the same level on opposite sides of the stem and midway between the leaf-scars or points of attachment of the leaves (Fig. 88e). These orifices, which serve as exit holes for the ants, shift their position from one pair of opposite sides of the stem to the alternating pair at each succeeding internode.

¹The following account of the anatomy and histology of this creeper is based upon the study of material (No. 743) collected by Lang and Chapin at Medje, July, 1914.

When the stem is split open it is found to be hollow. The central cavity is continuous from internode to internode, but is considerably constricted just above each node by a thicker peripheral layer of medullary tissue (Fig. 88, A-A). In addition to the nodal apertures which communicate with the exterior, there are numerous lateral internodal cavities which perforate the xylem and end blindly just under the bast. They are arranged symmetrically, one over the other, in longitudinal rows subtending the nodal apertures (Fig. 88*l*). In other words, there is an obvious and close correlation between the distribution of the lateral cavities and the phyllo-taxy of the plant.

In specimens which are inhabited by *Viticicola tessmanni* (Stitz) Wheeler, the conditions described above are characteristic of all portions of the stems and branches having fully elongated internodes. Only during the earlier stages in the differentiation of the nodes and internodes—in relatively close proximity to the growing-points or apical meristems—are they filled internally with medullary tissue and devoid of lateral cavities. The longitudinal and lateral cavities are also absent in very young plants that are not inhabited by ants. Such facts as these suggest that the central cavities and their bisymmetrically arranged lateral ramifications are excavated by the ants, which leave the supernodal constrictions or projecting rings of medullary tissue to separate the broods and prevent them from falling downward into the lower internodes.

In his field studies of *Vitex Staudtii*, Dr. Bequaert was unable to find any external evidences of preformed structures like the "prostomata" of *Cecropia*, described by Fritz Müller (1880-81), Schimper (1888) and others, which might account for the curiously symmetrical arrangement of the nodal apertures and lateral internodal cavities; nor was he able to find any clue to the functional significance of the latter. They are not used as receptacles for eggs, larvæ, or pupæ, nor do they contain coccids or other organisms. The only explanation that suggested itself was that they might be constructed for purposes of aëration. This suggestion is negatived, however, by the structure of those portions of the "bark" which cover the outer ends of the supposed lateral excavations. There are no lenticels or patches of aërenchyma suitably located, through which air might readily penetrate into the interior. On the contrary, the overlying tissues are compact and devoid of conspicuous intercellular spaces, and, in old stems, there are thick disks of impervious sclerenchyma situated just opposite the ends of the lateral cavities (Pl. XXXII, fig. 4).

Lateral Cavities

In stout, dry stems and branches of *Vitex Staudtii*, the lateral cavities appear superficially to be galleries or pits excavated in the woody portion of the "central cylinder," or stele. As is shown in Pl. XXXII, fig. 4, they perforate the xylem and end blindly on a general level with the cambium. However, a more detailed and critical study of the histology of the tissues figured in this photomicrograph indicates conclusively that the cavities are not mere tunnels in the normal xylem, since there is no fringe of chewed and broken prosenchyma such as occurs in galleries excavated by wood-boring insects. The outer two-thirds of the cavity are jacketed by the remains of a layer of very thin-walled, unlignified cells (*TPa*). In the xylem this layer rests upon heavily lignified, thick-walled parenchyma (*LXmPa*) which, in turn, merges more or less abruptly into prosenchyma (*XmPr*) (Pl. XXXII, fig. 3). That these layers were not formed subsequent to the excavation of a cavity in the prosenchyma is shown by the fact that there is no unconformity—torn or broken libriform fibers—between the parenchyma and surrounding prosenchyma.

What, then, is the mode of formation of the lateral cavities? Are they preformed structures or galleries excavated from a pre-existing core of delicate tissue? The section, illustrated in Pl. XXXIII, fig. 1, which was made from a freshly cut stem preserved in alcohol is of considerable interest in this connection. The soft tissues are *in situ* and have not contracted and collapsed as in preparations made from dried specimens. The lateral cavity does not extend to the general level of the surface of the woody cylinder, but its convex end subtends a plug of soft tissue (*CT*) which projects into the xylem. The inner portion of this intruding mass of unlignified tissue is jacketed laterally by an extension of the layer of thin-walled, unlignified cells (*TPa*) previously mentioned, which resemble in size, shape, and orientation those of the adjoining layer of heavily lignified xylem-parenchyma (*LXmPa*). Externally, the central core is constricted more or less by the phloem and cortex, into which it gradually merges. Since its outer margin is somewhat convex and its inner surface is concave, it forms a roughly dome-shaped layer of considerable thickness. Dark-colored, elongated, conducting cells, ramifying from the phloem (*Pm*), proliferate through it, and, as is shown in Pl. XXXIII, fig. 2, the thin-walled isodiametric cells which form its ground mass are arranged more or less symmetrically in radiating rows. This is particularly true of its inner portion (*NL*), where the cells are very much smaller and of more uniform size and shape (Pl. XXXIII,

fig. 4). In examining the photomicrographs, it should be noted that the concave inner margin of this intruding core of delicate, unligified tissue is irregularly serrate (Pl. XXXIII, figs. 2 and 4) and shows unmistakable evidences of having been gnawed by the ants. Small chunks of tissue have been nipped out of the surface, leaving a fringe of torn and injured cells. This suggests that the lateral cavities are not natural gaps in the woody portion of the central cylinder but are galleries excavated by the ants in peculiar cores of delicate, unligified tissue, that are symmetrically distributed in certain radii of the stem.

The question presents itself, accordingly, as to whether these parenchymatous areas of the xylem are normal features in the anatomy of *Vitex Staudtii* or traumatic structures produced by the ants. They have the appearance of abnormalities and resemble certain complex zoöcecidia or so-called prosoplasmatic galls, with their histological differentiation into "nutritive," "mechanical," and "conducting" tissues. The similarity becomes very striking indeed when these peculiar structures are studied microchemically. The cells—septate libriform fibers and parenchyma—of the normal xylem are packed with starch (Pl. XXXIII, figs. 1 and 3) and are separated from the core of thin-walled tissue by the layer of thick-walled, heavily lignified parenchyma corresponding to a mechanical layer, which is devoid of starch, as is the thin-walled unligified parenchyma which adjoins it. The dome-shaped, central core of soft tissue is abundantly supplied with proteins and fats, which reach their highest concentration in the cloudy protoplasts of the small, regularly arranged cells of the inner zone (Pl. XXXII, fig. 5). In stout stems the cap or disk of sclerenchyma, which is formed by the periderm and overlies the soft core of unligified tissue (Pl. XXXII, fig. 4), corresponds to a second mechanical layer. It is evident, accordingly, that in the tissues which surround the lateral cavities there are the equivalents of a "starch layer," a "protein-fat," or "nutritive" layer, two "mechanical" layers, and a ramifying system of conducting cells. As in certain insect galls,¹ the starch and protein-fat containing tissues are separated by a layer of heavily lignified cells, and the second, mechanical or "protective" layer is situated near the exterior. However, in view of the fact that ants are not known to produce gall-heteroplasias, particularly of this highly differentiated and structurally complex type, more critical evidence is required before these structures can safely be considered to be of traumatic origin and due solely to the activity of the ants.

¹Compare cynipid and other prosoplasmatic zoocecidia described by Houard (1903), Küster (1903, 1911), Cosens (1912), and others.

I have already stated that the lateral cavities do not contain coccids or the larvæ of gall-making insects; this is true of young as of old, stout stems and branches. Furthermore, the tissues in question do not appear to contain bacteria or other micro-organisms. In very young, tender stems, just subsequent to the formation of the longitudinal central cavity, the flat sides of the stele, or so-called fibrovascular cylinder, are prosenchymatous and devoid of gaps or oval patches of delicate tissue, and the peripheral layer of medullary tissue is homogeneous and entire (Pl. XXXII, fig. 2). At a little later stage in the differentiation of the internodes, the lateral cavities make their appearance. Macroscopic and microscopic studies of the tissues in the interior of such internodes reveal very clearly the mode of origin of the oval lateral pits. Patches of the medullary tissue are ripped and torn away and the prosenchyma is perforated, revealing the cambium. The exposed portion of the latter divides actively, producing callus, which projects toward the interior of the stem (Pl. XXXIV, fig. 2). With further increase in the size of the stem, the cavities, which are somewhat irregular at first with jagged margins, are enlarged by the removal of additional elements of the medulla and prosenchymatous xylem and are smoothed till they finally assume their characteristic symmetrical, oval outlines (Pl. XXXII, fig. 3). These facts indicate very clearly not only that the lateral cavities are excavated by the ants, but that the peculiar tissues that surround them are abnormalities, comparable to zoëccidia or heteroplasmatic galls.

Although the heteroplasias are relatively simple at their inception, they soon become complex and highly differentiated from the histological point of view. Thus, at first, there is merely a simple callus, which projects into a perforation in the prosenchymatous xylem. This is accompanied by more or less hypertrophy of the cells of the overlying cortex and a slight retardation in the development of the subepidermal periderm (Pl. XXXIV, fig. 2 and Pl. XXX, fig. 2). As growth proceeds, other structural abnormalities make their appearance. Owing to traumatic stimuli, the peripheral layer of meristematic cells of the cambium, adjoining the callus, ceases to form prosenchymatous xylem and lays down thick-walled, heavily lignified parenchyma next to the prosenchyma and thin-walled, unligified tissue next to the callus. As the mass of the callus increases, extensions of the phloem proliferate through it, accelerating a flow of nutritive substances to its innermost portions. When the overlying periderm is differentiated, it remains for a time unmodified or only slightly modified in structure (Pl. XXXIII, fig. 1),

but later forms a disk of very dense, heavily lignified tissue, or sclerenchymatous cap, opposite the central core of delicate cells (Pl. XXXII, fig. 4). As the woody cylinder increases in diameter, the ants continually, *i. e.*, at relatively frequent intervals, excavate the inner margin of the intruding mass of callus, for if they do not do so the lateral cavities become occluded by wound-wood. Such occluded galleries are of common occurrence, particularly in long internodes having very numerous heteroplasias and small broods.

The next question to be considered then is, why are these zoöcecidia so symmetrically arranged in obvious correlation with the decussate phyllotaxy of the plant? Pl. XXX, fig. 1 illustrates a transverse section of a young internode, cut just ABOVE a node (at the level *A-A* of Fig. 88). The four sides of the quadrilateral stem are histologically similar, with one notable exception. The vessels or principal water-conducting passageways are largely concentrated in one pair of opposite sides of the stele. A similar condition is shown in Pl. XXX, fig. 2, a transverse section cut just BELOW the node (at the level *B-B*, Fig. 88), but the vessels in this case are aggregated in the alternating pair of sides. In other words, the principal water-conducting passageways in each internode are largely confined to those sides of the stele which are to pass out to the leaves at the next (higher) node. Therefore, their orientation changes at each succeeding internode in accordance with the decussate phyllotaxy of the plant. As shown in Pl. XXX, fig. 2, the lateral cavities are excavated in the sides of the stele which are poorly supplied with vessels. Furthermore, they are located in those portions of the xylem which are devoid even of a narrow fringe of small primary tracheæ (*PT*).

Why should the ants select these radii of the stem for the construction of the lateral pits? Of course, the breaking of the conduits would certainly interfere with the normal flow of water to the leaves, and, inasmuch as in vines and lianas the area of water-conducting tissue is relatively small in proportion to the area of transpiring leaf-surface, this might affect the normal physiological processes of the plant and even lead to the drying up of the leaves. It seems probable, however, that the ants avoid the vessels because when the conduits are ruptured there is an excessive flow of water. That the cambia on the four sides of the stem are equally capable of producing the hyperplasias, is indicated by the fact that when the ants make an excavation in the wrong surface, as occasionally happens, it results in the formation of a heteroplasia which resembles those that occur so abundantly in the alternating pair of surfaces.

Exit Apertures

In stout stems the inner portions of the exit holes resemble structurally those of the lateral pits which subtend them. The prosenchymatous xylem is jacketed by thick-walled, lignified parenchyma which is covered in turn by a layer of thin-walled, unligified tissue. The outer surface of the xylem surrounding the exit gallery, however, is depressed considerably below the general level of the woody cylinder (Pl. XXXIV, fig. 1). In this depression rests a peculiar ring of sclerenchyma (Pl. XXXIV, figs. 1 and 3) which extends to the outer surface of the stem and usually projects considerably beyond it, so that the nodal apertures have externally an embossed or crater-like profile. These rings of extremely dense, tough tissue make their appearance in young stems and undoubtedly tend to prevent the cambium and cortex from forming lateral callus which would seal the exit hole unless periodically removed by the ants.¹ Similar structures may be formed in the internodal portions of the plant whenever, as occasionally occurs, one of the lateral galleries is extended beyond the cambium, through the cortex and epidermis, to the exterior of the stem. Under these circumstances the usual sclerenchymatous disk (Pl. XXXII, fig. 4) is replaced by a projecting ring of sclerenchyma (Pl. XXXIV, fig. 1).

I have already called attention to the bisymmetrical arrangement of these nodal apertures and their obvious relation to the decussate phyllotaxy of *Vitex Staudtii*. At each node there are two exit holes excavated on opposite sides of the stem and at approximately the same level (Fig. 88). It is interesting to note in this connection that in the verbenaceous myrmecophyte, *Clerodendron fistulosum*, described and figured by Beccari (1884-86), the nodal apertures² are located just below the points of attachment of the leaves, in the alternating pair of surfaces of the stem. This striking contrast in the location of the exit holes in the two myrmecophytes is due apparently to differences in their nodal and internodal anatomy. In *Vitex Staudtii* two entire sides of the stem pass out into the pair of opposite leaves at the node. These sides—"leaf traces"—which are considerably narrower than the alternate pair (Pl. XXX, fig. 2) become more and more abundantly supplied with vessels in the vicinity of the node. With the passing out of the leaf-traces, leaving two large gaps in the stele, there is an abrupt transition in the remaining sides of the stele from xylem that is nearly devoid of vessels to woody

¹In a number of other myrmecophytes that I have studied the exits or entrances soon become blocked by callus and ultimately by wound-wood unless kept open by the ants.

²Schimper (1888) questions Beccari's statement that these apertures arise spontaneously and considers that they are excavated by ants.

tissue that is crowded with water-conducting passageways. Therefore, the most favorable situations for the location of the nodal apertures are to be found midway between the points of attachment of the leaves and just below the level where these structural transitions occur. In the stems of certain species of *Clerodendron*, on the other hand, the elements of the xylem are differentiated and distributed in such a manner that the "prostomata" subtend the bases of the leaves.

The gaps left in the woody cylinder by the exit of leaf-traces are filled with very delicate, soft tissue. *A priori*, it would seem as if these gaps would be the most favorable places for the excavation of the exits. It should be noted, however, that if they were so utilized there would inevitably result a destruction of the axillary buds and the formation of lateral branches be prevented. In *Vitex Staudtii*, although the ants occasionally remove a portion of the tissue, I have never seen a node in which they had cut their way through to the exterior. Yet, as soon as the buds form branches, the cavities in the latter are found to communicate freely with those of the main axes. From the point of view of insect behavior, it would be extremely interesting to discover whether there are structures or substances in the buds which prevent their destruction by the ants.

Origin of the Central Cavities

Many of the earlier investigators assumed that the central chambers of various myrmecodomatia are excavated by ants. Thus, Rumphius (1750) and others considered that the anastomosing galleries in the "pseudobulbs" of *Myrmecodia* and *Hydnophytum* are constructed by ants. There is a considerable element of danger, however, in making hasty generalizations in regard to the origin of these structures. Forbes (1880), Treub (1883), and others have shown that the domatia of *Myrmecodia* and *Hydnophytum* occur normally in plants from which ants are entirely excluded. Furthermore, it is well known that plants, both herbaceous and arborescent, having hollow internodes are widely distributed in both temperate and tropical regions.

It has been stated that the stems of very young seedling plants of *Vitex Staudtii*, which are not inhabited by *Viticicola*, are filled with medullary tissue, whereas those of older plants, which are occupied by the ants, are hollow except in the vicinity of the growing points. This might be considered to indicate that the domatia are excavated by *Viticicola*. It must be admitted, however, that evidence of this character is purely circumstantial and not necessarily conclusive. Hollow internodes

may be present in large, vigorous adult shoots when they are entirely absent in smaller stems, such as are frequently formed during the earlier stages in the ontogeny of the plant or under unfavorable growth conditions. Furthermore, in examining herbarium material of other representatives of this genus, I find that, although certain species possess solid stems, others normally have well-developed central cavities in their core of medullary tissue (Pl. XXXI, fig. 1). The structure of the delicate, immature internodes of ant-inhabited specimens of *Vitex Staudtii* is of interest in this connection. Pl. XXXII, fig. 1 illustrates a transverse section of such an internode cut a relatively short distance behind the terminal growing-point. The medulla is not homogeneous but consists of an oval central core of very large, thin-walled cells and a peripheral layer of denser medullary tissue, which is richly protoplasmic. As the stem increases in diameter the oval core of delicate tissue shrivels up and is trimmed away by the ants leaving the oval cavity shown in Pl. XXXII, fig. 2. From the point of view of this histological evidence, it seems probable that in *Vitex Staudtii* there is an inherent tendency towards the formation of hollow stems and branches. Whether the ants accelerate the formation of the central cavities, as has been shown by Fiebrig (1909) to be the case in *Cecropia*, can only be determined by careful field observations.

Cuviera

A number of species of the African, rubiaceous genus *Cuviera* are myrmecophytic and characterized by having elongated, spindle-shaped swellings on the branches (Figs. 99 and 100) which are inhabited by ants.¹ Two lots of these myrmecodomatia, one collected by Dr. Bequaert at Stanleyville, March 8, 1915 and the other (No. 1031) by Messrs. Lang and Chapin at Kunga near Malela, July, 1915, were sent to me for anatomical study. The former, occupied by *Crematogaster africana* subspecies *laurenti* variety *zeta* (Forel), were obtained from *Cuviera angolensis* Hiern; the latter, inhabited by *Crematogaster impressiceps* variety *frontalis* Santschi, from an unidentified species of *Cuviera*.

The myrmecodomatia of the two species are similar and differ only in certain minor morphological and histological details. Externally, those of *Cuviera angolensis* are shorter, slimmer, and of a deep olive green color, whereas the others are longer, stouter, and of a reddish green color.² The color differences are due largely to differences in the internal

¹See Schumann (1891).

²In making these comparisons I am dealing with material preserved in alcohol.