

Studies of the holoparasite *Tristerix aphyllus* (Loranthaceae) infecting *Trichocereus chilensis* (Cactaceae)

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Many of the large columnar cacti *Trichocereus chilensis* near Santiago are infected by *Tristerix aphyllus*. This is one of the most highly reduced seed plants known: as it is an endoparasite, inflorescences are the only parts of the plant ever to emerge from the host, all the rest exists as an endophytic haustorial system; roots, vegetative stems, and leaves are not produced. After infection, the parasite spreads through the thick cortex of the host, reaching the vascular cambium and conducting tissues. It continues to grow intrusively throughout all tissues of the host shoot. In its invasive stage it occurs as a "myceliumlike" mass of uniseriate filaments that grow between host cells but only rarely enter them. Later growth is by longitudinal cell division that produces irregular parenchymatous strands. Eventually, xylem and phloem differentiate in the endophytic strands; the phloem is like that of other seed plants, but the xylem is almost pure parenchyma, with only occasional, idioblastic tracheary elements. Strands close to the epidermis of the host are able to produce adventitious flower buds that emerge through either soft regions in the epidermis (the areoles) or through accidental breaks in it. The flower stalk may persist, forming a small perennial inflorescence that has well-developed xylem, phloem, and periderm but is without leaves. Host cells appear healthy and normal, with no sign of damage caused by the presence of the parasite.

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Près de Santiago, plusieurs des gros cactus en forme de colonne appartenant au *Trichocereus chilensis* sont infectés par le *Tristerix aphyllus*. Cette dernière plante est l'une des angiospermes les plus réduites: comme il s'agit d'un endoparasite, l'inflorescence est la seule partie de la plante à émerger de l'hôte, le reste se réduisant à un système haustorien endophyte; il n'y pas de racines et de tiges végétatives, ni de feuilles. Après l'infection, la plante parasite se répand à travers le cortex épais de l'hôte et atteint le cambium et les tissus vasculaires. Elle continue ensuite à croître à travers tous les tissus de l'hôte. Au cours du stade de l'invasion, la plante parasite se présente comme une masse "d'aspect mycélien" de filaments unisériés qui croissent entre les cellules de l'hôte mais n'y a pénétré que rarement. Plus tard, la croissance s'effectue par des divisions cellulaires longitudinales qui produisent des cordons parenchymateux irréguliers. Du xylème et du phloème finissent par se différencier dans les cordons endophytes; le phloème est semblable à celui des autres angiospermes, mais le xylème est presque un pur parenchyme, avec seulement quelques éléments vasculaires épars. Les cordons parenchymateux situés à proximité de l'épiderme de l'hôte ont la capacité de produire des boutons floraux adventifs qui émergent soit à travers les régions tendres de l'épiderme (les aréoles), soit par des brisures accidentelles de l'épiderme. Le pédoncule floral peut persister: il forme alors une petite inflorescence pérennante, possédant du xylème, du phloème et un périoderme, mais dépourvue de feuilles. Les cellules de l'hôte paraissent saines et normales et ne présentent aucun signe de dommage dû à la présence du parasite.

[Traduit par le journal]

Introduction

The relationships of host plant and parasite have many interesting aspects, one of which is the modified anatomy and physiology of the parasite. Obviously, as parasites evolve from hemiparasitic to holoparasitic, their metabolism must be extensively modified. Likewise, exophytic parasites such as *Cuscuta*, *Phoradendron*, or *Striga*, having more or less normal stems and leaves (Kuijt 1969), are much less modified than the endoparasites such as *Rafflesia* or *Corynea* in which only the flowers or inflorescences emerge from the host (Kuijt 1969). Such endoparasites are among the most fascinating of all plants because they have so few of what we expect to be essential plant parts: they have no vegetative stems, leaves, or roots, and presumably their physiology and developmental metabolism are also highly altered. Endoparasites have been particularly difficult to study because they typically grow in remote sites and are not easily maintained in botanical gardens, or they infect hosts whose tissues are woody and hard, being very difficult technically.

The endoparasite *Tristerix aphyllus* (Figs. 2 and 3) does not suffer from these problems: it grows near Santiago and

infects the cactus *Trichocereus chilensis*, a plant that is almost pure parenchyma. This has made it extremely easy to collect and study *T. aphyllus* and its relationship with *T. chilensis*, and because cactus tissue is easily grown in culture, physiological—developmental studies should be possible. An early study (Reiche 1904) indicated that *T. aphyllus* has an extremely modified endophytic system.

Furthermore, this case of parasitism is of special interest because the host is unusual in being composed of large amounts of compact, persistent chlorenchyma which can serve as a source of carbohydrates and water for years, whereas more "typical" hosts usually are composed of large amounts of woody heterotrophic tissues maintained by conduction of nutrients from the leafy, autotrophic branch tips. Such hosts are typified by considerable acropetal transport of water and basipetal transport of carbohydrates; in *T. chilensis*, the rates of transport are unknown, but probably are small. Unlike most other parasites, *T. aphyllus* grows immersed in a large soft "storehouse" of nutrients and water, and it should not be surprising that it has lost most normal vegetative anatomy and physiology. The first step in studying this highly unusual plant

was an examination of its anatomy and development, which is reported here.

Materials and methods

Specimens of *Trichocereus chilensis* (Colla) Br. & R. infected with *Tristerix aphyllus* (D.C.) Barlow & Weins were collected at two localities, Llay Llay and Til Til, both about 100 km northwest of Santiago. Tissues were fixed in Formalin – alcohol – acetic acid (FAA) (50% EtOH) overnight, then processed by standard paraffin techniques. The slides were stained in special formulations designed to enhance the staining of cactus tissues: overnight in safranin (1 g safranin, 50 mL 2-methoxyethanol, 2 mL Formalin, 1 g sodium acetate, 50 mL 50% EtOH), rinsed in water, treated for 5 min with 1% chromic acid in water, rinsed in water, rapidly passed through an EtOH dehydration series, then treated for 8 s with Fast Green (0.5 g Fast Green, 50 mL absolute (abs) EtOH, 25 mL xylene, 25 mL clove oil; stir overnight and filter before use), rinsed in abs EtOH, rinsed in a mixture of 25% clove oil, 33% xylene, and 42% abs EtOH, then cleared for 10 min in a mixture of 2 parts clove oil, 1 part xylene, and 1 part abs EtOH. This is based on a modification of a schedule by Boke (1952).

For scanning electron microscopy (SEM), the material was fixed by standard procedures and observed with a JEOL scanning electron microscope at 25 kV.

Results

The host

Trichocereus chilensis is a tall columnar cactus that branches near its base, producing a plant composed of 3–10 vertical branches each ca. 12–15 cm in diameter and 2–4 m tall (Fig. 1). The surface of the branches is composed of 12–16 low ribs (Fig. 3) that contain the areoles (spine clusters = short shoots) which are separated from each other vertically by ca. 3 cm. Each areole has one prominent central spine ca. 2–12 cm long, and 8–12 radial spines, also of variable length. Each young areole has a dormant apical meristem capable of producing either a flower or a branch. The surface of the plant is composed of a tough thin epidermis that covers a thick hypodermis made of six to eight layers of cells with extremely thick walls (Fig. 4). Interior to the hypodermis is a parenchymatous cortex composed of several (10–15) exterior layers of columnar palisade chlorenchyma cells and an inner region of nonchlorophyllous parenchyma; the entire cortex is ca. 3–4 cm thick. All parenchyma cells, in both the cortex and the pith, are extensively interconnected by prominent primary pit fields.

The stele is composed of numerous small bundles: although a normal vascular cambium does form, there is very little secondary growth, and the bundles remain distinct for years, forming a solid wood only in very old plants. Each bundle contains (in centripetal order): a cap of primary phloem fibers, primary phloem, secondary phloem, vascular cambium, secondary xylem, and primary xylem (Fig. 7). The phloem consists of sieve tube members with large sieve plates (ca. 25 μ m

diam; Figs. 8 and 9), companion cells, and nonsieve-element parenchyma; the only sclerenchyma is a cap (ca. six cell layers) of living septate primary phloem fibers (Fig. 7). Xylem consists of a mixture of vessel elements with mostly simple perforation plates (but with occasional bars across the perforation), parenchyma, and fibers. All cells of the secondary xylem have thick lignified walls, and both the fibers as well as the parenchyma are living, with easily visible cytoplasm and nuclei. Narrow rays are occasionally present in the bundles, but the large medullary rays constitute the bulk of the radial system. These are initially pure primary parenchyma, but with the establishment of the vascular cambium, these rays also grow secondarily as the interfascicular vascular cambium deposits large, thick-walled parenchyma cells (Figs. 10 and 21).

Interior to the stele is the pith, composed of thin-walled parenchyma with a system of fine vascular bundles, especially near its outer edge. The pith is ca. 7 cm in diameter.

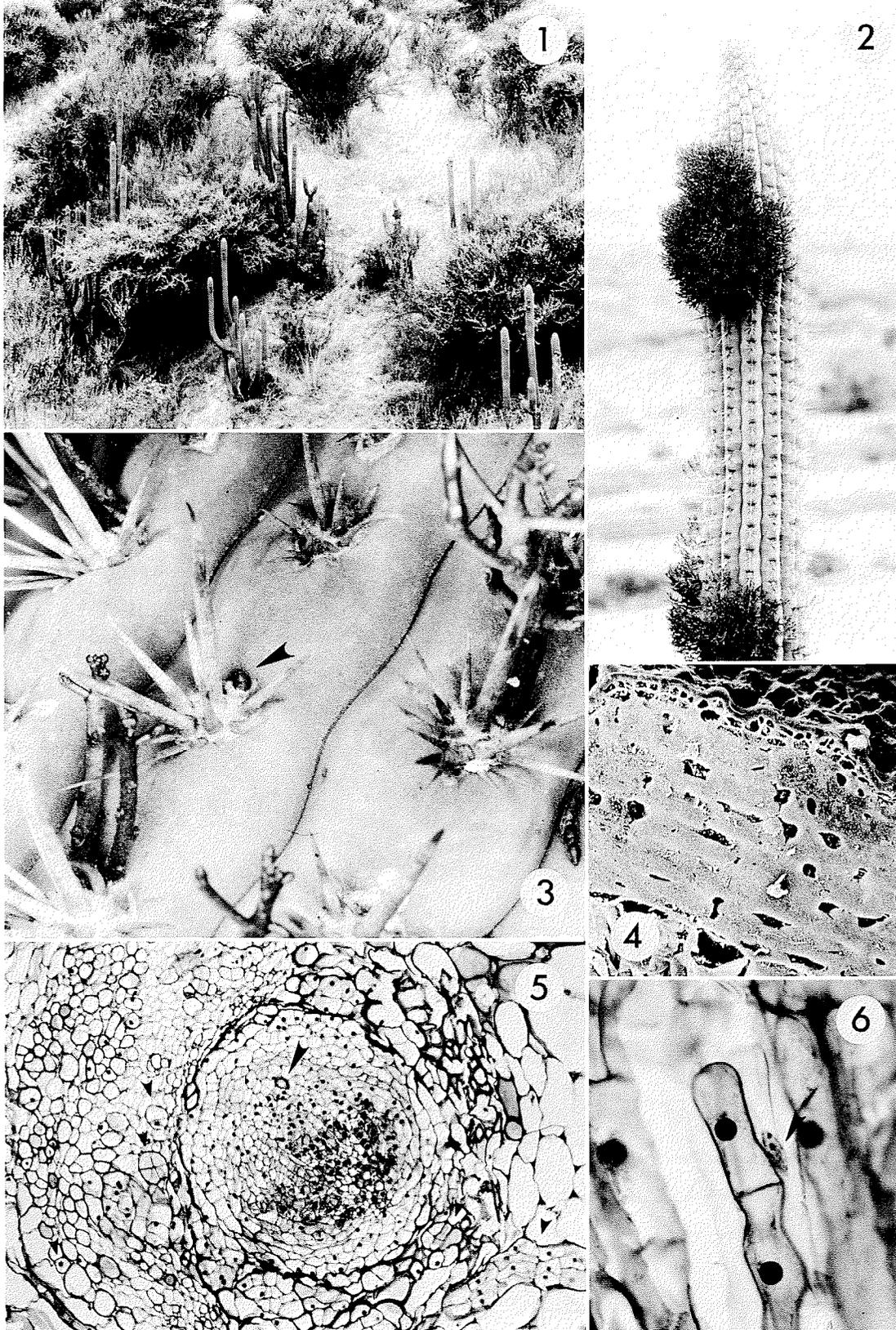
Early growth of *Tristerix*

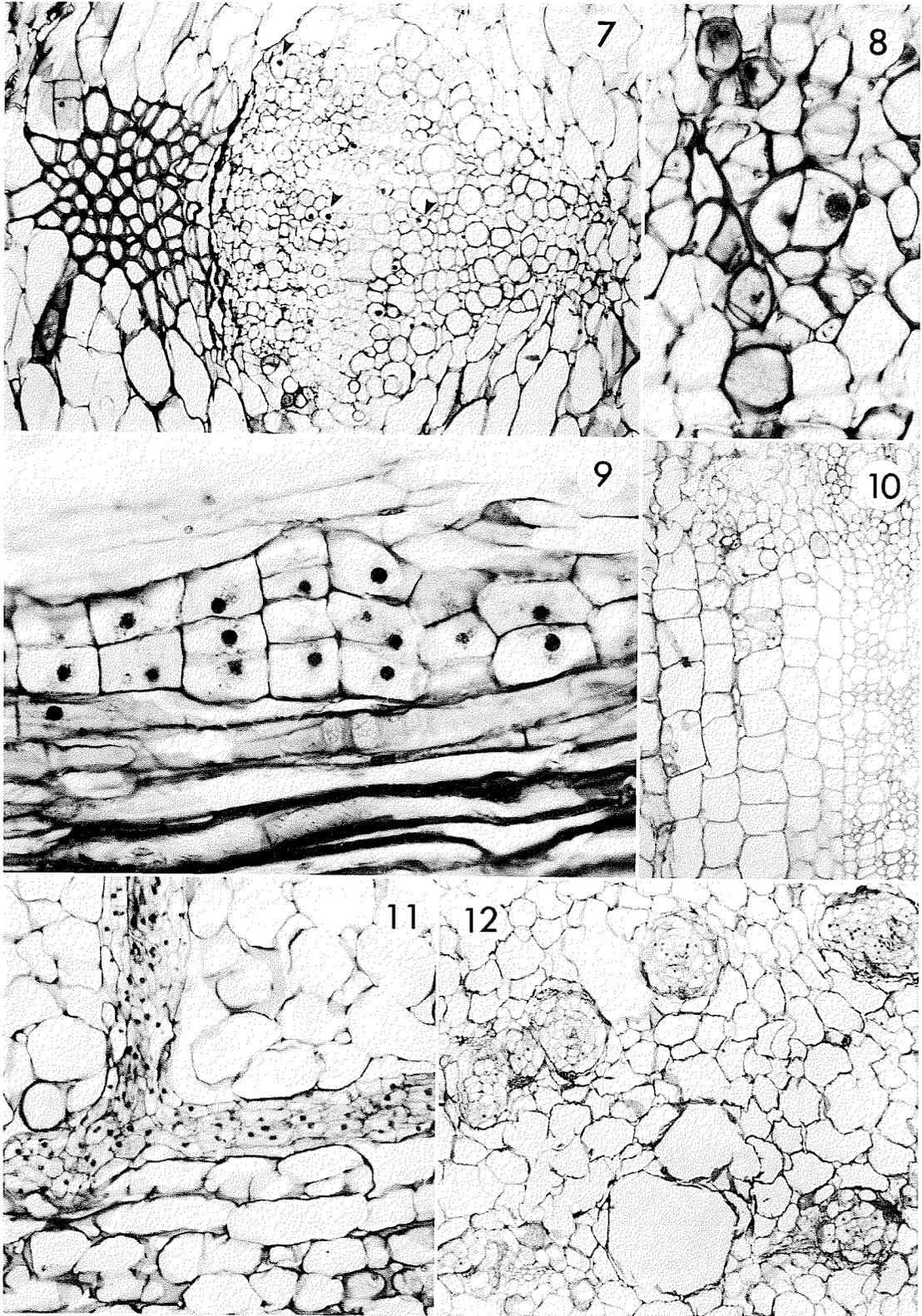
Germination and infection have not yet been encountered; the following is a description of established flowering plants. The endophytic portion of *Tristerix aphyllus* is present as a highly ramified and anastomosing network of parenchymatous tissues (Figs. 11 and 12), which occur in all parts of the host stem: cortex, primary, and secondary vascular tissues, including the vascular cambium, and in the pith. The parasite is capable of growing (i) intrusively as it invades previously uninfected host tissues and (ii) symplastically in coordination with an infected vascular cambium. With the stain technique used the nuclei of *Tristerix* stained a dense red while the nuclei of *Trichocereus* stained a light pink, making the cells of the two plants instantly distinguishable (Fig. 6).

The nature of the intrusive growth depends on the nature of the tissue being invaded: when *T. aphyllus* grows downward into mature parenchymatous cortical tissues, it grows as ramifying, uniseriate filaments which strongly resemble fungal hyphae (Figs. 6, 13, and 14). The older cactus cortex is aerenchymatous with an extensive system of interconnected air spaces and parenchyma cells that are rather thick walled; the whole tissue is quite hard (similar to a potato tuber). In these lower areas, *T. aphyllus* is found only as fine strands in the intercellular spaces, and the surrounding host cells are completely nondeformed by the parasite (Fig. 15); parasitic growth appears to be controlled purely by available open space, with the parasite having no ability to crush or penetrate host parenchyma cells.

On the upper edge of the endophyte, growth is also by uniseriate filaments, but the host tissues, being younger than those just described, are also softer and are deformed and displaced somewhat by the parasite. Apparently *T. chilensis* grows compensatorily because even though the parasite cells are cylindrical and fairly large, the host cells are only mildly deformed, not crushed or destroyed (Figs. 7, 8, 13, and 14).

FIGS. 1–6. Fig. 1. Habit of *Trichocereus chilensis* growing near Llay Llay. Note especially the branched, columnar growth form. Fig. 2. *Trichocereus chilensis* infected by *Tristerix aphyllus*; each of the large floral masses is composed of hundreds of small inflorescences like those of Fig. 3. $\times 0.1$. Fig. 3. Many areoles (spine clusters) are visible, most with one or two *T. aphyllus* floral branches. A *T. aphyllus* floral branch is just emerging from the central areole (arrow). $\times 0.5$. Fig. 4. The epidermis of *T. chilensis* is composed of thin-walled small cells, but the cells of the hypodermis are very thick-walled and constitute an extremely tough resistant barrier to either infection or flowering of the parasite. $\times 150$. Fig. 5. A large, mature strand of *T. aphyllus* growing in the phloem of a vascular bundle of *T. chilensis*. Host xylem is in upper left; parasite xylem is the tissue immediately surrounding its idioblastic tracheide (large arrow); parasite phloem is the dark, cytoplasmic cells just to the lower right of parasite xylem. Parasite "cortex" surrounds these two vascular tissues. Note that there are numerous small strands and filaments of the parasite mixed in the host phloem and cortex (small arrows). $\times 92$. Fig. 6. The tip of a growing hyphalike filament of *T. aphyllus*. Note the large, prominent, dark nuclei of the parasite, compared with the smaller, more lightly stained companion cell nucleus (arrow) of the host. $\times 465$.





The *T. aphyllus* filaments may be fairly straight, but typically they follow an irregular course, as though lines of weakness in the host were of primary importance (Figs. 13, 14, 25 and 26). As is true of the downward-growing filaments, they may be either branched or not, and frequently fill intercellular spaces, sometimes to the point of filling all available spaces and forming a pseudoparenchymatous matrix around the true parenchyma of the host (Fig. 16). This endophytic parasite tissue can be very large; several sections of *T. chilensis* approximately 0.5 m long were dissected, revealing that strands within them were interconnected, apparently being parts of a single *T. aphyllus* plant. In some branches of *T. chilensis*, the infection extended as much as 3 m, so the parasite was invading a volume of as much as 53 000 cm³.

The *T. aphyllus* filaments are most concentrated in the vascular tissues, especially the phloem; when the developing phloem is invaded, the resulting tissue has a completely normal appearance in that the host phloem is not crushed, but rather the nonconducting parenchyma that is typically present in phloem happens to be mostly parasite (Figs. 7 and 8); host sieve tube members, companion cells, and nonconducting parenchyma are all present with normal morphology. Individual cells can be identified as host or parasite only by observing the nucleus (Figs. 6 and 9) or by finding the large sieve plates: present in *T. chilensis* (Figs. 8 and 9). Similarly, in the xylem many of the axial parenchyma cells are actually filaments of the parasite (Figs. 7 and 17). They are easily identified by their thin non-lignified walls: they stain green whereas the true xylem parenchyma has slightly thickened, red walls. Unlike other cell types, developing tracheary elements seem to be sensitive to the parasite and are markedly deformed by the filaments; however, this does not inhibit the further development of the element, and normal secondary walls are deposited. Short "fingers" (Fig. 19) or "bubbles" (Fig. 17) of parasite may be completely embedded within a tracheary element, and many examples of tylosislike multiple invasions of single elements were observed (Fig. 20). Typically one of the many "tyloses" would contain a *T. aphyllus* nucleus. This cellular invasion is even more common in the thick-walled parenchyma cells laid down in the medullary rays (Figs. 10, 21, and 22).

The parasitic filaments are much less abundant in the vascular cambium, and those present are oriented radially, whereas in the xylem and phloem the filaments are predominantly axial in orientation (Figs. 23, 25, and 26). The presence of *T. aphyllus* filaments in the vascular cambium results in sympastic growth, because the parasite cells grow in a manner coordinated with that of the vascular cambium; as in most cacti, the vascular cambium of *T. chilensis* grows slowly, producing only 0.1 or 0.2 mm of secondary xylem per year. As new xylem and phloem are generated, they are invaded by sur-

rounding parasite filaments. In rare instances, the invasion is massive enough to totally disrupt the region, but usually the vascular cambium continues to deposit secondary xylem and phloem for (apparently) many years after establishment of the parasite within it (Figs. 18 and 33). Whereas all secondary phloem of a vascular bundle appeared to have a uniform distribution of parasite within it, in the secondary xylem the parasite was frequently present as tangential bands, separated from each other by regions of uninfected axial xylem. This suggests that the phloem can be invaded continuously, but the xylem is invaded episodically, producing tangential bands of heavily infected xylem that alternate with regions with few parasite filaments. Each of these infected bands was interconnected by parasite filaments running through the host rays.

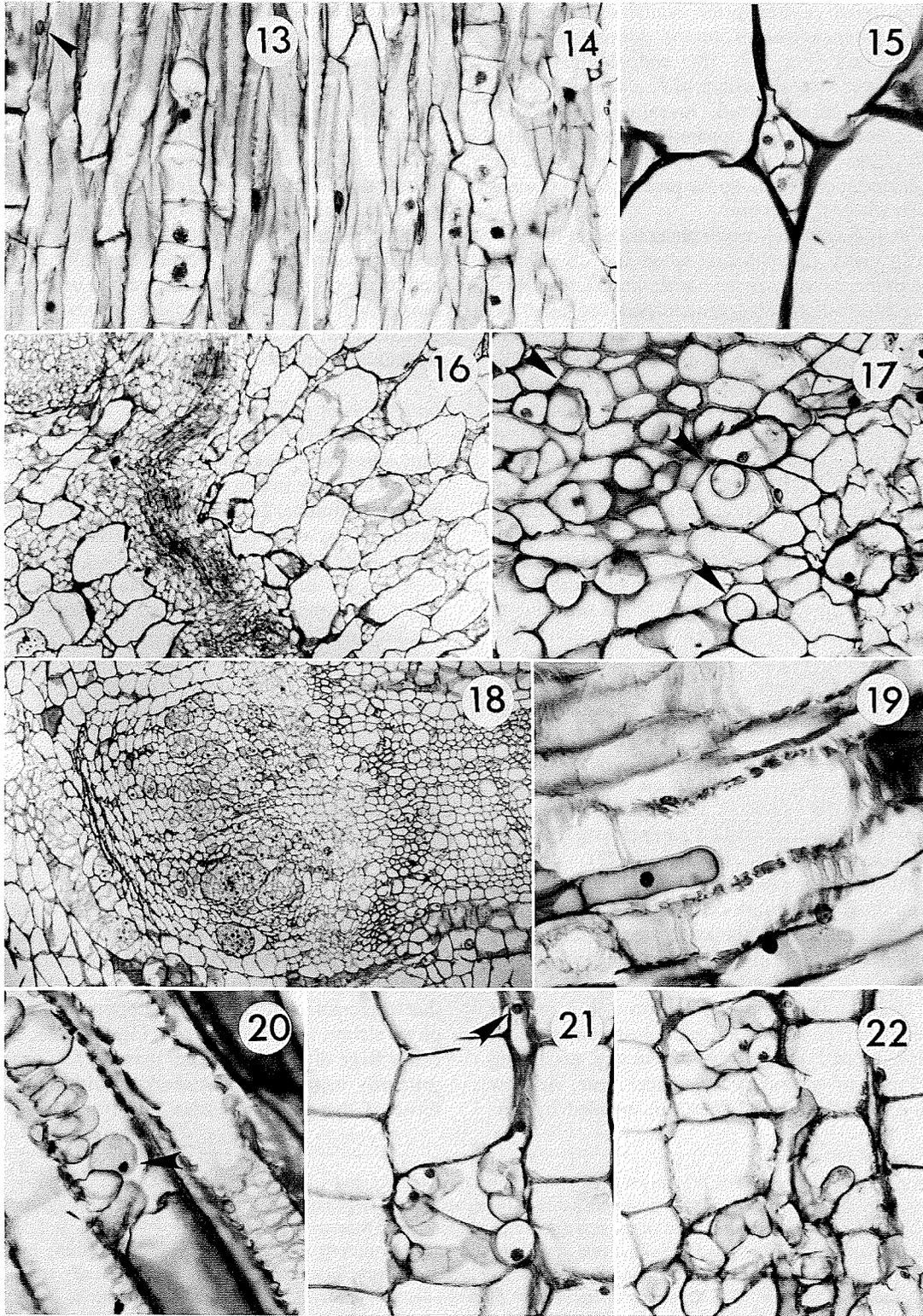
In brief summary, once established the parasite grows acropetally, basipetally and circumferentially in all host tissues, and lines of physical weakness seem to be important in guiding the growth, especially in older tissues. There is no apical meristem but rather a myceliumlike growth front of uniseriate hyphalike filaments.

Later growth of *Tristerix*

The intrusive filaments remain uniseriate for only a short time apparently, because they are usually less than 1 or 2 mm long. Cells only five or six cells back from the terminal cell can begin longitudinal division (Fig. 27). This is not highly coordinated: in any region, certain cells may have divided several times while adjacent cells have divided only once or not at all. Whereas the initiation of these divisions is irregular, the orientation is extremely precise: all divisions are longitudinal, creating very obvious packets of cells (Fig. 9). Ultimately all cells of the filaments undergo these longitudinal divisions, producing multiseriate parenchymatous filaments that have the same irregular longitudinal undulations that the original uniseriate filaments had (Figs. 11, 12, 16, 18, 24, and 29).

In older mature host tissues, this radial growth of the parasite can occur only to the extent allowed by the available intercellular space, so the filaments remain quite slender. In immature soft tissues, the radial growth can be such that the filaments attain a diameter of 840 μm (mean of 14 largest filaments, 630 μm ; Figs. 5 and 37). Although almost all filaments undergo radial growth, the extent is highly variable from filament to filament, such that of four or five adjacent filaments one or two may become large while the others attain diameters of only 30 to 40 μm (Figs. 5 and 18; the transcambial filaments that interconnect intraxylem endophyte with intraphloem endophyte always remain uniseriate; Figs. 23, 25, 26, and 28). The really large diameters (greater than ca. 500 μm) seem to occur primarily in the strands embedded within the phloem, while those of the pith and cortex remain somewhat smaller, and

FIGS. 7–12. Fig. 7. Vascular bundle of *Trichocereus chilensis* with a recent infection by *Tristerix aphyllus*. This bundle shows the morphology typical of uninfected *T. chilensis*; the parasite is distinguishable only because of its large nuclei (arrows). $\times 92$. Fig. 8. Magnification of infected phloem. The two central cells are parasite, most of the rest are cactus phloem, with four sieve plates visible. $\times 381$. Fig. 9. Longitudinal section of host phloem and a young parasite strand. *Tristerix aphyllus* grows in width by orderly longitudinal divisions, resulting in tiers of cells; the rate of division varies, such that some tiers contain four cells, others only two. This facilitates recognition of parasite cells versus host cells when nuclei are not in the plane of section. Note the two prominent sieve plates in the host phloem. Arrows indicate host nuclei. $\times 280$. Fig. 10. Cross section of *T. chilensis* xylem, showing the regular "conducting" xylem on the right with vessels, and on the left is the cambium-derived thick-walled parenchyma of the enlarging medullary rays. Many of these cells have been invaded and display one or several parasite inclusions (see Figs. 21 and 22 for higher magnification). $\times 73$. Fig. 11. Longitudinal section of a young strand of *T. aphyllus* in the host cortex. At this stage its cells have divided enough to form a parenchymatous mass, but no vascular tissue has formed. A cross section of this stage is shown in Fig. 12. $\times 104$. Fig. 12. Cross section of *T. aphyllus* strands at a stage before vascular differentiation. The three very large cells in the host cortex are mucilage cells. $\times 59$.



those of the xylem are smallest, apparently being restricted by the maturation and hardening of the host's lignified elements. This radial growth, like the initial intrusive growth, seems to be accompanied by some compensatory growth by the host, because even the largest parasite strands, those with diameters equivalent to 10–20 cactus cells, are not surrounded by layers of crushed cactus tissues; some cells may be slightly flattened and have an epithelial appearance, but they are all alive, with intact protoplasm and nucleus (Figs. 5, 31, and 34). Several instances were observed in which the surrounding host parenchyma was induced to expand greatly, forming a sheath around the parasite strand (Fig. 30). If two or three adjacent filaments enlarge greatly, they may trap islands of host parenchyma between them, producing a complex strand that is a mixture of the two plants; the trapped cactus parenchyma persists indefinitely, appearing healthy and normal (Fig. 31).

Once the parasite strands reach a certain diameter (ca. 120–180 μm), differentiation of tissues begins. The first step is the appearance of a layer of very narrow cells with elongate, narrow nuclei; these cells soon collapse completely, forming a dark irregular layer in the center of the strand (Figs. 31 and 34). Its purpose and significance are unknown. In strands that are slightly larger, a differentiation of vascular tissue is obvious: the center of the strand is occupied by a single collateral bundle of vascular tissue, the outer parts of the strand remaining parenchymatous and having the appearance of a cortex (Fig. 5). The vascular bundle is wedge-shaped in cross section, with the broad portion of the wedge being composed of xylem and the narrow portion being phloem. The bundle is oriented such that the phloem is proximal to the zone of collapsed cells. In large compound strands formed by the merger of several enlarging filaments (Fig. 36), each of the original filaments produces a vascular bundle, so a compound strand may have two to five vascular bundles. Although each of these bundles is oriented with regard to its appropriate collapsed zone, they are not oriented with regard to the other bundles of the same strand, which thus has an extremely irregular unorganized appearance. The vascular bundles of the endophyte strands, likewise, have no orientation with regard to any host tissues. More importantly, no direct connection with any of the host vascular tissue was observed: they are always separated by layers of "cortex" parenchyma and sometimes also by the collapsed zones. Within the compound strands, the vascular bundle of each component strand may contact or merge with the vascular bundle of the other component strands.

The phloem of the *T. aphyllus* endophyte appears to be completely normal, with short sieve tube members with horizontal sieve plates with fine sieve pores. Companion cells and nonconducting parenchyma are also present, but neither phloem fibers nor sclereids were observed. The fact that the phloem is adjacent to the collapsed zone suggests that at least part of the collapsed zone might be degenerated protophloem.

The xylem of the *T. aphyllus* endophyte is almost pure parenchyma (Fig. 5), and tracheary elements are rare. In the strands of endophyte embedded in the host phloem, tracheary elements are present only as occasional idioblasts: there are single isolated tracheary elements not in contact with others, and conduction is obviously impossible. However, in the endophytic strands immediately below the exophytic floral branches, there is a greater quantity of conducting cells, actually forming continuous rows, possibly being capable of conducting water (Figs. 35, 36).

An identifiable undisputable vascular cambium was never found in any endophyte vascular bundle; the cells immediately adjacent to the phloem resembled the xylem and were not arranged in uniform layers. Although the xylem and phloem tend to be arranged in rows, the rows are never as uniform and regular as would be expected from a vascular cambium; rather they have much the appearance of primary tissues derived from a procambium. As the vascular bundle develops and enlarges, the outer cortexlike portion of the strand enlarges and is not crushed (Figs. 5, 36, and 37). It may produce small isolated clusters of sclereids, and some of its cells contain many prominent starch grains (Fig. 32).

These vascular bundles never become very large: in the endophytic strands below the largest exophytic inflorescences that we found, the xylem was only 17 cells deep and the phloem just 11 (Fig. 36). Even in these largest oldest plants the anatomy never became like that of more "typical" seed plants: no endophyte was ever found with a vascular cambium or round central mass of xylem surrounded by phloem. As mentioned above, the largest strands are the compound ones in the axial phloem of the host. Endophytes located in either the pith or cortical parenchyma or in the host leaf trace phloem could occasionally become large and develop vascular tissue; conversely, many strands undergo very little radial growth, remaining as fine strands of parenchyma.

Tristerix aphyllus has no system of "sinkers" (special connections between the haustorial tissues in the host vascular tissue and the exophytic portion) as are reported for several

FIGS. 13–22. Fig. 13. Longitudinal section of *Trichocereus chilensis* phloem, showing a young invasive *Tristerix aphyllus* filament. Compare the sizes of the two nuclei (cactus nucleus indicated by arrow), and note the normal appearance of the phloem, with prominent sieve plates; the parasite has not crushed or destroyed any host tissues; the two seem to grow in a coordinated manner. $\times 164$. Fig. 14. As in Fig. 13, but this filament has branched. $\times 164$. Fig. 15. A slender strand of *T. aphyllus* growing between four host cortical cells. The thickened walls of these mature parenchyma cells are nondeformable, confining the parasite to existing intercellular spaces. $\times 184$. Fig. 16. Cortical parenchyma that was invaded while younger than that of Fig. 15. One filament has expanded laterally to form a strand which shows the beginning of vascular differentiation. The rest of the filaments have remained uniseriate but have proliferated to the point of filling every possible intercellular space. The growth of the parasite may have forced some separation of host cells; *T. chilensis* cortex usually does not have so much space between cells. $\times 44$. Fig. 17. Cross section of host xylem showing several cells (arrows) invaded by the parasite. $\times 231$. Fig. 18. A large vascular bundle of *T. chilensis* which has been infected. Note the large number of strands of *T. aphyllus* in the phloem; the xylem likewise is heavily infested, but the cells are difficult to distinguish in a black and white photograph. The number and size of the strands suggest that this bundle has been infected for quite some time, yet the cambium appears to be functioning normally. $\times 54$. Fig. 19. This vessel element was invaded when young and has reacted by depositing a solid, lignified wall around the filament, while the rest of the element formed normal intervascular pitting. The parasite has remained alive. $\times 280$. Fig. 20. Tylosislike invasions of a vessel element, one of which contains a nucleus (arrow). Because these have not been covered by host secondary wall (as in Fig. 19), they probably invaded the vessel element after it had matured. $\times 242$. Fig. 21. Thick-walled medullary parenchyma cells that have been invaded by parasite filaments, one of which extends upward between cells (arrow). This host cell was living, its nucleus was present in a different section. $\times 191$. Fig. 22. As in Fig. 21, but showing the disruption that may result from extensive invasion. $\times 175$.

other parasites. The surface of the *T. chilensis* is covered with areoles that are actually short shoots whose leaves are modified into spines (Fig. 3). Each short shoot is connected to the vascular bundles of the stele by a ring-shaped set of ca. 15–30 leaf traces that cross the wide (3–4 cm) cortical parenchyma (Figs. 37 and 38). Usually the phloem (sometimes also the neighboring parenchyma) of each of these leaf traces is infected by *T. aphyllus* strands which can become quite large, each producing its own vascular bundle. These can have the appearance of sinkers, but the size and complexity of the parasite strand seems more correlated with the size of the host trace rather than the presence of an exophyte; also, these leaf traces can ramify, contributing to the system of cortical traces and these are also infected by *T. aphyllus* (Figs. 11 and 12).

To summarize briefly, a plant of *T. aphyllus* such as is shown in Figs. 2 and 3 may have an extensive three-dimensional myceliumlike endophytic portion that can extend as much as 2–3 m along the length of the host shoot, be restricted primarily to one side of the host or completely encircle it, and be present in all host tissues (except the spines and hypodermis probably). In a cross section through the host in a region of mature endophyte, it would be possible to encounter uniseriate filaments, thin parenchymatous strands, somewhat larger strands with a collapsed zone and vascular tissue, and large compound strands composed of several smaller strands that had merged by chance as they grew into each other, possibly trapping islands of host parenchyma which remain alive.

Discussion

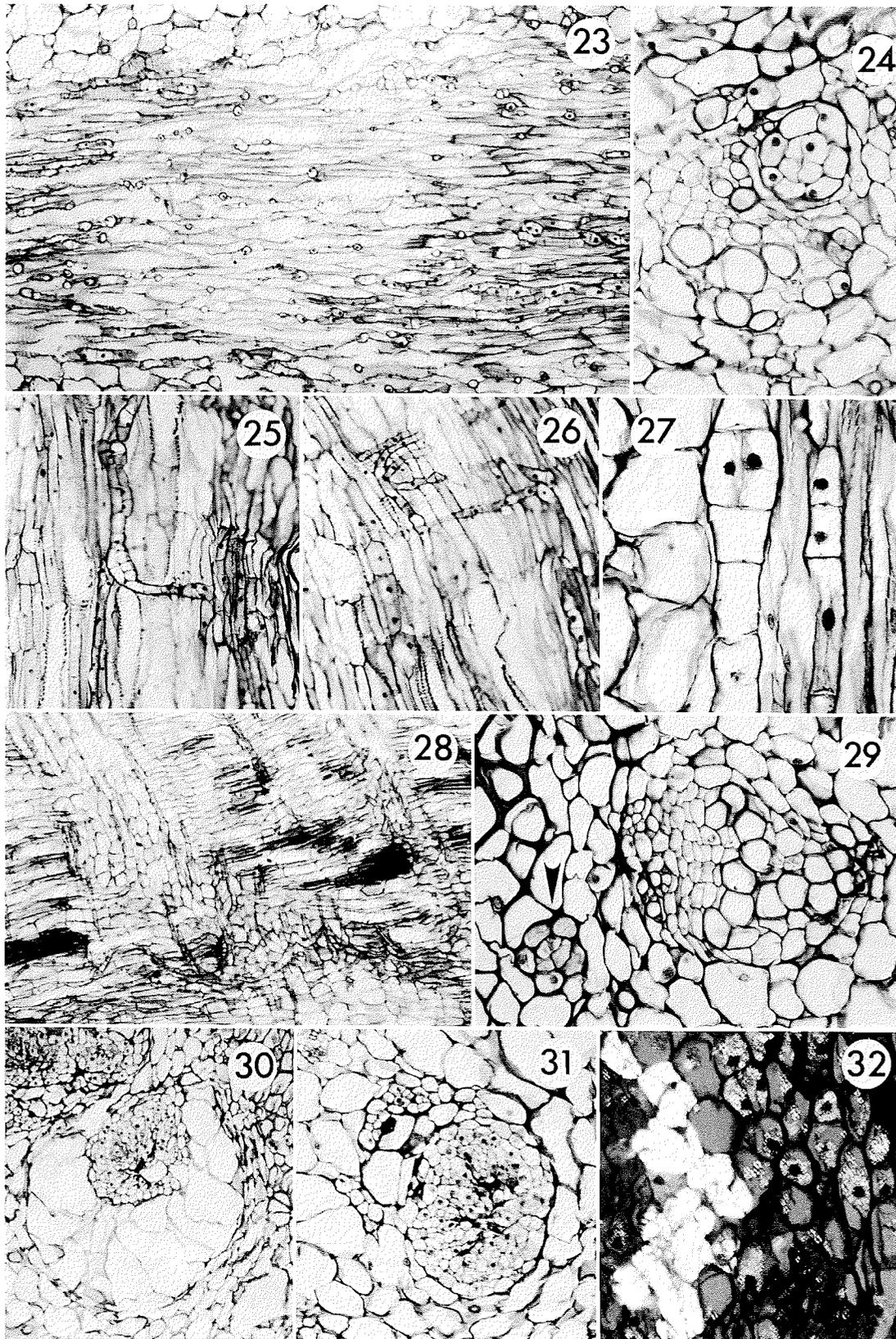
Tristerix aphyllus is obviously one of the most highly reduced seed plants known: it completely lacks most of the

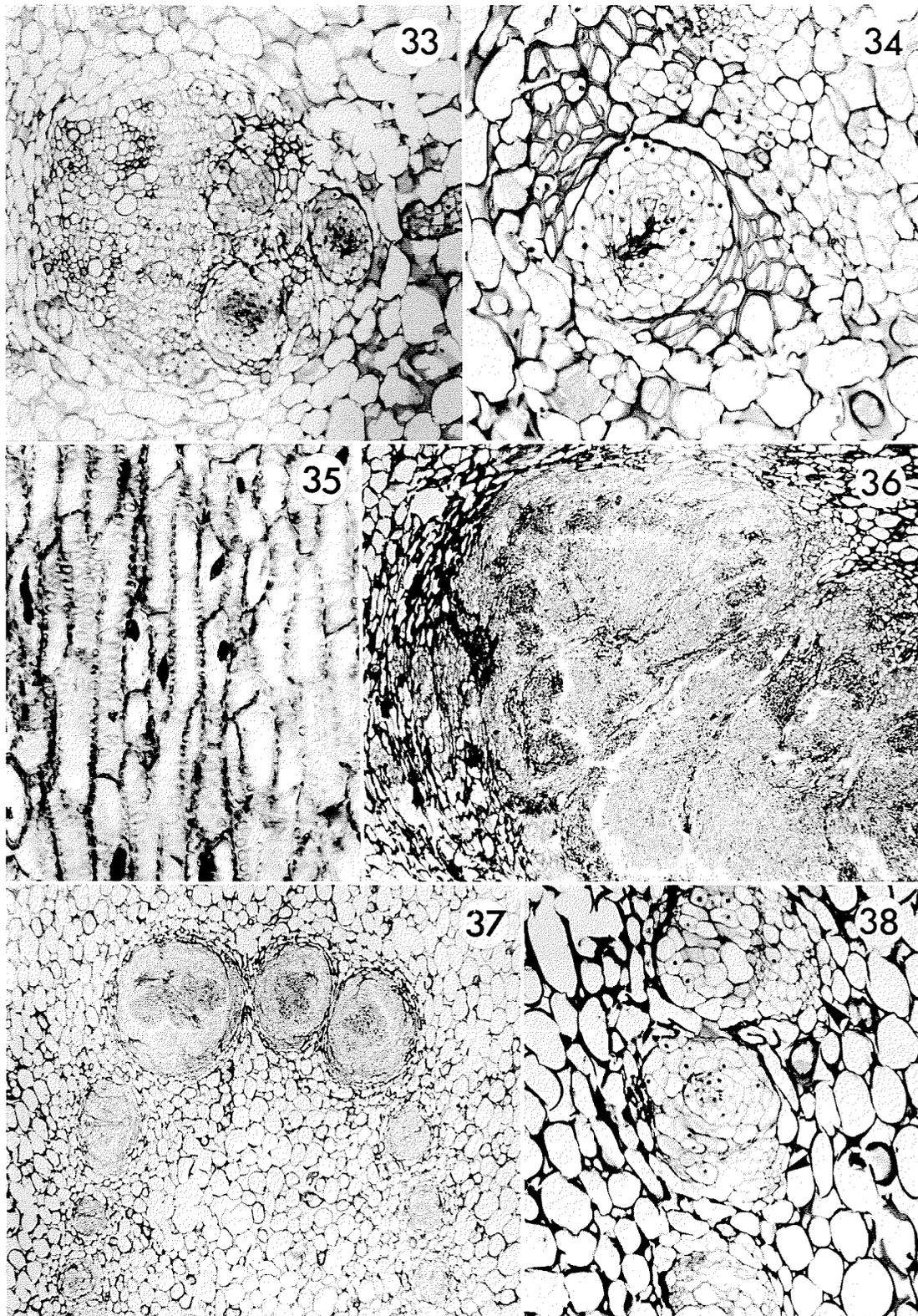
organs and tissues that are considered to virtually define spermatophytes; it has no roots, and the only stems ever produced are those of the small inflorescences; leaf primordia are produced in these inflorescences, but development apparently stops early and the primordia do not mature into leaves. The only normal organs produced are flowers and inflorescences. Such reduction is rare, being found elsewhere only in the Rafflesiaceae (Dell et al. 1982; Kuijt 1969; Rutherford 1970), Viscaceae, and Santalaceae (J. Kuijt, personal communication). All other members of the Loranthaceae, especially other species of *Tristerix*, have large well-developed exophytic portions with green photosynthetic leaves and stems with normal primary and secondary growth. It is interesting that such total suppression of normal development has occurred, especially when one considers the implications for physiology and morphogenesis. It is easy to think that the three primary functions of typical stems and leaves are photosynthesis, conduction, and support, three functions which are unnecessary in an endoparasite. But shoot systems do much more than this; they also (i) produce the buds that develop into flowers, (ii) control the growth and orientation of their tissues, and (iii) sense the environment and produce (as well as respond to) hormones and other morphogenic signals.

Obviously, with the endophyte being so totally modified, the normal mechanism for producing flower buds is absent, and they must arise adventitiously from the parenchymatous strands. Although naturally occurring adventitious buds are not rare in spermatophytes, these are usually vegetative buds and are produced on organs of a much more typical morphology than the endophyte of *T. aphyllus*. The only other plants that give rise directly to adventitious flower buds (of which we are aware) occur in a few other parasites and *Stichianthus*

FIGS. 23–32. Fig. 23. An oblique longitudinal section showing host xylem on left, vascular cambium in the center and phloem on the right (the picture is lying on its side). The endophyte filaments are oriented axially in the xylem and phloem and are thus cut longitudinally, but they are radially oriented in the cambium. See Figs. 25 and 26 for radial sections. Note that the transcambial strands are uniseriate. $\times 58$. Fig. 24. Cross section of a very young, infected vascular bundle. The *Tristerix aphyllus* filament has just begun longitudinal division and is only four cells wide. This material was taken from a very young region of the *Trichocereus chilensis* shoot, and parasite filaments had probably invaded its apical meristem. $\times 177$. Fig. 25. A radial section of infected vascular tissue, showing the transcambial interconnection of an intraxylem endophyte strand with an intraphloem endophyte strand. Compare with Figs. 23, 26, and 28. $\times 71$. Fig. 26. Similar to Fig. 25, but demonstrating that the transcambial filaments can branch, thus increasing the infection of the cambium. $\times 71$. Fig. 27. A young filament, just beginning longitudinal cell division. $\times 173$. Fig. 28. Transcambial strands in an old section of *T. chilensis*. This vascular bundle was very large, with mature parasite strands, yet the transcambial filaments remained uniseriate. Xylem on bottom, phloem on top; the stem's axis is oriented horizontally. $\times 33$. Fig. 29. Initial stages of lateral growth in a *T. aphyllus* filament (arrow) located in cortex near a vascular bundle. Although there were several filaments in this region, the vascular bundle itself was not infected. $\times 171$. Fig. 30. This *T. aphyllus* strand has induced the adjacent host cortex cells to enlarge, forming a prominent sheath. $\times 58$. Fig. 31. Note the irregular outline of this strand: the bulk of it, on the right, has probably developed from one or two filaments; the two "arms" extending to the left have developed from separate filaments and encircle several host cortex cells. If the filaments of the arms had enlarged also, a complex strand would have resulted, with patches of host tissue trapped inside it. $\times 93$. Fig. 32. This is the cortexlike region of a large mature strand, similar to that in Fig. 5. With polarized light it is possible to see starch grains and sclereids. $\times 45$.

FIGS. 33–38. Fig. 33. A host vascular bundle in which several filaments have begun developing into large strands. Also, note the strand in the cortex that is "approaching" one of the axial strands. $\times 58$. Fig. 34. A strand in which the collapsed zone has formed, but before vascular differentiation has begun. This strand is located within the cap of phloem fibers and had pushed them aside, indicating that the parasite invaded this area before the cells became lignified. $\times 104$. Fig. 35. Wood of a very large endophyte strand such as that shown in Fig. 36. It is rare to find contiguous tracheary elements in the strands embedded in the phloem of the axial bundles, but xylem such as shown here frequently does develop just below large exophytic floral branches. $\times 255$. Fig. 36. A portion of a complex strand in the outer cortex below a large exophytic floral branch. Although the xylem tends to be centrally located, note that it is really organized as small patches, with numerous collapsed areas separating them. Perhaps as many as 13 distinct strands constitute this portion. The cortexlike region still persists, and this extensive expansion has crushed neighboring host cells. The white areas are cracks in the preparation. $\times 23$. Fig. 37. Tangential section of the cactus, producing a cross section of the infected leaf traces. Ten traces are visible, all infected; as the leaf traces branch and anastomose, so do the infecting parasite strands. The three upper strands have already developed vascular tissue, but the lower strands have not yet formed collapsed zones. The host vascular tissue of the leaf traces are so small as to be indistinguishable (see Fig. 38). With further development, these separate strands would have merged into a complex strand such as that in Fig. 36. $\times 57$. Fig. 38. Higher magnification of several strands as in Fig. 37. These have not yet formed the collapsed zone. Even at this magnification, the host tracheary elements are so small as to be difficult to see (arrows). $\times 92$.





(Rubiaceae; Winkler 1931). From tissue culture experiments it has been established that in certain species or varieties, vegetative buds can be induced artificially. But this is possible in only a few species at present; in the majority, this morphogenic trigger is apparently so complex as to have not yet been discovered, even though a great deal of effort has been expended. The capacity of *T. aphyllus* to produce flowers adventitiously (especially only adventitiously) probably represents a major metabolic modification.

The second major function of shoot (and root) systems, to control the normal establishment and development of tissues, has likewise been highly modified. The vegetative growth and morphogenesis of *T. aphyllus* can perhaps be best understood as two phases: opportunistic and controlled. The actual invasive growth has many aspects that seem to be directed more by opportunity than by *T. aphyllus* itself: in the more lignified or harder parts of the host, *T. aphyllus* grows primarily in areas of weakness or free intercellular spaces. In softer areas of the host, much of the orientation of the parasite matches that of the host; if any control is being exerted over *T. aphyllus*, *Trichocereus chilensis* is the one exerting it. The largest best-developed strands of *T. aphyllus* occur in the axial phloem, a region that should be richest in nutrients and water; the strands in the inner cortex and pith (tissues poor in nutrients and devoid of stored starch) are the most slender and least developed, something that would be consistent with a poor food supply. Such differences in the growth of separate parts of the same plant suggest that very little conduction is occurring, and growth depends on the conditions presented by the host. Morphogenesis, as opposed to invasive growth, appears to be more orderly and to be controlled by *Tristerix* itself. Transcambial strands are oriented radially and do not undergo any increase in diameter; the strands are of a uniform diameter for short distances, they are not just lumps of callus; vascular tissue development is spatially oriented within the strands and begins when the strands attain a critical diameter. At present it would not be useful to speculate as to whether these control mechanisms are completely new and unique to *T. aphyllus* or whether they are modifications of preexisting control mechanisms that occurred in the stems and (or) roots of the ancestral species. Certainly the mechanism for tip growth must be new; growth by apical cell division in uniseriate filaments may be common in algae and fungi, but we do not know of instances in which it occurs in seed plants (unless an occasional trichome) except in other highly modified endophytes, such as *Arceuthobium* (Thoday and Johnson 1930), *Cuscuta* (Dörr 1969, 1972), and members of Rafflesiaceae (Kuijt 1969).

In considering the morphogenesis just described, the natural first assumption is that *T. aphyllus*, like any plant, is controlling its own development. But *T. aphyllus*, being an endoparasite, is unusual in that its entire ecosystem is the interior of one single other plant; furthermore, consider that *T. aphyllus* is physically located within the gradients and fluxes of host hormones and metabolites that are controlling the morphogenesis of *T. chilensis*. The opportunity for "foreign" control is unique. Similarly, *T. chilensis* is exposed to whatever hormonal mechanisms that *T. aphyllus* might have. It is, therefore, especially interesting that the cactus growth and differentiation are so completely normal: even the cells and tissues of the host immediately adjacent to those of *T. aphyllus* develop normally, with signs of disruption being extremely rare.

Just like any other plant, *T. aphyllus* must be capable of sensing the environmental conditions so that growth, flow-

ering, and dormancy occur at the proper times. This aspect of the loss of the vegetative shoot system is especially interesting considering that leaves are typically important in the synthesis of auxins, cytokinins, and gibberellins; that they detect day length and thereby control flowering and dormancy; and that their chloroplasts produce essential components for lipid synthesis. Certainly many other taxa have become leafless, retaining only leaf primordia and apparently transferring the functions of the leaves to the stems, and the family of the host, the Cactaceae, is the classic example of this. But neither the Cactaceae nor many other plants have become leafless, stemless, and rootless. Because the endophytic strands are the only vegetative part of *T. aphyllus*, the typically leaf-based functions must have been transferred to it, but the situation is even more exotic, because any environmental information arriving at the endophyte must have been "filtered" through the host first. Thus seasonality might be detected by light-dark periods that *T. aphyllus* could possibly measure itself (since *T. chilensis* tissues are not opaque), or it might detect seasonality by responding to the hormone-growth response of the host. The advent of the rainy season could be detected only by sensing the hydration of the host tissues. Because the physical body of a plant is the expression of its control mechanisms, it cannot be disputed that those of *T. aphyllus* have been highly modified, as have been those of members of the Rafflesiaceae. But the modifications in this family seem to have occurred in the evolution of the whole family, whereas *T. aphyllus* is distinct not only in its family, but also in its genus; the modifications must have occurred very rapidly.

Cactus tissues can easily be sterilized and maintained in culture; experiments are in progress to study the physiology of *T. aphyllus* and will be reported in future papers. Finally, it must be mentioned that a previous paper on *T. aphyllus* (Thiselton-Dyer 1901) actually describes the wound periderm that *T. chilensis* deposits around the tunnels of beetle larvae. Although an interesting paper, it has nothing to do with *T. aphyllus*.

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- BOKE, N. H. 1952. Leaf and areole development in *Coryphantha*. *Am. J. Bot.* **39**: 134–145.
- DELL, B., J. KUO, and A. H. BURBIDGE. 1982. Anatomy of *Pilosyles hamiltonii* C. A. Gardner (Rafflesiaceae) in stems of *Daviesia*. *Aust. J. Bot.* **30**: 1–9.
- DÖRR, I. 1969. Feinstruktur intrazellulär wachsender *Cuscuta-Hyphen*. *Protoplasma*, **67**: 123–137.
- . 1972. Der Anschluß der *Cuscuta-Hyphen* an die Siebröhren ihrer Wirtspflanzen. *Protoplasma*, **75**: 167–184.
- KUIJT, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, CA.
- REICHE, K. 1904. Bau und Leben der chilenischen Loranthacee *Phrygilanthus aphyllus*. *Flora* (Jena, 1818–1965), **93**: 271–297.
- RUTHERFORD, R. J. 1970. The anatomy and cytology of *Pilosyles thurberi* Gray (Rafflesiaceae). *Aliso*, **7**: 263–288.
- THISLTON-DYER, W. T. 1901. Morphological notes. IV. The haustorium of *Loranthus aphyllus*. *Ann. Bot. (London)*, **15**: 749–757.
- THODAY, D., and E. T. JOHNSON. 1930. On *Arceuthobium pusillum*, Peck. I. The endophytic system. *Ann. Bot. (London)*, **44**: 393–413.
- WINKLER, H. 1931. Über die eigenartige Stellung der Blüten bei der Rubiacee *Stichianthus minutiflorus* Valet. *Planta*, **13**: 85–101.