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# Histological and Histochemical Study of the Roots of Zamia floridana and the Endophytic Alga Contained In Them

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# Histological and Histochemical Study of the Roots of *Zamia floridana* and the Endophytic Alga Contained In Them

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*Abstract.* Some of the epigean roots are found to contain an endophytic blue-green alga, *Anabaena cycadearum;* others are free of algae but are later invaded. Normal roots and algal-free roots possess a diarch protostele; algal-infected roots have a triarch protostele. The normal root has an epidermis which in the other roots is sloughed off and replaced by a leathery phelloderm. The algal-infected root contains in the mid-cortex a wide area filled with algal cells and spanned at infrequent intervals by starch-free layer, radially elongated cortical cells. The algal-free root contains a starch-free layer of cells in the mid-cortex which are believed to elongate radially after algal penetration has taken place. The elongate cells are high in sulfhydryl protein content and contain conspicuous globules of acidic lipids. The similarity between the elongate cortical cells and the algal cells in their preferential uptake of Bennett's red sulfhydryl reagent and in the retention of safranin red when counterstaining points strongly to a definite chemical similarity between these host cells and the endophytic alga.

The presence of an endophytic blue-green alga within the swollen epigean roots of cycads has been recognized for well over three quarters of a century. The condition was first reported by Reinke (1872), who described the relationship as parasitism. The parasite he determined to be a blue-green alga which he called *Anabaena cycadearum* because of its almost universal occurrence within the roots of cycads. Later workers investigating the same situation were divided in their opinions as to whether the condition represented true parasitism or symbiosis. A review of the literature pointed out that various workers were also in disagreement with some very basic morphological aspects of the alga-cycad complex. This need for clarification motivated additional research into the problem, part of which is described in this account.

## DESCRIPTION OF ROOTS

Examination of the roots of *Zamia floridana* reveals that there are three major lateral root types present:

(1) The "normal" root, which does not differ from the typical lateral roots of other higher plants.

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(2) The algal-infected root, which in gross aspect grows to the surface of the soil and there projects well above the soil surface, forming a compact structure of short, thick, finger-like roots resembling a sea coral superficially and commonly referred to as "coralloid" roots (Chaudhuri and Akhtar, 1931; Horejsi, 1910; Life, 1901; Schneider, 1894; Spratt, 1911, 1915; Watanabe, 1924; Winter, 1935; Zach, 1910). Each stubby individual root making up the coralloid root complex has a common point of origin at the tip of a normal lateral root with numerous other similar thick, shortened roots, and each member displays one to several dichotomous branches. When an algal-infected root is broken off, it reveals in cross section a concentric green ring lying equidistant between the center of the root and its periphery.

(3) The algal-free coralloid root, which is similar to the algal-infected coralloid in that it also displays dichotomy and consists of short, finger-like projections jutting above the soil surface, but is different in that it displays fewer root members and has no interior green ring in cross section.

## Histological Studies

**Methods.** A comparative histological examination of all three root types was made by killing and fixing the roots in a formalin-acetic acid-alcohol solution and staining the microtomed sections with safranin red followed by fast green according to the procedure outlined by Johansen (1940). The most satisfactory results were obtained by placing root segments in the killing-fixing solution under vacuum in order to exhaust air pockets particularly prevalent in the algal-infected roots. Optimum thickness for examination of the sections was 10$\mu$. Both transverse and longitudinal sections were made, but only transverse sections will be discussed.

**Description of Cross Sections.** Cross sections of the normal root revealed a diarch protostele, whose xylem elements had retained the red dye, surrounded by cortical and epidermal cells whose walls had taken on a green color.

The algal-free coralloid displayed a close anatomical resemblance to the normal root except for a notable increase in the size of the cortical cells and the replacement of epidermis with a peripheral corky tissue. The algal-free root possessed a red-staining diarch protostele, a cortex taking up the fast green dye preferentially, and a phelloderm two to four cells in thickness which retained the safranin red.

The algal-infected coralloid root possessed a triarch protostele bordered by green-walled cortical cells with a red peripheral phel-

loderm. The cortex was remarkable because it was divided into two distinct zones—and inner zone adjacent to the stele and an outer zone lying beneath the peripheral phelloderm. These inner and outer cortical zones were abruptly separated from each other by a wide area completely filled with algal cells which often retained the red stain and contrasted strongly with the two green-staining cortical zones on either side. Close examination showed the algae to be occupying an interstitial cavity the breadth of which was spanned by a few infrequent cortical cells which had greatly elongated in a radial direction to connect the inner and outer cortical zones at scattered points. These elongate cells, unlike the adjacent cortical cells in the inner and outer border zones, often retained the safranin red as did the algal cells with which they were surrounded, suggesting some chemical affinities with the invading algae rather than the ordinary cortical cells.

## Histochemical Tests

In all the following tests the use of freshly collected plant material was strictly adhered to, since histochemical detection of cell and tissue constituents is dependent upon the use of intact living material. As Pearse (1954) pointed out, grossly erroneous results can be obtained by careless selection of material, since dead, wounded, or dying cells undergo definite chemical changes and produce various autolytic and sub-mortem by-products.

Freehand sectioning with a sharp razor blade was used unless otherwise noted, and such sections were immediately treated with the proper reagents in order to circumvent any oxidation or autolysis of tissues. Similarly, the chemical reagents were freshly prepared to insure their proper action.

**Starch.** Since freehand sectioning of the roots displaced the starch granules, the freezing microtome was used in the test for starch. Propane gas was used as the freezing agent, and frozen root sections were cut 30$\mu$ to 45$\mu$ thick. The starch test, using a solution of IKI, indicated that starch was present in considerable quantities in all three root types and tended to accumulate in those cortical cells nearest the central core, becoming less abundant toward the root periphery. In the algal-free root a starch-free zone, consisting of a single layer of cortical cells, could be demonstrated in the mid-cortex (Figure 1). The cells on either side of this layer possessed starch in considerable quantities. When the algal-infected root was examined, the elongate cortical cells, also consisting of a single layer in the mid-cortex, were found to be starch-free. This would suggest the presence in algal-free roots of a specialized layer of cortical cells which are capable of radial elongation and of spanning the cavity in the mid-cortex when invaded by algae.

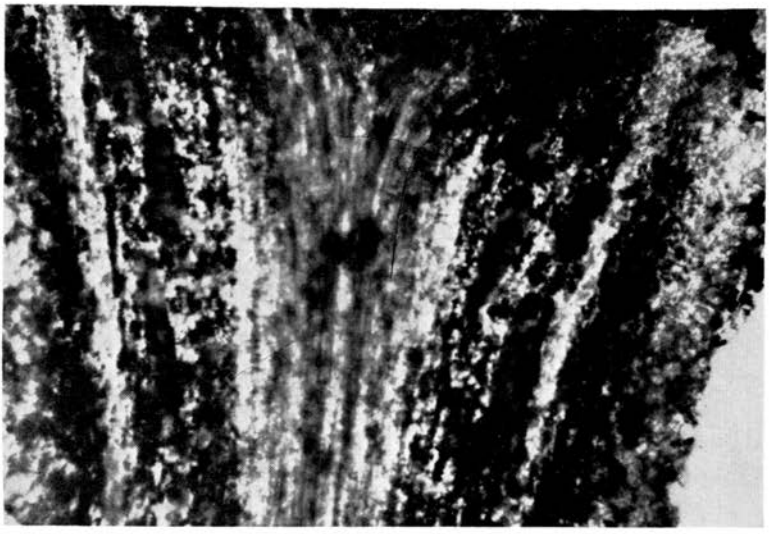

Figure 1. Algal-free coralloid root displaying the single cell layer of starch-free cells in the mid-cortex on either side of the central cylinder.

**Lipids.** The roots containing the blue-green algae possessed oil globules in the cells of the inner cortex and within the elongate cortical cells spanning the algal cavity, whereas the other root types were free of such globules. When the common lipid stains Sudan III, Sudan IV, Sudan Black B, or Nile blue sulfate were applied to the root sections, they were incapable of staining the globules because the ethylene glycol, ethyl alcohol, or diacetin solvents also dissolved the fragile oil globules. For this reason it was necessary to modify these methods by preparing a 1 percent aqueous solution of Nile blue sulfate which was used to distinguish neutral from acidic lipids, since neutral lipids stain pink and acidic lipids become blue. The acquisition of a distinct blue color by the oil globules was intepreted as denoting the presence of acidic lipids.

**Glycogen.** The presence of glycogen within the algal cells was demonstrated by staining with potassium iodide solution (IKI). Since other substances (proteins and amyloids) might intefere with a positive test for glycogen, confirmatory tests were run (Pearse, 1954). Glycogen is hydrolyzed by the enzyme ptyalin. Therefore, if IKI is applied to algal cells receiving no preliminary ptyalin, any glycogen they contain should remain intact and take on a brownish-yellow stain. On the other hand, a control group of algal cells previously treated with ptyalin should not react to IKI but should retain their blue-green color. By using this confirmatory test it was demonstrated that glycogen was present in the cells of *Anabaena cycadearum*.

**Proteins.** The problem of detecting native proteins in plant tissues has received little attention. This has been due chiefly to the fact that so few reagents show enough specificity to protein groups alone to assure their detection in tissues. Recently Bennett (1951) devised a method for detection of sulfur-containing proteins in animal tissues by the use of 1-(chloromercuriophenylazo)-2-naphthol, to which is ascribed a high protein specificity when sulfhydryl groups are present. This reagent was applied to hand-cut cross sections of all three types of roots. It was soon obvious that the reagent required a longer up-take time for plant tissue than that suggested by Bennett for animal tissues, and that only the algal-infected roots showed promise of absorbing it in detectable quantities. At the end of 14 days, examination of root sections revealed an up-take of the reagent by both the algal cells and the elongate cortical cells spanning the algal cavity. The marked intensity of color of these algal and host cells is interpreted as evidence of the presence of significant amounts of sulfhydryl proteins within them. It further infers an interaction between the elongate host cells and the algae with which they are in proximity.

## Discussion

A consideration of the morphology of the alga-cycad relationship indicates certain peculiarities somewhat unique to this problem. Seldom has such a definite internal delimiting zone been detected in a host plant for its invader, and seldom has the invader been an alga. Although some particular plant pathogens may be restricted to definite tissues or certain intercellular areas, there has been no suggestion of a "preparatory" zone existing in the plant to accommodate a future invader as has been suggested in the roots of *Z. floridana*. The presence of a starch-free zone consisting of a single layer of cells in the mid-cortex of algal-free coralloids seems highly suggestive of a future site for the accommodation of invading algal cells. This is especially so when we consider that the mid-cortex of algal-infected coralloids contains an intercellular cavity filled with living *Anabaena* interspersed with a single layer of starch-free, elongate cortical cells spanning this gap. There is little reason to doubt that these elongate cells have had their origin from the single layer of starch-free parenchymal cells in the mid-cortex of the algal-free coralloid. The ability of these specialized cells to elongate in response to the presence of the invading algal cells makes them distinctive.

This physical difference seems to have a chemical counterpart even from the rough results of this study. It has been shown that a rather fundamental chemical difference exists between the elongate cortical cells and the ordinary parenchymal cells of the cortex of algal-

infected roots. This was demonstrated by the fact that the elongate cells retained safranin red after a counterstain of fast green caused the remaining parenchymal cells to become green. This chemical difference was verified by demonstrating the proteinaceous nature of the bridging elongate cells; these cells assumed a deep red color in comparison to the surrounding cortical cells when the red sulfhydryl reagent was applied.

The presence of lipid bodies within the cytoplasm of the elongate cells is not at all understood and can not be commented upon at this time.

Both histological examination and histochemical tests infer that some interaction takes place between *A. cyadearum* and the elongate cortical cells of the host root, since both algal cells and host cells show similar preferential uptake of the protein-detecting red sulfhydryl reagent and both show similar affinities for the same dye.

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