

# THE CORALLOID ROOTS OF *MACROZAMIA COMMUNIS* L. JOHNSON

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## Summary

*Macrozamia communis* exhibits root dimorphism, possessing both normal and coralloid roots. The latter are pneumatophores in which an algal zone may be present or absent. In the coralloid roots the root cap tissue was interpreted as forming a secondary cortex which persisted throughout the life of the root. Underlying the secondary cortex was a transformed epidermis, the cells of which, following the establishment of algae within the intercellular spaces, elongated radially to form an algal zone easily visible to the eye. *Nostoc*-like blue-green algae were isolated from this zone. In the absence of algal infection the transformed epidermis remained inconspicuous. The root cap anatomy of the normal roots was similar to that described for other gymnosperms. The tips of the alga-free coralloid roots were characteristically located near the soil surface. The alga-containing coralloid roots were less strictly negatively geotropic and were found at depths up to 30 cm below the soil surface.

## I. INTRODUCTION

The coralloid roots of *Macrozamia communis* L. Johnson (syn. *M. spiralis* Miq.), containing endophytic blue-green algae, were shown by Bergersen, Kennedy, and Wittmann (1965) to fix nitrogen. These negatively geotropic roots have been little studied. Working with *M. spiralis*, McLuckie (1922) concluded that the coralloid roots represented "tubercles" formed in response to bacterial infection and that no algal zone was present. Later Schaede (1944), who also studied *M. spiralis*, observed an algal zone but not bacteria. Both authors agreed that lenticels were absent.

Since Reinke (1872, quoted by McLuckie 1922) first described the association between blue-green algae and the coralloid roots of *Cycas revoluta* many papers have dealt with similar structures in other members of the Cycadaceae. This literature is most recently reviewed by Schaede (1944). Typically the blue-green algae in cycad coralloid roots are found localized in a discrete algal zone, which is enclosed by an outer cortex, absent in normal roots. McLuckie (1922) has theorized that the outer cortex corresponds to a persistent root cap. This theory has not been supported by later workers and Schaede (1944) is emphatic that no root cap is present on the coralloid roots. Goebel (1932), without anatomical examination, has further suggested that the cycad coralloid roots were formed primarily as pneumatophores.

The factors responsible for the initiation of coralloid roots are considered uncertain. McLuckie (1922) and Watanabe (1924) have suggested that initiation was induced respectively by bacteria and anaerobic conditions. As algae, fungi, and bacteria were not consistently observed in coralloid roots, Goebel (1932) and Schaede (1944) have tentatively put forward the view that microorganisms were not responsible for coralloid root initiation.

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In the present study a description is made of the morphology, anatomy, and development of *M. communis* coralloid roots. Schaede (1944) has called attention to the need for interpretation of the unusual cell layer in coralloid roots which ultimately forms the algal zone. This, together with the interpretation of the outer cortex, has been the primary objective of the current investigation.

## II. MATERIALS AND METHODS

*Macrozamia* plants and coralloid roots were collected near Durras Lake, N.S.W., and near the roadside of the Clyde Mountain Road, Bateman's Bay, N.S.W. Seedlings were grown by two methods: they were either washed and kept enclosed in plastic bags or they were potted in a glasshouse.

Anatomical studies were made on roots fixed in Navashin's fixative for 48 hr or in formalin-acetic acid-alcohol for 24 hr. The roots were embedded in paraffin wax and microtome sections were mounted directly from 70% alcohol into either acetic acid-iron haematoxylin (Wittmann 1962) and liquid glucose (glucose syrup) (1:1 v/v) or into acetic acid-iron haematoxylin combined with the clearing agent chloral hydrate (Wittmann 1965). Carbol fuchsin and acid fuchsin-aurantia (Gurr 1956) were used as bacterial stains. Schulze's chlor-zinc-iodine was used to detect starch grains.

## III. RESULTS

### (a) *Development and Anatomy of Normal Roots*

In *Macrozamia* the primary root of the germinating seedling grows at the expense of the stored material within the female prothallus, soon becoming fleshy and stout in outline. Secondary root development follows (Plate 1, Fig. 1), the first pair of roots being highly modified. The normal roots have a diarch stele, develop a typical gymnosperm root cap (Plate 2, Fig. 2), and, in other respects, conform to previous description of the normal cycad root (Chamberlain 1935). As in other cycads (Watanabe 1924), the primary root is strongly contractile.

### (b) *Development and Anatomy of Coralloid Roots*

Highly modified roots arise from the pericycle of the diarch stele of the primary roots and hypocotyl (Plate 1, Fig. 1) and (more rarely) on plagiogeotropic secondary and tertiary roots (Plate 1, Fig. 7). Young coralloid roots can be recognized at a very early stage, due to their characteristic outer cortex and the rounded appearance of their tips (Plate 1, Fig. 1). In the normal root, the meristematic region lies under the root cap which is ultimately shed to expose the root epidermis (Plate 2, Fig. 2). In the coralloid roots the meristematic region, by contrast, extends and becomes terminal (Plate 2, Fig. 1), while the root cap material appears to persist throughout the life of the root, forming a structure identical with that of the secondary cortex described by previous workers (Schaede 1944). Initially this secondary cortex overlies the protoderm, a layer of cells analogous in origin to the epidermis of normal roots (Plate 2, Figs. 1 and 3). It is this single layer of cells which forms the algal zone of later stages (Plate 3, Figs. 1-6). In the absence of algal infection this layer remains comparatively inconspicuous (Plate 2, Figs. 1 and 3).

Lenticels were observed very infrequently in young coralloid roots and normal roots. However, they were particularly abundant in the older coralloid roots following periderm development and algal establishment (Plate 1, Figs. 3-5).

#### (c) *Development of the Algal Zone*

Following algal infection, the cells of the transformed epidermis enlarge radially to the root axis as algae fill the intercellular spaces (Plate 3, Figs. 1-6) and soon the zone is easily visible to the eye (Plate 1, Figs. 3 and 4). The extracellular distribution of the endophyte was shown by tangential section (Plate 3, Fig. 6) of the algal zone shown in transverse section in Plate 3, Figure 5. The blue-green algae isolated from this zone (Plate 2, Figs. 4 and 5) could be considered as belonging to either the genus *Nostoc* or *Anabaena*. No attempt has been made to clarify further the taxonomic position of the endophyte. The coralloid roots become thicker after algal infection and lose their strict negative geotropism (Plate 1, Figs. 5 and 7). Infected coralloid roots were found at depths down to 30 cm, perhaps due to the activity of the strongly contractile primary root.

#### IV. DISCUSSION

The coralloid roots commonly observed on *M. communis* have been clearly shown to fall into two categories, depending on whether or not an algal zone is present. The two types of coralloid root can readily be distinguished by their external appearances (Plate 1, Figs. 2-7). To some extent the conflicting views of McLuckie (1922) and Schaede (1944) can be reconciled if it is supposed that they studied respectively alga-free and alga-containing coralloid roots. However, the fact that both authors also failed to observe lenticels and that Schaede (1944) detected only a pale algal zone would lead to the further conclusion that only very young coralloid roots were examined. Neither Schaede (1944) nor the present authors were successful in repeating the observation of McLuckie (1922) that bacteria were present inside the living cells of coralloid roots. The conclusion of McLuckie (1922) that the coralloid roots are bacterial "tubercles" is therefore questionable and it appears more appropriate to interpret the coralloid roots as an inherent feature of the *Macrozamia* plant.

The term "pneumatophore" for the coralloid roots would appear justified by the fact that they are true negatively geotropic roots, adapted for gas exchange. Although lenticels are rare in the young coralloid roots, gas exchange could readily occur through the loosely packed cells of the outer cortex. Older coralloid roots are encased in a thick periderm (Plate 3, Figs. 1 and 4) and develop lenticels (Plate 1, Figs. 3-5). The outer cortex is interpreted as being derived from persistent root cap tissue which may now be added to the wide range of other tissues including epidermis, cortex, phloem, and pericycle, which can form periderm (Esau 1965). Air passage through the coralloid roots of *Cycas* has been demonstrated by Life (1901). It is interesting to speculate that the coralloid roots have evolved first as pneumatophores and that at a later stage they acquired blue-green algal endosymbionts.

Although the characteristic zones of the cycad coralloid root have been named for many years, only McLuckie (1922) has attempted to account for their structure. He claimed that the outer cortex of the coralloid roots was analogous to the velamen

of the aerial roots of some orchids, indicating that these structures were derived from root cap tissue. Further information was not given to show how this theory was derived and subsequently Schaede (1944) has denied that root caps exist on coralloid roots and no attempt has been made to interpret the origin of the algal zone.

In the present study, cytological staining techniques were employed to reveal differences between the meristematic regions of coralloid and normal root tips. The normal root had a meristematic pattern (Plate 2, Fig. 2) which conforms to previous descriptions of gymnosperm roots, in which the root cap cells were ultimately shed, exposing the root epidermis. In the coralloid root tip on the other hand, the meristem became more terminal (Plate 2, Fig. 1), root cap cells were differentiated weakly, if at all, but retained meristematic activity longer than did their counterparts in the normal root. This enabled the formation of a secondary cortex which persisted on the coralloid root, an interpretation shown diagrammatically in Figure 1.

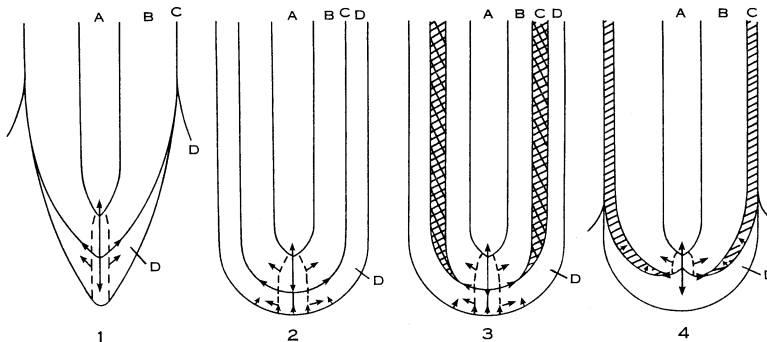


Fig. 1.—Diagrammatic representation of the development of: 1, *Macrozamia* normal root; 2, *Macrozamia* alga-free coralloid root; 3, *Macrozamia* alga-containing coralloid root; and 4, orchid aerial root tip. A, stele; B, cortex; C, epidermis; D, root cap.

It could also be suggested that the outer cortex corresponds to a multiple epidermis, as has been shown by Engard (1944) and many earlier workers for the velamen of orchid roots. Comparison reveals that while the velamen is formed from the protoderm (Engard 1944), cells of the outer cortex of coralloid roots are clearly formed exterior to the protoderm (Plate 2, Figs. 1 and 3). Another important point of difference is that both orchid aerial roots (Fig. 1) and *Macrozamia* normal roots (Plate 2, Fig. 2) possess a well-differentiated root cap which is shed in the normal manner. By contrast, the corresponding structure in *Macrozamia* coralloid roots remains meristematic at the tip and is persistent through later stages of root development. The "multiple epidermal" origin of the outer cortex in *Macrozamia* is therefore rejected in favour of the explanation that the outer cortex is formed from root cap tissue.

This latter explanation implies that the outer cortex encloses normal root structure. Thus the cell layer immediately under the outer cortex would correspond to a transformed epidermis. Significantly perhaps, after algal infection, cells of this transformed epidermis can elongate to form the algal zone in a manner analogous

to that of the root epidermis of mycorrhizal-infected plant roots. Without algal infection the transformed epidermis remained a comparatively inconspicuous layer. The mode of algal infection is not known and at this stage it can only be surmised that the algae gain entry to the transformed epidermis through the loosely packed cells covering the tip of the pneumatophore.

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#### EXPLANATION OF PLATES 1–3

##### PLATE 1

- Fig. 1.—Germinating seedling showing the first stage of lateral root formation. *A*, the first paired lateral roots arising from the pericycle of the diarch stele—these roots have dome-shaped tips and represent the first stage in the development of coralloid roots; *B*, normal lateral roots with pointed tips.  $\times 0.45$ .
- Fig. 2.—Seedlings with fully developed alga-free coralloid roots. Level *A* corresponds to soil surface. Dichotomous branching tends to occur at clearly defined levels, *A* and *B*.  $\times 0.2$ .
- Figs. 3 and 4.—Coralloid root sliced transversely and longitudinally to show the algal zone (*A*), outer cortex (*B*), and lenticel (*C*).  $\times 4.5$  (approx.).
- Fig. 5.—Alga-containing coralloid roots with abundant lenticels.  $\times 0.45$
- Fig. 6.—Alga-free coralloid roots showing papillate nature of the outer surface. Seedlings grown under very favourable glasshouse conditions.  $\times 0.35$ .
- Fig. 7.—Young plants showing the effect of lowering the soil level. The alga-free coralloid roots left protruding above soil level (*A*) have shrivelled and are dying. Those lower down are elongating towards the new soil level. Alga-free coralloid root (*B*) originates from normal lateral root. In central position (*C*) are alga-containing coralloid roots which have characteristically lost their strict negative geotropism.  $\times 0.35$ .

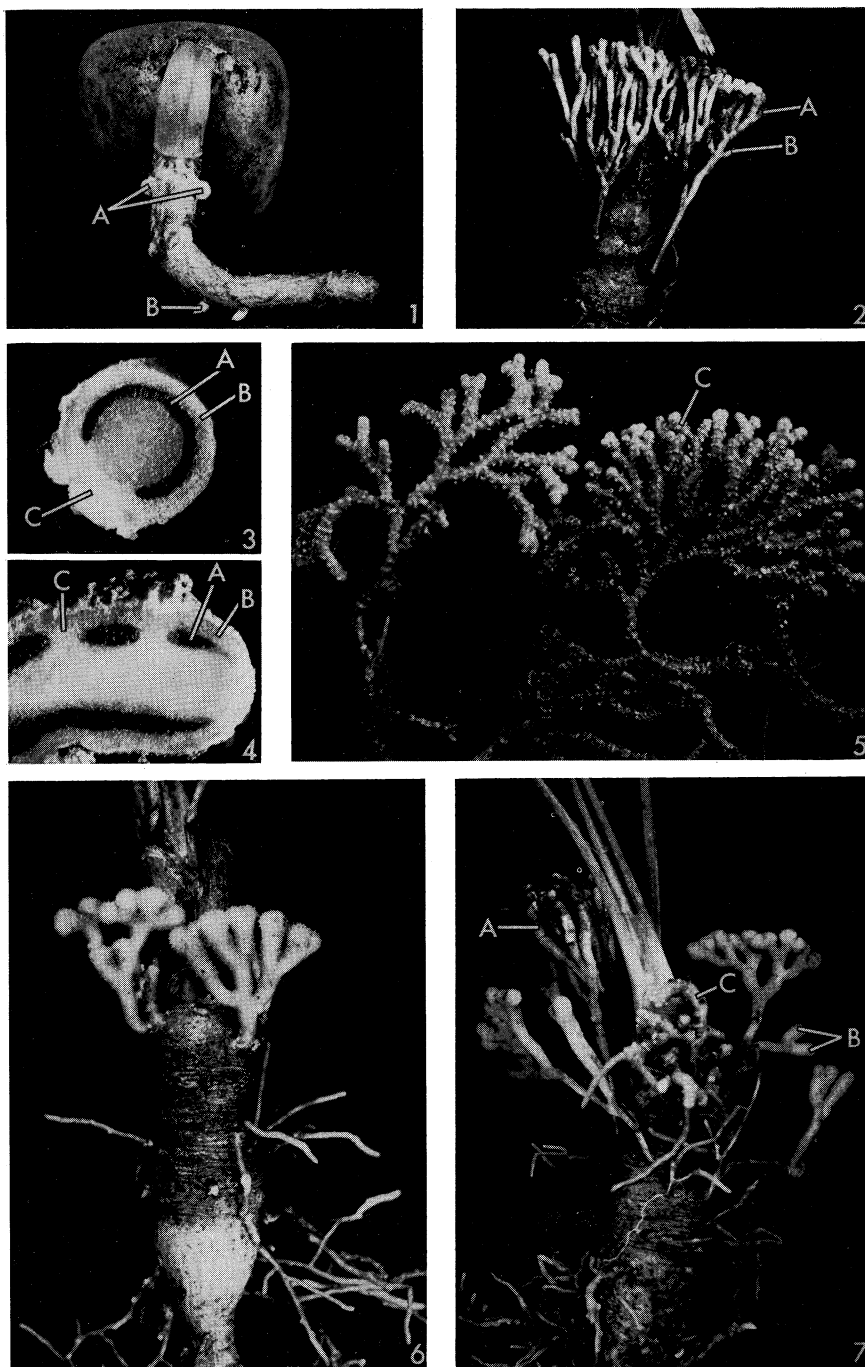
## PLATE 2

- Fig. 1.—Median longitudinal section through an alga-free coralloid root. Protoderm layer (*A*) is indicated by rows of nuclei located midway between the central stele and the outer papillose layer. Dark tannin-containing cells are evident in Figures 1 and 3.  $\times 25$ .
- Fig. 2.—Median longitudinal section of normal root showing the root cap (*A*) with material sloughing off to expose the root epidermis (*B*).  $\times 15$ .
- Fig. 3.—Transverse section through an alga-free coralloid root showing layer (*A*) corresponding to the epidermis of normal roots.  $\times 35$ .
- Figs. 4 and 5.—Blue-green algae belonging to the family Nostocaceae obtained from the algal zone and cultured on an agar medium. Hydrolysed and stained with acetic acid-haematoxylin.  $\times 625$ .

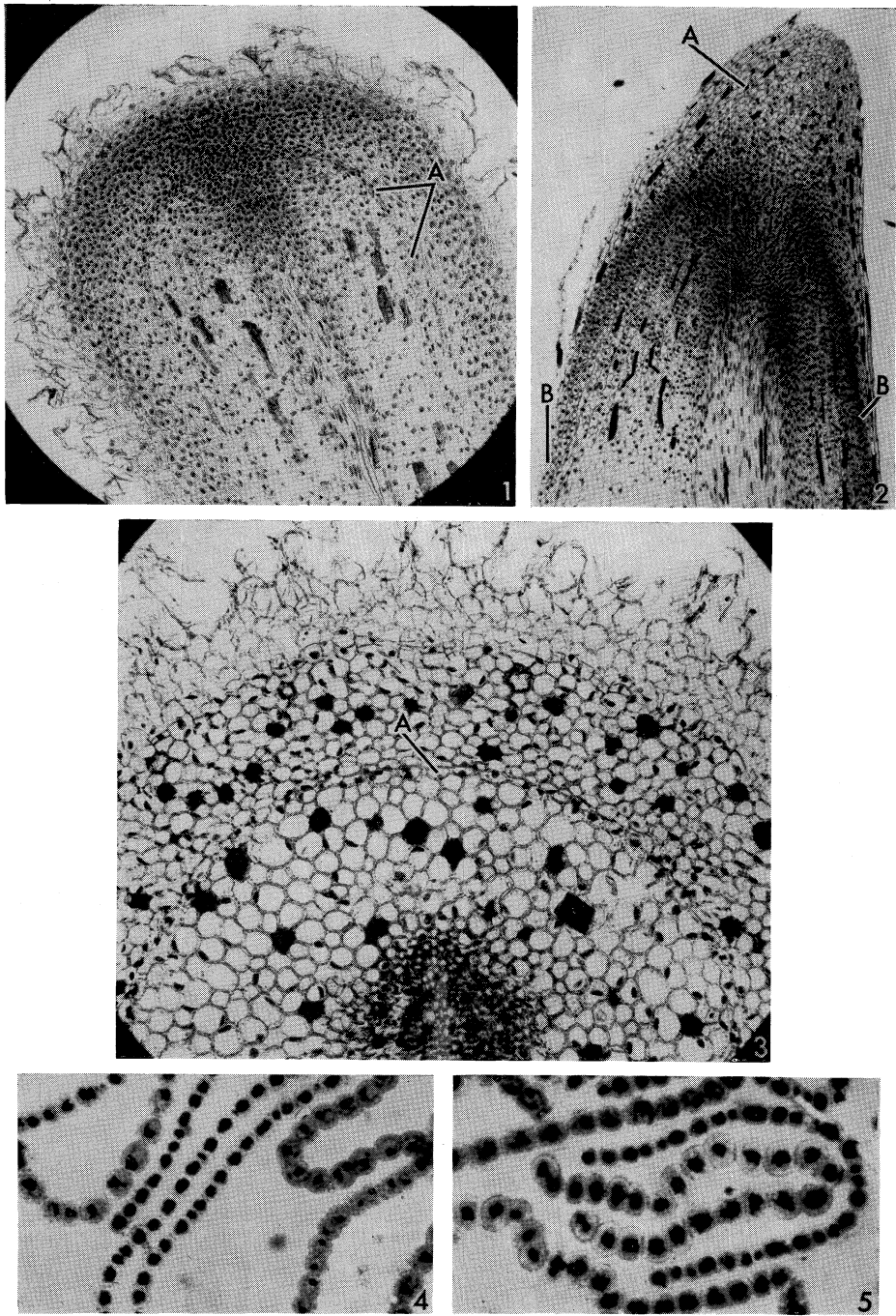
## PLATE 3

- Fig. 1.—Median longitudinal section of alga-containing coralloid root, showing the periderm (*A*), outer cortex (*B*), algal zone (*C*), inner cortex (*D*), and central stele (*E*).  $\times 7.5$ .
- Figs. 2 and 3.—Radial longitudinal section through the algal zone close to the tip of the root. Intercellular spaces (*A*) decreasing in size towards the tip. In Figure 3 algae were removed during processing of sections. Stained nuclei are evident in these and subsequent figures. Magnifications: Figure 2,  $\times 35$ ; Figure 3,  $\times 140$ .
- Fig. 4.—Transverse section through a well-established algal zone (*A*), with periderm (*B*) enclosing the outer cortex (*C*).  $\times 20$ .
- Fig. 5.—As in Figure 4, showing algae in intercellular spaces (*A*) between modified epidermal cells (*B*).  $\times 145$ .
- Fig. 6.—Tangential longitudinal section through the algal zone showing the densely packed algal cells (*A*) filling the intercellular spaces between the modified epidermal cells (*B*).  $\times 125$ .

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