

## Apical control of growth in a filamentous red alga (Ceramiaceae, Rhodophyta)

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The undescribed ceramiacean red alga used in this work is closely related to *Ceramium*. The alga is pseudodichotomously branched with alternate axes being dominant. Removal of the apical tip from the primary indeterminate axis increased the production of adventitious branches from pericentral cells behind the apex. This effect was not reversed with the addition of exogenous IAA to the growth medium in a concentration range of  $10^{-9}$  M to  $10^{-4}$  M. Removal of the apical tip from the subordinate axis did not influence adventitious branch production.

**Keywords:** Adventitious branches, apical control, Ceramiaceae, indole-3-acetic acid, primary indeterminate axis, subordinate axis.

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### Introduction

The complex and elaborate patterns of cell enlargement and symmetry found in filamentous Rhodophyceae are a reflection of rigid control of growth and development (Dixon 1971). Little is known about the internal mechanisms which modify this development but apical dominance may be a key to understanding morphogenesis and differentiation of red algal cells (Garbary *et al.* 1988).

The apex in higher plants is a self-determining region controlling differentiation (Hillman 1984). Apical dominance is the control exerted by the apices of the shoot over development and positioning of lateral buds on the same or different shoots. When the effect of apical dominance is strong, there is little or no lateral bud outgrowth. If apical dominance is weak, the more basal lateral shoots grow to a greater length than those situated nearer the apex. Thus the branching pattern, and hence plant form, is determined to a large extent by apical control (Cline 1991). Excision of the apical portion is a simple method of eliminating apical dominance (Hillman 1984).

Apical dominance has been reported in a number of Rhodophyta. Removal of the apices of *Antithamnion defectum* Kylin disrupted the pattern of band elongation on the axial cells, showing that regulation of cell wall deposition bands was under apical control. The more distant the cell was from the apex, the more likely it was to have disrupted bands. There is also a hierarchy of control with the apical cell of the main axis being dominant and less dominance being shown by the apical cells of indeterminate laterals (Garbary *et al.* 1988). Removal of the apical cell induced lateral branch formation in the red algae *Apoglossum ruscifolium* Kylin (Abelard & L'Hardy-Halos 1975 in Buggeln 1981), *Pterothamnion plumula* (as *Antithamnion plumula* (Ellis) Thur; L'Hardy-Halos 1971 in Buggeln 1981) and the brown alga *Ascophyllum nodosum* (L.) LeJol (Moss 1970 in Buggeln 1981). Lateral branch elongation was accelerated by removal of the apical cell in *Sphacelaria cirrhosa* C.Ag. (Ducreux 1977 in Buggeln 1981). An example of weaker apical dominance was reported in *Griffithsia pacifica* Kylin where there was increasing potential to produce new shoot cells the further the axial cell was from the apex (Duffield *et al.* 1972).

The most common theory for the control of apical dominance in higher plants involves auxin, although evidence for the direct role it plays are inconclusive. Cytokinins may also play a sec-

ondary role, being able to stimulate lateral bud outgrowth after the removal of auxin (Phillips 1975; Cline 1991).

There are numerous reports of seaweeds containing similar plant growth regulators (PGR) as those found in vascular plants and of exogenous application of the PGR's affecting algal growth and development (Bradley 1991). It still remains to be demonstrated whether growth substances like auxins, provide the actual control mechanism for apical dominance in red algae (Garbary *et al.* 1988). However, there are some examples where exogenously applied auxin had an effect on algae. Application of auxin to the brown alga *Fucus vesiculosus* L. appears to inhibit lateral bud formation (Moss 1965) while addition of indole-3-acetic acid (IAA) to apical segments of primary laterals from *Sargassum muticum* (Yendo) Fensholt decreased growth of the primary laterals (Chamberlain *et al.* 1979).

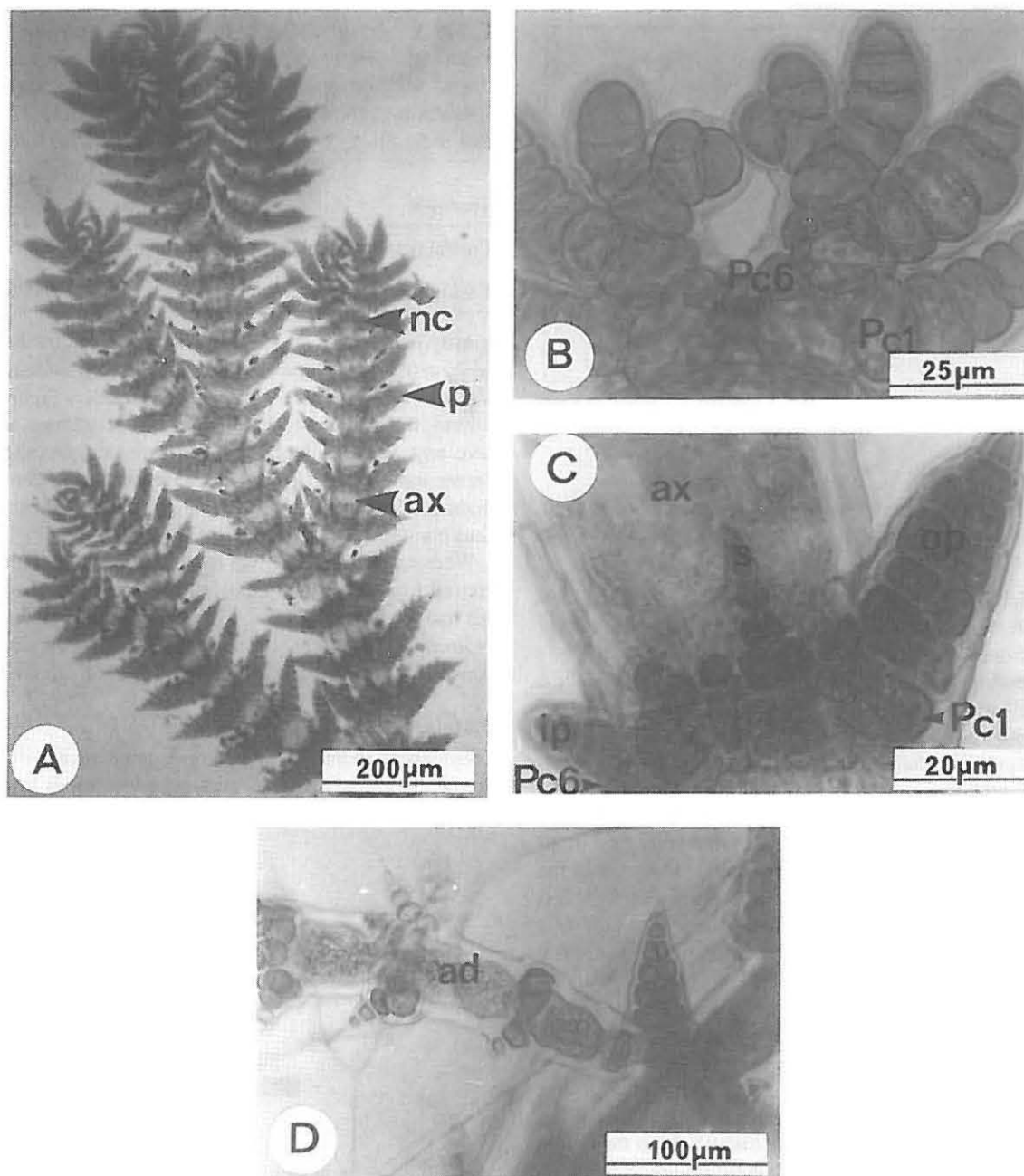
The experimental organism used in this study will be described elsewhere (R.E. Norris pers. comm.). In this work, it is referred to as Ceramiaceae indet. The aims of the work described in this paper were to establish if apical dominance plays a role in controlling growth and morphogenesis in Ceramiaceae indet. and to investigate the possible role of IAA in the process.

### Materials and Methods

The male gametophyte of Ceramiaceae indet. was collected in a seaweed washed ashore at Trafalgar Beach on the KwaZulu-Natal south coast, South Africa on 28 September 1985 and brought into unialgal culture by R.E. Norris. The alga has since been kept in culture in the Department of Botany, University of Natal Pietermaritzburg. It is maintained in Provasoli's Enriched Seawater (PES; McLachlan 1973) at 20°C, in a 16 h light: 8 h dark photoregime and illuminated with incandescent and fluorescent light at  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ . A voucher specimen of the clonal material used in this study has been deposited in the University of Natal herbarium (NU 10678).

The growth rate of Ceramiaceae indet. grown under experimental conditions was measured daily by recording the number of axial cells making up the thallus. There were five replicates.

The smallest possible portion (4–8 axial cells) of the apical tip of the primary axis was removed under sterile conditions using a sewing needle which had been sharpened to a cutting edge using a diamond. It was not possible to remove only the apical cell with the equipment available. The thalli were subsequently transferred to a watch-glass and rinsed twice in sterilised PES to remove any extruded cytoplasm. Replicates were placed into separate compart-



**Figure 1** A. Gross morphology of the originally collected wild Ceramiaceae indet. showing pseudodichotomous branching with alternate branches becoming dominant. Paired pinnae (p) arise at each cortical band (nc) between the axial cells (ax). B. Apical tip region of Ceramiaceae indet. showing dividing apical cells. The development of the larger pinnae from Pc1 on the outer surface of the thallus and the smaller pinnae from Pc6 on the inner surface are also apparent. C. A cortical band region in Ceramiaceae indet. showing the axial cells (ax) and cortical branches of determinate growth. The outer pinnae (op) develop from Pc1, the inner pinnae (ip) from Pc6 and the spines (s) from the other pericentral cells. D. Adventitious branch (ad) arising from Pc1. The axial cells and nodal cortical bands are less developed in the adventitious branch than in the parent branch.

ments in repli-dishes with each compartment containing 4 ml PES. The repli-dishes were closed with parafilm and placed in growth cabinets under the conditions described above. There were five replicates per treatment for each experiment. The experiment was terminated after 10 days as it became difficult to accurately count the number of axial cells. Intact plants were used as control replicates and cultured under the same conditions as outlined above.

The number of adventitious branches and the number of axial cells making up the original thallus and any new growth along this thallus were recorded on day 0 and again on day 10. The ratio of number of adventitious branches: number of axial cells on day 10 was determined. Significant differences were revealed using the Kruskal Wallis One-Way Analysis of Variance and Multiple Range

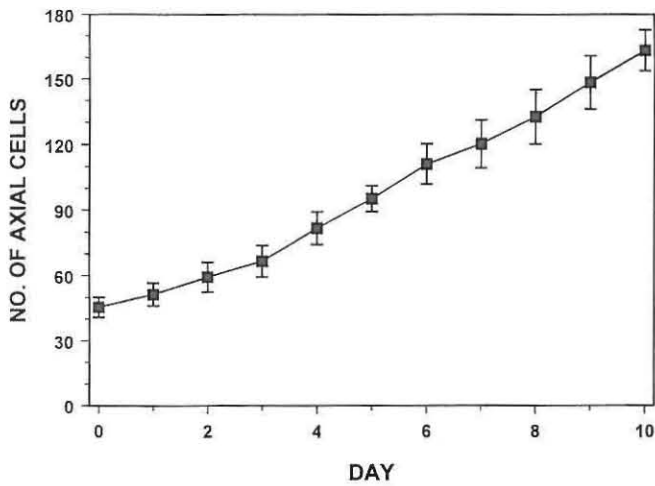
Test (Tukey).

The above experiment was repeated but with the apical tips of subordinate axes being removed instead of the apical tip of the primary axis. Measurements were taken on day 0 and on day 7.

In a third experiment, the primary apical tips were excised and added to PES containing IAA in the concentration range of  $10^{-9}$  M to  $10^{-4}$  M. There were two control treatments, one with the apical tips intact and one with the primary apical tip excised. Measurements were taken on day 0 and on day 10.

## Results

The growth pattern of Ceramiaceae indet. is similar to *Ceramium* species with an uniaxial thallus organisation and differentiation



**Figure 2** The growth rate as determined by the number of axial cells in the thallus of Ceramiaceae indet. when grown under the conditions described above.

between axial and cortical cells. However, Ceramiaceae indet. has a unique morphology with distichously arranged paired pinnae arising from the cortical bands (Figure 1A).

The dome-shaped apical cell divides horizontally to produce a subapical cell which enlarges to form an axial cell (Figure 1B). Pseudodichotomous branches are initiated by oblique divisions of the apical cell and alternate branches become dominant.

Six pericentral cells make up each nodal cortication. The two largest pericentral cells, Pc1 and Pc6, situated on the outer and inner faces of the thallus as determined by the preceding pseudodichotomy, give rise to the paired pinnae. This results in the characteristic distichous arrangement of the pinnae. Pc's 2-5 give rise to uniseriate spines. Most of the cortical band growth is acropetal (Figure 1C). The cultured material showed less cortical band growth than the wild material (Figure 1A) although it remained similar in all other respects.

Adventitious branches of unlimited growth develop from nodal cortications on the more mature parts of the thallus. They have a distinctive morphology with axial cells being smaller than those on the pseudodichotomous branches and the pinnae being greatly reduced in size. With increasing distance from their point of initiation, the morphology of the adventitious branch resumes its normal appearance with larger axial cells, more developed cortical bands and pseudodichotomous branching (Figure 1D).

Ceramiaceae indet. grew quickly in culture (Figure 2), doubling the number of axial cells in the thallus every four to five days. This meant that the subsequent experiments had to be of a fairly short duration to make recording of the data accurate.

After ten days there was a significant difference in the ratio of number of adventitious branches: number of axial cells between the control replicates and those with apical tips excised from the

**Table 1** Adventitious branch formation in Ceramiaceae indet. after the apical tips were excised from the primary axes. Measurements were taken after 10 days growth. Results are shown as mean  $\pm$  standard error. Different letters indicate significant differences at the level  $P < 0.001$

Treatment	No. adventitious branches: no. of axial cells
Control (intact apical tip)	0.011 $\pm$ 0.004 <sup>a</sup>
Excised apical tip	0.409 $\pm$ 0.031 <sup>b</sup>

**Table 2** Adventitious branch formation in Ceramiaceae indet. after the apical tips were excised from subordinate axes. Measurements were taken after seven days. Results are shown as mean  $\pm$  standard error. Same letters indicate no significant differences at the level  $P < 0.1$

Treatment	No. adventitious branches: no. of axial cells
Control (intact apical tip)	0.012 $\pm$ 0.005 <sup>a</sup>
Excised apical tip	0.017 $\pm$ 0.005 <sup>a</sup>

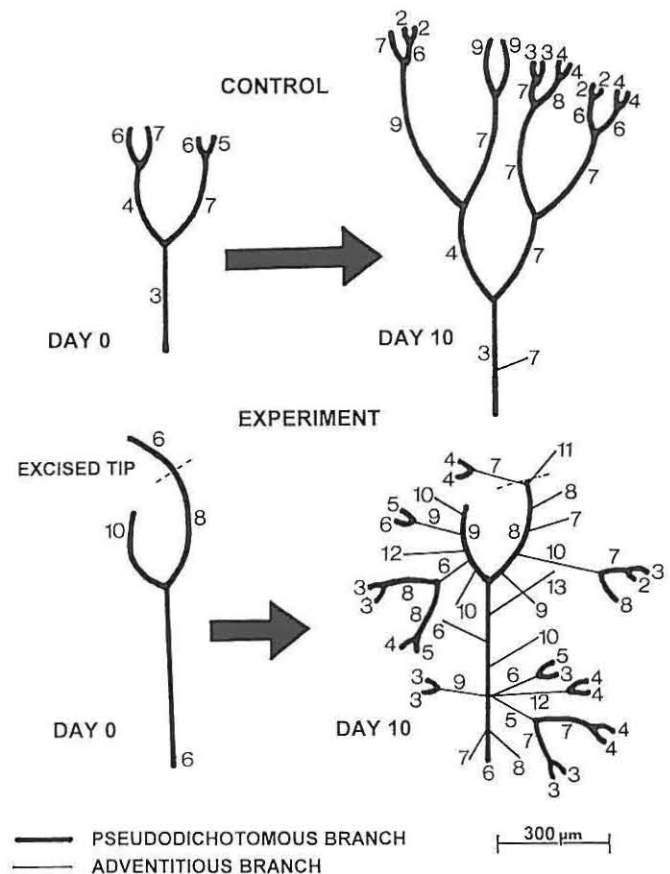
primary axis (Table 1). The adventitious branches also had more axial cells per segment than the true pseudodichotomous branches, although the axial cells were smaller (Figure 3).

Removal of the apical tip from the subordinate axis did not have any significant effect on growth and morphogenesis in Ceramiaceae indet. Growth proceeded in the normal pseudodichotomous manner without an increase in the number of adventitious branches (Table 2; Figure 4).

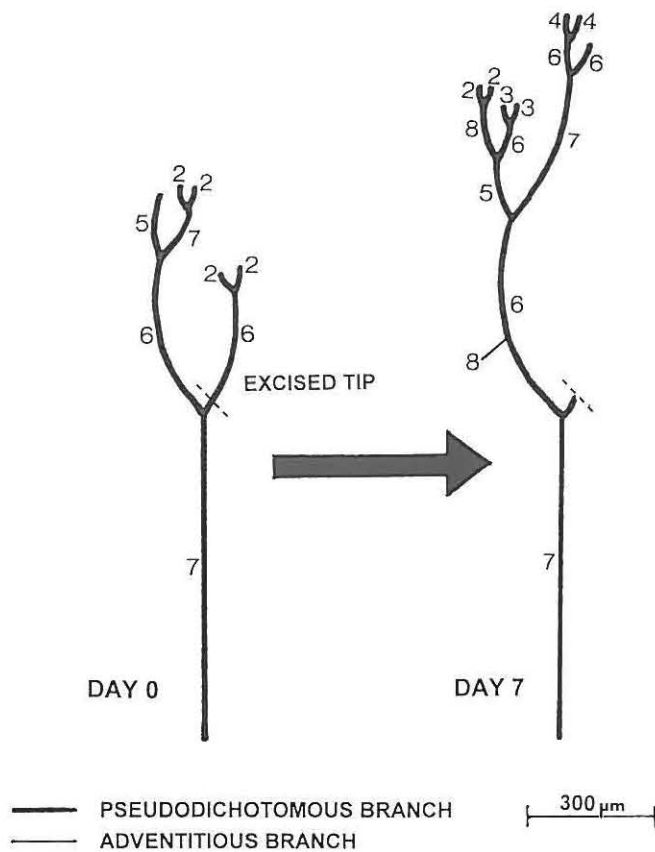
When exogenous IAA was applied to material with apical tips excised from the primary axis, the control replicates with intact tips had significantly fewer adventitious branches than the other treatments. All other treatments including the control with excised tips, had significantly higher ratios (Table 3).

**Discussion**

Ceramiaceae indet. displays apical dominance with the apical tips of the primary axis suppressing adventitious branch formation. Removal of the apex of the subordinate axis had no effect



**Figure 3** Diagram showing the growth of Ceramiaceae indet. after removal of the apical tip from the primary axis. Values indicate the number of axial cells per branch.



**Figure 4** Diagram showing the growth of Ceramiaceae indet. after removal of the apical tip from the subordinate axis. Values indicate the number of axial cells per branch.

on adventitious branch formation, implying that the dominant apex exerts total control over adventitious branch formation. This suggests some form of intercalary communication with growth regulating substances moving along filaments (Waaland 1990).

Apical dominance is thought to be a survival mechanism in higher plants, ensuring growth by providing a reservoir of meristems when the apical shoot is damaged; e.g. by foraging animals

**Table 3** Adventitious branch formation in Ceramiaceae indet. after the apical tips were excised from the primary axes. Measurements were recorded after the algae had been growing in various IAA concentrations for 10 days. Results represent mean  $\pm$  standard error. Different letters indicate significant differences at the level  $p < 0.001$

Treatment	No. adventitious branches: no. of axial cells
Control + tip	0.021 $\pm$ 0.005 <sup>a</sup>
Control - tip	0.227 $\pm$ 0.045 <sup>b</sup>
10 <sup>-9</sup> M IAA	0.230 $\pm$ 0.036 <sup>b</sup>
10 <sup>-8</sup> M IAA	0.214 $\pm$ 0.050 <sup>b</sup>
10 <sup>-7</sup> M IAA	0.263 $\pm$ 0.074 <sup>b</sup>
10 <sup>-6</sup> M IAA	0.290 $\pm$ 0.084 <sup>b</sup>
10 <sup>-5</sup> M IAA	0.553 $\pm$ 0.118 <sup>c</sup>
10 <sup>-4</sup> M IAA	0.315 $\pm$ 0.060 <sup>b</sup>

or wind (Phillips 1975; Cline 1991). In other *Ceramium* species, adventitious branches may develop in apparently normal specimens but occur most frequently in specimens which have been grazed by animals or damaged in some other way (Dixon 1960). This appears to be a similar survival mechanism as in higher plants. This may also apply to Ceramiaceae indet., as growth was able to continue after removal of the dominant apical tip. Although the adventitious branches had an unusual morphology with smaller axial cells and greatly reduced cortical bands and pinnae, once they had undergone one pseudodichotomous division, they resumed the typical morphology.

Exogenous application of IAA to Ceramiaceae indet. did not reverse the effect of removal of the dominant apical tip, suggesting that apical dominance is not controlled by IAA but by some other factor. IAA uptake would have to be positively confirmed, possibly using radiolabelled isotopes before any definite conclusions can be made on the role, if any, that auxin plays in growth and development in Ceramiaceae indet.

While apical dominance plays a role in determining the growth form in Ceramiaceae indet., with the apical tip of the primary indeterminate axis suppressing adventitious branch formation, it was not possible to speculate on the role IAA might play in this control. The apical tips of secondary indeterminate axes do not exert any apical control.

#### Acknowledgements

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