STUDIES ON THE REGULATION OF ALGAL GROWTH BY GIBBERELLIN

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Abstract

CCC and Amo-1618, at relatively high concentrations only, inhibited the growth of excised branch apices of the red alga *Hypnea musciformis*. Neither GA_3 nor GA_7 stimulated growth of the alga in the presence or absence of these compounds, and gibberellin-like material extracted from *H. musciformis* also failed to stimulate growth. However, both gibberellins stimulated the growth of slow-growing, but not fast-growing, branch apices of the related red alga *Gracilaria verucosa*. It is concluded that endogenous gibberellins may not regulate the growth of *H. musciformis*, but this is likely to be a peculiarity of this species and not a general phenomenon in red algae.

CCC inhibited the growth of gametophytes of the brown alga *Ecklonia radiata*, and GA_3 significantly overcame this inhibition in a manner compatible with the concept of CCC inhibiting gibberellin biosynthesis. The complement of acidic, ethyl acetate-soluble gibberellins extracted from those regions of *E. radiata* sporophytes active in cell division was chromatographically similar, but differed from those extracted from a relatively quiescent region of the alga. These data support previous conclusions that endogenous gibberellins are involved in the growth regulation of *E. radiata*.

I. Introduction

Previous work has demonstrated that acidic material with gibberellin-like biological activity in d1 and d5 maize can be extracted from the red alga *Hypnea musciformis* (Wulf.) Lamour, the green alga *Enteromorpha prolifera* (O. F. Muller) J. Ag., and the brown alga *Ecklonia radiata* (C. Ag.) J. Ag. (Jennings and McComb 1967; Jennings 1968). In addition, the growth of both *E. radiata* and *Ent. prolifera* was shown to respond in a similar manner to both gibberellic acid and to fractions extracted from the same species which had been found to have biological activity in dwarf maize. On the basis of this evidence it was considered that endogenous gibberellins may be involved in the growth regulation of *E. radiata* and *Ent. prolifera*. Comparable data were not available at that time for the growth regulation of *H. musciformis*. It is the purpose of this paper to present information concerning this point for *H. musciformis*, and for a related red alga *Gracilaria verucosa* (Hudson) Papenfuss. Also, additional data are given in support of the previous conclusions with *E. radiata*.

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II. Materials and Methods

Plants of *H. musciformis* and *E. radiata* were collected for culture experiments from shallow sublittoral reefs at Cottesloe Ocean beach, W.A., during May–June and February respectively, and *G. verucosa* was collected from the shallow sublittoral in the Swan R., W.A., estuary during March to May.

Apical branch segments of the red algae (each 2 cm in length), were cut from freshly collected plants and shaken vigorously in filtered sea or estuary water. The segments were then cultured in Petri dishes containing 40 ml of filtered sea or estuary water, five or six segments per dish. As both species are uniaxial members of the Gigartinales, cell division and cell elongation processes are largely confined to the apical and subapical regions of branches, which were included in these 2 cm long segments. Gametophytes of *E. radiata* were obtained, cultured, and their growth analysed in the manner described by Jennings (1967, 1968), for an 11-day period. Cultures were maintained at 22°C and were exposed to continuous light of approximately 50 f.c. intensity, provided by "warm white" fluorescent tubes. This low light intensity was found convenient to use for the red algae, as rapid bleaching occurs at higher intensities due to photodestruction of phycoerythrin. It was also considered satisfactory for the culture of gametophytes of *E. radiata*, as both the growth rate and pattern of control plants was similar to that of plants grown under a light regime of 1500 f.c. intensity (Jennings 1967). Experiments with the red algae were conducted for between 2 and 7 days. Culture solutions were changed every 2–3 days.

The following chemicals, in aqueous solution, were included: gibberellic acid (GA_3; British Drug Houses), gibberellin A_7 (GA_7; Nutritional Biochemicals), 2-chloroethyltrimethylammonium chloride (CCC; British Drug Houses), 4-hydroxy-5-isopropyl-2-methylphenyltrimethyl ammonium chloride piperidine-1-carboxylate (Amo-1618; Nutritional Biochemicals), tributyl-2,4-dichlorobenzylphosphonium chloride (Phosfon D; Carolina Biological Supply).

Sporophytes of *E. radiata* were collected during February and acidic, ethyl acetate-soluble material was extracted from 500 g fresh weight of blade, transition region, and stipe. These extracts were prepared, chromatographed, and bioassayed with d5 maize as described previously (Jennings 1968).

Throughout, the significance of differences from control values was assessed by an analysis of variance.

Table 1

EFFECT OF VARIOUS CONCENTRATIONS OF GA_3 AND GA_7 ON THE GROWTH OF EXCISED APICAL SEGMENTS OF *H. MUSCIFORMIS*

Growth is expressed as the mean daily growth per segment. No data differed from the control at $P \leqslant 0 \cdot 1$

GA_3 Concn. (mg/l)	Growth per Segment (mm/day)	GA_7 Concn. (mg/l)	Growth per Segment (mm/day)
0*	0·92	0*	0·96
0·001	0·88	0·01	0·91
0·01	0·98	0·05	1·04
0·1	0·92	0·5	0·98
1·0	0·95		

* Control.

III. Results

(a) *Culture Experiments with* H. musciformis

From Table 1 it is clear that neither GA_3 nor GA_7, over a wide range of concentrations, was able to influence the growth of branch apices of *H. musciformis*, though quite vigorous growth did occur in culture. This experiment was repeated twice with GA_3 and once with GA_7, yielding similar results. An eluate of the R_F 0·3–0·4 fraction of a chromatogram of an acidic extract from 100 g fresh weight of *H. musciformis*,

shown to be biologically active in d1 and d5 maize (Jennings and McComb 1967) also failed to elicit a growth response (mean daily growth per segment for treated and control plants 0·75 mm/day).

In a further effort to elucidate this point several compounds which are reported to block gibberellin biosynthesis in higher plants and in the fungus *Fusarium*, were applied to cultures. CCC inhibits gibberellin biosynthesis in *Fusarium* (Kende, Ninneman, and Lang 1963; Ninneman *et al.* 1964; Cross and Myers 1969), and inhibits the conversion of mevalonate to kaurene in a cell-free system from pea (Anderson and Moore 1967). Amo-1618 inhibits gibberellin biosynthesis in *Fusarium* (Cross and Myers 1969), in pea seeds, and *Echinocystis* endosperm (Dennis, Upper, and West 1965; Anderson and Moore 1967). Phosfon D inhibits kaurene production in the *Echinocystis* system of Dennis, Upper, and West (1965). The rationale of the experiments described here was that a growth inhibition produced by these compounds at concentrations of a similar order of magnitude to those used by the above workers, which could at least in part be overcome by gibberellin, would be evidence for the participation of endogenous gibberellins in growth control of the alga.

From Table 2 it can be seen that at the highest concentration of CCC and Amo-1618 tested growth was significantly reduced, while Phosfon D was without effect. The expansion kinetics are represented in Figure 1. The Amo-1618 inhibition

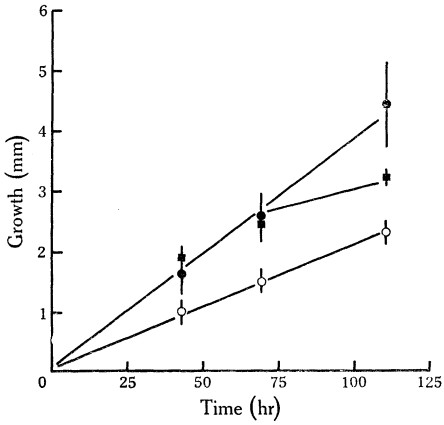

Fig. 1.—Growth kinetics of apical segments of *G. verucosa* treated with CCC (○) and Amo-1618 (■), both growth retardants at a concentration of 200 mg/l, and an untreated control (●). Vertical bars represent standard errors.

only became apparent after 69 hr, while CCC effected growth almost immediately. It was noticed at 69 hr that apices treated with Amo-1618 had lost some of their red pigmentation (due to R-phycoerythrin), while CCC-treated plants appeared normal. At the end of the experiment the plants were homogenized in 1% NaCl using a ten-Broek homogenizor and centrifuged at 40,000 *g* for 10 min. Phycoerythrin was estimated colorimetrically at 550 nm. Results are given in the following tabulation:

	Control	Plants Treated with Amo-1618	Plants Treated with CCC
Phycoerythrin content			
Optical density units/segment	0·056	0·024	0·052
Optical density units/gram	4·7	2·8	4·9

Thus plants treated with Amo-1618 contained far less phycoerythrin than control or CCC-treated plants, and effects arising from this phenomenon, rather than a direct

inhibition of any pathway of gibberellin biosynthesis, may have been responsible for the growth inhibition in Amo-1618 cultures only after 69 hr.

Results of experiments in which GA_3 and GA_7 were applied to cultures simultaneously with inhibitory concentrations of CCC and Amo-1618 are also presented in Table 2. Neither gibberellin was effective in reversing the growth inhibition to a

TABLE 2

EFFECT OF VARIOUS CONCENTRATIONS OF CCC AND AMO-1618 BOTH IN THE PRESENCE AND ABSENCE OF GA_3 AND GA_7, AND OF PHOSPHON D ALONE, ON THE GROWTH OF APICAL SEGMENTS OF *H. MUSCIFORMIS*

Growth is expressed as the mean daily growth per segment. No hormonal treatment reversed the effect of an inhibitor at $P \leqslant 0 \cdot 05$

Inhibitor (+ Hormone) Present	Inhibitor Concn. (mg/l)	Hormone Concn. (mg/l)	Growth per Segment (mm/day)	Inhibitor (+ Hormone) Present	Inhibitor Concn. (mg/l)	Hormone Concn. (mg/l)	Growth per Segment (mm/day)
CCC	0†	—	0·85	Amo-1618	0†	—	0·96
	5·0	—	0·90		20	—	0·91
	500	—	1·05		200	—	0·70*
	750	—	0·44**				
	0†	—	1·13		0†	—	1·13
	750	—	0·87*		200	—	0·67*
(+GA_3)	750	0·05	0·67*	(+GA_3)	200	0·05	0·73*
	0†	—	0·96		0†	—	0·96
	750	—	0·50**		200	—	0·70*
(+GA_7)	750	0·5	0·59*	(+GA_7)	200	0·5	0·83
				Phosphon D	0†	—	0·79
					0·05	—	0·76
					0·5	—	0·80

* Values differing significantly from the control at $0 \cdot 05 \geqslant P > 0 \cdot 01$.

** Values differing significantly from the control at $0 \cdot 01 \geqslant P > 0 \cdot 001$.

† Controls.

significant extent; neither did they reverse the decline in phycoerythrin content, as the following results show:

	Control Plants	Plants Treated with: Amo-1618	Amo-1618 +GA_3	Amo-1618 +GA_7
Phycoerythrin content (optical density units/gram)	2·9	1·5	1·5	1·4

(*b*) *Culture Experiments with* G. verucosa

Because of some of the negative results obtained from experiments with *H. musciformis*, similar experiments were run with *G. verucosa* which, like the former species, is a uniaxial member of the Gigartinales (Fritsch 1952). Unfortunately it was not feasible to attempt extraction of gibberellin-like compounds from this species because a high degree of epiphytic contamination by other algae precluded collection of sufficient "clean" material.

It can be seen in Table 3 that plants treated with GA_3 and GA_7 on occasions grew at a greater rate than the controls. This growth stimulation was most pronounced

TABLE 3

EFFECT OF VARIOUS CONCENTRATIONS OF GA_3 AND GA_7 ON THE GROWTH OF APICAL SEGMENTS OF *G. VERUCOSA*

Growth is expressed as mean daily growth per segment. Results of three separate experiments with each hormone are given

GA_3 Concn. (mg/l)	Growth per Segment (mm/day)			GA_7 Concn. (mg/l)	Growth per Segment (mm/day)		
	Expt. 1	Expt. 2	Expt. 3		Expt. 1	Expt. 2	Expt. 3
0†	2·0	1·63	1·13	0†	3·30	1·13	1·10
0·02	2·15	2·04	—	0·05	—	—	1·12
0·05	—	—	1·50**	0·5	3·0	1·57**	1·52
0·5	1·65	1·65	—	1·0	—	—	1·18

* Values differing significantly from the control at $0 \cdot 05 \geqslant P > 0 \cdot 01$.
** Values differing significantly from the control at $P \leqslant 0 \cdot 001$.
† Controls.

with the slower-growing plants. A similar observation has been made in seven separate experiments with the cytokinin kinetin (Jennings, unpublished data). Analysis of variance for the mean daily growth rate of control plants from 15 separate experiments

TABLE 4

EFFECT OF VARIOUS CONCENTRATIONS OF CCC AND AMO-1618, BOTH IN THE PRESENCE AND ABSENCE OF GA_3 AND GA_7, ON THE GROWTH OF APICAL SEGMENTS OF *G. VERUCOSA*

Growth is expressed as mean daily growth per segment. No hormone treatment reversed the effect on an inhibitor at $P \leqslant 0 \cdot 05$

Inhibitor (+ Hormone) Present	Inhibitor Concn. (mg/l)	Hormone Concn. (mg/l)	Growth per Segment (mm/day)	Inhibitor (+ Hormone) Present	Inhibitor Concn. (mg/l)	Hormone Concn. (mg/l)	Growth per Segment (mm/day)
CCC	0†	—	1·48	Amo-1618	0†	—	1·57
	50	—	1·78		10	—	1·83
	500	—	1·14*		100	—	1·20**
	750	—	0·64***				
					0†	—	1·57
	0†	—	1·48		100	—	1·20**
	500	—	1·14*	($+GA_3$)	100	0·05	1·17**
($+GA_3$)	500	0·05	0·92**	($+GA_7$)	100	0·5	1·26
($+GA_7$)	500	0·5	1·18				

* Values differing significantly from the control at $0 \cdot 05 \geqslant P > 0 \cdot 01$.
** Values differing significantly from the control at $0 \cdot 01 \geqslant P > 0 \cdot 001$.
*** Values differing significantly from the control at $P \leqslant 0 \cdot 001$.
† Controls.

indicates that significant differences existed, and hence that all experiments could not be considered to have been conducted with plants from the same "growth rate"

population. On the other hand the "slow-growing" plants, i.e. plants with a mean daily growth rate less than a standard error below the overall mean growth rate (calculated from control growth rates for all 15 experiments), were shown by analysis of variance not to differ significantly. These plants were therefore grouped together and tested for significant gibberellin effects. For this purpose all GA_3 and GA_7 results were bulked together and considered as a single gibberellin treatment. It was found that growth per segment for slow-growing plants treated with gibberellin was 1·38 mm/day (significantly different from control, 1·13 mm/day, at 5% level) and for fast-growing plants 2·02 mm/day (control, 2·29 mm/day). Thus gibberellin treatment significantly stimulated the growth of slow-growing but not fast-growing plants of *G. verucosa*. The fast-growing group may not be considered in the same manner, as it is statistically heterogeneous, but it is evident that growth was not stimulated. Similar results have been obtained with kinetin.

As with *H. musciformis* both CCC and Amo-1618 substantially inhibited the growth of *G. verucosa*, and neither GA_3 nor GA_7 was able to reverse this inhibition (Table 4).

(c) *Culture Experiments with* E. radiata *Gametophytes*

Using the rationale already outlined with respect to *H. musciformis* the possibility of an interaction between GA_3 and CCC was investigated. Owing to the difficulty in obtaining gametophytes of *E. radiata* it was not possible to determine the effect of a range of concentrations of CCC on growth, but from experience with other algae a concentration of CCC of 500 mg/l was chosen. The effects of CCC at this concentration alone and in combination with GA_3 at a concentration of 0·05 mg/l on total length, cell length, and cell number of 10-day old *E. radiata* gametophytes are presented in the following tabulation:

Compound Present	Total Length (μm)	Cell No.	Cell Length (μm)
Control	35·5	2·1	16·9
CCC	15·0**	1·1*	13·6
GA_3	36·1	2·1	17·2
CCC+GA_3	23·6*†	1·7‡	13·8

* Value differs from control at $0·05 \geqslant P > 0·01$.

** Value differs from control at $P \leqslant 0·01$.

† Value differs from CCC alone treatment at $0·05 \geqslant P > 0·01$.

‡ Value differs from CCC alone treatment at $0·01 \geqslant P > 0·001$.

Growth kinetics are presented in Figure 2. Growth of *E. radiata* gametophytes occurs in four phases, consisting of cell extension during zoospore germination and followed in turn by cell division, cell extension, and finally further cell division (Jennings 1967, 1969*b*). This situation is also apparent in Figure 2. CCC markedly inhibited growth, particularly that due to cell division, which was completely blocked for most of the culture period. Cell elongation was not significantly inhibited, but occurred out of phase with control plants. Though GA_3 was without effect by itself in this experiment, it caused a significant reversal of the CCC-induced growth inhibition ($P = 0·02$), and

this was mainly due to an increased number of cells. Both the first and second cell division periods were significantly stimulated.

(*d*) *Distribution of Gibberellin-like Materials in* E. radiata *Sporophytes*

If endogenous gibberellins are involved in the growth regulation of *E. radiata* sporophytes, as suggested by Jennings (1968), one might expect qualitative or quantitative differences or both in gibberellin content in the various tissues, particularly as these tissues display marked differences in growth capacity and pattern. Limited data from higher plants suggest that there are qualitative (Wheeler 1962) and quantitative (Radley 1958; Wheeler 1960, 1962) differences in acidic gibberellin-like

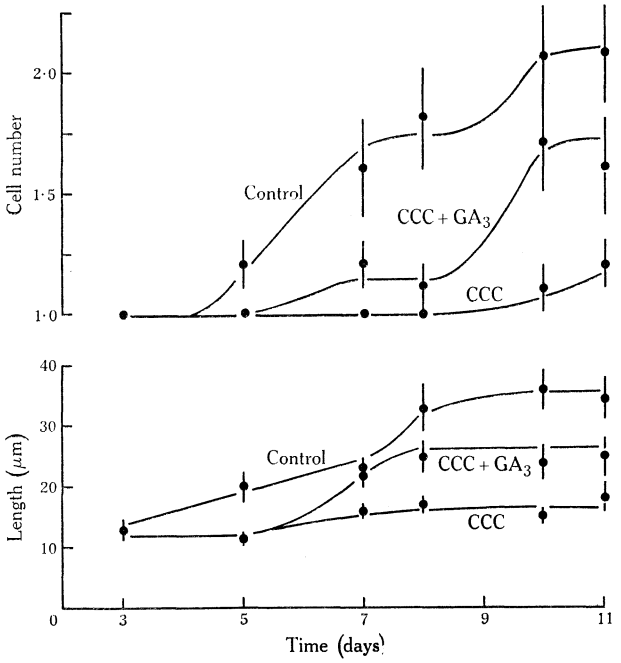

Fig. 2.—Growth kinetics of gametophytes of *E. radiata* treated with CCC (500 mg/l) and CCC (500 mg/l) + GA_3 (0·05 mg/l), and an untreated control. Vertical bars represent standard errors.

compounds extracted from various plant parts. To this end the acidic, ethyl acetate-soluble fraction was extracted from blade, transition region, and stipe tissue of *E. radiata*, chromatographed on paper in n-butanol–1·5N NH_4OH, and bioassayed with d5 maize. Results are presented in Figure 3. Gibberellin-like material was detected in all parts of the alga. The activity profile of the transition region resembled that of the blade, with a single slow-running band of active material between R_F 0·1 and 0·4. Active material was present at similar R_F values in the stipe extract, but in addition there occurred a faster-running band of low activity. Similar results have been obtained on another occasion. The slower-running zone from each extract may have

contained similar gibberellin-like material, and so there is justification for comparing concentrations from the bioassay data. If these are expressed in terms of GA_3-equivalents the approximate concentrations are—blade 1·6 μg/kg, transition region 1·5 μg/kg, stipe 0·2 μg/kg. These values may be underestimates as gibberellin antagonists may be co-extracted with acidic gibberellin-like materials, and they also occur at higher concentrations in the blade (Jennings 1969*a*). Any underestimation would therefore be greatest for the blade extract.

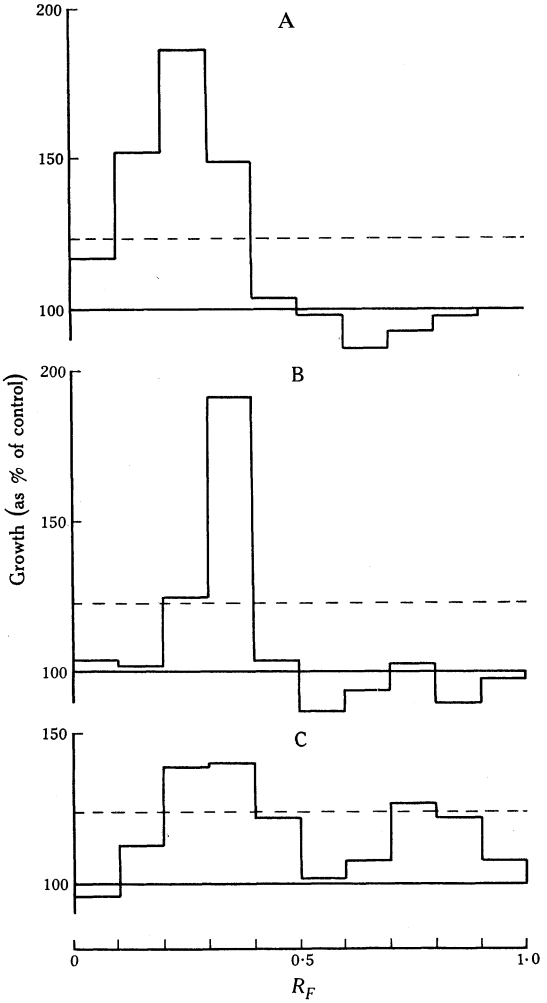

Fig. 3.—Bioassay with d5 maize of chromatograms of acidic, ethyl acetate-soluble extracts from blade tissue (*A*), transition region tissue (*B*), and stipe tissue (*C*) of *E. radiata* sporophytes. Values above the dotted line differ significantly from the control at $P \leqslant 0 \cdot 05$.

IV. Discussion

Though gibberellin-like material can be extracted from *H. musciformis* (Jennings and McComb 1967), the evidence that neither GA_3 nor GA_7 is able to influence the growth of the alga both in the presence and absence of CCC and Amo-1618, suggests that endogenous gibberellins may not regulate growth of the plant. This is supported

by the observation that material shown to be active in dwarf maize, also failed to influence growth of *H. musciformis*, though it was extracted from the same species. The growth inhibition caused by CCC and Amo-1618 was probably not due to an effect on gibberellin biosynthesis. The minimum concentration of these compounds required to inhibit growth were considerably in excess of the minimum concentration required to inhibit gibberellin biosynthesis in higher plants and *Fusarium* (Kende, Ninneman, and Lang 1963; Ninneman *et al.* 1964; Dennis, Upper, and West 1965; Anderson and Moore 1967; Cross and Myers 1967), and Amo-1618 inhibited growth only after an effect on the photosynthetic biliprotein, R-phycoerythrin, was manifest. However, the data do not preclude the possibility that the endogenous gibberellins cannot be substituted by GA_3 and GA_7. Mention should also be made of the possibility that limitations on a possible gibberellin response may have been imposed by the culture conditions. However, this does not seem likely as vigorous growth did occur in culture and similar apices are responsive to cytokinin (Jennings 1969*b*). It should also be mentioned that this plant is the only one of five species of algae which have failed to respond to gibberellin under these conditions of culture. In addition there does not appear to be any reason to suspect that excission might preclude response to gibberellin, as studies involving the ^{14}C-labelled photosynthetic assimilate indicate that organic translocation does not occur in this alga (Jennings 1966); a conclusion which is not at all surprising when anatomical and environmental considerations are taken into account (e.g. Fritsch 1952).

The probable failure of endogenous gibberellins to participate in the growth control of *H. musciformis* is likely to be a peculiarity of this plant rather than a general situation in red algae, as the growth of slow-growing plants of *G. verucosa* was stimulated by hormonal concentrations of GA_3 and GA_7. Several species of *Porphyra* are also reported to respond to gibberellin (Kinoshita and Teramoto 1958; Iwasaki 1965). The failure of GA_3 and GA_7 to reverse the CCC- and Amo-1618-induced growth inhibition probably reflects a lack of specificity of these compounds in inhibiting gibberellin biosynthesis in *H. musciformis*.

The evidence that GA_3 was able to interact positively with CCC, and partially overcome the growth inhibition caused by this compound with gametophytes of *E. radiata*, supports the concept that inhibition to gibberellin biosynthesis was at least partially responsible for the reduced growth in the presence of CCC. Though GA_3 was not stimulatory by itself in this experiment, in the presence of CCC it stimulated the same growth processes previously found to be sensitive to gibberellin (Jennings 1968). This clearly implies a function for endogenous gibberellins in the growth regulation of *E. radiata*. This interpretation is further supported by results from the extraction experiments with sporophytes, where high levels of gibberellin-like materials (R_F 0·1–0·4) were extracted from the blade and transition region, but not the stipe. In *E. radiata* the blade and transition region tissues have the greatest growth activity (Fritsch 1952; Lindauer, Chapman, and Aiken 1961).

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