

Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop growth

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Received 17 August 1988. Revised February 1989

Key words: foliar spray, kelp, *Phaseolus vulgaris*, phytohormone

Abstract

In 1983 and 1984 field plot experiments were established to assess the effects of a foliar applied (2 or 4 L ha⁻¹ × four applications per season) kelp *Macrocystis integrifolia*, concentrate on growth and nutrition of bean, *Phaseolus vulgaris*. A commercial kelp concentrate, prepared from *Ecklonia maxima*, was also used as a test comparison. In the first year a phytohormonal extract of the *M. integrifolia* concentrate, designed to extract the cytokinin, auxin and gibberellin phytohormones, was also applied to the crop to test the thesis that these phytohormones are active constituents. In each of the two field seasons the kelp concentrates increased harvestable bean yields on average by 24%. The phytohormonal extract also increased yields, but was less effective than the kelp concentrate itself. Bioassay results demonstrated the presence of phytohormone-like substances in this crude extract.

Introduction

Various kelps have been processed commercially for worldwide use as crop foliar sprays. Much of the early research has been reviewed by Abetz (1980). In the late 1970's a kelp (*Ecklonia maxima* (Osbeck) Papenfuss) concentrate called "Kelpak 66", for use as crop foliar spray was manufactured by Kelp Products Ltd. of South Africa. The South African process involves physical disintegration of raw fresh kelp by milling and high pressure (> 40,000 kPa) homogenization to reduce the particle size of the kelp to approximately 50 µm (South African Patent 78/3281). Selected crop responses include increased seed germination and plant growth and altered development and nutrition (Featonby-Smith and van Staden, 1983a; 1983b; Kotze and Joubert, 1980; Nelson and van Staden, 1984a; 1984b). Cytokinin-like substances have also been detected in the kelp concentrate and are suspected to be active constituents (Featonby-Smith and van Staden, 1983b; Finnie and van Staden, 1985), although 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor to the

phytohormone ethylene, is also implicated (Nelson and van Staden, 1984b and 1985).

Field studies were designed to investigate the potential use of the kelp, *Macrocystis integrifolia* Bory, processed as a concentrate for use as a kelp foliar spray on bean (*Phaseolus vulgaris* L.) crop productivity. The *M. integrifolia* kelp concentrate was compared to a crude phytohormonal extract of the *M. integrifolia* concentrate. This was a test of the hypothesis that natural growth promoting phytohormones, such as auxin, gibberellin and cytokinin, may be active constituents. This phytohormonal extract was subsequently bioassayed for the presence of growth promoting phytohormones cytokinin, auxin and gibberellin-like substances. The commercially available South African kelp (*E. maxima*) concentrate, "Kelpak 66", was applied as an already documented and effective comparison.

Material and methods

Design of experiment

1983 Field trial. On May 13, 1983 fresh kelp, *M.*

integrifolia, was harvested near Execution Rock, just west of the Bamfield Marine Station, Vancouver Is., British Columbia (N latitude 48°49'; W longitude 125°11'). The rapidly growing apical portion of the plant was harvested, counting twelve laminae back from the scimitar apex (top 1.5 m). Approximately 24 healthy apical portions of the plant were selected from the kelp bed and immediately hand shredded to pieces less than 4 cm in size. The mulched kelp was mixed thoroughly and 1-kg portions placed in ziplock plastic bags, air-vented and put on ice in a cooler and transported back to the marine station. The kelp was then immediately frozen (-15°C) and transported the next day on ice to the University of British Columbia, where it was transferred to a freezer at -70°C.

The X-press (AB Biotec) was used to process the kelp into a concentrate resulting in the disintegration of plant cell walls and membranes and reducing particle size. The concentrate or mash (M) was then subjected to a series of extraction procedures to produce the rude kelp phytohormonal extract (E). The extraction procedure (Fig. 1) was devised using common methods employed in phytohormonal analysis and is based upon the recovery coefficients of the various phytohormones for particular solvent at defined pH as described by Murakami (1970), Mann and Jawroski (1970), Hemberg (1974), Atsumi *et al.* (1976), Cihá *et al.* (1977) and Walton *et al.* (1979). Both the kelp concentrate (M) and its phytohormonal extract (E) were stored at -70°C prior to use as foliar spray treatments discussed later in the text. In separate extractions FRACTIONS I, II and III were then further purified and/or chromatographed prior to bioassay detections.

Gibberellin bioassay. Fraction I was rotary-evaporated under vacuum at 35°C until near dryness, taken up in three separate 3 mL volumes of 80% ethanol, applied (5 cm strip) to Whatman 3 MM chromatography paper, developed (10 cm height) in ascending solvent (isopropanol: NH₄OH:water 10:1:1), left to air dry and prepared for the rice seedling bioassay as described by Murakami (1970).

Cytokinin bioassay. Fractions I and II were combined and rotary-evaporated under vacuum at 35°C until near dryness, taken up in 50 mL of 80%

ethanol acidified (0.1 M HCl) to pH 2.5, applied to a Dowex-50W cation exchange column (bed volume 135 mL) and run at 4°C using methods similar to those described by van Staden (1976). The collected eluate was rotary-evaporated until near dryness and applied to silica gel plate (Kieselgel 60 F; Merck) and developed in an ascending solvent (Chloroform:methanol 17:1) as described by Rademacher and Graebe (1984). Developed plates were cut into 10 equal width strips (R_r 0 to 1.0), gel scraped off into 10 mL test tubes and eluted 3 × with 2 mL 100% methanol, each of which was centrifuged and decanted. The 6 mL collected was split into three aliquots by placing 2 mL into 5.5 cm petri dishes containing two #1 Whatman filter papers. Prepared standards were also included at this time. Each dish was then air dried in a fume hood for 16 h. After drying 2.0 mL of 0.013 M sodium phosphate buffer (pH 6.3) was added to each dish and the Cytokinin Amaranthus Cotyledon Beta-Cyanin Production bioassay (Biddington and Thomas, 1973) was conducted.

Auxin bioassay. Fraction III was rotary evaporated under vacuum at 35°C to near dryness, taken up in three 3 mL volume each of which was applied to a silica gel plate (Kieselgel 60 F 254; Merck) and developed (10 cm) in an ascending solvent (isopropanol:NH₄OH:water 10:1:1) as described by Rademacher and Graebe (1984). The developed plate was cut into 10 equal width strips (R_r 0 to 1.0), gel scraped off into 10 mL test tubes, eluted once in 3 mL of acetone and centrifuged. The acetone eluate was decanted into a 10 mL test tube and dried in a stream of N₂. The silica gel residue was then washed in 6 mL of 0.01 M KH₂PO₄ buffer (pH 6.4) containing 2% sucrose and centrifuged. The eluates were then combined for each R_r. Each 6 mL eluate was split into three 2 mL aliquots and placed in 3.5 cm petri dishes. Prepared standards in buffer/sucrose were also included at this time. The Auxin Avena Coleoptile Straight Growth Test Bioassay was then performed according to Nitsch and Nitsch (1956).

Known natural phytohormones were also chromatographed as outlined and used as reference comparisons to detected activities in the kelp phytohormonal fractions. All standards and references were obtained from Sigma Chemical Co., St. Louis, Missouri.

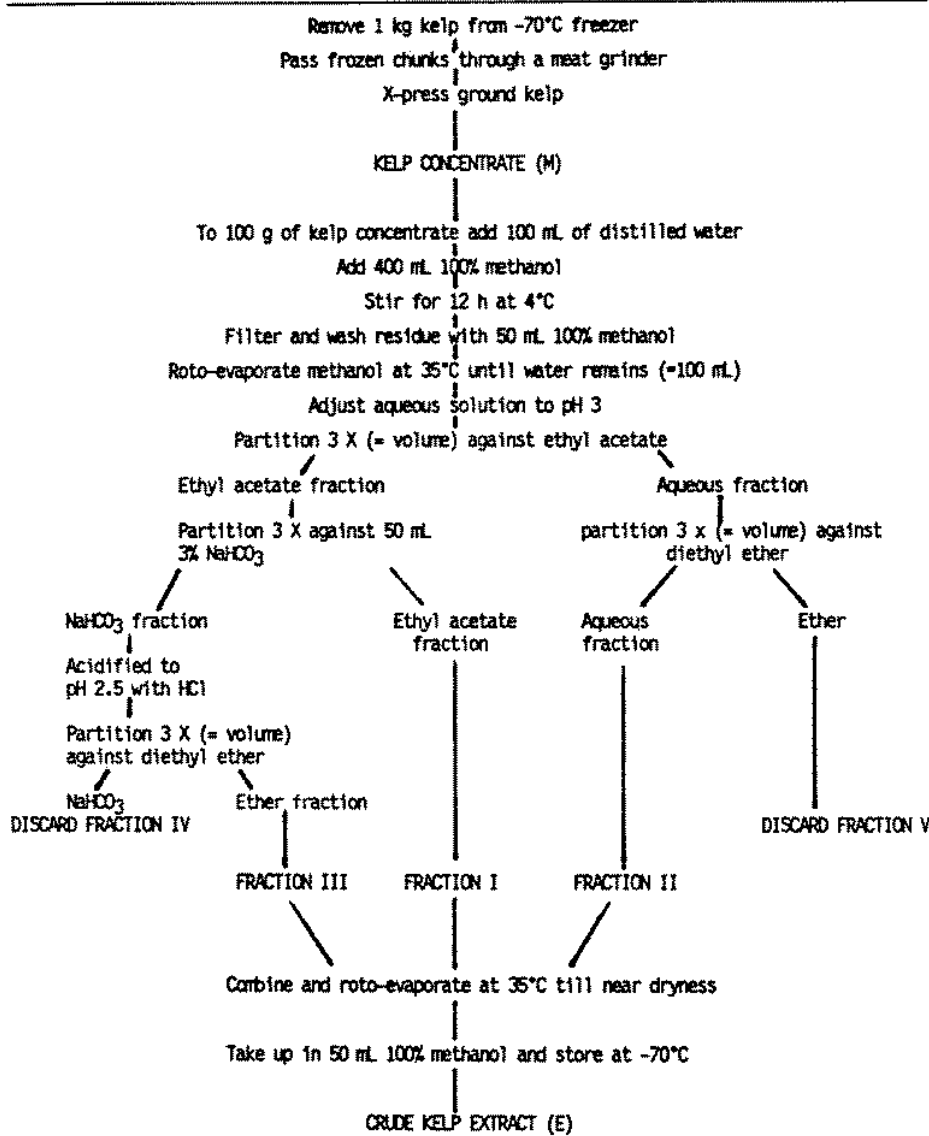


Fig. 1. Flow chart for the preparation of the *M. integrifolia* kelp concentrate (M) and extract (E).

The *M. integrifolia* kelp concentrate (M) and its extract (E) were thawed just prior to being applied to the crop foliage at 2 and 4 L ha⁻¹ (kelp concentrate equivalent) diluted with water 1:500 and 1:250 (W/V), respectively. The 4 L ha⁻¹ applications for M and E are referred to as M1 and E1 and the 2-L ha⁻¹ applications are referred to as M2 and E2, respectively. The South African, *E. maxima*, kelp concentrate "Kelpak 66" (K) (manufactured

by Kelp Products Ltd., P.O. Box 465, Cape Town 8000, South Africa), was applied at 4 L ha⁻¹, diluted 1:250 (W/V) with water. The controls (C) were sprayed with an equivalent volume of water. All foliar treatments were applied to each of the plots, described later in the text, using a "Solo" backpack sprayer on days 21, 36, 50 and 64 after sowing. Canopy temperatures at the time of spraying were 15°, 22°, 23° and 21°C, respectively.

1984 Field trial. Kelp (*M. integrifolia*) was harvested on May 14, 1984 using the same methods and area of kelp harvest described in the 1983 field trial investigation. Four of the treatments in the 1983 field trial, which included the control, X-pressed kelp (*M. integrifolia*) concentrate (applied at 2 and 4 L ha⁻¹) and the commercial kelp concentrate "Kelpak 66" (applied at 4 L ha⁻¹) were repeated in the 1984 field trial. The X-press method and the storage conditions of the kelp concentrate or mash (M) were described for the 1983 field trial investigation. An alternative method, which would be more appropriate for the commercial manufacture of a kelp concentrate from *M. integrifolia*, was also devised and field tested. The alternative method allowed better control of the particle size of the concentrate through centrifugal dispersion and high pressure homogenization of the kelp. The four step dispersion/homogenization method of producing the kelp concentrate in this investigation was: 1) mincing the kelp with a meat grinder which reduced the kelp to a particle size of approximately 1.0 cm in diameter; 2) dissolving the preservative (sodium benzoate) and buffer (mono-ammonium phosphate buffer) in water (9 parts kelp slurry to 1 part water containing the preservatives and buffer by volume) and mixing to give a final concentration of 0.1% preservative and 0.3% buffer; 3) subjecting the kelp slurry containing the buffer and preservatives to high speed centrifugal dispersion (Brinkman Manual) which reduced and further liquified the kelp to particles of less than 1 mm in size; and 4) subjecting the liquified kelp to high pressure (25,000 kPa) homogenization using a single stage homogenizer (Gaulin Manual) which reduced the particle size of the kelp to approximately 50 to 100 µm.

This homogenized *M. integrifolia* kelp or "SeaSpray" (S) was then bottled and stored at room temperature (20°C) prior to its use in the field investigation. Both of the *M. integrifolia* concentrates (S and M) were foliar applied to the crop at rates of 2 and 4 L ha⁻¹ diluted with water 1:500 and 1:250 (W/V), respectively. The S was adjusted to M equivalent because of the additional water in the processing procedure. The 4 L ha⁻¹ application for S and M are referred to as S1 and M1 and the 2 L ha⁻¹ applications are referred to as S2 and M2. The South African commercially produced kelp (*E. maxima*) concentrate, Kelpak 66, was applied at

4 L ha⁻¹ (K) diluted 1:250 (W/V) with water. The control (C) was sprayed with an equivalent volume of water. All foliar treatments were applied to plots replicated three times within each of two blocks using a "Solo" backpack sprayer at mid-afternoon on days 14, 28, 42 and 56 after sowing. Canopy temperatures at the time of spraying were 22°, 20°, 20° and 18°C, respectively.

The plots were located on Reynelda Farms, Westham Island, Ladner, British Columbia (N latitude 49°05'; W longitude 123°10'). The soil was described for a previous kelp soil amendment field experiment (Temple and Bomke, 1988). In 1983 each plot measured 2.4 × 4.0 m (spraying area) with four rows of inoculated (*Rhizobium leguminosarum* biovar *phaseoli*) bush beans (*P. vulgaris* cv. Galamor), spaced 0.6 m apart, planted to a depth of 3.5 cm on June 15, 1983. The total plot area measured 36.0 × 9.0 m with two rows of plots (12 in each) separated by a 1.0 m walkway. A boundary row of beans separated each of the plot spraying areas. Plots were split into 4 blocks (2 blocks per row of plots) using a randomized complete block design for each of the 6 foliar treatments. At sowing soil pH, %C, %N, available (mg kg⁻¹) P, K, Ca, Mg, Fe, Cu, Mn and Zn were 4.6, 2.7, 0.28, 60, 280, 970, 140, 170, 10, 54 and 5.0 respectively. In 1984 the crop was seeded on July 13, 1984. Each plot measured 4.8 × 6.0 m (spraying area) with 8 rows spaced 0.6 m apart with a boundary row placed between each of the plots. The total plot area was 54.4 × 20.0 m with three rows of plots (12 in each) separated by 1.0 m walkways running between the length of the plots. At sowing, soil pH, %C, %N, available P, K, Ca, Mg, Fe, Cu, Mn and Zn (mg kg⁻¹) were 5.6, 1.9, 0.11, 50, 220, 1500, 260, 160, 9.5, 54 and 5.5, respectively. Methods of soil analysis are discussed later in the text. Prior to seeding, 200 kg ha⁻¹ of 0-0-60 was broadcast and incorporated and during seeding 300 kg ha⁻¹ of 11-51-0 was banded 5 cm to the side and below the seed.

At harvest, 8 September 1983 and 29 September 1984, 2.0-m strips were systematically harvested from the middle area of each of the two centre rows of each plot. The harvested plant material (combined leaf and stem and marketable beans) was weighed and randomly sampled for dry weight analysis using the methods described by Temple and Bomke, 1988. Measured crop growth respon-

ses at maturity included fresh and dry shoot, leaf and stem and bean yields, harvest index and fresh/dry weight ratios.

Statistical analysis. In 1983 plant growth and development variables of the samples taken at harvest were subjected to analysis of variance with treatment means separated into single degree of freedom comparisons of C vs (M1 + M2 + E1 + E2 + K), K vs (M1 + M2 + E1 + E2), (M1 + M2) vs (E1 + E2), (M1 + E1) vs (M2 + E2) and (M1 + E2) vs (M2 + E1). In 1984 plant growth and development variables of samples taken at harvest were subjected to analysis of variance with treatment means separated into single degree of freedom comparisons of C vs (M1 + M2 + S1 + K), K vs (M1 + M2 + S1 + S2), (M1 + M2) vs (S1 + S2), (M1 + S1) vs (M2 + S2), and (M1 + S2) vs (M2 + S1). Significance was at the 5% level and coefficients of variation (CV) given.

Plant and soil analyses. Kelp samples were analyzed for total N, P, K, Ca, Mg, Fe, Cu, Mn and Zn concentrations using methods described by Temple and Bomke, 1988. The *M. integrifolia* and *E. maxima* kelp concentrates were dried at 70°C for dry weight determination and ground using a mortar and pestle. The *M. integrifolia* phytohormonal extract (E) was also analyzed for the above elements by placing 5.00 mL (the equivalence of 10 g of dry kelp) in a digestion tube, drying off the methanol with a stream of air and then proceeding with the digestion. All plant elemental concentrations were expressed on a dry weight basis.

Each of the air dried soil samples was analyzed for pH, %C, total %N and available P, K, Ca, Mg, Fe, Cu, Mn and Zn (mg kg⁻¹). The methods of analysis for soil pH, %C, available P and %N were described by Temple and Bomke, 1988. Available soil K, Ca and Mg were determined by extracting 5.000 g of soil with 50.0 mL of Morgan's Extraction Solution using methods described by Greweling and Peech (1965). Available Fe, Cu, Mn and Zn were determined by extracting 10.000 g of soil with 50.0 mL of 0.1M HCl using methods described by Fiskell (1965). Soil extract concentrations of K, Ca, Mg, Fe, Cu, Mn and Zn were determined using a Perkin and Elmer 330 atomic absorption spectrophotometer.

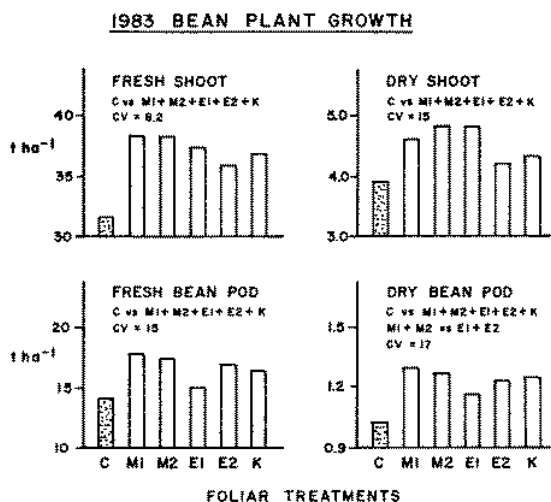


Fig. 2. Foliar treatments include: control (C); *M. integrifolia* kelp concentrate produced with an X-Press and applied at 4 L ha⁻¹ (M1) and 2 L ha⁻¹ (M2); the *M. integrifolia* phytohormonal extract applied at a kelp concentrate weight equivalence of 4 L ha⁻¹ (E1) and 2 L ha⁻¹ (E2) and the *E. maxima* kelp concentrate applied at 2 L ha⁻¹ (K). Significant contrasts and coefficients of variation are given.

Results and discussion

Under 1983 and 1984 field conditions both methods of producing the *M. integrifolia* kelp concentrate and *E. maxima* kelp concentrates (S, M and K) and the phytohormonal extract (E), applied at either 2 or 4 L ha⁻¹, as a foliar spray, were effective in increasing fresh and dry marketable bean pod yields (Fig. 2 and 3). In 1983 the *M. integrifolia* kelp concentrate (M) was more effective in increasing dry bean pod yields than its phytohormonal extract (E).

Cytokinin, gibberellin and auxin-like substances were detected in the chromatographic fractions of the *M. integrifolia* phytohormonal extract (Fig. 4). Active Rf values for cytokinin (FRACTIONS I and II) matched closely those of zeatin (Z) or its riboside (ZR) and isopentenyl adenine (IPA) and its riboside (IPAR). Active Rf values for gibberellin (FRACTION I) were detected in the broad central region of the chromatograph (0.4 to 0.8) with the known gibberellin GA₃ chromatographing from Rf 0.5 to 0.7 of this region. Active Rf values for auxin (FRACTION III) match closely that of indole-

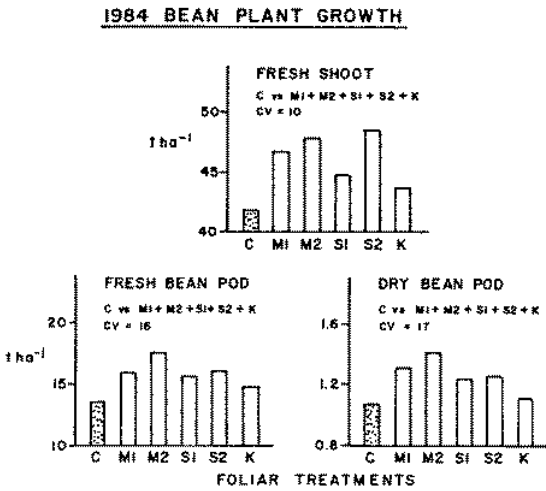


Fig. 3. Foliar treatments include: control (C); *M. integrifolia* kelp concentrate produced with an X-Press and applied at 4 L ha^{-1} (M1) and 2 L ha^{-1} (M2); the *M. integrifolia* kelp concentrate produced by the dispersion/homogenization method and applied at 4 L ha^{-1} (S1) and 2 L ha^{-1} (S2) and the *E. maxima* kelp concentrate applied at 2 L ha^{-1} (K). Significant contrasts and coefficients of variation are given.

acetic acid (IAA). Cytokinin and auxin-like activity was not detected in either of the two discard fractions (FRACTIONS IV and V), although some gibberellin-like activity was detected.

The effectiveness of the phytohormonal extract in promoting the bean yields supports, in part, the thesis that phytohormones or organic compounds may be active constituents of the *M. integrifolia* kelp concentrate. Research efforts directed at further purification and fractionation of the various phytohormones using physiochemical methods of analysis with known extraction efficiencies prior to foliar application are warranted.

According to Finnie and van Staden (1985) the concentration of *E. maxima* concentrate in solution, prior to application to tomato roots, is an important factor controlling the growth regulating efficacy. A 1:100 dilution (kelp concentrate:water) was inhibitory, while higher dilutions (1:400 to 1:600) were growth promoting. Upon chromatographing the *E. maxima* kelp concentrate these researchers were also able to demonstrate that several growth regulating substances were present in the concentrate, each of which elicited different plant growth and developmental responses. Featonby-Smith and van Staden (1987) also

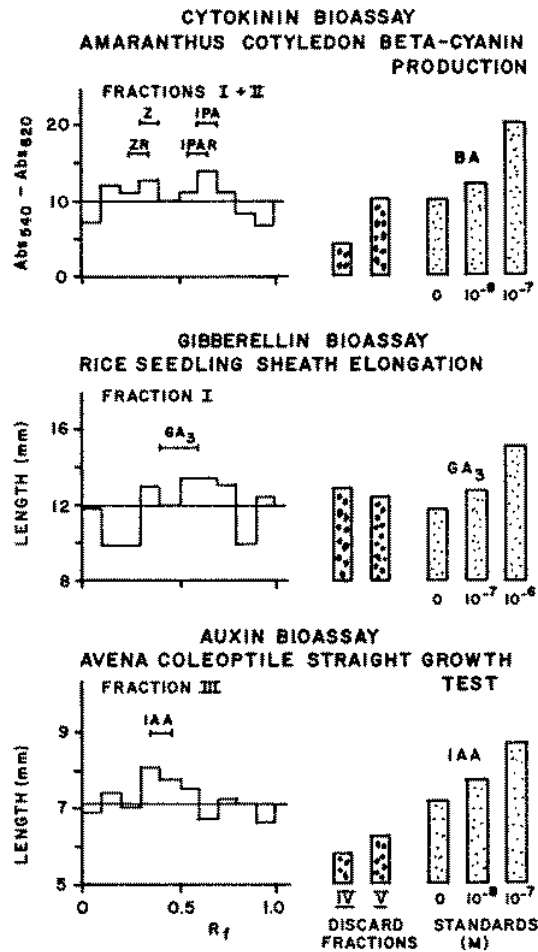


Fig. 4. Histograms are the phytohormonal bioassay activities of the 10 eluted chromatograph sections (R_f) of FRACTIONS I, II and/or III, described in Fig. 1. of the *M. integrifolia* foliar extract (E) applied in the 1983 field trial. Shaded bars are activity levels for the discard FRACTIONS IV and V and standards (M) used (BA, benzyl adenine; GA₃, gibberellic acid 3 and IAA, indoleacetic acid). Horizontal bars above the histograms are the R_f regions for the chromatographed standards (Z, zeatin; ZR, Zeatin riboside; IPA, isopentenyl adenine; IPAR, isopentenyl adenine riboside; GA₃, gibberellic acid 3; IAA, indoleacetic acid).

demonstrated differences in barley grain yield components (number of ears and number of grains ear⁻¹) with varying dilutions of the *E. maxima* concentrate in solution (1:250 and 1:500 dilutions). In this investigation the differences in marketable dry bean yields between the *M. integrifolia* kelp concentrate (M) and its phytohormonal extract (E)

Table 1. Elemental composition of dry kelp concentrates and foliar elemental application to crop area

Element	Elemental concentrations of concentrates			Elemental applications			1983 Average bean shoot uptake
	Unit	<i>M. integrifolia</i>	<i>E. maxima</i>	Unit	<i>M. integrifolia</i>	<i>E. maxima</i>	
N	%	1.00	32.6	kg ha ⁻¹	0.0081	1.14	84.4
P	%	0.23	4.3	kg ha ⁻¹	0.0019	0.15	9.2
K	%	8.5	3.3	kg ha ⁻¹	0.070	0.080	85
Ca	%	1.2	0.21	kg ha ⁻¹	0.010	0.004	47
Mg	%	0.82	0.10	kg ha ⁻¹	0.006	0.002	9.4
Fe	mg kg ⁻¹	560	5	g ha ⁻¹	0.460	0.009	620
Cu	mg kg ⁻¹	4	4	g ha ⁻¹	0.003	0.007	31
Mn	mg kg ⁻¹	10	3	g ha ⁻¹	0.008	0.005	390
Zn	mg kg ⁻¹	21	10	g h ⁻¹	0.017	0.017	99
Dry matter	%	10.2	22.0	kg ha ⁻¹	0.81	1.76	

may also be related to altered concentrations of one or more active growth regulating substances. Such an effect could have been caused by (a) low or varying extraction efficiency for particular growth regulating substances from the concentrate, (b) omission of growth regulating substances from the extract, (c) presence of inhibitory substances and/or (d) deactivation of growth regulating substances during the extraction procedure.

The *E. maxima* concentrate was found in this investigation to have relatively high dry weight N and P concentrations, while nutrient levels in the *M. integrifolia* phytohormonal extract were below the limits of detection (Table 1). It is doubtful, however, that the small quantities of nutrient elements applied to the foliage either via the pure kelp concentrates or "Kelpak 66" would significantly contribute to the total shoot mineral nutrient requirement.

Blunden (1977) examined the elemental composition of various kelp extracts and concluded that the quantities of kelp foliar spray applied could not supply a significant portion of the annual requirements of macronutrients to a crop, but could perhaps supply an amount of limiting nutrient to correct a marginal deficiency only. Kotze and Joubert (1980) in their investigation on rye and cabbage concluded that because kelp foliar sprays were found to be effective only upon soils which were fertilized, the response could not be attributed to the mineral nutrition of the kelp. Finnie and van Staden (1985) have also demonstrated that the effectiveness of a kelp foliar spray to promote growth is lost upon ashing of the kelp concentrate. Although it is doubtful that the heightened responses obtained from the use of kelp

foliar sprays could be attributed to the mineral constituents of the kelp, it has yet to be demonstrated that synergistic effects between its mineral nutrient elements and growth regulating substances do not exist. Recent investigations have documented synergistic effects on plant growth to foliar applications of various elements with phytohormones (Marschner, 1982; Mengel and Kirkby, 1982; Neuman and Nooden, 1983).

Conclusions

Under 1983 and 1984 field conditions both the *M. integrifolia* and *E. maxima* kelp concentrates, applied at either 2 or 4 L ha⁻¹ as foliar sprays increased the yields of marketable beans. A crude phytohormonal extract of *M. integrifolia* was also effective in increasing marketable bean yields, although less effective than its pure kelp concentrate. Cytokinin, auxin and gibberellin-like substances were detected in the chromatographic fraction of the *M. integrifolia* phytohormonal extract.

Acknowledgements

Many thanks must be given to the Reynolds family of Reynelda Farm and the Science Council of BC for their contribution to this investigation.

References

- AB Biotec The X-press Method for Disintegration of Cells. Biotec Inc., Parklawn Dr., Rockville, Maryland, USA.

- Abetz P 1980 Seaweed extracts: have they a place in Australian agriculture or horticulture? *J. Aust. Inst. Agric. Sci.* 46, 23-29.
- Atsumi S, Kuraishi S and Hayashi T 1976 An improvement of auxin extraction procedure and its application to cultured plant cells. *Planta* 129, 245-247.
- Biddington N L and Thomas T H 1973 A modified amaranthus bioassay for rapid determination of cytokinins in plant extracts. *Planta* 111, 183-186.
- Blunden G 1977 Cytokinin activity of seaweed extracts *In Marine Natural Products and Chemistry*. Eds. D J Faulkner and W H Fenical. pp 337-343. Plenum Pub. Corp., New York.
- Brinkman Manual Brinkman Instrument Division, 50 Galaxy Blvd., Rexdale, Ont., Canada.
- Ciha A J, Brenner M L and Brun W A 1977 Rapid separation and quantification of abscisic acid from plant tissue using high pressure liquid chromatography. *Plant Physiol.* 59, 821-826.
- Featonby-Smith B C and van Staden J 1983a The effects of seaweed concentrate and fertilizer on growth of *Beta vulgaris*. *Z. Pflanzenphysiol. Bd.* 112, 115-162.
- Featonby-Smith B C and van Staden J 1983b The effect of seaweed concentrate on the growth of tomato plants in nematode-infested soil. *Scientia Hort.* 20, 137-146.
- Finnie J F and van Staden J 1985 Effect of seaweed concentrate and applied hormone on *in vitro* tomato roots. *J. Plant Physiol.* 120, 215-22.
- Featonby-Smith B C and van Staden J 1987 Effects of seaweed concentrate on grain yield in barley. *S. Afr. J. Bot.* 53, 125-128.
- Fiskell J G A 1965 Copper. *In Methods of Soil Analysis*. Vol. II, Agronomy 9. Ed. C A Black. pp 1078-1089. Amer. Soc. of Agron., Madison, Wis., USA.
- Gaulin Manual Gaulin Laboratory Homogenizer. Manton-Gaulin Manufacturing Co. Inc., 44 Gaulin Street, Everett, Mass., USA.
- Greweling T and Peech M 1965 Chemical Soil Test. New York State College of Agriculture. Bulletin 960.
- Hemberg T 1974 Partitioning of cytokinins between ethyl acetate and acid water phases. *Physiol. Plant.* 32, 191-192.
- Kotze W A G and Joubert J 1980 Influence of foliar spraying with seaweed products on the growth and mineral nutrition of rye and cabbage. *Eisenberg J.* 4, 17-20.
- Mann J D and Jaworski E G 1970 Minimizing loss of indoleacetic acid during purification of plant extracts. *Planta* 92, 285-291.
- Marschner H 1982 Effects of mineral nutrition on phytohormone balance in plants. *In Plant Nutrition*. Ed. A Scaife. pp 354-359. 1982 Proc. Int. Plant Nutrition Colloquium, Vol. I. Commonwealth Agriculture Bureau.
- Mengel K and Kirkby E A 1982 Principles of Plant Nutrition. 3rd ed. International Potash Institute, Switzerland.
- Murakami Y 1970 New rice seedling test for gibberellins: Microdrop method. *JARQ* 5, 5-9.
- Nelson W R and van Staden J 1985 1-aminocyclopropane-1-carboxylic acid in seaweed concentrate. *Bot. Mar.* 28, 415-417.
- Nelson W R and van Staden J 1984a The effects of seaweed concentrate on growth of nutrient-stressed greenhouse cucumbers. *Hort. Sci.* 19, 81-82.
- Nelson W R and van Staden J 1984b The effects of seaweed concentrate on wheat culm. *J. Plant Physiol.* 115, 433-437.
- Neumann P M and Nooden L D 1983 Interaction of mineral and cytokinin supply in control of leaf senescence and seed growth in soybean explants. *J. Plant Nutr.* 6, 735-742.
- Nitsch J P and Nitsch C 1956 Studies on the growth of coleoptile and first internode sections: A new, sensitive, straight-growth test for auxin. *Plant Physiol.* 31, 94-111.
- Rademacher W and Graebe J E 1984 Isolation and analysis by gas liquid chromatography of auxins, gibberellins, cytokinins and abscisic acid from a single sample of plant material. *Ber. Deutsch. Bot. Ges. Bd.* 97, 75-85.
- South Africa Patent (78/3281) 1978 Wet algae processing system.
- Temple W D and Bomke A A 1988 Effects of kelp *Macrocystis integrifolia* on soil chemical properties and crop responses. *Plant and Soil* 105, 213-222.
- van Staden J 1976 Extraction and recovery of cytokinin glucosides by means of a cation exchange resin. *Physiol. Plant.* 38, 240-242.
- Walton D, Dashek W and Galson E 1979 A radioimmunoassay for abscisic acid. *Planta* 146, 139-145.