

# Effects of Cytokinin-Containing Seaweed Extract on *Phaseolus lunatus* L.: Influence of Nutrient Availability and Apex Removal

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We examined the effect of a cytokinin-containing extract of the marine alga *Ascophyllum nodosum* on the growth of immature *Phaseolus lunatus* under conditions of high and low nutrient availability, and the response of decapitated *Phaseolus lunatus* to the extract. Under low nutrient availability, few differences between control and treated plants were found. Therefore the extract did not provide supplementary nutrients to these plants. Under higher nutrient availability, plants receiving the more concentrated extract treatments did not produce as much new growth as control plants and plants receiving lower concentrations. For plants with apical meristems removed, control plants grew taller and produced more new leaf tissue than *Ascophyllum nodosum*-extract treated plants. However, treatment with the extract resulted in plants with greater specific leaf mass compared with controls. Given these results, this particular seaweed extract was not beneficial for stimulating the growth of immature *Phaseolus lunatus* or promoting their compensation for damage.

## Introduction

Liquid extracts derived from marine algae have been used over the past forty years on a variety of crops to promote plant growth and development (Crouch and Van Staden 1994). Interest in these seaweed concentrates (SWC) in agricultural systems has focused on their use as an inexpensive source of naturally occurring plant growth regulators. Because the concentrations of minerals and nutrients present in these products are low relative to crop requirements, much of the benefit from applications of SWC has been attributed to the presence of plant hormones, especially cytokinins (Blunden 1977, Verkleij 1992). Various seaweed concentrates contain significant amounts of cytokinins in addition to other phytohormones (Crouch and Van Staden 1993).

Endogenous cytokinins are physiologically important to the growth and development of plants. High levels of cytokinins promote cell division (Weaver 1972, Elliott 1982, Taiz and Zeiger 1991) and delay senescence (Nooden and Leopold 1978). Endogenous cytokinins also occur at high levels in the leaves of plants recovering from herbivory (Trumble *et al.* 1993) or artificial damage (Wang *et al.* 1977, Palmer *et al.* 1981).

Exogenous applications of cytokinins reportedly improve plant vigor in adverse environmental conditions (Senn *et al.* 1961, Mooney and Van Staden 1985, Beckett and Van Staden 1991). Therefore, exogenous applications of cytokinins or cytokinin-containing products could increase the growth of nutrient stressed plants or recovery of plants from damage. However, data on the efficacy of these SWC are

conflicting, with exogenous applications of SWC producing positive, neutral, or inhibitory effects on the plants tested (Humphries 1958, Blunden and Wildgoose 1977, Crouch and Van Staden 1994, Hedin and McCarty 1994). Among the factors that can account for these differences are the source and concentration of products, methods of application, plant species or variety tested, and the plant characteristics measured.

Our objectives were to compare the growth of nutrient stressed and unstressed lima beans (*Phaseolus lunatus* L.) treated with different rates and application methods of a cytokinin-containing product derived from the brown alga *Ascophyllum nodosum* (L.) LeJol., and the response of *Phaseolus lunatus* to applications of that product following removal of the apical meristem (decapitation).

## Materials and Methods

### Plant material

Seeds of *Phaseolus lunatus* ('Henderson Bush') were germinated in containers filled with vermiculite. After primary leaf expansion began, individual seedlings were transplanted to 10-cm × 10-cm pots containing UC soil mix (Matkin and Chandler 1957). Plants were maintained in greenhouses and were watered daily. All experiments began when the first trifoliate leaves had expanded fully.

### Seaweed concentrate (SWC)

We used a commercially available SWC, Cytokin<sup>®</sup> (Plant Biotech Inc., Corrales, NM, U. S. A.). Cytokin

is derived from the brown alga *Ascophyllum nodosum* through a heated alkaline hydrolysis process. This product contains a cytokinin activity equivalent to 0.01% kinetin, as determined by mung bean root bioassays and HPLC assays. In addition, cytokin contains trace amounts of nitrogen (2%), phosphoric acid (1%), potash (6%), and calcium (1%). For all treatments, the SWC was diluted to appropriate levels (vol./vol.) in an aqueous solution of 0.5% Tween 80 as an added surfactant.

### Seaweed concentrate (SWC) and nutrient treatments

This experiment was arranged as a  $5 \times 2$  factorial, with 5 levels of SWC treatments and 2 levels of nutrient availability. Four treatments were foliar applications (0, 0.2%, 0.4%, and 1% SWC), and the fifth treatment was a 0.4% SWC root drench. Eight plants were initially assigned to each of these  $5 \times 2$  treatment levels. Plants receiving foliar sprays were sprayed to the point of run-off with a manual atomizer. Plants treated with the root drench received 20 mL of SWC solution weekly. Nutrient treatments were also provided weekly. Each week, one half of the plants per SWC treatment received 25 mL of a 15N-12.9P-12.5K fertilizer solution (3 mg/mL) (Stern's Miracle Grow®, Port Washington, NY, U.S.A.). The remaining plants received no supplemental fertilization.

### Apex removal

Lima bean plants were grown as described above. All plants received weekly fertilizer treatments (25 mL of a 15N-12.9P-12.5K fertilizer solution, 3 mg/mL). Following expansion of the first trifoliolate leaves, plant stems were severed with a razor blade, 2.5 cm above the first trifoliolate leaf node. The next day, plants were sprayed with either a 0.4% solution of SWC, or a control solution prepared using the techniques discussed previously. Plants received three more applications on a weekly basis, and were harvested one week after the final application.

For all experiments, stem height and leaf number were recorded weekly. Plants were destructively harvested one week following the final SWC treatments. At harvest, the wet mass of stems and leaves were determined. Leaf area was measured with a LiCor Model 3000 leaf area meter (LiCor, Omaha, NE, U.S.A.). Stems, leaves and roots were then dried at 60 °C for seven days, after which their dry masses were determined. Biomass was determined as the ratio of dry mass to wet mass.

Data were analyzed with the SuperAnova statistical package (Abacus Concepts, Berkeley, CA, U.S.A.). Independent variables were, level of SWC, nutrient level, and a covariate, the initial plant height. For the decapitated plant experiment, independent variables were, SWC treatment and initial

plant height. Means and their standard errors are reported. Separation of treatment means are based on least-squares means t-tests.

## Results

### Effect of SWC and nutrient availability on *Phaseolus lunatus*

The seaweed extract inhibited stem elongation and leaf expansion of *Phaseolus lunatus*. No concentration of the seaweed extract resulted in increased plant size compared to controls, and differences among SWC treatments were more pronounced with the addition of nutrients. In the unfertilized treatments, control plants and plants receiving foliar applications gave similar responses while the plants receiving the root drench tended to be significantly smaller. Unfertilized plants receiving the 1.0% treatment were similar to controls for most traits, but were not significantly greater. Among plants receiving supplemental fertilization treatments, the SWC controls and 0.2% treated plants gave similar responses while plants receiving the root drench were the most different.

Stem elongation over the four weeks of the experiment differed significantly among the SWC treatments ( $F = 2.8$ ,  $df = 4, 68$ ,  $P = 0.035$ , Table I). Among fertilized plants, final stem height was greatest for the control group and the most dilute extract concentration (0.2%) and least in plants receiving the root application of SWC and the highest foliar application concentration (1.0%). A similar pattern in final stem height occurred among plants that did not receive supplemental nutrients, although only the 1.0% foliar- and 0.4% root drench-treated plants were significantly different (Table I).

The same trends of retarded plant growth with more concentrated applications of SWC and less inhibition with the more dilute concentrations were evident for the leaf area per plant. There was a significant interaction between SWC and fertilizer treatment ( $F = 10.3$ ,  $df = 4, 68$ ,  $P < 0.0001$ ) with the addition of fertilizer substantially increasing total leaf area only for plants receiving lower concentrations of SWC (Table I). The difference in leaf area between fertilized and unfertilized plants in the 1.0% SWC treatment was less than 2%, and only 40% for plants receiving the 0.4% root drench treatment. In contrast fertilized plants in the 0.4% foliar treatment had over 70% more leaf area than unfertilized plants, and fertilized plants in the other two treatments (0.2% and control) had well over twice the leaf area of unfertilized plants.

Wet mass and dry mass of leaves varied in a similar pattern to leaf area (Table I). However, wet mass per unit area did not differ with respect to SWC treatment ( $F = 0.7$ ,  $df = 4, 68$ ,  $P > 0.56$ ), but this ratio was significantly less for fertilized plants than for un-

fertilized ones ( $F = 19.3$ ,  $df = 1, 68$ ,  $P < 0.0001$ , Table I). While the ratio of wet mass to leaf area did not differ, the ratio of leaf dry mass to leaf area (specific leaf mass) was significantly related to SWC and fertilization treatments ( $F = 5.2$ ,  $df = 4, 68$ ,  $P < 0.0001$ , Table I). Except for the 1.0% SWC treatment, the amount of biomass per unit area was greater for unfertilized plants compared to fertilized plants and increased according to SWC. Plants receiving the root drench had the highest specific leaf mass (Table I).

Root development was also affected by the SWC treatments. As before, fertilization enhanced the effect of SWC. Root dry masses showed the same pattern as that for leaves and stems, with no level of SWC yielding plants with greater dry root masses than controls (Table I). Fertilization did not affect root mass for plants in the control, 0.2%, or 1.0% SWC treatments. However, fertilization resulted in significantly smaller root systems in plants receiving the root drench and 0.4% foliar applications.

Table I. Morphological traits of *Phaseolus lunatus* plants following four weekly treatments with different levels of cytokinin-containing seaweed concentrate, and two levels of nutrient supplementation. Means ( $\pm$  SE) within a column followed by a common letter are not significantly different ( $P > 0.05$ , least squares means t-tests).

SWC treatment	Stem height (cm)		Leaf area (cm <sup>2</sup> )		Leaf wet mass (mg)		Leaf dry mass (mg)		Leaf wet mass/area (mg/cm <sup>2</sup> )	
	Nutrient addition									
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Control	86.7 $\pm$ 6.4 a	65.4 $\pm$ 4.9 ab	735.8 $\pm$ 63.9 a	347.8 $\pm$ 29.5 ab	8580 $\pm$ 790 a	4590 $\pm$ 360 ab	1030 $\pm$ 90 a	610 $\pm$ 42 a	11.7 $\pm$ 0.3 a	13.2 $\pm$ 0.2 a
0.2% foliar	80.9 $\pm$ 3.6 a	64.6 $\pm$ 4.8 ab	696.4 $\pm$ 48.4 a	241.1 $\pm$ 16.2 bc	8230 $\pm$ 450 a	3230 $\pm$ 230 bc	1100 $\pm$ 90 a	470 $\pm$ 40 ab	12.0 $\pm$ 0.3 a	13.4 $\pm$ 0.3 a
0.4% foliar	73.2 $\pm$ 5.1 b	64.6 $\pm$ 4.8 ab	538.9 $\pm$ 54.0 b	315.6 $\pm$ 55.4 b	6300 $\pm$ 640 b	4230 $\pm$ 740 ab	770 $\pm$ 70 b	610 $\pm$ 110 a	11.7 $\pm$ 0.1 a	13.4 $\pm$ 0.1 a
1.0% foliar	62.7 $\pm$ 10.2 b	71.3 $\pm$ 2.6 a	445.9 $\pm$ 49.1 b	455.0 $\pm$ 49.5 a	5350 $\pm$ 560 b	5470 $\pm$ 580 a	630 $\pm$ 60 bc	630 $\pm$ 80 a	12.1 $\pm$ 0.4 a	12.0 $\pm$ 0.1 b
0.4% root	60.7 $\pm$ 7.6 c	51.5 $\pm$ 7.3 c	240.5 $\pm$ 35.8 c	171.8 $\pm$ 11.5 c	3060 $\pm$ 480 c	2160 $\pm$ 170 c	440 $\pm$ 70 c	370 $\pm$ 30 b	12.0 $\pm$ 0.9 a	12.5 $\pm$ 0.3 ab

Table I. (Continued)

SWC treatment	Leaf dry mass/area (mg/cm <sup>2</sup> )		Stem dry mass (mg)		Root dry mass (mg)		Per cent biomass	
	Nutrient addition							
	Yes	No	Yes	No	Yes	No	Yes	No
Control	0.141 $\pm$ 0.006 a	0.177 $\pm$ 0.007 c	470 $\pm$ 50 a	420 $\pm$ 30 ab	220 $\pm$ 30 a	250 $\pm$ 20 a	12.1 $\pm$ 0.4 a	13.3 $\pm$ 0.4 ab
0.2% foliar	0.157 $\pm$ 0.005 a	0.194 $\pm$ 0.009 b	530 $\pm$ 60 a	320 $\pm$ 30 bc	240 $\pm$ 30 a	220 $\pm$ 10 a	13.2 $\pm$ 0.6 a	14.5 $\pm$ 0.6 a
0.4% foliar	0.144 $\pm$ 0.006 a	0.190 $\pm$ 0.007 bc	330 $\pm$ 30 b	440 $\pm$ 70 a	140 $\pm$ 20 b	270 $\pm$ 40 a	12.3 $\pm$ 0.5 a	14.2 $\pm$ 0.4 a
1.0% foliar	0.145 $\pm$ 0.006 a	0.138 $\pm$ 0.003 d	290 $\pm$ 20 b	300 $\pm$ 40 c	140 $\pm$ 30 b	130 $\pm$ 20 b	12.0 $\pm$ 0.5 a	11.5 $\pm$ 0.5 b
0.4% root	0.185 $\pm$ 0.010 b	0.214 $\pm$ 0.009 a	270 $\pm$ 20 b	310 $\pm$ 30 c	40 $\pm$ 10 c	120 $\pm$ 30 b	16.5 $\pm$ 2.3 b	17.1 $\pm$ 0.6 c

### Effect of SWC on artificially decapitated plants

Foliar application of SWC did not stimulate plant recovery from artificial damage (i. e. apex removal). As with undamaged plants, artificially damaged plants treated with SWC allocated more resources to existing tissues and added new vegetative tissue more slowly than untreated controls. Final stem height was significantly greater for controls ( $84.7 \pm 4.1$  cm) than for SWC treated plants ( $69.1 \pm 4.1$  cm,  $F = 7.6$ ,  $df = 1, 33$ ,  $P < 0.01$ ). Application of the SWC also resulted in plants having less leaf area than controls ( $F = 4.8$ ,  $df = 1, 33$ ,  $P < 0.035$ , Table II). Despite the greater leaf area of control plants, there was no difference in leaf wet mass ( $F = 1.2$ ,  $df = 1, 33$ ,  $P = 0.28$ ) or leaf dry mass ( $F = 0.4$ ,  $df = 1, 33$ ,  $P = 0.55$ , Table II). Consequently, SWC-treated plants had significantly greater specific leaf masses than untreated plants ( $F = 16.1$ ,  $df = 1, 33$ ,  $P < 0.0001$ ).

The physiological effect of SWC-treatment is evident in the differences among primary leaves, which were expanded fully when testing began, of treated and untreated plants. The primary leaves of SWC-treated plants had greater biomass ( $14.6 \pm 0.3\%$ ) than controls ( $13.3 \pm 0.3\%$ ,  $F = 7.4$ ,  $df = 1, 33$ ,  $P = 0.01$ ), and greater specific leaf mass than control plants ( $2.00 \pm 0.04$  mg/cm<sup>2</sup> vs.  $1.77 \pm 0.04$ ,  $F = 14.1$ ,  $df = 1, 33$ ,  $P = 0.0009$ ).

While SWC-treated plants ( $135.7 \pm 15.2$  cm<sup>2</sup>) added less new leaf area than controls ( $179.1 \pm 15.2$  cm<sup>2</sup>,  $F = 3.9$ ,  $df = 1, 33$ ,  $P = 0.05$ ), the mass/area ratio of those new leaves was greater for SWC-treated plants than for the controls (wet mass:  $11.1 \pm 0.1$  mg/cm<sup>2</sup> vs.  $10.3 \pm 0.1$ ,  $F = 22.1$ ,  $df = 1, 33$ ,  $P < 0.001$ ; dry mass:  $1.59 \pm 0.03$  mg/cm<sup>2</sup> vs.  $1.44 \pm 0.03$ ,  $F = 12.7$ ,  $df = 1, 33$ ,  $P = 0.001$ ).

### Discussion

Applications of this particular SWC had a marked effect on most morphological characteristics of *Phaseolus lunatus* that we examined, indicating that this extract of *Ascophyllum nodosum* is biologically

active and alters the development of immature *Phaseolus lunatus*. However, the SWC tended to retard rather than stimulate new growth. No concentration of the SWC resulted in plants that were larger or had greater leaf area than untreated control plants. Our results indicate that the SWC did not provide sufficient nutrients for enhancing plant growth, but the SWC does possess other biologically active compounds which tend to inhibit growth. The interaction between SWC and fertilization treatment for many characteristics suggests that plants do not utilize fully the bioregulators in the extract, except with sufficient nutrient availability. Then the application of this particular SWC results in *Phaseolus lunatus* allocating more resources to existing tissue at the expense of producing new growth. These results strongly suggest that cytokinins are a biologically active component of this SWC (Neumann 1988), as has been found for other SWCs (Crouch and Van Staden 1993).

Under the high nutrient treatment, the effect of the SWC was most pronounced when applied as a root drench. Plants can take up biologically active compounds both through root and foliar applications of seaweed concentrates (Nelson and Van Staden 1984, 1986). For *Phaseolus lunatus*, the root application probably delivered the greatest amount of SWC to plants. Because of this, and because responses to foliar applications generally followed a trend according to the concentrations of the seaweed extract, differences in SWC treatments probably result from a dose response and do not result from differences between foliar and root uptake by *Phaseolus lunatus*.

As a result of reports that SWCs act as biostimulants for many systems (Crouch and Van Staden 1994), we tested the response of artificially damaged *Phaseolus lunatus* to SWC treatments to determine if treated plants would recover from the damage faster than untreated plants. However, as demonstrated for the undamaged plants, SWC treated plants did not add new tissue as rapidly as untreated controls. The lower amount of new leaf area added by treated plants and the fact that treated plants had greater specific leaf mass ratios (dry mass/area), especially in fully expanded primary leaves, are indicative that this

Table II. Morphological traits of decapitated *Phaseolus lunatus* after four weekly treatments with either a 0.4% foliar application of a cytokinin-containing seaweed concentrate or a control spray. Plants were decapitated 2.5 cm above the first trifoliate leaf node. Means ( $\pm$  SE) within a column, followed by a common letter are not significantly different ( $P > 0.05$ , least squares means t-tests).

SWC treatment	Stem height (cm)	Leaf area (cm <sup>2</sup> )	Leaf wet mass (mg)	Leaf dry mass (mg)	Leaf wet mass/area (mg/cm <sup>2</sup> )	Leaf dry mass/area (mg/cm <sup>2</sup> )	Stem dry mass (mg)	Root dry mass (mg)	Per cent biomass
Control	$84.7 \pm 3.4$ a	$386.6 \pm 19.0$ a	$4220 \pm 210$ a	$580 \pm 30$ a	$10.9 \pm 0.1$ a	$0.151 \pm 0.003$ a	$240 \pm 10$ a	$140 \pm 10$ a	$13.9 \pm 0.2$ a
Treated	$69.1 \pm 4.3$ b	$348.0 \pm 20.0$ b	$4090 \pm 260$ a	$590 \pm 30$ a	$11.7 \pm 0.1$ b	$0.169 \pm 0.003$ b	$230 \pm 10$ a	$120 \pm 10$ a	$14.4 \pm 0.2$ a

*Ascophyllum nodosum* extract contains biologically active cytokinins. Cytokinins are known to promote cell division (Miller 1961) and to accumulate in primary leaves of decapitated *Phaseolus vulgaris* L. plants (Wang *et al.* 1977, Palmer *et al.* 1981). However, cytokinins also inhibit leaf senescence by blocking export of photosynthates to new tissue, and stimulating translocation of resources to treated leaves (Mothes 1961, Leopold and Kawase 1964). Because these processes are dependent on levels of other phytohormones (Elliott 1982), the most important effect of SWC applications could be in altering the balance of hormones in plants.

Such an alteration in hormone balance may account for variable responses to applications of *Ascophyllum nodosum* extracts. El-Sayed (1991) reported that lower rates of cytokinin sprayed on *Phaseolus vulgaris* increased plant growth relative to controls. While a 0.4% concentration of a different *Ascophyllum nodosum* extract from the one we tested increased leaf mass of spinach (*Spinacia oleracea* L.), it did not promote photosynthesis (Cassan *et al.* 1992). Higher concentrations of another *Ascophyllum nodosum* extract resulted in stunted plants of *Euphorbia pulcherrima* Willdenow (Senn and Skelton 1969). Increasing concentrations of *Ascophyllum nodosum* extracts are

increasingly detrimental to the growth of *Geranium* sp. (Senn and Skelton 1969, Senn and Kingman 1978). Hedin and McCarty (1991) found no consistent benefit or detriment in applying two levels of another SWC preparation to cotton (*Gossypium hirsutum* L.).

These disparate findings point out the need for careful evaluation of the use of SWCs in agricultural systems. However, our results indicate that applications of the *Ascophyllum nodosum* extract we tested are not beneficial to immature *Phaseolus lunatus*. This particular product was not beneficial in alleviating nutrient stress, promoting growth under adequate nutrient availability, or in aiding plant recovery from artificial damage.

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