THE EFFECT OF SEAWEED CONCENTRATE ON PLANT GROWTH

By

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Cogito, ergo sum. I think, therefore I am.

(Le Discours de la Méthode)

René Descartes (1596-1650)

PREFACE

I hereby declare that this thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of Natal, Pietermaritzburg, except where the work of others is acknowledged, is the result of my own investigation.

Ian James Crouch

December, 1990

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ABSTRACT

The application of seaweed concentrates to plants has been shown to enhance growth and improve yield parameters. How these natural products elicit their beneficial responses is still unclear. While many of the growth responses have been attributed to cytokinins, it is obvious that this group of plant hormones cannot account for all the beneficial effects incurred from seaweed use. This study was therefore initiated to investigate the effects of a commercial seaweed concentrate (Kelpak) on several aspects of plant growth and development.

Tentative determination of plant growth regulators in the seaweed concentrate (SWC) using bioassay systems, indicated the presence of compounds with gibberellin- , abscisic acid- and auxin-like properties. Tentative identification of the auxins present in the SWC and *Ecklonia maxima* using High Performance Liquid Chromatography revealed the presence of tryptophan, indole-3-acetamide, indole-3-acetic acid, indole-3-carboxylic acid and indole-3-acetaldehyde.

The effect of SWC on the growth of nodal potato explants cultured *in vitro* was examined. 0.2% SWC significantly accelerated shoot growth and development. When applied at a concentration of 0.4% the number of axillary shoots per node increased. This treatment also stimulated the development of potato tubers on the shoots.

The SWC was also shown to enhance the growth of tomato (*Lycopersicon esculentum* Mill.) roots cultured *in vitro*. Filtration of the SWC indicated a promotory filtrate phase and an inhibitory cell wall phase. The application of the SWC to nematode-infested roots, cultured *in vitro*, reduced the degree of infestation in susceptible roots but induced host/parasite compatibility in a resistant variety.

One of the most pronounced effects noted with seaweed application was the promotion of adventitious roots on several species of garden plants. The application of similar dilutions to *Eucalyptus* cuttings increased the average root mass but had little effect on the number of roots initiated per cutting. The rooting factors, purified by HPLC, were tentatively identified as indole-3-acetamide, indole-3-acetic acid, indole-3-carboxylic acid or indole-3-acetaldehyde by co-chromatography with authentic standards.

Finally, the effect of seaweed concentrate on the growth of tomato plants grown in nematode-infested soil was investigated. SWC applied as a soil drench, improved plant vigour, significantly increased shoot and root fresh weights and resulted in a marked reduction in the number of nematode galls per unit length and per unit weight of root. Plants treated with a foliar spray of SWC were invariably the first to produce ripe fruit. Total yield was improved by over 10%. Ashing the SWC indicated that the active constituents are possibly of an organic nature. Filtering the SWC confirmed earlier reports that promotory and inhibitory compounds are present in the concentrate. Chromatographic separation of the SWC into 10 Rf zones indicated the presence of several components with growth regulatory properties. It was found that the same fractions that improved plant growth also reduced nematode infestation.

The significance of these findings and the possible relationship between the endogenous plant growth regulators in *Ecklonia maxima* and the effect of the SWC on plant growth is discussed.

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GENERAL INTRODUCTION AND OBJECTIVES

Seaweeds have been utilized in agriculture for many centuries. Commercial exploitation of seaweed as a plant additive has however, met with variable success owing to conflicting reports on the value of seaweed for crop improvement. Reports in recent studies that seaweed concentrates increase plant vigour and yield have resulted in a renewed interest in the modern day application of commercial preparations.

Because of the many adverse effects of synthetic fertilizers upon the environment there is a need for natural sources of fertilizers and soil ameliorants. This, combined with the ability of seaweeds to improve plant growth, their ease of application and their relatively low costs, has led to an increased interest in natural seaweed products.

Although studies have revealed many interesting factors concerning seaweed as a plant stimulant, a great deal of research has yet to be undertaken before all the factors responsible for improved plant growth are known. Initially it was thought that certain constituents in seaweeds improve soil structure making conditions more suitable for root growth (MILTON, 1964). A better developed root system would enhance water and mineral uptake by the plants, resulting in improved growth. At this time, the presence of endogenous trace elements in seaweeds was also thought to explain some of the beneficial effects obtained through the use of seaweed preparations (FRANCKI, 1960a). However, if the concentration at which these products are applied is taken into consideration then it is clear that the level of mineral elements in the seaweed mixture would be too low to have any serious effect on plant growth (ABETZ, 1980).

In view of the low rates of application necessary to elicit a physiological response it was therefore suggested that organic compounds rather than mineral elements are responsible for yield increases. Recent research has shown that seaweed products

contain certain plant growth regulators and at present many of the observed effects are ascribed to these constituents. That plant hormones, and in particular cytokinins, may be involved was suggested by BOOTH (1966). This conclusion was reached as many of the responses obtained from seaweed application were found to be similar to those following the application of cytokinins to plants. Further evidence supporting this hypothesis was the detection of cytokinin-like activity in a number of marine algae and later in commercial seaweed preparations.

Although cytokinins have been identified in seaweed products it, seems unlikely that they are the only beneficial growth substances involved, - particularly in view of the wide range of physiological processes affected by seaweed application. This has therefore led to an emphasis on research directed at identifying and isolating other plant growth regulators in seaweed extracts.

In this study, the effects of a commercially available seaweed concentrate on different aspects of plant growth were examined. The main areas of research included:

1. A study to determine the presence of plant growth regulators in processed *Ecklonia maxima* (Osbeck) Papenfuss material and the tentative identification of auxins in the lamina, stipe and holdfast regions of the alga;

2. A study to examine the effect of seaweed concentrate (SWC) on the growth of potato (*Solanum tuberosum* L.) shoots cultured *in vitro*;

3. Studies investigating the effect of SWC on the growth of isolated tomato roots cultured *in vitro*;

4. Research providing evidence for a rooting factor in the SWC; and,

5. Studies determining the effect of SWC on the growth of nematode-infested tomato (*Lycopersicon esculentum* Mill.) plants.

CHAPTER 1

LITERATURE REVIEW

1.1 History of Seaweed Utilization

Reports of the use of marine algae as fertilizers date back to the Ancient Greeks and Vikings. Where supplies near coastlines are plentiful, historical records show the long term use of seaweed as animal fodder and as a soil conditioner (STEPHENSON, 1974a). Early references to the use of seaweed as a manure have been found in the Orient. By the 12th Century the large brown algae were used for manuring on the coastal lands of France, Ireland, Scotland and Normandy (BOOTH, 1965). Today, probably the largest application of seaweed as manure is along the entire coast of Brittany (CHAPMAN & CHAPMAN, 1980). During the 17th Century, the first industry involving seaweeds, the Kelp trade, developed. The algae of Europe used for the Kelp trade were species of *Laminaria*, *Fucus* and *Ascophyllum nodosum* (L.) Le Jolis. These seaweeds provided a source of soda (*Fucus* and *Ascophyllum*), iodine (*Laminaria*) and later, in the 19th Century - products such as ammonia and potash (SENN & KINGMAN, 1978). Before seaweeds could be used as an economic manure for inland farmers, they had to be treated in some way. This led to the granting of the first patent in 1856 for a dried seaweed manure (Gardissal). In 1912 the first alkaline liquid seaweed extract (Penkals) was patented.

With the development of chemical fertilizers in the late 1880's, seaweed as a form of soil enrichment began to decrease in popularity. In recent times, detrimental effects of synthetic products upon the environment became apparent and natural bio-degradable fertilizers received renewed attention.

The marketing of 'Biomisation Fluid' or 'Baby Bio', and 'Biohumus' followed shortly afterward. These were produced by the aqueous extraction of seaweed and peat. Other products became available in the 1960's: Marinure, Sea Born, Seahorse, Sea Magic 3 (S.M.3), Trident and a product made by Algea Produckter A/S (BOOTH, 1969). The manufacture of these extracts was by simple extraction or by high pressure alkaline extraction of large brown seaweeds. Their application by foliar spraying resulted in worldwide reports of improvements in plant vigour and yield. This led to a sharp increase in seaweed research as attempts were made to establish the regulatory action of the different seaweed products.

1.2 Seaweed Application in Agriculture

1.2.1 Direct application of harvested seaweed

The utilization of natural, unprocessed seaweed in agriculture is largely limited to coastal areas where it is still commonly used as an animal fodder and as a soil additive.

As an animal feed seaweed mainly supplements protein intake, being similar in protein content to good quality hay (BLACK, 1955; BELEAU, HEIDELBAUGH & VAN DYKE, 1975). Seaweed as an animal fodder is valuable as a source of trace elements (SMITH, 1961; JENSEN, 1971), vitamins and vitamin precursors including carotenoids and xanthophylls (CHAPMAN & CHAPMAN, 1980). This makes them valuable as a food supplement on mineral deficient pasture land. Nutritional investigations show that animal fodder supplemented with up to ten percent seaweed can be beneficial to some animals (CHAPMAN, 1970). Experiments have shown that seaweeds are unsuitable as a source of food energy for horses, cattle, sheep, swine and poultry. The polysaccharides contained in seaweed are too difficult for these animals to

digest. Yet, BLACK (1955) noted that some ruminants can digest them partially but only after the development of a compatible microflora.

The addition of algal polysaccharides to animal feeds may bind and remove harmful metal pollutants from the intestinal tracts (SKORYNA & TANAKA, 1969; TANAKA, HURLBURT, ANGELHOFF & SKORYNA, 1971). MYKLESTAT (1979) has related heavy metal exchange in marine algae to the ion exchange properties of these polysaccharides.

1.2.2 Seaweed meals and liquid seaweed extracts

Seaweed treatment of crops has grown in popularity and led to the development of many processed seaweed products. These can be placed into three groups: meals for supplementing soil in large volumes or for blending into defined rooting media for glasshouse crops, powdered or liquid extracts, and concentrates employed as root dips, soil drenches and as foliar sprays (BOOTH, 1969; SENN 1987; METTING, RAYBURN & REYNAUD, 1988).

Seaweed meal applied to the soil serves two main functions. As a fertilizer it promotes plant growth through the slow release of mineral nutrients and as a soil conditioner it improves aeration and aggregate stability. Generally, unprocessed seaweeds have a similar nitrogen content to many animal manures, less phosphorus, but more potassium, total salts and readily available micronutrients (STEPHENSON, 1974a; SENN & KINGMAN, 1978). The soil conditioning property of seaweed is attributed to alginic acid which comprises about one-third of its carbohydrate content. QUASTEL & WEBLEY (1947) proved that alginates enhance the crumb structure and moisture retaining characteristics of light soils and can ameliorate the sticky nature of clay soils.

In temperate agriculture and horticulture, most seaweed products are manufactured from brown algae harvested from temperate waters. The three species most commonly used as supplemental fertilizers and soil conditioners are *Ascophyllum*

nodosum (L.) Le Jolis, *Ecklonia maxima* (Osbeck) Papenf. and *Fucus vesiculosis* (L.) C. Agardh. Less commonly employed are species of *Laminaria* and *Sargassum* (BLUNDEN, CHALLEN & WOODS, 1968; BLUNDEN, 1977). Although these algae belong to the class Phaeophyceae it is probable that their size and availability govern their choice rather than by any specific determination of chemical suitability (MOONEY & VAN STADEN, 1985).

Liquid extracts of seaweeds are made by several processes that include: stirring macerated seaweed in a vat containing hot water; acidic or alkaline hydrolysis with or without steam; and the pressure burst technique. In the latter method, employed in South Africa, a liquid seaweed concentrate is produced without resorting to chemical or heat extraction. The use of pressure to break down the structural components of the cell allows the release of practically all the seaweed's essential constituents, including plant growth regulators (NELSON, 1985).

Seaweed meal and liquid extracts are produced from the same kinds of seaweed and therefore have certain qualities in common. For example, both provide at least traces of many mineral elements (STEPHENSON, 1968) and both, by stimulating the action of soil bacteria, help make phosphorus and potassium available to plants (MILTON, 1964; CAIOZZI, PEIRANO, RAUCH & ZUNINO, 1968). The main difference between these two types of products is the time it takes to elicit a response. Seaweed meal may take months to become fully effective because the carbohydrate material it contains has to be broken down by soil bacteria before it can be used by the plant. In liquid extracts, the cellulosic constituents of the seaweed are already broken down or removed (depending on the extraction process) and so the released constituents can be absorbed through both the leaves and roots of a plant thus becoming effective more quickly. The dried meal, with its slow release of alginic acid and other polyuronides, is a better soil conditioner than liquid seaweed extracts (CHAPMAN & CHAPMAN, 1980). STEPHENSON (1974a) justifies its use for crops such as potatoes, asparagus, flowers, fruit and hops but not for cereals.

Since the 1960's, commercial use of concentrated seaweed extracts as foliar sprays, seed dips and soil drenches has increased far more rapidly than the use of meal applied as a soil additive. This might be attributed to the relative ease of transporting and storing extracts and concentrates compared to meals and to the efficiency with which biologically active constituents are made available when products are applied directly rather than blended with the bulk of the soil.

1.3 Effect of Commercial Seaweed Preparations on Plants

The responses of plants to seaweed application are many and varied (Table 1.1). These include higher yields; increased nutrient uptake and changes in plant tissue composition; increased resistance to frost, fungal diseases and insect attack; longer shelf life of fruit and better seed germination.

In many trials, the experimental plan and degree of replication makes it difficult to determine whether differences between treatments are statistically significant. Erratic results may arise from any of the following: climatic and soil conditions of field experiments, botanical and geographical source of seaweed, method of seaweed processing, and form and application of seaweed product. Although ample documentation demonstrates the scope of seaweed research, the identity of the active constituents and their mode and mechanisms of action are still not known. Initially it was thought that the many beneficial effects of seaweed were derived from either the chelating properties of certain of the constituents (LYNN, 1972), or improved absorption of trace elements by the plants (FRANCKI, 1960a, 1960b; AITKEN & SENN, 1965; OFFERMANS, 1968; SENN & KINGMAN, 1978). Current research however, suggests that certain plant growth substances in the concentrates may be the active constituents. To assess these views, it is necessary to examine the organic and inorganic constituents of seaweeds.

Table 1.1 The effect of applied commercial seaweed products on plant growth.

Key to growth parameters affected:

1 Plant yield
2 Shoot dry weight
3 Root dry weight
4 Total dry weight
5 Delayed fruit senescence
6 Increased frost resistance
7 Increased nutrient uptake
8 Better seed germination
9 Fruit mass
10 Average seed mass
11 Increased root growth
12 Reduced incidence of pathogen and insect attack
13 Enhanced fertilizer use efficacy
14 Reduction in nematode infestation
15 Other

\+ = positive effect through seaweed application
0 = no effect through seaweed application
\- = negative effect through seaweed application

Abreviations:

CD = cutting dip
FD = fruit dip
FS = foliar spray
IV = *in vitro*
RD = root dip
SD = seed dip
SF = soil flush
SM = seaweed meal
TC = tissue culture

PLANT SPECIES	APPLIED SEAWEED OR COMMERCIAL PREPARATION			Plant yield	Shoot dry weight	Root dry weight	Total dry weight	Delayed fruit senescence	Increased frost resistance	Increased nutrient uptake	Better seed germination	Fruit mass	Average seed mass	Increased root growth	Insect & pathogen control	Enhanced fertilizer use	Reduction in nematodes	Other	ADDITIONAL COMMENTS	REFERENCES
	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Allium cepa (Onion)	*Laminaria saccharina*	(SF)	1:100				+												Ashed extract significantly increased wet and dry weight of onion.	Blunden et al., 1968.
			1:100 (ashed)				+													
Allium cepa (Spring onion)	*L. saccharina*	(SF)	1:100				-												Ashed extract significantly increased wet and dry weight of spring onion.	Blunden et al., 1968.
			1:100 (ashed)				+													
Allium porrum (Leek)	*L. saccharina*	(SF)	1:100				-												Ashed extract significantly increased wet and dry weight of leek.	Blunden et al., 1968.
			1:100 (ashed)				+													
Apium graveolens (Celery) cv. Avonpearl	Maxicrop *(Ascophyllum nodosum)*	(FS)		+												+			Increase of 18% over nitro-chalk treated heads when SWC used in conjunction with the fertilizer.	Stephenson, 1974b.
cv. Lathom Blanching				+												+				
Arachis hypogaea (Groundnuts)	Brown seaweeds (manure)	(SM)	5 kg N per acre	+						+						+			Enhanced utilization of mineral nutrients. Increased N & fat content of pods.	Bokil et al., 1972.

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
cv. Tanut 74	Cytex			+															Cytex = Water soluble algal extract	Ketring & Schubert, 1981.
cv. Sellie	Kelpak *(Ecklonia maxima)*	(FS)	1:400 (2dm ha^{-1})	+									+						Seed mass increased by 65%. Increased protein content	Featonby-Smith & van Staden, 1987a.
cv. Tanut 74	Cytex			+															Cytex = Water soluble algal extract	Ketring & Schubert, 1981.
Beta brasiliensis	*L. saccharina*	(SF)	1:100	+			+												Ashed treatments significantly better than controls.	Blunden et al., 1968.
			1:100 (ashed)	+			+													
Beta vulgaris (Beetroot) Effect on salt tolerance	*Durvillea antarctica*	(SM)	1.25:100	-														-	Plants pale and stunted. As beetroot is salt tolerant, detrimental effects not due to salt stress.	Francki, 1960a.
(Sugar Beet)	*A. nodosum*		5 cwt per acre	+														+	Seaweed extract increased sugar content. 13.5% yield increase.	Booth, 1966.
	Maxicrop														+				Reduction in aphids.	Stephenson, 1966.
(Sugar Beet) 13 Varieties	Sea Magic 3 (S.M.3)	(FS)	1:100	+		+													Significant increase in root sucrose content. Amino-nitrogen and potassium contents	Blunden et al., 1979.

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
																			significantly reduced.	
(Swiss Chard)	Kelpak	(FS)	1:500	+	+	+	+									+			Seaweed extract in combination with a fertilizer increased all parameters further.	Featonby-Smith & van Staden, 1983a.
(Table Beet) cv. Detroit Dark Red cv. Medium Top cv. Mono-King Explorer	Cytex	(SD)	1:100								+								Seaweed extract significantly improved seed germination over a range of temperatures (10?C - 30?C).	Wilczek & Ng, 1982.
Brassica oleracea (Cabbage)	*E. maxima*	(FS)	1:330		0	+				+						+			Root growth doubled. Significant reduction in calcium uptake.	Kotze & Joubert, 1980
		(FS)	1:1000		0	+										+			Root growth increased by 20%.	
cv. Capitata	Kelpak	(RD)	1:250		0		0													Aldworth & van Staden, 1987.
		(RD)	1:500	+	+	+	+												Plant vigour improved.	
Brassica oleracea cv. Yaralong 66 (Cauliflower)	Maxicrop	(FS)	1:170	0														+	Significant increase in curd diameter.	Abetz & Young, 1983.
Brassica rapa (Turnip) cv. Snowball	Maxicrop	(FS)	1:120	+		+									+				A 70% reduction of powdery mildew.	Stephenson, 1966. Booth, 1964a.
Callistemon citrinus (Bottlebrush)	Kelpak	(CD)	1:10											+					Initiated >130% more roots.	Crouch & van Staden, 1990a.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Capsicum annuum (Peppers) cv. Cal. Wonder	S.M.3	(SF)	2.3 l ha^{-1}	+				+											Yield increase of 26%	Blunden, 1972.
	A. nodosum	(SF)	1:50							+									Increased utilization of B, Cu, Fe, Mn and Zn.	Lynn, 1972.
			1:100							+										
	S.M.3		7.5 mg l^{-1} kinetin equivalents					+											Significant reduction in the rate of reddening from 26 to 59 days.	Blunden et al., 1978.
	Marinure							0												
(Sweet Peppers)		(SM)	50 kg ha^{-1}	+						+								+	Significant increase in yield. Increased initiation of flower buds.	Senn & Kingman, 1978.
	A. nodosum	(SM)	100 kg ha^{-1}	+																
Chrysanthemum sp.	Maxicrop	(FS)	1:200												+				Red spider mite and aphid populations significantly reduced.	Stephenson, 1966.
Citrullus lantanus (Ambrosia melon)	*A. nodosum*	(SM)								+									Increased uptake of Mg, N, Ca. Sugar content increased by 2-3%.	Aitken & Senn, 1965.
Cucumis melo																				
Citrus aurantium cv. Valencia (Sour Orange)	*A. nodosum*	(SM)	672 kg ha^{-1}									0					0		Populations of *Tylenchulus semipenetrans* not significantly reduced.	Tarjan, 1977.
Citrus latifolia (Limes) cv. Persian	S.M.3 Marinure		30 mg l^{-1} kinetin equivalents					+											Significant retardation in the rate of degreening.	Blunden et al., 1978

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES		Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Citrus lemon (Lemon) Seedlings		*A. nodosum*	(SM) (FS)	448 kg ha^{-1} 2.24 kg ha^{-1} (in 379 l water)				+										+ +		Significant decreases in *Pratylenchus coffeae* populations. Reduced numbers of *Radopholus similis.*	Tarjan, 1977.
	cv. Euraka	Kelpak	(FS)	1:400							-									When applied in combination with zinc sulphate ,Kelpak did not improve the foliar uptake of zinc.	Beckett & van Staden, 1988.
Citrus sinensis (Grapefruit seedlings)		*A. nodosum*																	+	Application of seaweed extract to nutrient deficient cultures, lessened or nullified deficiency effect.	Aitken, 1964
(Oranges) Seedlings	cv. Pineapple	*A. nodosum*	(SF)	1:25															+	Seaweed extract supplied enough Mg, Mn, Zn and B to carry on near normal respiration.	Senn & Skelton, 1969.
	cv. Hamlin	S.M.3	(FS)	1:500	+															Small but consistent increases in yield.	Blunden, 1972.
	cv. Parson Brown	S.M.3	(FS)	1:500	+																
	cv. Pineapple	S.M.3	(FS)	11.2 l ha^{-1}	+																
Cucumis melo (Melon)		*A. nodosum*	(SM)													+				Reduced mildew infection.	Senn et al., 1961.
Cucumis sativus (Cucumbers)		Dswe Algifert *A. nodosum*	(FS)	1:400	+				+											Yield increased by over 40%. Shelf-life increased from 14-21 days.	Povolny, 1969a.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
	Dswe Algifert	(FS)		+											+				Yield increase of about 17%. Increased profit of 146%. Reduced incidence of red spider mite.	Povolny, 1971.
5 Weeks after transplanting cv. Pepinova	Kelpak	(RD)	1:250			+	+												Seaweed extract applied to nutrient-stressed plants.	Nelson & van Staden, 1984a
		(FS)	1:500	+		+	+													
		(RD+FS)				0	0													
10 Weeks after transplanting		(RD)	1:250			0	0			+									Significant increase in nitrogen and phosphorus content.	
		(FS)	1:500			+	+			+										
		(RD+FS)				+	+			+										
	Maxicrop	(FS)	1:200												+				Reduced numbers of Red spider mite.	Stephenson, 1966.
Daucus carota (Carrot)	Maxicrop	(FS)	70 l ha^{-1}	+															Almost 100% increase in yield after foliar spray application.	Stephenson, 1968.
Dianthus deltoides (Carnation)	Kelpak	(CD)	1:10											+					Initiated about 50% more roots than control cuttings.	Crouch & van Staden, 1990a.
Euphorbia pulcherrima (Poinsettia)	*A. nodosum*	(SM)	1:200															+	Greater number of flowers; prolonged life of blooms.	Senn & Kingman, 1978.
	Sea Born	(SF)	1:5		-														Stunted plants.	Senn & Skelton, 1969.
	A. nodosum		1:25		+														Lower metabolic activity.	
			1:50		+															
		(SM)	300		+														Higher metabolic activity.	
			500		0															
			900		+															

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Evolvulus glomeratus	Kelpak	(CD)	1:10											+					Initiated >60% more roots than control cuttings.	Crouch & van Staden, 1990a.
Festuca rubra (Creeping Red fescue)	S.M.3	(SD)	1:100								+								Drying the seed after the seaweed extract treatment negated the beneficial effects.	Button & Noyes, 1964.
		(SD)	1:200								+									
		(SD)	1:20								-									
Fragaria vesca cv. Cambridge Favorite. (Strawberry)	*A. nodosum*		1:100	+															Significant increase in yield of 30%.	Stephenson, 1966.
	Maxicrop	(FS)	1:120												+				*Botrytis* infection reduced from 22.5% to 4.6%.	Stephenson, 1968.
cv. Cambridge Favorite.	Maxicrop			+															Yield increased between 19-133%.	Booth, 1973.
cv. Merton	Maxicrop		1:50	+															Seaweed treatment resulted in an average increase of about 10%. Earlier ripening and better fruit quality increased income per acre by 14.3%.	Booth, 1973.
cv. Senga Precons			45 g acre^{-1}	0																
cv. Kennedy				+																
cv. Gorella I				+																
cv. Gorella II				+																
cv. Albert Ier				+																
cv. Senga Gigana				0																
cv. Vola				+																
cv. Cambridge Favorite.	Maxicrop			+															Mean yield increase of 44%.	Booth, 1974.
Fragaria x ananassa Duchesne	Maxicrop	(FS)	1:120	+											+				*Botrytis* infected only 1.7% of fruit on treated plants	Stephenson, 1966.

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
																			compared to 12.9% on control plants. Yield increase of about 30%.	
Geranium sp.	*A. nodosum*	(SM)	1:20 to 1:2.5		+														Respiratory activity of leaves measured on a dry weight basis.	Senn & Skelton, 1969.
	A. nodosum	(SM)	5, 10, 20, & 40% per vol soil.				-											-	Increase in dry weight % of plant tissue - decrease in water content. Greatly reduced number & size of flowers.	Senn & Kingman, 1978.
Gladiolus sp. cv. Leewenhorst	S.M.3	(SF)	1:100	+															Significant increase in corm wet weights with seaweed application.	Blunden, 1972.
Glycine max. (Soybean)	*A. nodosum*	(FS)		+															Significant increase in protein content.	Senn & Kingman, 1978.
Gossypium herbaceum (Cotton)	*A. nodosum*	(SD)	1:25 to 1:50															+	Increased respiratory activity of seed.	Aitken & Senn, 1965.
Hibiscus esculentus (Ladies Finger)	*Hypnea spp.*	(SM)		+															Yield 73% better than farmyard manure. Peak of fruiting one month earlier.	Thivy, 1959.
Hordeum vulgare cv. Clipper (Winter Barley)	Kelpak	(FS) (FS) (SF)	1:250 1:500 1:250										+ + +						Grain mass per plant increased in the order of 50% in all treat-	Featonby-Smith & van Staden, 1987b.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
		(SF) (SD)	1:500 1:250										+						ments. SWC treated plants resulted in a greater number of fertile spikelets per ear.	
Humulus lupulus (Hop) cv. OR 55	Maxicrop	(FS)																+	Alpha acid content increased by: 35.2%	Booth, 1974.
cv. W.G.V.		(FS)																+	15.4%.	
cv. O.T. 48		(FS)																+	23.5%	
cv. O.R.S.		(FS)																+	22.8%.	
Ilex crenata rotundifolia (Holly)	*A. nodosum*	(RD)	100% 1:25 for 5 sec.											- +					Retarded rooting. Increased degree of rooting and respiration rate.	Senn & Kingman, 1978.
Ilex opaca cv. Hume No.2 (American holly)	*A. nodosum*	(SD)	1:25 to 1:50															+	Increased respiratory activity of seed.	Aitken & Senn, 1965.
Impatiens auricoma	Kelpak	(CD)	1:10											+					Initiated >100% more roots than control cuttings.	Crouch & van Staden, 1990a.
Ipomoea batates (Sweet Potatoes) cv. Centennial cv. Carogold	*A. nodosum*	(SM)		+						+						+			Increased uptake of Mg, N, and Ca. Seaweed meal in combination with 5-10-10 fertilizer increased the meal by 100%.	Senn & Kingman, 1978.
Lactuca sativa (Lettuce)	*L. saccharina*	(SF)	1:100				-													Blunden et al., 1968.

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
			1:100 (ashed)				+												Ashed extract significantly increased dry weight.	
Seedlings	Seasol		high												+				SWC reduced 'damping-off' caused by *Pythium*.	Braid, 1978.
cv. Greendale	Maxicrop	(FS)	1:200	+											+			+	Significant decrease in number of diseased lettuces or those that failed to form hearts. Significant increase in weight and mean heart diameter.	Abetz & Young, 1983.
cv. Winter Crisp (Nutrient deficient conditions)	Kelpak	(SF)	1:250	0						0										
			1:500	0						0										
(Optimal nutrient conditions)			1:250	+						+									SWC significantly increased the amounts and concentration of Ca, K, and Mg in the lettuce leaves.	Crouch et al., 1990.
			1:500	+						+										
Lavandula vera (Lavander)	Kelpak	(CD)	1:10											+					Initiated about 18% more roots on cuttings.	Crouch & van Staden, 1990a.
Ligustrum lucidum (Glossy Privet)	*A. nodosum*	(SD)	1:25								+								Seaweed extract increased respiratory activity in the seeds.	Senn & Skelton, 1969.
Lycopersicon esculentum (Tomato) cv. Potentate	*Pachymenia himantophora*	(SM)	1.25:100							+									Seaweed extract application resulted in the release of un-	Francki, 1960a, 1964.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES		Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
																				available manganese from the soil. (Slightly toxic)	
		Durvillea antartica	(SM)	1.25:100	-						+									Depressed nitrogen availability.	
Effect of salt stress	cv. Potentate	*Durvillea antartica*	(SM)	1.25:100															-	Leaf yellowing. Detrimental effect thought not to be due to salt stress. (See Beetroot)	Francki, 1960a.
Seedlings	cv. Potentate	*Pachymenia himantophora*	(SM)	1.25:100	+												+			Significant increase in dry weight. Highly significant (1% level) when applied with ammonium nitrate.	Francki, 1960b.
		Durvillea antartica	(SM)	1.25:100	+												+			Highly significant (1% level) when applied with ammonium nitrate.	
		A. nodosum	(SM)							+										Seaweed treated plants withstood temperatures of 29 F.	Senn et al., 1961.
		Extract and commercial starter solution.							-											Resulted in increased incidence of blossom-end rot.	Aitken & Senn, 1965.
			(SF)	1:25									+								
			(FS)+(SF)						-												
	cv. Ailsa	Maxicrop	(SD)	1:120												+				Damping-off	Stephenson,

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Craig																			reduced by 45%.	1966; Booth, 1964.
cv. Manapal Trellis	S.M.3	(SF)	2.3 l ha^{-1}	+															Tomato yield increased by 20%.	Blunden, 1972.
Pots dipped in seaweed dilution.	Dswe Algifert	Pot dip	1:100				+												Overall dry weight of plants increased by 36%.	Povolny, 1974.
Seedlings	Maxicrop	(FS)	1:200	+															Tomato yield increased by 37%.	Stephenson, 1974b.
	Dswe Algifert	(FS)						+											Pre-harvest application of seaweed extracts decreased fruit losses after 4 weeks by 45%.	Povolny, 1976.
	A. nodosum	(FS)		+															Significant increase in soluble solids.	Senn & Kingman, 1978.
Seedlings	*A. nodosum*	(SM)	1:30 soil					+											Increased cold hardness.	
		(SM)	50 kg ha^{-1}	+															Significant increase in yield.	
			100 kg ha^{-1}	+																
Tomatoes grown in nematode-infested soil.	Kelpak	(FS)	1:500	+	+	+	+					+							SWC applied as a flush increased the number of nematodes in the soil but reduced the number within the roots.	Featonby-smith & van Staden, 1983b.
		(SF)	1:500	+	+	+	+					+					+			

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
In vitro cultures. cv. Moneymaker	Kelpak	(IV)	1:100 1:400 1:500											+ +					Measurement of root length and number of lateral roots.	Finnie & van Staden, 1985.
Seedlings cv. Karino	Kelpak	(FS)	1:100 1:20 1:100 + Trelmix 1:20 + Trelmix	0 0															No effect on uptake of foliar applied Cu and Mn in plants receiving no macro-nutrients. Zn uptake significantly enhanced.	Beckett & van Staden, 1990a.
cv. Karino	Kelpak	(SF)	1:400-1:100	+	+	+	+					+					+		Average fruit fresh weight increased by 30%.	Crouch & van Staden, 1990a.
Malus sylvestris (Apples) cv. Sturmer Delicious	Maxicrop														+				Reduced red spider mite population by 50%.	Slade, 1967.
(Detached shoots)	Maxicrop	(FS)	1:50												+				Significantly reduced red spider mite.	Stephenson, 1966. Stephenson, 1968.
(Stored apples)																				
cv. Cox Orange	Dswe	(FS)	0.8:100					+											Seaweed treatment resulted in a 4.3% loss of fruit compared to 39.2% of controls.	Povolny, 1969 b; c.
M. pumila	Algifert																			
cv. Nonnetit		(FS)	0.8:100					+												
cv. Goldparm		(FS)	0.8:100					0												
cv. Grendier	Maxicrop			+															Apple yield increased by 96% when expressed as fruit fresh weight.	Booth, 1974.
cv. Starking	Agral	(FS)	1:500	0				0		+									Significant increase in Mn uptake.	De Villiers et al., 1983.
cv. Golden	Agral	(FS)	1:500	0				0		0										

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Delicious Seedlings	Agral	(FS)	1:500							-									Significant decrease in zinc uptake.	De Villiers et al., 1983.
Mangifera indica (Mango)	S.M.3	(FD)	7.5 mg l^{-1} kinetin equivalents					-											Significant increase in rate of ripening.	Blunden et al., 1978.
	Marinure	(FD)	30 mg l^{-1} kinetin equivalents					-											Significant increase in rate of ripening.	Blunden et al., 1978.
Musa sp. (Bananas)	S.M.3	(FS)	1.14 l ha^{-1}	+						+		+							Marked increase in manganese uptake. Yield increase of 12%.	Blunden, 1972.
M. acuminata	S.M.3	(FD)	30 ppm					-											Significant increase in rate of ripening.	Blunden et al., 1978.
Nandina domesticum (Heavenly Bamboo)	*A. nodosum*	(SD)	1:25 1:5								+								Increased respiratory activity in the seeds.	Senn & Skelton, 1969.
Nicotana tobacum (Aromatic Tobacco)	*A. nodosum*	(FS) Seaweed dust																+	Higher initial nitrogen content in leaves reflected as increased alkaloids.	Aitken & Senn, 1965.
		(SD)	1:25 to 1:50															+	Increased respiratory activity in seeds.	
	A. nodosum	(SM)	500 lb $acre^{-1}$ 100 lb $acre^{-1}$															-	Significant reduction in respiratory activity in leaves.	Senn & Skelton, 1969.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Pennisetum typhoids (Pearl Millet)	Brown seaweeds (manure)	(SM)	5 & 10 kg N acre^{-1}	+						+						+			N & phosphoric acid content increased. All parameters increased further in combination with fertilizer.	Bokil et al., 1972.
Persea americana *Persia gratissima* (Avocados)	S.M.3 Marinure	(FD)	2.5-30 ppm kinetin equivalents					0											No significant effect on ripening time.	Blunden et al., 1978.
Phaseolus vulgaris (Bean) cv. Wintergreen	Kelpak	Leaf wetted	1:100															+		
			1:500															+	SWC did not inhibit the uptake of a selective broad-leaf herbicide (MCPA).	Erasmus et al., 1982.
Seedlings	Agral	(FS)	1:500							+									Significant increase in calcium content.	De Villiers et al., 1983.
	Kelpak	(FS)	1:500			+	+					+		+		+		+	Seaweed extract in combination with a fertilizer increased all parameters further. Significant increase in cytokinin levels.	Featonby-Smith & van Staden, 1984a.
cv. Galamor	*Macrocystis integrifolia*	(SM)	7.5 to 60 t ha^{-1}	+	+					+									Increasing seaweed concentration resulted in significant increases in N, K, Cl, Fe & Zn concentrations.	Temple & Bomke, 1988.
			120 t ha^{-1}	-							-								Reduced seedling emergence and yield.	
cv. Galamor	SeaSpray	(FS)	4 l ha^{-1}	+	+														Kelp concentrates	Temple &

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
	M. integrifolia (concentrate)		2 l ha^{-1}	+	+														significantly increased yield by 24%.	Bomke, 1989.
		(FS)	4 l ha^{-1}	+	+															
	M. integrifolia (extract)		2 l ha^{-1}	+	+															
	Kelpak	(FS)	1:250 4 l ha^{-1}	+	+															
Plants grown in cv. Galamor 3 soil regimes a) dry soil (-120 to -150 kPa) b) field capacity (-30 to -50 kPa) c) wet soil (0 to -10 kPa)	SeaSpray	(FS)	1:250	+	+	+	+												SeaSpray gave best results when plants grown in dry soils. Kelpak enhanced growth in all soils. Effects on different growth parameters varied with soil regime and applied seaweed.	Temple et al., 1989.
	Kelpak	(FS)	1:250	+	+	+	+													
Pinus alba (White Pine)	*A. nodosum*	(SD)	1:25 to 1:50															+	Significantly increased respiratory activity.	Aitken & Senn, 1965.
Pinus taeda (Loblolly Pine)	*A. nodosum*	(SD)	1:100 1:25 1:5															+ + +	Significantly increased respiratory activity.	Senn & Skelton, 1969.
Pisum sativum (Pea)	*A. nodosum*	(SD)	1:25 to 1:50															+	Significantly increased respiratory activity.	Aitken & Senn, 1965.
	D. antarctica	(SM)	1.25:100	-															Significantly inhibited growth of plants.	Francki, 1964.
Plants grown in fields previously supplied	*M. integrifolia*	(SM)	7.5 to 120 t ha^{-1} Applied previous		-					+									Leaf and stem yields decreased with increasing concentrations of kelp.	Temple & Bomke, 1988.

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
with kelp.			season.																Increase in Na and K concentrations in leaf and stem.	
Prunus armeniaca (Apricot)	Dswe Algifert	(FS)						+											Seaweed extract reduced fruit storage losses from 30% to 12%.	Povolny, 1972.
Prunus domestica (Plum)	Maxicrop	(FS)																	Increase of	Booth, 1974.
cv. Burbank																		+	40%	
cv. Wyedale																		+	62%	
cv. Laxton's Cropper																		+	86% of flowers that reached maturity.	
Prunus persica (Peach)		(FS)						+							+				Reduced incidence of brown rot, from 3.7 to 1.5%. Sixteen days after harvest 32.2% of controls rotten, compared to about 15% from seaweed treated trees.	Driggers & Marucci, 1964.
cv. Sullivan Elbert								+											Significant increase in shelf-life by application of pre-harvest sprays.	Senn & Skelton, 1966.
cv. Rio-Oso -Gem	Sea Born *A. nodosum*	(FS)	1:100					+											Over 50% of control fruit unmarketable; Less than 20% of seaweed treated fruit rotten.	Skelton & Senn, 1969.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
cv. Harvest Gold		(FS)	1:100					+											Seaweed treatment reduced number of rotten peaches by almost 40%.	Skelton & Senn, 1969.
		(SM)	0.9 kg per tree					+												
cv. Jerseyland		(FS)	1:100					+												
cv. Blake		(FS)	1:100					+											Three sprays, at the beginning of the growing season resulted in the greatest extension of shelf-life.	Skelton & Senn, 1969.
	Dwse Algifert	(FS)						+											Reduced storage losses by 35%.	Povolny, 1972.
cv. Harvest Golden	*A. nodosum*	(SM)	0.9 kg per tree					+											Seaweed meal applied to the soil resulted in a 45% decrease in rotten peaches.	Senn & Kingman, 1978.
cv. Van Riebeeck	Agral	(FS)	1:500	0				0		+									Significant increase in phosphorus content.	De Villiers et al., 1983.
Pyrus communis (Pears)	S.M.3 Marinure	(FD)	2.5-30 ppm kinetin equivalents					0											No significant effect on ripening time.	Blunden et al., 1978.
Raphanus sativus (Radish)		(SM)	20 cwt acre^{-1}	0							+								Hastened germination and increased % emergence.	Simpson & Hayes, 1958.
	Laminaria cloustoni Edmondst	(SM)	20 cwt acre^{-1}	0																
	A. nodosum	(SD)	1:25 to 1:50															+	Increased respiratory activity of seeds.	Aitken & Senn, 1965.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
Ribes nigrum (Blackcurrent) cv. Westwick	Maxicrop	(FS)	1:450	+														0	Was not able to reduce damage from sulphur sprays. Sulphur sprays used to control eriophyid gall mite.	Smith & Clarke, 1967.
cv. Baldwin	Maxicrop	(FS)		+												+			Seaweed + nitrogen increased yield by about 20%.	Booth, 1973.
	Maxicrop + Nitrogen	(FS)		+												+			Yield increased by 19.4%. Commercial foliar feeds only increased yield by 7.5%.	Booth, 1974.
Rhododendron maximum	*A. nodosum*	(RD)	1:50; 1:25 for 5 sec.											+					Increased rooting percentage.	Senn & Kingman, 1978.
																		+	Prevented leaf abscission.	
			100%											-					Retarded rooting.	
Secale cereale (Rye)	*E. maxima*	(FS)	1:330		0	+				+						+			Up tp 85% increase in shoot dry weight.	Kotze & Joubert, 1980.
			1:500		+					+						+				
			1:1000		+	+				+						+			Significant increase in CA, Mg, K, & P. Increased uptake of Zn and Cu.	
Sinapis alba (Mustard)	S.M.3	(SD)	1:100				+												About 13% yield increase in dry weight; and 60% in wet weight.	Challen & Hemingway, 1966.

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
	L. digitata			+														+	Greater increase in wet weight compared to dry weight.	Blunden et al., 1968.
	L. saccharina		0-2% w/v solids	+																
	A. nodosum			+																
	Fucus versiculosus			+																
	S.M.3	(SD)	1:100	+																Stephenson, 1968.
Solanum melongeria (Aubergines)	S.M.3 Marinure	(FD)	2.5-30 ppm kinetin equivalents					0											No significant effect on ripening time.	Blunden et al., 1978.
Solanum tuberosum (Potato)			10 gal $acre^{-1}$	+						+									Increase in N, P, k, Ca, Mg and Fe content.	Booth, 1966.
cv. La Soda	S.M.3	(FS)	1:100	+															Yield increased by 37%.	Blunden, 1972.
cv. King Edward	S.M.3	(FS)	1:100 1:200 ($1122\ l\ ha^{-1}$)	+												+			Highly significant increases in yield when seaweed applied in combination with fertilizer (15:15:19).	Blunden & Wildgoose, 1977
cv. Pentland Dell				0																
cv. van de Plank	Kelpak	(TC)	1;250 1:500															+ +	The SWC enhanced the growth of the plantlets and induced tuber formation.	Crouch, (Chapter 3)
Tagetes patula Janie (Dwarf Marigold)	Kelpak	(FS)	2cm 1:1		+	+	+											+	Increased number of flowers and plant size.	Aldworth & van Staden, 1987.
Triticum aestivum		(FS)	1:100				+													Blunden et al.,

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES		Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
(Wheat)		*L. Saccharina*																			1968.
				1:100 (ashed)				+												Ashed extract significantly increased wet and dry weight.	
(Winter Wheat)																					
	cv. Cappelle-Desprez	Maxicrop	(SF)	3 l acre^{-1}	+															Yield increase of 8%	Stephenson, 1974.
				1 gall acre^{-1}	+															Yield increase of 10.7%	
(Wheat)	cv. Inia	Kelpak	Leaves wetted	1:100															0	Effect of SWC on the uptake of a selective broad-leaf weed killer SWC did not promote uptake.	Erasmus et al., 1982.
				1:500																	
	cv. Inia	Kelpak	(FS)	1:100	+														+	SWC increased culm thickness by more than twice that of controls. Result of increased cell size.	Nelson & van Staden, 1984b.
			(SF)	1:100	+														+		
			(SF)	1:500	+														+		
	cv. Inia	Kelpak	(FS)	1:250	+															Water stressed conditions for all treatments.	Mooney & van Staden, 1985.
			(FS)	1:500	0																
			(FS)	1:750	0		+														
			(FS)	1:250	+															Undroughted conditions for all treatments.	Mooney & van Staden, 1985.
			(FS)	1:500	0																
			(FS)	1:750	0		+														
			(SF)	1:100										+							
	cv. Inia	Kelpak	(FS)	0.01 cm		0	+	0			+			0						Significant increase in the number of spikelets; kernels; culm diameter and length and kernel nitrogen content.	Nelson & van Staden, 1986.
				0.05 cm		+	+	+			+			+					+		
				1cm	+	+	+	+			+			+					+		
				2cm		+	+	+			+			+					+		

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
cv. SST 66	Kelpak	(RF)	1:400	+									+						the SWC had no effect on the yield of wheat receiving an adequate nutrient supply but significantly increased the yield of nutrient stressed plants.	Beckett & van Staden, 1990b.
		(RF)	1:400 (ashed)	+									+							
cv. SST 66	Kelpak	(RF)	1:400	+									+	+					The SWC had no significant effect on the yield of wheat receiving an adequate K supply, but significantly increased the yield of K stressed plants.	Beckett & van Staden, 1989.
		(RF)	1:400 (ashed)	+									+	+						
Vicia faba (Broad Beans)	*A. nodosum*														+				Wingless aphids reproduced less on seaweed treated leaves.	
Vitex agnus-castrus	Kelpak	(CD)	1:10											+					Initiated over 300% more roots on cuttings.	Crouch & van Staden, 1990a.
Vitis vinifera (Grape) cv. Pinot Meunier	Maxicrop	(FS)		+												+			Significant increase in yield.	Booth, 1973.
cv. Pinot Meunier	Maxicrop	(FS)	Sprayed over 2 seasons	+															Yield increased by 12 %.	Stephenson, 1974b.
(Champagne Wine)			Only 1 season	+															Yield increased by 7.8%.	

Table 1.1 The effect of applied commercial seaweed products on plant growth (Continued)

PLANT SPECIES		Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
		A. nodosum	(FS)		+															Significant increase of soluble solids.	Senn & Kingman, 1978.
	cv. Barlinka cv. Alphonse Lava	Agral	(FS)	1:500	0						0									No effect on mineral nutrition	De Villiers et al., 1983.
	cv. Cardinal	Goemill *A. nodosum*	(FS)	8.5 ml l^{-1}															+	Absence of major structural aberrations and non-toxicity of product at recommended doses. Cells have responded to treatment and observed effects appear to be reversible.	Pellegrini et al., 1987.
Zea mays (Maize, Corn)		*A. nodosum.*	(SM)	50 kg ha^{-1} 100 kg ha^{-1}	+									+						Significant increase in: yield; sugar content, number of of ears per plot; and mass of ears.	Aitken & Senn, 1965 Senn & Kingman, 1978.
	cv. PNR 473	Kelpak	(RF)	1:300				-										+		*Pratylenchus zeae* populations 21.5 - 31.2% lower when treated with SWC.	De Waele et al, 1988.
			(RF)	1:600				0										+			
			(RF)	1:1200				+										+			
			(TC)	1:100											0			+		SWC significantly suppressed the reproduction of *Pratylenchus zeae* by 47.1-63.1%.	De Waele et al, 1988.
			(TC)	1:200											0			+			
			(TC)	1:500											0			+			
Sweet Corn	cv. Golden Bantam	S.M.3	(FS)	5.6 l ha^{-1}	+														+	Seaweed application resulted in a 42% increase in tassled	Blunden, 1972.

Table 1.1 **The effect of applied commercial seaweed products on plant growth (Continued)**

PLANT SPECIES	Name	Application	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	COMMENTS	REFERENCES
																			ears and a 56% increase in ripe ears.	
Zinnia elegans (Zinnia)	*A. nodosum*	(SD)	1:25 to 1:50															+	Increased respiratory activity in seeds.	Aitken & Senn, 1965.
Miscellaneous Kentucky blue-grass	*A. nodosum*	(SM)	50 kg ha^{-1}												+				*Fusarium roseum* tricinctum reduced by 48%.	Darrah & Hall, 1976.
Perennial ryegrass fairway turf	*A. nodosum*	(SM)	50 kg ha^{-1}														+		*Paratylenchis sp.* (Pin nematode) reduced by 98%.	Darrah & Hall, 1976.
																	+		Decrease in nematode *(Pratylenchis sp.)* populations.	
Grass	Algistim	(FS)	11.22 l ha^{-1}															+	Significant increase in crude protein content.	Blunden, 1977.
Established turf	Maxicrop	(FS)	5 pts $acre^{-1}$ (1:30)	+															Yield incease of 15%. Effect reduced with time.	Stephenson, 1974b.
Paddock	Maxicrop	(FS)	8 pts $acre^{-1}$ (1:25)							+									Improved mineral nutrition.	Stephenson, 1974b.
Fruit Orchard	Maxicrop	(FS)	1:50												+				Significantly reduced Red spider mite	Stephenson, 1966.

1.4 Chemical Composition of Seaweeds

Table 1.2 lists the typical chemical analyses of three commercially available seaweed products (Kelpak 66, Maxicrop and Seasol). Kelpak 66 is prepared from the brown alga *Ecklonia maxima* while both Maxicrop and Seasol from *Ascophyllum nodosum*.

Marine algae contain all major and minor plant nutrients (STEPHENSON, 1968) and trace elements (BOOTH, 1964b; YAMAMOTO & ISHIBASHI, 1972; YAMAMOTO, OTSUKA, OKAZAKI & OKAMOTO, 1979). In view of the fact that seaweed contains these mineral elements, their possible involvement in the observed growth responses cannot be disregarded. However, the presence of these inorganic constituents as a possible explanation for improved plant growth is not adequate because the amount of seaweed applied to crops contain too few of these elements to elicit the beneficial responses that have been observed (BLUNDEN, 1977). TEMPLE & BOMKE (1989) suggested that seaweed application to crops could however, supply an amount of limiting nutrient to correct a marginal deficiency.

At least seventeen of the common amino acids are reported to occur in seaweeds (COULSON, 1953; FOWDEN, 1962; PELLEGRINI, 1968, 1969; HUVE & PELLEGRINI, 1969; MUNDA & GUBENSEK, 1975), of which aspartic acid, glutamic acid and alanine are known to be present in species of commercial importance. Marine algae are rich in carbohydrates which act as chelating agents. Alginic acid, laminarin and mannitol contained in commercial seaweed preparations represent nearly half of the total carbohydrate content. Seaweeds also support a wide range of vitamins (HUNDIN & ERICSON, 1956; TEERI & BEIBER, 1958; KANAZAWA, 1963; GÜVEN, GÜLER & YÜCEL, 1976) including vitamins C, B_1 (thiamine), B_2 (Riboflavin), B_{12}, D_3, E, K, niacin, and pantothenic, folic and folinic acids. Although vitamin A is not present, the presence of its precursor ß-carotene and another possible precursor, fucoxanthin has been demonstrated (STEPHENSON, 1968).

Table 1.2 Typical chemical analyses of three commercially available seaweed products.

Constituents	Kelpak 66 (mass per cubic decimeter)	Maxicrop Multiple Concentrate	Seasol Liquid (OM content > 14.5 pH 9.0 -10.5)
Macro/Micro Nutrients			
Protein	3g	-	-
Carbohydrates (alginates, laminarin, mannitol)	16.9g	-	-
Nitrogen	3.6g	3.5g	1.80%
Phosphorus	8.2g	1.2g	0.18%
Potassium	7.2g	5.5g	2.55%
Barium	1.9g	-	-
Boron	0.24g	11mg	0.5ppm
Calcium	800mg	500mg	0.20%
Cobalt	.3mg	14mg	-
Copper	.2mg	50mg	54ppm
Fluorine	.4mg	-	-
Iodine	8.6mg	2.5g	-
Iron	13.6mg	1.1g	24ppm
Magnesium	200mg	1.4g	0.16%
Manganese	8.4mg	44mg	3ppm
Molybdenum	0.38mg	14mg	3ppm
Nickel	0.34mg	24mg	-
Sodium	800mg	-	-
Strontium	0.4mg	-	-
Sulphur	0.64mg	1.4g	0.14%
Zinc	4.2mg	180g	15ppm
Vitamins			
B_1	0.08mg	-	-
B_2	0.08mg	-	-
C	20mg	-	-
E	0.68mg	-	-
Amino Acids			
Alanine	280mg	-	-
Valine	150mg	-	-
Glycine	140mg	-	-
Isoleucine	92mg	-	-
Leucine	180mg	-	-
Proline	184mg	-	-
Threonine	152mg	-	-
Serine	208mg	-	-
Methionine	72mg	-	-
Hydroxyproline	36mg	-	-
Phenylalanine	8mg	-	-
Aspartic Acid	316mg	-	-
Glutamic acid	20mg	-	-
Tyrosine	332mg	-	-
Ornithine	20mg	-	-
Lysine	272mg	-	-
Arginine	16mg	-	-
Growth Regulators			
Auxins	-	-	-
Cytokinins	0.031mg	-	-
Gibberellins	-	-	-

Apart from the organic and inorganic constituents listed above, evidence suggests the presence of substances of a more stimulatory and antibiotic nature. RHINEHART & SHIELD (1978), HOPPE & LEVRING (1982) and FENICAL (1982) have recently reviewed the work on antibiotic substances obtained from marine algae. Antibacterial activity (SCREENIVASA RAO & PAREKH, 1981; PESANDO & CARAM, 1984; PADMINI SCREENIVASA RAO, SCREENIVASA RAO & KARMARKAR, 1986, 1988; OKAMI, 1982) as well as antiviral activity (EHRESSMANN, DEIG, HATCH, DISALVO & VEDROS, 1977; SCREENIVASA RAO & SHELAT, 1982; PESANDO & CARAM, 1984) is also well documented for seaweeds.

The rate of application of seaweed extract is low in terms of solid content. The active compound or compounds must therefore be effective in very low concentrations (BRAIN, CHALOPIN, TURNER, BLUNDEN & WILDGOOSE, 1973). Various organic compounds known to elicit strong physiological responses in low doses are the plant growth regulators (PGR's).

1.5 Plant Growth Regulatory Activity of Seaweeds

There has been much speculation about the amount and type of growth regulatory substances, especially plant growth hormones, which exist in seaweeds. Much of this speculation arose due to observations made when bioassays, performed to determine the presence of plant hormone-like substances, resulted in positive responses from seaweed material. In recent years it has been postulated that the presence of endogenous PGR's in commercial seaweed products play a significant role in the expression of beneficial effects.

1.5.1 The occurrence of auxins, gibberellins and abscisic acid in marine algae

Auxins or auxin-like compounds occur endogenously in many marine algae (SCHIEWER & LIBBERT, 1965, SCHIEWER, 1967; AUGIER, 1976a) but their activity in commercial seaweed

products is regarded as low (WILLIAMS, BRAIN, BLUNDEN, WILDGOOSE & JEWERS, 1976). Indole acetic acid (IAA) has been identified (KINGMAN & MOORE, 1982; SANDERSON & JAMESON, 1986) as have some related compounds (SUMERA & CAJIPE, 1981).

The presence of gibberellin-like substances in seaweeds is well documented (BENTLY, 1960; RADLEY, 1961; KATO, PURVES & PHINNEY, 1962; MOWAT, 1963, 1964, 1965; JENNINGS & McCOMB, 1967; JENNINGS, 1968; GUPTA & SHUKLA, 1969; AUGIER, 1974a, 1976b; HUSSAIN & BONEY, 1973; TAYLOR & WILKINSON, 1977; WILDGOOSE, BLUNDEN & JEWERS, 1978). At least two compounds have been recorded that behave like the gibberellins GA_3 and GA_7, although these may be vitamins A_1 and A_4 (STEPHENSON, 1968). A terpenoid, α-tocopherol a major component of the E group of vitamins, may mimic gibberellin activity (JENSEN, 1969; GOPOLA, 1984).

In 1973, HUSSIAN & BONEY demonstrated the presence of water soluble growth inhibitors in *Laminaria digitata* and *A. nodosum*. Gas-liquid chromatographic (GLC) analysis of these substances showed, in one case, properties similar to abscisic acid (ABA). The occurrence of ABA in *A. nodosum* has since been confirmed by KINGMAN & MOORE (1982).

1.5.2 The occurrence of cytokinins in marine algae

Most crop responses to seaweed are thought to be due primarily to cytokinins, a diverse group of plant hormones that influence cell division (BOOTH, 1966). The detection of cytokinin-like activity in several commercial seaweed products supports this view.

The occurrence of cytokinin-like substances in algae is very well documented (BENTLY-MOWAT & REID, 1968; HUSSIAN & BONEY, 1969; JENNINGS, 1969a; AUGIER, 1972, 1974a, 1974b; AUGIER & HARADA, 1972, 1973; VAN STADEN & BREEN, 1973; MOONEY & VAN STADEN, 1986). Recently MOONEY & VAN STADEN (1987) showed the presence of several cytokinins in the brown alga *Sargassum heterophyllum* (Turn.) J. Ag.. Using high performance liquid chromatographic (HPLC) analysis *trans*-zeatin, *trans*-ribosylzeatin,

dihydrozeatin, and iso-pentenyladenine were tentatively identified. Cytokinin-like activity in seawater has also been demonstrated (PEDERSON & FRIDBORG, 1972; KENTZER, SYNAK, BURKIEWICZ & BANAS, 1980). PEDERSON (1973), using combined gas chromatography-mass spectrometry (GC-MS) techniques, identified a cytokinin present in seawater taken from the *Fucus* - *Ascophyllum* zone as 6-(3-methyl-2-butenylamino) purine.

1.5.3 The occurrence of cytokinins in commercial seaweed preparations

BRAIN, CHALOPIN, TURNER, BLUNDEN & WILDGOOSE (1973) were the first to detect high cytokinin-like activity in a commercially available seaweed extract (S.M.3). They detected cytokinin-like substances by *in vitro* growth of carrot explants in a cytokinin free medium containing the seaweed extract. Results indicated that seaweed extract has a cytokinin activity capable of producing physiological change, even when applied at the low concentrations used under field conditions.

The presence of cytokinin-like substances in commercial seaweed preparations has been confirmed both by bioassay and analytical instrumentation (Table 1.3). BLUNDEN & WILDGOOSE (1977) using the radish leaf bioassay found the cytokinin-like activity of seaweed extract (S.M.3) to be equivalent to 125 mg l^{-1} kinetin in aqueous solution. In 1978, BLUNDEN, JONES & PASSAM detected cytokinin-like substances in three commercial seaweed extracts (S.M.3, Marinure and Algistim). Combining different solvent techniques with GLC, KINGMAN & MOORE (1982) revealed the presence of several plant growth regulators including cytokinin-like compounds in an *Ascophyllum* product. FEATONBY-SMITH & VAN STADEN (1983b, 1984a, 1984b) and FINNIE & VAN STADEN (1985) demonstrated that an *Ecklonia* product (Kelpak 66) contained compounds with properties similar to zeatin. Using a mass spectrometric stable isotope dilution method, TAY, MACLEOD, PALNI & LETHAM (1985) and TAY, PALNI & MACLEOD (1987) identified and quantified several cytokinins in a seaweed extract (Seasol). These included *trans*-zeatin, *trans*-zeatin riboside and their dihydro derivatives; iso-pentenyladenine; iso-pentenyladenosine and several cytokinin glucosides.

Table 1.3 **Cytokinins in Commercial seaweed preparations. (PC = paper chromatography, HPLC = high performance liquid chromatography, GLC = gas liquid chromatography, NMR = proton nuclear magnetic resonance.**

Commercial Seaweed Preparation	Detection of activity and cytokinin-like compounds	Bioassay	Phytochemical methods						References
			Purification by filtration or fractionation	PC	HPLC	GLC	MS	NMR	
Sea Magic 3 (S.M.3) Species of Laminariaceae & Fuciaceae	Indications that the commercial aqueous seaweed extract contains compounds of a cytokinin nature.	a) Promotion of growth *in vitro* of carrot explants in a cytokinin-free medium b) Examination of growth promoting activity on a cytokinin requiring strain of *Atopa belladonna* c) Radish leaf bioassay (BENTLY-MOWAT & REID, 1968)							BRAIN, CHALOPIN, TURNER, BLUNDEN & WILDGOOSE, 1973.
S. M. 3	Close correlation between results achieved with the use of kinetin and a seaweed extract of equivalent cytokinin activity on the yield of potatoes.	Radish leaf expansion bioassay with kinetin as the reference compound (KURAISHI & OKUMURA, 1956).							BLUNDEN & WILDGOOSE, 1977.

Table 1.3 (Continued)

Commercial Seaweed Preparation	Detection of activity and cytokinin-like compounds	Bioassay	Phytochemical methods						References
			Purification by filtration or fractionation	PC	HPLC	GLC	MS	NMR	
S.M.3	All three compounds were shown to induce cytokinin-like growth.	Radish leaf expansion bioassay with kinetin as the reference compound (KURASHI & OKUMARA, 1956; BENTLY-MOWAT & REID, 1968).							BLUNDEN, JONES & PASSAM, 1978.
Ascophyllum nodosum (used in the preparation of Maxicrop).	Separation of some free cytokinins, purine bases and their ribosides (in addition to ABA and IAA).		+			+			KINGMAN & MOORE, 1982.
Kelpak 66 *Ecklonia maxima*	Cytokinin-like activity co-chromatographed with zeatin and ribosylzeatin. Tentative identification of *cis*- and-*trans*-ribosylzeatin, *trans*-zeatin, dihydrozeatin N^6 (Δ^2-isopentenyl) adenosine.	Soybean callus bioassay (MILLER, 1968).		+					FEATONBY-SMITH & VAN STADEN, 1983b.
		Soybean callus bioassay (MILLER, 1968).	+	+	+				FEATONBY-SMITH & VAN STADEN, 1984a,b.

Table 1.3 (Continued)

Commercial Seaweed Preparation	Detection of activity and cytokinin-like compounds	Bioassay	Phytochemical methods						References
			Purification by filtration or fractionation	PC	HPLC	GLC	MS	NMR	
	Cytokinin-like activity co-chromatographed with zeatin and ribosylzeatin.	Chromatographed fractions of SWC applied to *in vitro* cultured tomato roots.		+					FINNIE & VAN STADEN, 1985.
Seasol *Durvillea potatorum* (Tasmanian Giant Bull kelp).	Positive identification of: *trans*-zeatin; *trans*-zeatin riboside; their dihydroderivatives; N^6 (Δ^2-isopentenyladenine and isopentenyladenosine).	Soybean callus bioassay (MILLER, 1968).				+	+	+	TAY, MACLEOD, PALNI & LETHAM, 1985.
	Zeatin-O-glucoside; dihydrozeatin-O-glucoside; dihydrozeatin riboside-O-glucoside; zeatin riboside-O glucoside.	Soybean callus bioassay (MILLER, 1968).				+	+	+	TAY, PALNI & MACLEOD, 1987.
Maxicrop	Unequivocal identification of zeatin, dihydrozeatin, isopentenyladenine and isopentenyladenosine.	Tobacco callus bioassay (MURASHIGE & SKOOG, 1962).	+			+	+		SANDERSON & JAMESON, 1986.

Table 1.3 (Continued)

Commercial Seaweed Preparation	Detection of activity and cytokinin-like compounds	Bioassay	Phytochemical methods						References
			Purification by filtration or fractionation	PC	HPLC	GLC	MS	NMR	
Maxicrop (cont.)	Detection of cytokinin glucosides.								
Maxicrop S.M.3 Alginex Seamac	Identification of the following betaines: Glycinebetaine; γ-aminobutyric acid betaine; α-aminovaleric acid betaine.	Microbiological assay procedure based on measuring growth of *Klebsiella pneumoniae*.			+		+	+	BLUNDEN, CRIPPS, GORDON, MASON & TURNER, 1986.

1.6 Possible Explanations for the Stimulatory Activity in Seaweed Products

Initially improved plant growth through seaweed application was attributed to its soil conditioning properties. This may have some standing when seaweed is applied as a meal, but foliar application of seaweed concentrates in very small doses refutes this argument. It is more likely that seaweed products elicit a response by enhancing the uptake of mineral elements from the soil or through the regulatory action of plant growth substances in the seaweed. The magnitude of the growth responses following seaweed application suggest a possible additive effect of both nutrient uptake and of plant growth regulatory action.

While the principal active component in seaweed products is unknown, it is likely that several factors each play an important role. Of these, cytokinins have been singled out as of particular significance. The prime physiological responses to cytokinins that might be important in improving plant growth are their effects on protein synthesis and cell division (POZSAR, EL HAMMADY & KIRALY, 1967; MILLER, 1963), on nutrient mobilization, particularly the dominance of various organs for nutrients (LETHAM, 1978), senescence retardation (SHAW, BHATTACHARYA & QUICK, 1965), and inhibition of fungal infection (DEKKER, 1963). BLUNDEN & WILDGOOSE (1977) found close correlations between results obtained from the use of kinetin and a commercial seaweed extract in potato field trials. They suggested that the response elicited by the seaweed involved certain substances with cytokinin-like properties. Seaweed application is also known to increase chlorophyll content, fresh mass and leaf area of treated plants (FEATONBY-SMITH & VAN STADEN, 1983b). The application of a synthetic cytokinin (benzyladenine) to plant leaves (NAITO, TSUJI & HATAKEYAMA, 1978) has been shown to give similar results.

1.6.1 Improved root growth

A pronounced effect of seaweed application to plants is the development of a vigorous root system, which is often expressed as higher yields (BLUNDEN &

WILDGOOSE, 1977; BLUNDEN, WILDGOOSE & NICHOLSON, 1981; FEATONBY-SMITH & VAN STADEN, 1983a, 1984b; NELSON & VAN STADEN, 1984a). MILTON (1964) suggested that the degraded complexes of fucoidin, alginates and similar compounds present in seaweed extracts retain very strong polar groups and therefore enhance soil aggregate and crumb structure. This makes conditions more suitable for root growth. *Ascophyllum nodosum* and other algae commonly used for seaweed extracts contain considerable quantities of phenolic compounds and mannitol (BOOTH, 1969). These substances stimulate root development and exhibit growth promotory properties (POAPST & DUNKEE, 1967; JACKSON, 1965). FINNIE & VAN STADEN (1985) showed that application of low concentrations of seaweed concentrate to tomato (*Lycopersicon esculentum*) roots stimulated root extension and lateral root development. Concentrations of zeatin below 10^{-7} mimicked this effect. Ashing the extract lost this stimulatory effect implying that the regulatory effect is associated with an organic fraction rather than inorganic components. However, as ashing also removes nitrogen (BECKETT & VAN STADEN, 1990b) improved root growth may be a result of available nitrogen in SWC. High concentrations of either zeatin or the seaweed concentrate inhibited root growth. This agrees with the known inhibitory effects of cytokinins on root extension (STENLID, 1982; BIDDINGTON & DEARMAN, 1982/83) and lateral root formation (TORREY, 1976; WIGHTMAN, SCHNEIDER & THIMANN, 1980).

1.6.2 Increased nutrient uptake and changes in plant tissue composition

Improved mineral nutrition in plants using commercial seaweed preparations is well documented (FRANCKI, 1960a, 1960b; OFFERMANS, 1968; AITKEN & SENN, 1965; LYNN, 1972). An increased root system with its larger surface area available for mineral absorption is important, but other ways by which seaweed extracts promote mineral nutrition have been suggested. Research suggests that certain constituents in seaweed may play a role in the chelation of metals to give soluble complexes, thus increasing uptake of trace elements by plants (SENN & KINGMAN, 1978). It is also possible that applied hormones within the extract may act directly on the uptake mechanisms in the roots.

FRANCKI (1960a, 1960b) noted that leaves of tomato plants treated with seaweed meals and sprays contained more manganese than was present in the seaweed itself, concluding that the seaweed had released unavailable manganese from the soil. By adding the seaweed extract to mineral deficient solutions used on green peppers, LYNN (1972) showed improved utilization of boron, copper, iron, manganese, and zinc.

CROUCH, BECKETT & VAN STADEN (1990) found that the application of seaweed concentrate to lettuce plants receiving an adequate supply of nutrients enhanced the uptake of calcium, potassium and magnesium but had little effect on nutrient stressed plants.

Greater availability of nitrogen (BOOTH, 1966; CAIOZZI, PEIRANO, RAUCH & ZUNINO, 1968; SENN & KINGMAN, 1978; BECKETT & VAN STADEN, 1989, 1990b), phosphorus (BOOTH, 1966; CAIOZZI, PEIRANO, RAUCH & ZUNINO, 1968; DE VILLIERS, KOTZE & JOUBERT, 1983); potassium (BOOTH, 1966; BECKETT & VAN STADEN, 1989); calcium (SENN & KINGMAN, 1978; DE VILLIERS, KOTZE & JOUBERT, 1983); manganese (BLUNDEN, 1972); magnesium (SENN & KINGMAN, 1978); iron (BOOTH, 1966; CASTILLO, 1966) and zinc (BECKETT & VAN STADEN, 1990a) to plants after seaweed application has also been recorded.

Precisely how seaweed concentrates promote nutrient uptake is uncertain. The chelating ability of seaweed products can possibly be attributed to the ion exchange properties of constituent polysaccharides. For example, alginate which is composed of D-mannuronic and L-guluronic acid residues, can combine naturally with trace minerals preventing them from settling out, even in alkaline soils (MYKLESTAD, 1964, 1968, 1979). These trace elements therefore remain available to plants.

The role of hormones in the uptake of nutrients is still not fully understood. If cytokinins are the active constituents of seaweed extracts, then the question arises as to how they exert their effect on mineral nutrition if it is not simply a consequence of increasing the absorptive area and vigour of root systems. One possibility is that they may be directly involved with the mechanisms of nutrient uptake by stimulating ATP-ase activity in a way similar to that which has been

reported for auxins (HAGER, MENZIL & KRAUSS, 1971). ATP-ases are enzymes involved with the absorption of cations and anions by the root.

It is well known that plants are able to absorb nutrients through their foliage (KANNAN, 1986). Thus, increased mineral levels in plants treated with foliar applications of seaweed extract may have arisen via direct absorption through the leaves. Foliar application of trace elements is often particularly beneficial, as these elements tend to be immobile in the soil (TISDALE, NELSON & BEATON, 1985). The cuticle is the main barrier to nutrient uptake by leaves. Nutrients probably cross the cuticle by diffusion, and then enter the symplast of the epidermal cells by active uptake or facilitated diffusion (BECKETT & VAN STADEN, 1990a).

1.6.3 Increased resistance to fungal and bacterial diseases and insect attack

Improved mineral nutrition leads to healthier plants that are better at withstanding detrimental attacks by pests. Heightened resistance to fungal, bacterial and insect attack has been observed for a variety of plants treated with seaweed preparations (SENN, MARTIN, CRAWFORD & DERTING, 1961; BOOTH, 1964b; DRIGGERS & MARUCCI, 1964; AITKEN & SENN, 1965; BOOTH, 1966, 1969; STEPHENSON, 1966). Whether mechanisms other than improved vigour are involved cannot be ascertained. It is possible that treated plants are nutritionally unsuited to the development of the pathogens, or that the natural host resistance mechanisms are somehow strengthened thereby preventing the pathogen from invading the host tissue (ABETZ, 1980). Anti-fungal (KHALEAFA, KHARBOUSH, METWALLI, MOSHEN & SERWI, 1975), anti-bacterial (VACCA & WALSH, 1954; STEPHENSON, 1968; BOOTH, 1969) and anti-viral properties (POZSAR, EL HAMMADY & KIRALY, 1967; EHRESSMAN, DEIG & HATCH, 1979) within the seaweeds may also aid in the prevention of the harmful effects of some pathogens.

MITCHELL (1963) showed that laminarin (a component of many brown seaweeds and fungal cell walls) reduced the incidence of soil borne fungal diseases when added to

the soil and might be due to stimulation of hyperparisitic fungi, a form of biological control.

FEATONBY-SMITH & VAN STADEN (1983b) suggested that the cytokinin content in the extract may affect the resistance of plants to pests. Applied cytokinins, while not eliminating the pest itself, apparently allow the plant to increase its resistance to them.

1.6.4 Reduced nematode infestation in plants

There are reports that seaweed application can reduce the incidence of nematode infestation in plants (STEPHENSON, 1968; TARJAN, 1977; TARJAN & FREDERICH, 1983). FEATONBY-SMITH & VAN STADEN (1983b) found that tomato plants treated with seaweed extract resulted in a significant improvement in root growth and reduced root-knot nematode (*Meloidogyne incognita*) infestation. Many reports exist on the role of hormones, and in particular cytokinins, in nematode infestation and development in the roots of susceptible hosts (DROPKIN, HELGESON & UPPER, 1969; KOCHBA & SAMISH, 1971; SAWHNEY & WEBSTER, 1975). DROPKIN, HELGESON & UPPER (1969) found that higher concentrations of kinetin not only decreased larval penetration into the roots of tomato plants, but also inhibited the development of those that entered. BRUESKE & BERGESON (1972) observed that infestation of roots by *M. incognita* resulted in decreased cytokinin levels in the root exudate of tomatoes. FEATONBY-SMITH & VAN STADEN (1983b) suggested that this decrease in cytokinins may be responsible for the observed reduced shoot growth associated with nematode infestation. The application of seaweed concentrate to infected plants might be instrumental in overcoming this imbalance.

1.6.5 Increased fruit and seed yield

The application of commercial seaweed preparations to plants has been shown to increase fruit and seed yield (AITKEN & SENN, 1965; BLUNDEN, 1972; FEATONBY-SMITH & VAN STADEN, 1984a, 1987a, 1987b; NELSON & VAN STADEN, 1986). FEATONBY-SMITH & VAN STADEN (1984a) examined the effect of seaweed concentrate on bean yield and found that fruit of treated plants were significantly larger than fruit from control plants. The fruit from treated plants were also found to contain higher cytokinin levels indicating either an increased translocation of cytokinins from the roots (DAVEY & VAN STADEN, 1978; VONK, 1979), or the production of cytokinins within the fruit themselves (HAHN, DE ZACKS & KENDE, 1974). The increase may have resulted from the translocation of exogenous cytokinins from the seaweed concentrate, applied to the plant as a foliar spray.

Developing fruit require nutrients and metabolites in order to reach maturation (VAN STADEN & COOK, 1986). NOODEN & LEOPOLD (1978) demonstrated that during fruit development, the mobilization centre for photosynthesis shifts away from other organs to the developing fruit. VARGA & BRUINSMA (1974) reported that seeds and fruits have the potential to act as sinks for cytokinins. A build up of these PGR's in the reproductive organs at this time suggests their possible involvement in this process. FEATONBY-SMITH & VAN STADEN (1987a) correlated the effect of a synthetic cytokinin (benzyladenine) and a seaweed extract in fruit production in groundnut. Both treatments resulted in significant increases in the number and size of the fruit and seeds, indicating that cytokinins may be involved in the development of these reproductive plant parts. Exogenously applied cytokinins present in seaweed concentrates, in combination with high levels of endogenous cytokinins in the fruit, may therefore be instrumental in producing stronger physiological sinks and the preferential transport of nutrients into the fruit of treated plants.

1.6.6 Delayed senescence

The accumulation of nutrients and metabolites in a plant organ results in increased growth and delayed senescence. Application of cytokinins to plants increase fruit growth (WEAVER & VAN OVERBECK, 1963), and fruit immersed in cytokinin solutions can exhibit increased shelf life (BLUNDEN, JONES & PASSAM, 1978). Thus, increased cytokinin levels in fruit may contribute to the better quality and extended shelf life observed for seaweed treated crops (SKELTON & SENN, 1969; FEATONBY-SMITH & VAN STADEN, 1984a).

Delayed ripening of fruit through pre-harvest seaweed application has been noted for peaches (DRIGGERS & MARUCCI, 1964; SKELTON & SENN, 1969), apples (POVOLNY, 1969b, 1969c) and apricots (POVOLNY, 1972). BLUNDEN, JONES & PASSAM (1978) investigated the effects of post-harvest dipping of fruit into seaweed solutions and found a significant reduction in the rate of degreening of *Citrus* fruit. They also noted that some seaweed treatments increased fruit senescence rather than delayed it.

1.6.7 Increased seed germination

Seed germination and the breaking of dormancy are two other plant growth regulator effects that have been noted following the application of commercial seaweed preparations. VAN STADEN, OLATOYE & HALL (1973) implicated gibberellins, cytokinins and, occasionally, ethylene in this process. Promotion of germination of table beet (WILCZEK & NG, 1982) and of creeping red fescue grass (BUTTON & NOYES, 1964) by extracts of mixed species of brown algae have been reported. This effect has been attributed to cytokinins since gibberellins rapidly decompose once seaweeds are harvested (METTING, RAYBURN & REYNAUD, 1988).

1.6.8 Beneficial effects conferred by seaweed application that cannot be explained by cytokinins

Despite the probable value of cytokinin content of seaweed extracts, the observed levels of cytokinins are not sufficiently great to produce all the claimed beneficial effects of the extracts (NELSON & VAN STADEN, 1985; TAY, MACLEOD, PALNI & LETHAM, 1985). It also appears unlikely that cytokinins are the only growth substances active in commercial seaweed preparations, particularly in view of all the different physiological responses elicited through seaweed application.

BLUNDEN, ROGERS & BARWELL (1984) found major discrepancies in results obtained for the cytokinin contents of seaweed extracts when bioassayed by different methods. They therefore concluded that they must contain other bio-active compounds. One such group reminiscent of cytokinins are the betaines (WHEELER, 1973), which are considered to aid adaption to osmotic (WYN-JONES & STOREY, 1981) and frost stresses (SAKAI & YOSHIDA, 1968). BLUNDEN, CHALLEN & WOODS (1968) examined four commercially available products (SM3, Maxicrop, Alginex and Seamac) for their betaine content. They found several to be present, although in concentrations too minute to account for all implicated responses. Included was glycinebetaine which has been reported to have a role in frost tolerance (BLUNDEN & GORDON, 1986; BLUNDEN, EL BAROUNI, GORDON, McLEAN, & ROGERS, 1981).

Other effects not attributable to cytokinins include the enhancement of the efficiency of some herbicides and fertilizers (FEATONBY-SMITH & VAN STADEN, 1983a); and increased culm development and subsequent yield of wheat (NELSON & VAN STADEN, 1984b).

ERASMUS, NELSON & VAN STADEN (1982) investigated the effect of seaweed extract on foliar absorption of a radioactively-labelled selective herbicide ^{14}C MPCA (2-methyl-4-chlorophenoxyacetic acid). The applied extract did not appear to promote the uptake of herbicide in narrow-leaved plants (wheat) and did not inhibit the uptake in broad-leaved plants (beans). This indicated that the selectivity of the herbicide was not detrimentally affected when used in combination with the seaweed preparation.

NELSON & VAN STADEN (1984b, 1986) demonstrated that seaweed concentrate applied to wheat significantly increased culm diameter, total number of spikelets per ear and grain yield per ear and per plant. The increase in culm diameter was due mainly to an increase in cell size, particularly within the vascular bundles. BRUINSMA (1982) noted that substances affecting the endogenous gibberellin/ethylene balance produce similar effects. He found that ethylene-producing growth regulators such as DNOC (4,6-dinitro-o-cresol) (BRUINSMA, 1962) and CCC (2-chloroethyl- trimethylammonium chloride) (DE VOS, DILZ & BRUINSMA, 1967) increase the number of grains per ear and to delay senescence of the mature leaves (BRUINSMA, 1962). Similarities between these effects and those obtained with seaweed suggest that the extract may be acting to disrupt the endogenous gibberellin/ethylene balance in favour of ethylene. NELSON & VAN STADEN (1985) confirmed ethylene activity in seaweed treated plants by quantifying the presence of ACC (1-aminocyclopropane -1-carboxylic acid), an ethylene precursor, at 0.01 mmol dm^{-3} in an *Ecklonia* product.

1.7 Standardisation of results

The effects produced through the use of seaweed products varies considerably with the amount applied and with the time of application. If reproducible results are to be achieved through the use of these products, then it is necessary to establish the levels of PGR activity in the various commercial preparations. Because of fluctuating hormone levels, a reliable and simple procedure for routine assay of extracts is a major problem. MOONEY & VAN STADEN (1986) attributed variations within seaweed preparations to several factors:

a) the season during which the plants were harvested (MOONEY, 1983; FEATONBY-SMITH & VAN STADEN, 1984b);

b) stages of development of the algae (JENNINGS, 1969a; FEATONBY-SMITH, 1984);

c) the time during the lunar cycle at which harvesting was carried out (MOONEY & VAN STADEN, 1984a; FEATONBY-SMITH, 1984; HOFMAN, FEATONBY-SMITH & VAN STADEN, 1986);

d) different hormones within a particular group exhibiting different levels of activity (LETHAM, 1978); and

f) deactivation of endogenous growth regulating substances during the extraction process.

Fluctuations in cytokinin levels during the growth cycle have been noted for *Sargassum heterphyllum* (Turn.) J. Ag. (MOONEY & VAN STADEN, 1984b) and *Ecklonia maxima* (FEATONBY-SMITH & VAN STADEN, 1984b). Thus the levels of activity of seaweed preparations may differ in relation to the amounts of hormone present (MOONEY, 1983; MOONEY & VAN STADEN, 1984a, 1984b; FEATONBY-SMITH & VAN STADEN, 1984b). It is also possible that the extracts may contain substantial amounts of growth inhibitory materials that interfere with the bioassays (BLUNDEN, 1977). This would make it difficult to measure biological activity in a particular seaweed product. At present, the soybean callus bioassay (MILLER, 1965) and the radish leaf expansion bioassay (KURAISHI & OKUMURA, 1956; BENTLEY-MOWAT & REID, 1968) are the two systems most commonly used for determining cytokinin activity in seaweed preparations. Nevertheless, the use of accurate chemical assay methods, which are rapid and more reliable is necessary (BLUNDEN, 1977).

Crop responses to seaweed treatment vary considerably with the method, time, frequency and mode of application. Seaweed extract applied as a soil flush, foliar spray, seed dip or fruit dip may vary in degree of effectiveness depending on the age and species of plant under investigation. It is also possible that erratic results through seaweed application may be directly related to the age of the commercial seaweed preparation. In order to standardise seaweed trials, it is essential that these factors must be taken into consideration.

1.8 Conclusion

From the above account it is obvious that there is a vast body of information concerning the beneficial effects of seaweed products on plant growth. Although various compounds have been postulated as being the main active component(s) of seaweed products, no conclusive evidence has been produced for the superiority of one component over the other.

The use of commercial seaweed products in the horticultural sector is fast becoming an accepted practice. As more information about the composition, biological activity, and nutrient compositions of various formulations becomes available, seaweed products will probably be put to wider use in large-scale agriculture.

CHAPTER 2

DETERMINATION OF PLANT GROWTH REGULATORS IN SEAWEED CONCENTRATE AND THE TENTATIVE IDENTIFICATION OF AUXINS IN *ECKLONIA MAXIMA*.

2.1 Introduction

Many of the beneficial results following seaweed application have been attributed to the fertilizer value of the algae (Chapter 1.6.2). BLUNDEN (1977) examined the nutritional value of various kelp extracts and concluded that the quantity of nutrients supplied to crops in foliar sprays could not account for the enhanced plant growth. The small amounts (0.2 to 1.0%) of SWC applied to plants indicate the presence of active compounds with remarkable growth effects. Organic compounds known to have strong physiological effects at very low concentrations are the plant growth regulators. Although the occurrence of endogenous hormones in marine algae has been studied extensively (Chapter 1.5), little research has been conducted to identify and isolate these compounds in seaweed products. While many of the growth responses have been attributed to cytokinins, it is obvious that this group of plant hormones cannot account for all the beneficial effects incurred from the application of seaweed. This chapter examines the presence of plant growth regulators, other than cytokinins, in a SWC.

The analysis of plant growth regulatory substances in algal extracts involves several steps. Initially the extracts are tested for biological activity using bioassay systems. However, a critical assessment of the significance of an active compound depends on the unequivocal identification of that compound. Unequivocal identification can only be made by using physicochemical methods. Further research must therefore

involve accurate and quantitative techniques of sample purification (HPLC) and hormone identification (GC-MS).

The use of bioassays in detecting biological responses in plant extracts is well established. The usefulness of such assays is governed by their specificity; sensitivity; ease in measuring a detectable and relatively fast response; relative ease in setup and control; and absence in the bioassay plant material of the chemical(s) being tested. By indicating the response of a specific tissue, organ, cell or whole plant to the presence of a specific molecule, these assays are used to estimate the levels and/or degree of biological activity of a particular compound (HEUSER, 1988).

Although bioassays are a valuable tool in hormone research, several major problems are associated with their use in quantifying and identifying promotory or inhibitory substances. The following are of importance:

(i) Bioassays measure a developmental response in proportion to the amount of a chemical or substance. To obtain definitive results assay conditions must be strictly controlled.

(ii) Biological variation exists in all bioassay material. To reduce sample variability a considerable number of replications are required which can be tedious and time consuming.

(iii) In bioassays the physiological response to a substance in an impure extract or sample fraction is the sum of promotive and inhibitory responses that result from the various chemical constituents. Such interactions can confound the interpretation of results.

(HEUSER, 1988)

In the following study, specific bioassays were selected to test SWC for the presence of gibberellin, abscisic acid and auxin-like growth substances. As cytokinins and ACC (a precursor of ethylene) have already been reported to occur in Kelpak SWC (FEATONBY-SMITH & VAN STADEN, 1984b; FINNIE & VAN STADEN, 1985; NELSON & VAN STADEN, 1985), their detection was excluded from this investigation. An attempt was therefore

made to tentatively determine gibberellin-, ABA- and auxin-like compounds in SWC with a view to a better understanding of the mode and mechanisms of action of seaweeds on plant growth. As the auxin bioassays showed particularly good biological responses to the SWC, further tests were undertaken to tentatively identify indole-like compounds in both fresh and processed *Ecklonia maxima*. All results were statistically analyzed using a one way analysis of variance (where $P<0.05$).

2.2 Materials and Methods

2.2.1 Seaweed concentrate

The seaweed concentrate used in this study was "Kelpak". Kelpak is manufactured by Kelp Products (Pty) Ltd., Cape Town, Republic of South Africa from the stipes of the brown alga *Ecklonia maxima* (Osbeck) Papenfuss using a cell burst process. This process does not involve the use of heat, chemicals or dehydration which could affect some of the organic components of the concentrate.

2.2.2 Extraction and purification techniques

Extraction of the SWC

The SWC was extracted in 80% methanol (AR grade) for twelve hours at 10°C. The methanol fraction was separated from the cellular debris by filtration through Whatman Nº 42 filter paper and the filtrate reduced to dryness under vacuum at 35°C. The residue was then resuspended in either 100% methanol for storage, or in 100 mℓ phosphate buffer (pH 8.0) for solvent partitioning.

Preparation and extraction of fresh Ecklonia maxima

Freshly harvested *Ecklonia maxima*, flown up from Cape Town at 4°C, was separated immediately into holdfast, stipe and lamina regions. Each plant part was frozen in liquid nitrogen, lyophilised and finely milled. Dry, powdered samples were stored at minus 20°C for further analysis. Exact amounts of the samples were extracted in 80% methanol and prepared for separation and purification as described above for the SWC samples.

Gibberellin extraction and separation:

Extracted SWC was partitioned for gibberellins according to the procedure of HEDDEN (1987) (Figure 2.1). After the separation, five fractions were tested for gibberellin-like activity (Table 2.1).

Auxin and abscisic acid extraction and separation:

Extracted SWC was partitioned for auxins according to the procedure of SANDBERG, CROZIER & ERNSTSEN (1987) (Figure 2.2). (**Abbreviations:** Indole-3-ethanol (IEt), indole-3-methanol (IM), indole-3-aldehyde (IAld), indole-3-acetic acid (IAA), 5-hydroxy-3-indoleacetic acid (5-OH-IAA), 4-chloroindole-3-acetic acid (4-Cl-IAA), indole-3-carboxylic acid (ICA), indole-3-acetylaspartic acid (IAAsp), indole-3-pyruvic acid (IPyA), phenylacetic acid (PAA), indole-3-acetylglucose (IAGluc), 2-*O*-(indole-3-acetyl) *myo*-inositol (IAInos), 5-*O*-ß-L-arabinopyranosyl-2-*O*-(indole-3-acetyl)-*myo*-inositol (IAInos-arabinoside), 5-*O*-ß-L-galactopyranosyl-2-*O*-(indole-3-acetyl)-*myo*-inositol (IAInos-galactoside)).

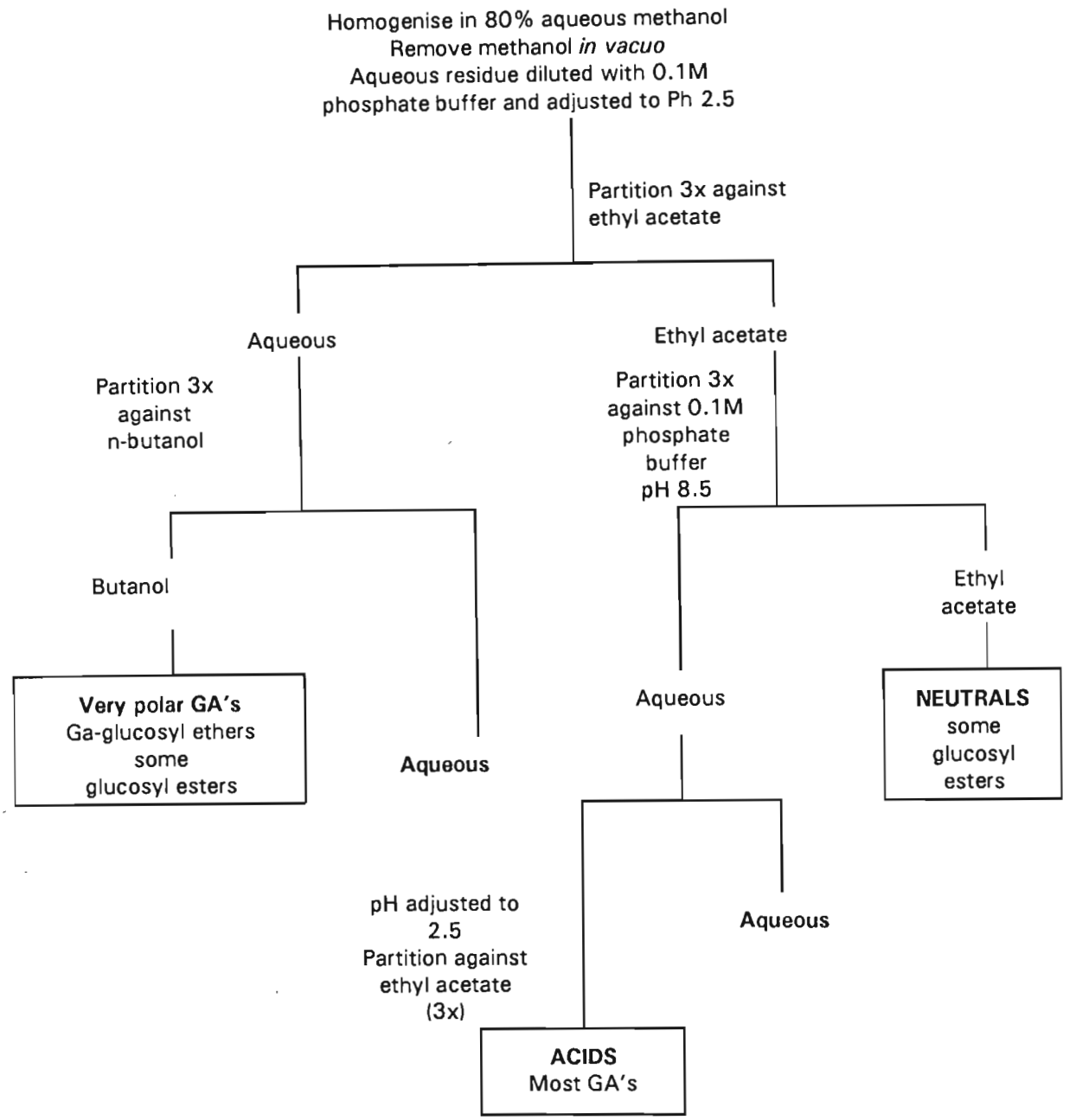

Figure 2.1 Partitioning procedure for the separation of seaweed concentrate into acidic, neutral/basic and 1-butanol-soluble (polar) fractions (HEDDEN, 1987).

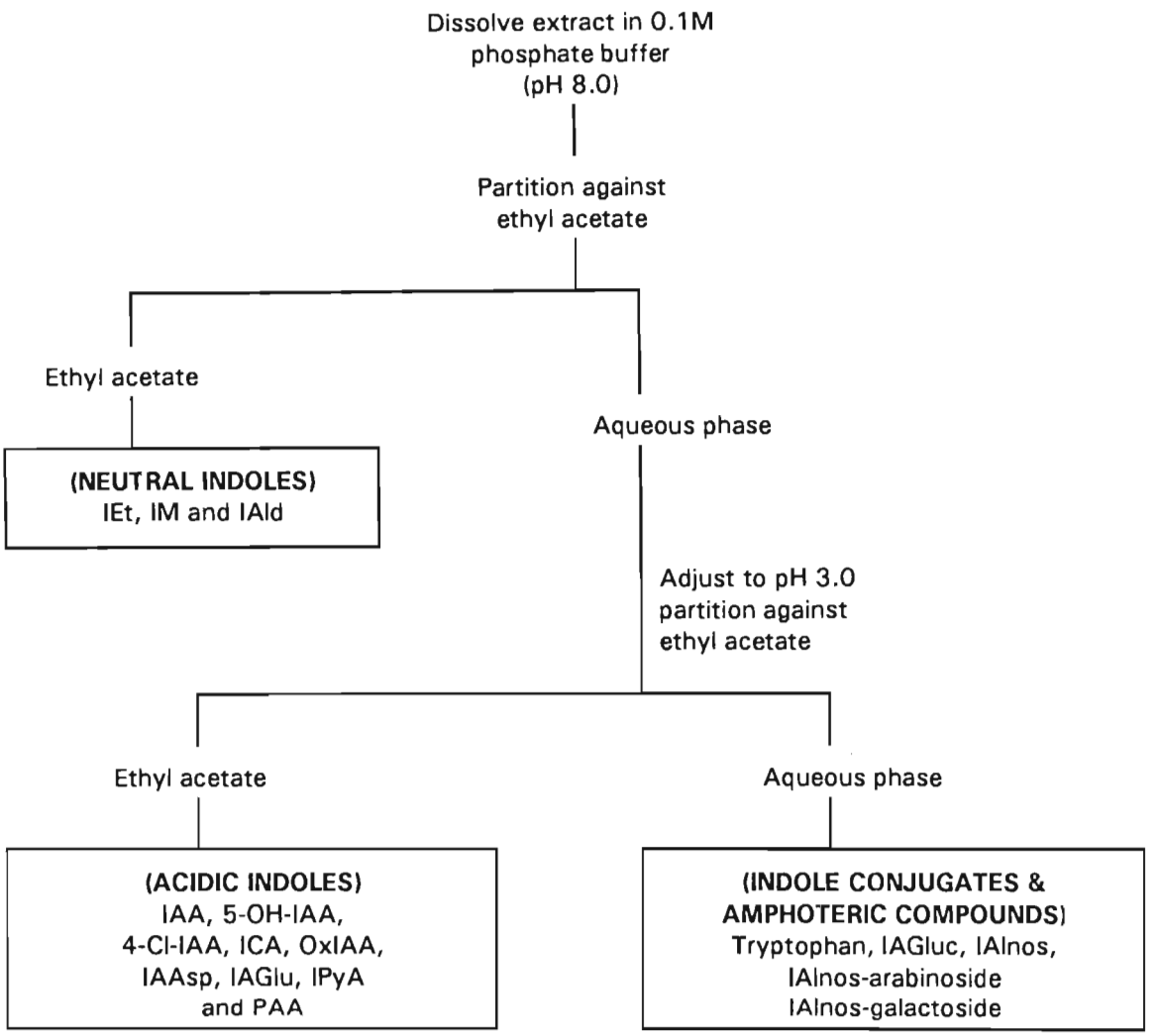

Figure 2.2 Partitioning procedure for the separation of indoles into neutral, acidic and conjugate fractions. Abbreviations in text (section 2.2.1) (SANDBERG, CROZIER & ERNSTSEN, 1987).

2.2.3 Chromatographic techniques

High performance liquid chromatography

Separation of processed and fresh *Ecklonia maxima* extracts was achieved by reversed phase high performance liquid chromatography (HPLC). The column used was a Hypersil 25 x 0.4 cm ODS (C18 bonded, 5 mm particle size) with a flow rate of 1.0 mℓ per minute maintained by a 3 500 p.s.i. single piston reciprocating pump. Absorbance was recorded with a Varian variable wavelength monitor at 280 nm which was fitted with a 8 $\mu\ell$ detector cell. Separation was achieved using a Varian 5 000 liquid chromatogram and the data output recorded using a Vista 4 000 data system. Extracts from the solvent partitioning were redissolved in HPLC grade methanol and 15 or 20 $\mu\ell$ aliquots injected into the chromatograph. The aqueous buffer consisted of 0.2 M acetic acid adjusted to pH 3.5 with triethylamine (LEE, MOK, MOK, GRIFFIN & SHAW, 1985). A linear gradient of methanol:aqueous buffer (20:80 to 80:20 over 45 minutes) was used for separation. Auxin and abscisic acid-like compounds were tentatively identified on the basis of co-chromatography with authentic standards. Aliquots of 1.0 mℓ corresponding to the retention time of the standards were collected for the SWC, air dried, and then assayed for biological activity.

Gas chromatography

Extracted samples were methylated with diazomethane (BLAU & KING, 1977) and analysed using a Varian 3700 gas chromatograph. Data was recorded on a Hewlett Packard 3380A integrator. After two minutes at 140°C, the column temperature was increased at a rate of 3°C minute^{-1} to 265°C and held for 5 minutes. One $\mu\ell$ of sample was injected onto an OV 17 column (2 m x 6 mm x 3 mm Chromosorb WHP, mesh range 80/100). Auxin and ABA-like compounds were tentatively identified on the basis of co-chromatography with authentic standards.

2.2.4 Biological and chemical assay techniques

Dwarf rice microdrop bioassay for gibberellins

The dwarf rice microdrop bioassay was performed as described by MURAKAMI (1968) with slight modifications. The dwarf cultivar 'Tanginbozu' of *Oryza sativa* was chosen because of its sensitivity to a wide range of gibberellins and because it exhibits no endogenous gibberellin-like activity. Seeds were surface sterilised in 0.3% sodium hypochlorite for 20 minutes, rinsed in running tap water for eight hours and left to soak in distilled water for 48 hours at 32°C. The water was changed after 24 hours. Germination was achieved by incubating the seeds on moist filter paper at 32°C. Germinated seeds of uniform coleoptile emergence were selected and a pair placed in each compartment of a repli-dish filled with 0.9% agar. Twenty seeds were planted for each treatment to be tested. The repli-dishes were placed in a plastic tray containing moistened towelling and covered by clear plastic wrap to maintain a high humidity. The trays were kept in a growth chamber with constant light (illumination supplied by cool white fluorescent tubes with a light intensity of 27 μ mole m^{-2} s^{-1}) at 32°C. After 48 hours the seedlings were treated with the test solutions. Five sets of GA_3 standards in the range 10^{-2} to 10^{2} μg ℓ^{-1} were used to construct a standard curve.

Two $\mu\ell$ of each treatment to be tested, containing 0.05% Tween 20 as a surfactant, were then applied with a microsyringe to the axil of the first leaf of each seedling. The repli-dishes were returned to the growth chamber and after 72 hours the length of the second leaf sheath measured. The experiment was repeated to confirm results.

Lettuce hypocotyl inhibition bioassay for ABA

The lettuce hypocotyl bioassay as described by BAKKEN & BOE (1982) was used to detect ABA-like compounds in the SWC. Lettuce seeds (*Lactuca sativa* cv Winter Crisp) were placed in Petri dishes (14 x 1.5 cm) on Whatman Nº 3 filter paper moistened with 15 mℓ of distilled water and incubated in a growth chamber with continuous light (illumination supplied by cool white fluorescent tubes with a light intensity of 27 μ mole m^{-2} s^{-1}) at 24 ± 2°C. After 48 hours seedlings were selected for uniform length and state of development and placed in Petri-dishes (6.0 x 1.5 cm) on Whatman Nº 1 filter paper moistened with three mℓ of test solution. Two replicates of each treatment and 0.001, 0.01, 0.1, 1.0, 10 and 100 μg $m\ell^{-1}$ authentic ABA standards were assayed with 12 plants per replication. Seedlings were incubated for four days in darkness at 24 ± 2°C. After incubation, hypocotyl lengths were measured and recorded.

Avena straight growth test for auxins

Oat seeds with their hulls removed were soaked in distilled water in the dark for two hours. After soaking, the seeds were rinsed two or three times and planted to a depth of 1.5 cm in moist vermiculite. The container was covered and placed in the dark at 26°C with the seeds being exposed to one hour of red light out of every 24. After approximately 70 hours, coleoptiles 20-30 mm in length were transferred to yellow-green light conditions where their tips and bases were removed. The decapitated coleoptiles were then incubated in the solution to be assayed and changes in length after 12 hours recorded. In this assay growth is proportional to the log of the IAA concentration (NITSCH & NITSCH, 1956).

Lettuce root growth test for auxins

For the root growth test, 30 lettuce seeds (*Lactuca sativa* cv Wintercrisp) were placed in 90 mm Pyrex Petri dishes containing two discs of Whatman Nº 1 filter paper and 5 mℓ of test solution. Dish covers were replaced, sealed with parafilm and the seeds incubated under white light (light intensity of 27 μ mole m^{-2} s^{-1}) at 20 to 23°C. The root lengths were measured after 80 hours. Each treatment was represented by three dishes. Growth inhibition at high concentrations and growth promotion at low concentrations is indicative of auxin activity (NITSCH & NITSCH, 1956; RAUB, CARDELLINA & SCHWEDE, 1987).

Mung bean root initiation bioassay for auxins

The SWC, extracted for auxins according to the procedure of SANDBERG, CROZIER & ERNSTSEN (1987) (Figure 2.2), was tested for auxin activity with the mung bean root initiation bioassay.

Using the standard procedure of HESS (1961a), mung beans (*Vigna mungo* L.) were surface sterilized for 20 min in 3.5% sodium hypochlorite, rinsed, then soaked in tap water for 6 hours. The seeds were planted in moist vermiculite in large trays (50 X 40 cm) and allowed to germinate at 26°C in a growth cabinet. After 9 days, uniform hypocotyl cuttings 12 cm in length with two primary leaves but cotyledons removed, were prepared from the seedlings. The cuttings were immediately transferred to vials (90 X 24 mm) filled to a depth of 6 cm with the respective test solutions. Six cuttings were placed in each vial and three vials, arranged in a randomized block, were used for each treatment. The vials were placed 5 cm apart, in trays and were left at 24 ± 3°C at a light intensity of 10.2 μmole $m^{-2}s^{-1}$ for 8 h. After this pulse treatment the bases of the cuttings were rinsed with tap water and the cuttings then transferred to clean vials containing water only, for 8 days, whereafter the number of roots formed were recorded. Counting was facilitated by the fact that roots formed in 4 rows.

Salkowski's Colour test for auxins

Although less sensitive than any of the bioassays, this simple chemical test is useful for detecting moderate levels of natural auxins such as IAA in test solutions. Auxin, in the presence of iron chloride in Salkowski's reagent, forms a red-coloured complex that can be quantitatively measured using a colorimeter (PAECH & TRACY, 1955).

Salkowski's reagent was prepared by adding 300 mℓ of concentrated sulphuric acid to 500 mℓ water followed by 15 mℓ 0.5M ferric chloride solution. The seaweed treatments were prepared by centrifuging 50 mℓ SWC at 3000 rpm for 10 minutes to remove wall debris, and diluting the supernatant liquid phase to the required concentrations. To conduct the colour test, two mℓ of the test solution was added to 8 mℓ Salkowski's reagent, mixed thoroughly and left for exactly 30 minutes. At this time the intensity of the colour was measured using a colorimeter (λ = 510 nm) and auxin concentrations estimated from a calibration curve. A dilution series of IAA over the range 40 mg ℓ^{-1} to 1 mg ℓ^{-1} was used to establish the curve.

2.3 Tentative Detection of Gibberellin and ABA-like Substances in SWC.

The Dwarf rice microdrop bioassay was used to test for gibberellins in the SWC. A lack of endogenous gibberellins in this rice cultivar results in limited shoot extension. The application of exogenous gibberellins to the seeds counter this deficiency and produce a linear curve for a log dose-log response (MURAKAMI, 1968). Abscisic acid-like compounds in the SWC were determined using the lettuce hypocotyl bioassay. In this assay, elongation of the hypocotyls is inversely correlated to the log concentration of ABA (BAKKEN & BOE, 1982). A promotion of hypocotyl extension may be indicative of gibberellins.

2.3.1 Experimental Procedure and Results

The SWC was extracted for gibberellins as outlined earlier (section 2.2.1). After solvent partitioning the five fractions (polar gibberellins, neutral, acidic, basic aqueous discard, and acidic aqueous discard) were tested for gibberellin- and ABA-like activity.

In the dwarf rice microdrop bioassay, a dilution series of authentic gibberellin (GA_3) produced a linear curve for a log dose-log response (Figure 2.3). The SWC treatments invariably promoted growth of the second leaf sheath in the order of 0.001 to 0.01 mg ℓ^{-1} GA_3 equivalents (Figure 2.4). Although these fractions showed more biological activity than non-treated seeds these results were never significant.

Table 2.1 The effect of fractionated SWC on the growth of lettuce seedlings.

TREATMENT	GROWTH RESPONSE
Polar GA's	No hypocotyl growth. Roots rotted, cotyledons expanded and of normal appearance.
Neutral GA's	No hypocotyl growth. Roots necrotic, enlarged cotyledons.
Acidic GA's, ABA	Hypocotyls significantly longer than controls. Seedlings of normal appearance.
Aqueous residue 1	Yellow seedlings. Necrotic roots. No hypocotyl or cotyledon growth.
Aqueous residue 2	Healthy roots. Cotyledons slightly expanded. Hypocotyl growth inhibited.
1.0 % SWC	Hypocotyls significantly longer than controls. Seedlings of normal appearance.

In the lettuce hypocotyl bioassay, a range of authentic ABA concentrations produced a linear curve for a log dose response (Figure 2.5). The morphological effects of the different SWC treatments are recorded in Table 2.1. Although the polar, neutral, and two aqueous fractions (Figure 2.6) all significantly inhibited seedling development

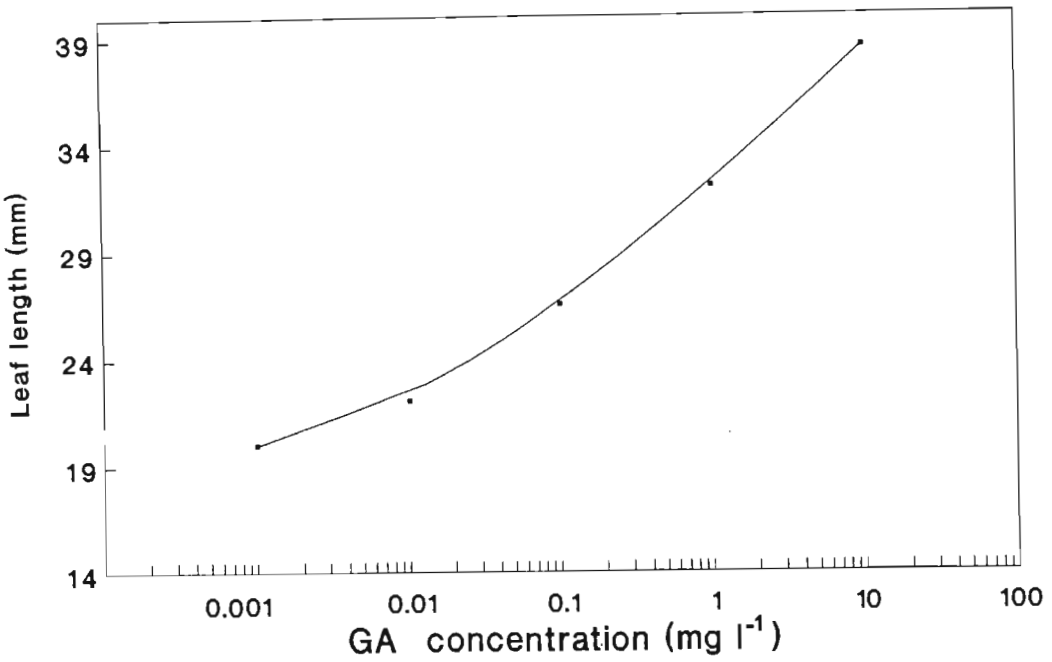

Figure 2.3 The effect of various concentrations of GA, on the growth of the second leaf sheath of dwarf rice seedlings.

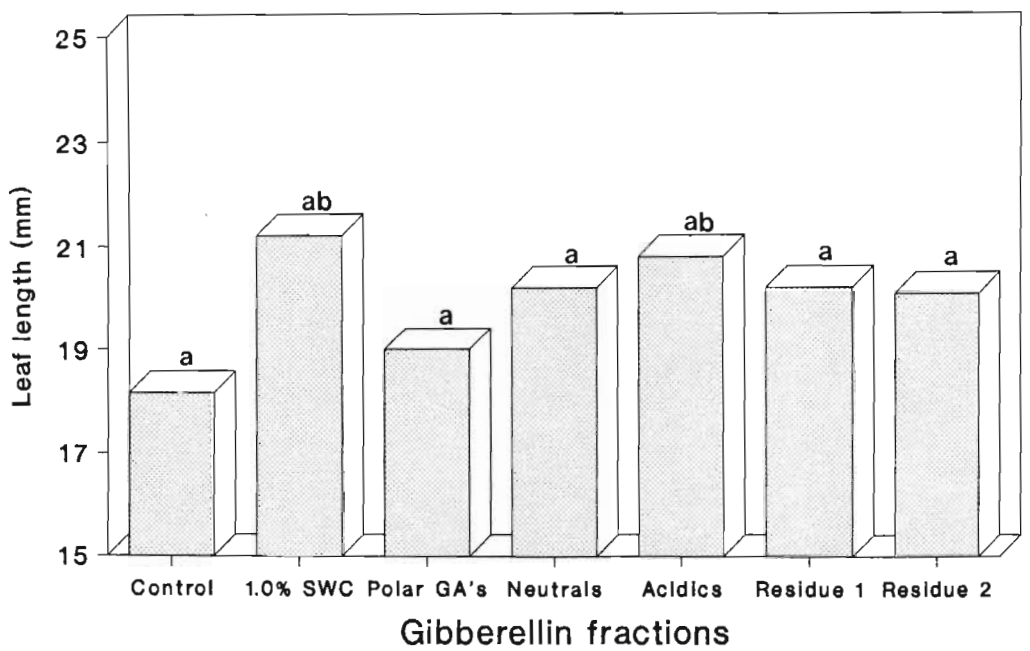

Figure 2.4 Detection of gibberellin-like activity in fractionated and 1.0% SWC. The SWC was solvent partitioned according to HEDDEN (1987) and biological activity determined using the dwarf rice bioassay.

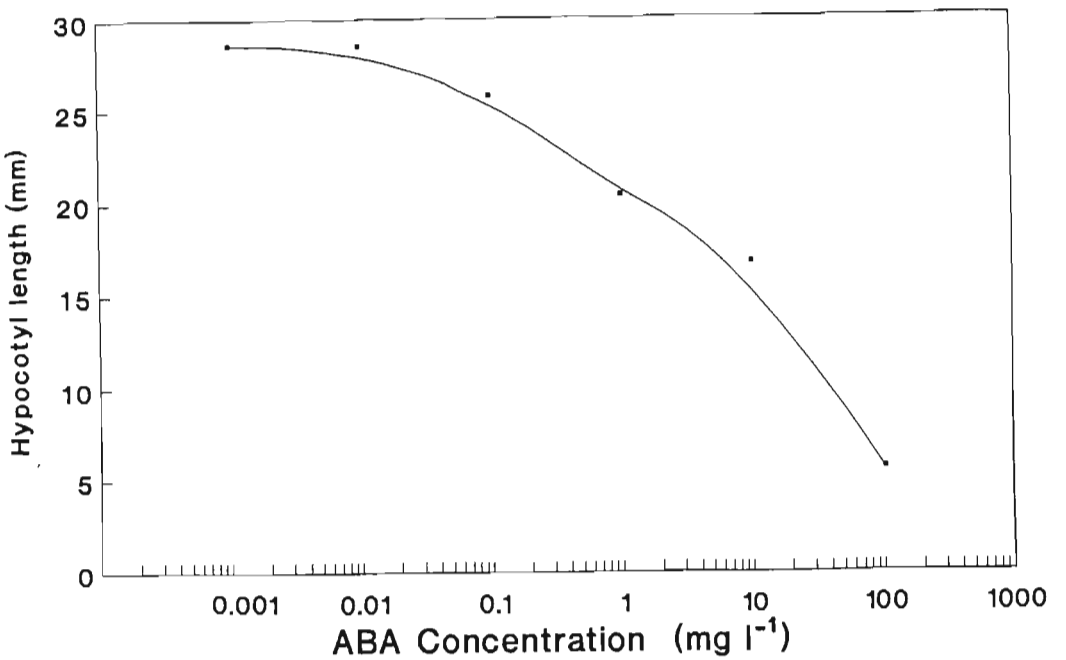

Figure 2.5 The effect of abscisic acid on the inhibition of lettuce hypocotyls four days after treatment.

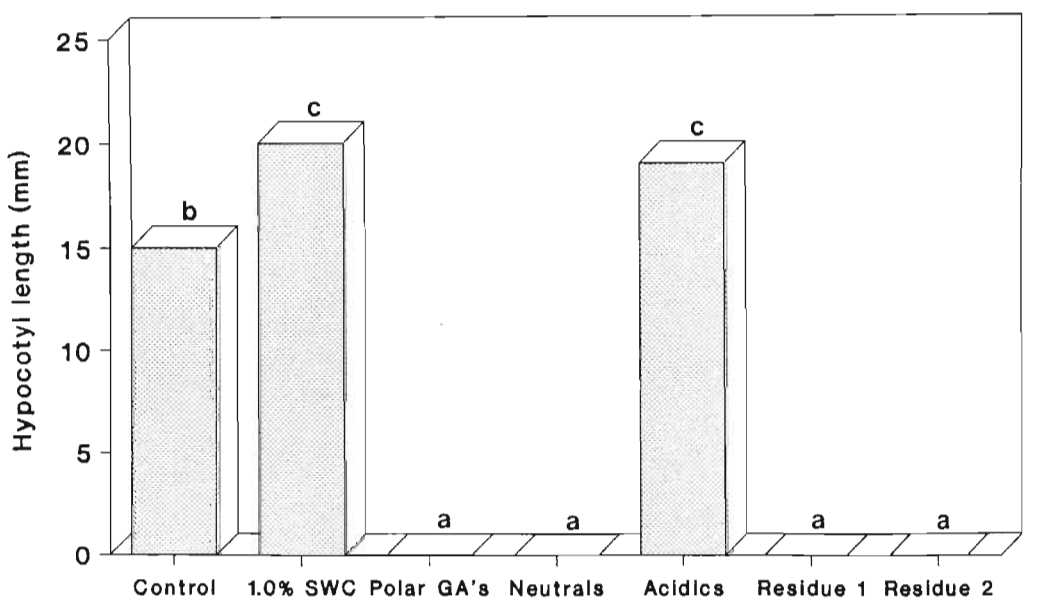

Figure 2.6 Detection of ABA-like activity in fractionated and 1.0% SWC. The SWC was solvent partitioned according to HEDDEN (1987) and biological activity determined using the lettuce hypocotyl bioassay.

and hypocotyl length, only the acidic aqueous fraction was not detrimental to the plant tissue. Hypocotyls of seeds treated with the 'acidic ABA fraction' were significantly longer than non-treated seeds. As this fraction did not show reduced hypocotyl extension, the inhibitory responses noted in the other seaweed fractions are possibly not due to ABA action. This evidence suggests that inhibitors other than ABA may be involved.

To tentatively determine the presence of abscisic acid in the SWC, extracts were purified with high performance liquid chromatography and ABA detected on the gas chromatograph. Both these analytical techniques indicated the presence of compounds that co-chromatographed with ABA.

2.4 Determination of auxin-like compounds in SWC.

Some of the growth responses following seaweed application can possibly be explained by the presence and action of auxins. Several assays were therefore performed to test the SWC for auxin-like activity. These included the *Avena* coleoptile straight growth test, the lettuce root growth test, the mung bean root initiation bioassay, and, a chemical assay (Salkowski's reaction).

2.4.1 Experimental Procedure and Results

With each of these bioassay systems, various concentrations of SWC were compared to a known auxin dilution series.

In the *Avena* coleoptile bioassay, growth of the coleoptiles was proportional to the log of the IAA concentration (Figure 2.7). The SWC almost invariably increased coleoptile length (Figure 2.8) ($P<0.05$), although 10 mℓ ℓ^{-1} was toxic and killed the

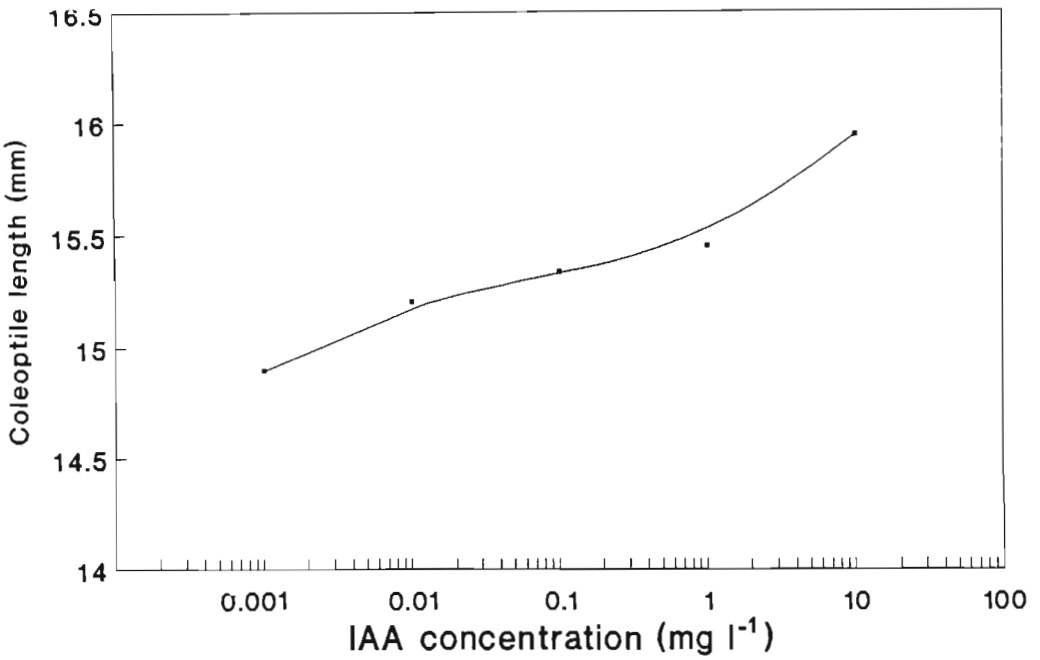

Figure 2.7 The effect of indole-3-acetic acid (IAA) on the extension of *Avena* coleoptiles.

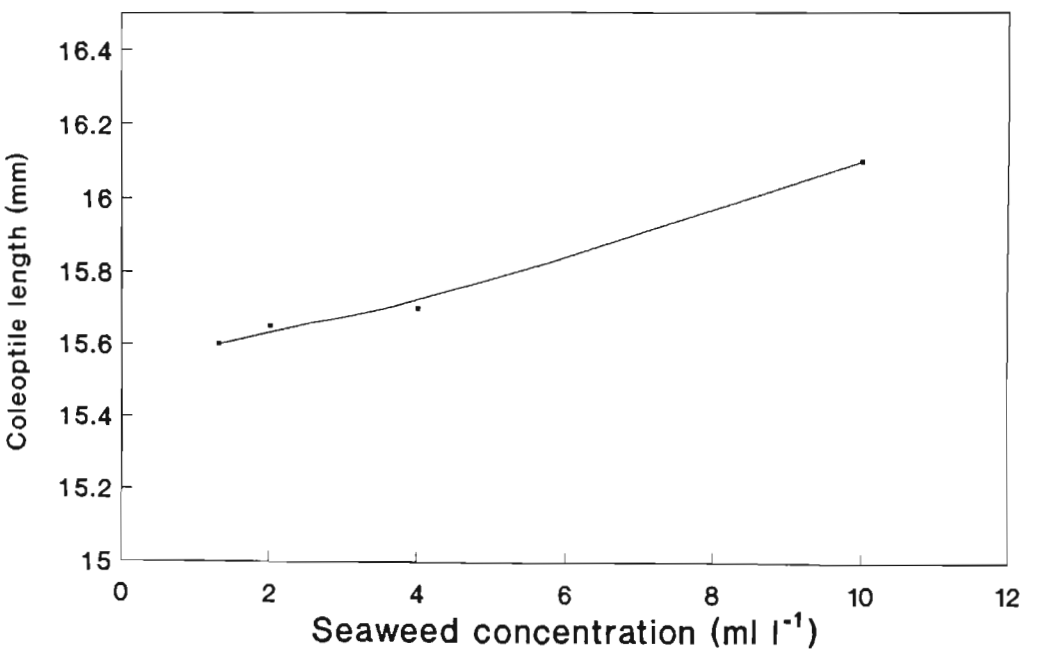

Figure 2.8 Determination of auxin-like activity in SWC using the *Avena* coleoptile bioassay as a test system.

tissue. A concentration of 1.3 mℓ ℓ^{-1} SWC gave an equivalent biological response as 1.0 mg ℓ^{-1} IAA (Figure 2.7).

The results of the lettuce root growth test were not entirely supportive of auxin activity. No concentration of authentic IAA (Figure 2.9) or SWC (Figure 2.10) significantly enhanced root growth although IAA at a concentration of 1×10^{-9} slightly increased root length. Concentrations of SWC above 10% were toxic to the seeds and root growth was severely inhibited. Concentrations below this resulted in healthy seedlings, with reduced root growth. These results suggest that either certain inhibitors are affecting root development or that the SWC contains high levels of auxins.

Table 2.2 Auxin equivalents for SWC as determined by the mung bean rooting bioassay.

	Number of Roots	IBA Equivalents
Control	5	0
50 % SWC	0	N/A
10 % SWC	42	$> 10^{-4}$
1.0 % SWC	17	$> 10^{-4}$

The most pronounced auxin-like responses were detected with the mung bean root initiation bioassay. When treated with a range of IBA concentrations, the mung bean cuttings gave a linear response (Figure 2.11). IAA and 2,4-D (2,4-dichlorophenoxy acetic acid) did not root cuttings. A significant rooting response was obtained with 1.0% and 10% SWC (Figure 2.12). A 50% concentration of SWC was toxic to the cuttings. The result of the above bioassay expressed as auxin (IBA) equivalents is recorded in Table 2.2. Ten percent SWC gave rise to three times as many roots as the most effective concentration of IBA.

Using the mung bean bioassay system, SWC fractions, extracted for indoles as outlined in section 2.1.2, were tested for auxin activity. Most of the root initiation

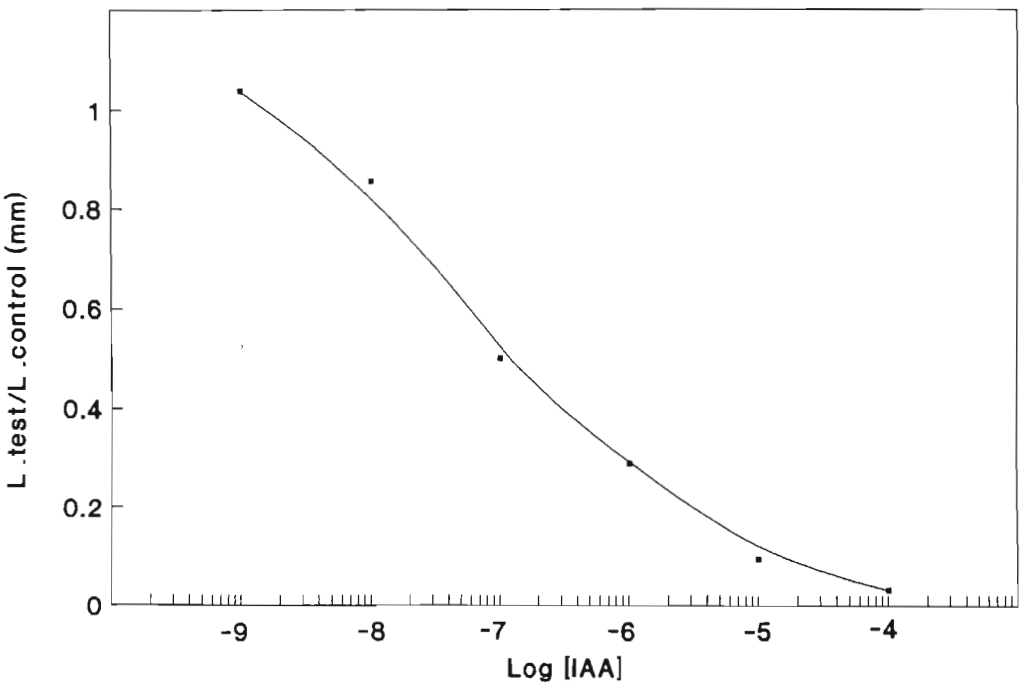

Figure 2.9 The effect of indole-3-acetic acid (IAA) on the length of lettuce roots.

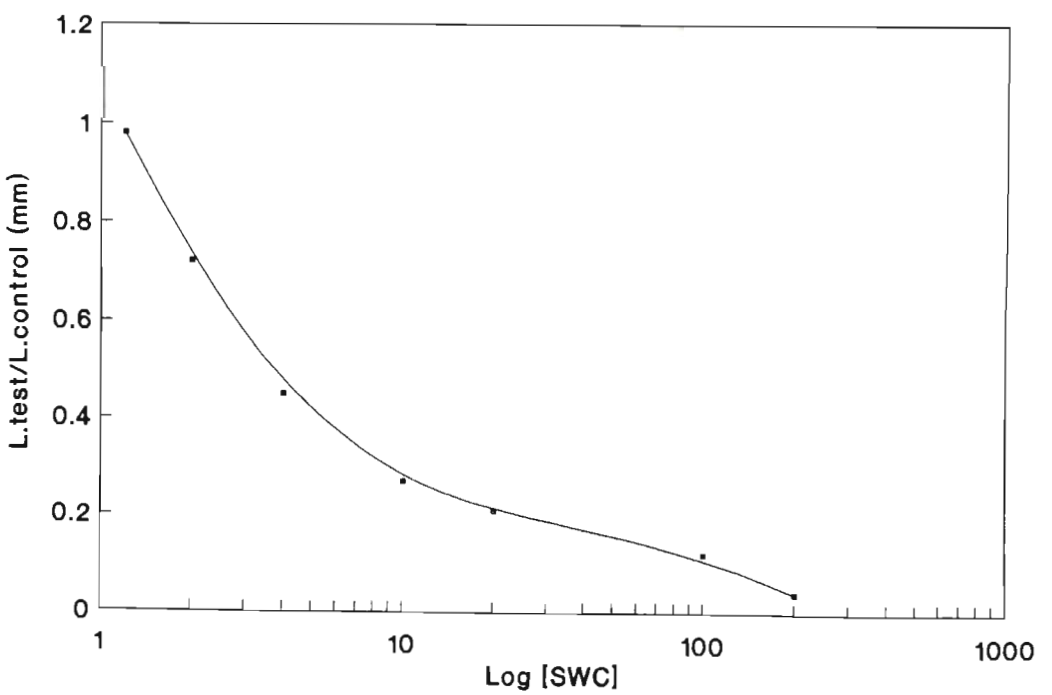

Figure 2.10 Detection of auxin-like activity in SWC using the lettuce root growth bioassay to test for biological activity.

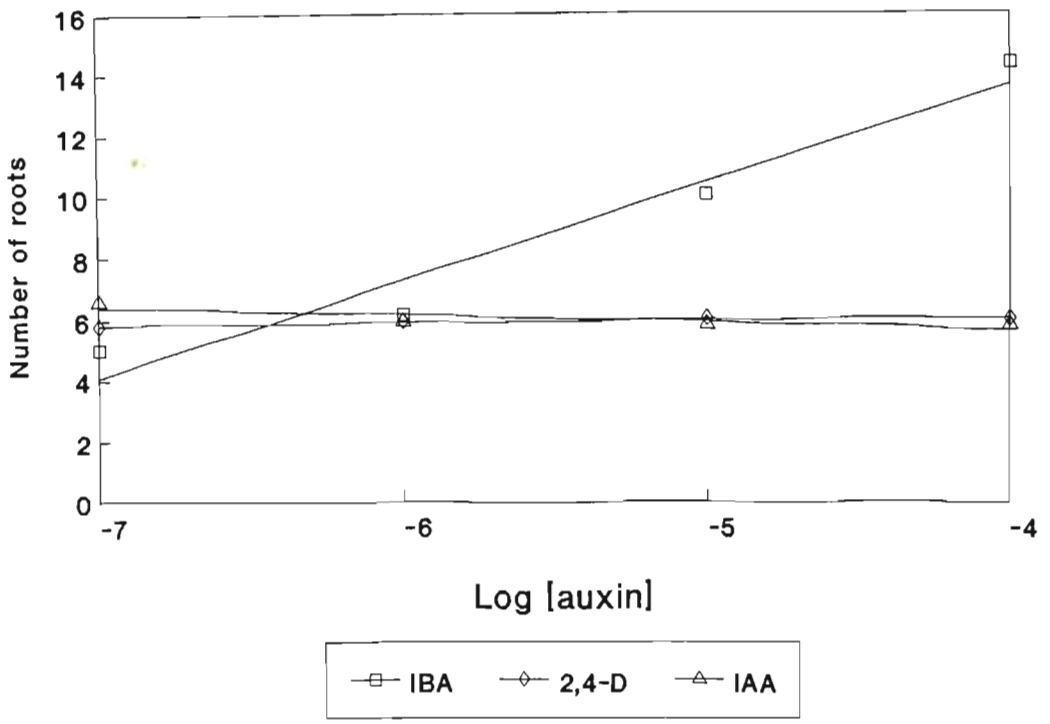

Figure 2.11 The effect of IBA, IAA and 2,4-D on the initiation of roots on mung bean cuttings 8 days after an 18 hour pulse treatment.

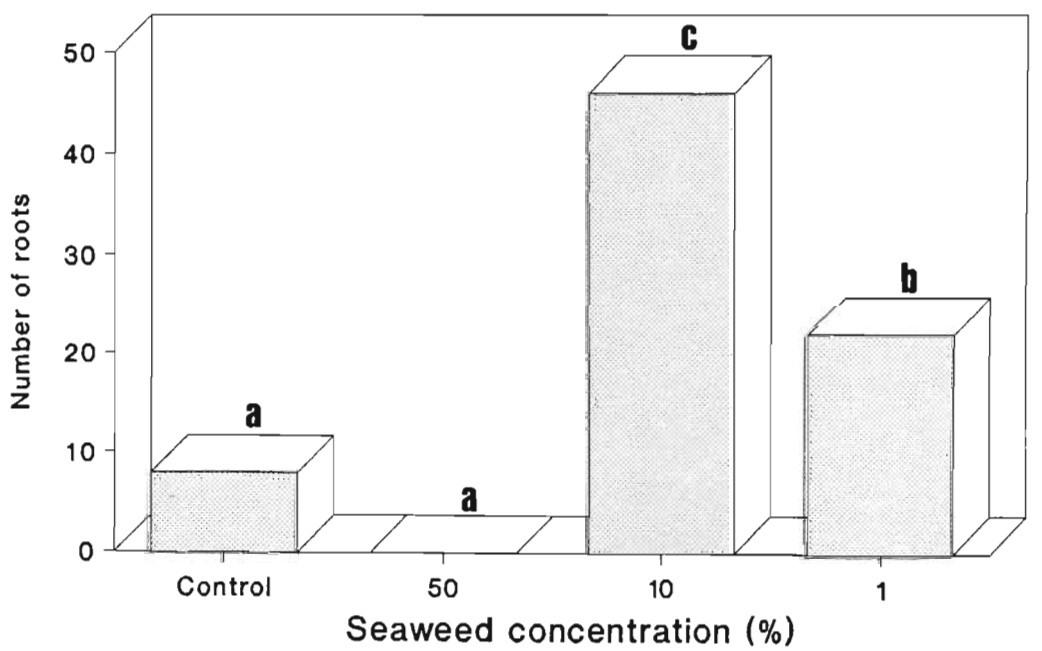

Figure 2.12 The effect of SWC on the rooting of mung bean cuttings 8 days after an 8 hour pulse treatment. Bars with the same letter are not significantly different.

activity was found in the neutral fraction (Figure 2.13). The significance of this result is discussed in detail in Chapter Five.

With Salkowski's colour test, a range of authentic IAA concentrations produced a linear curve when plotted against per cent transmittance (Figure 2.14). Only the highest concentration of SWC gave any colour reaction indicating that the amount of auxin present in the seaweed was too small to effectively elicit a colour response. This was to be expected as the assay is insensitive to concentrations of auxin below 10 mg ℓ^{-1}.

2.5 Tentative identification of the auxins present in fresh and processed *Ecklonia maxima*

In the above experiments, routine auxin bioassays responded positively to SWC, suggesting the possible presence of auxin or auxin-like compounds in Kelpak. Although the occurrence of endogenous hormones in marine algae has been extensively studied, there have been few attempts at identifying the auxins present in marine algae and commercial SWCs. Thus, the second part of this chapter reports on attempts to assess the identity of auxins in fresh and processed *Ecklonia maxima.*

Ten gram samples of freeze dried and powdered holdfast, stipe and lamina material from *Ecklonia maxima* plants were solvent partitioned for auxins as described earlier by SANDBERG, CROZIER & ERNSTSEN (1987) (section 2.4) and tentative identification of these compounds attempted following separation using HPLC and GLC. The presence of IAA and related compounds in SWC was determined in a similar fashion using 500 mℓ of SWC.

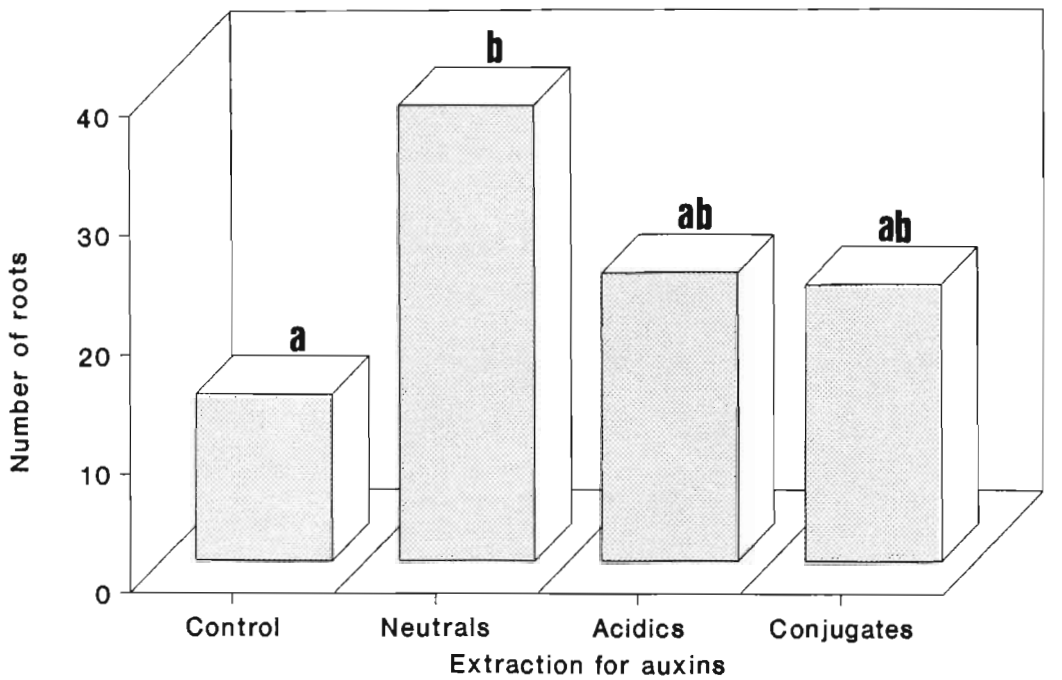

Figure 2.13 Detection of auxin-like activity in SWC following solvent partitioning for auxins. Bars with the same letter are not significantly different.

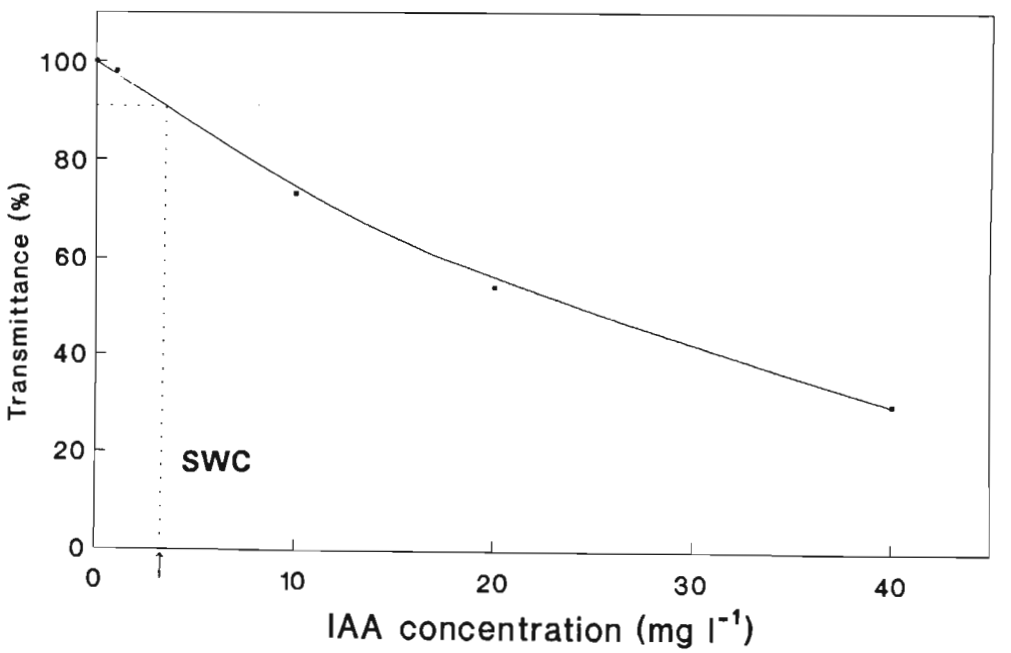

Figure 2.14 Detection of indoles in SWC using Salkowski's colour test for auxins.

2.5.1 Results

When authentic samples of L-tryptophan; indole-3-acetic acid (IAA); indole-3-butyric acid (IBA); indole-3-acetaldehyde (IAAld); indole-3-acetamide (IAcet); indole-3-propionic acid (IPA); indole-3-carboxylic acid (ICA); and 4-trans-abscisic acid (ABA) (Figure 2.15) were separated by solvent partitioning (SANDBERG, CROZIER & ERNSTSEN 1987) it was found that separation was incomplete (Figure 2.16). The 'acidic and neutral indole' fractions retained the majority of the authentic indoles, and in particular the acidic indoles. The aqueous or 'indole conjugate' fraction contained only tryptophan. IAcet was only detected in the 'neutral fraction'.

HPLC indicated the presence of compounds, in extracts of the SWC and the holdfast, stipe and lamina of *E. maxima*, with retention times corresponding to some of those of the standards (Figures 2.17 to 2.20).

In all the seaweed samples, most of the auxin-like compounds were detected in the 'neutral indole' fraction. The aqueous residue fraction almost invariably had a prominent peak with a retention time of 8.5 minutes which co-eluted with tryptophan. A compound that closely resembled IAcet was detected in the 'neutral indole' fraction at 16.3 minutes. Minor peaks were observed in the 'acidic indole' fraction at a retention time of 23 to 25 minutes which co-eluted with IAA, ICA, IPA, or other closely related compounds. A compound that co-eluted with ABA was detected in both the 'acidic and neutral indole' fractions at a retention time of 29 minutes.

GLC of methylated extracts of SWC and *E. maxima* holdfast, stipe and lamina material are shown in Figures 2.21 to 2.25. Only the 'neutral indole' fractions showed prominent peaks. 'Acidic indole' fractions invariably had only minor peaks even at a high attenuation while the aqueous fractions yielded very few minor peaks. Regardless of plant part, detectable compounds were similar for each of the three fractions. Indole-3-acetic acid and indole-3-carboxylic acid were tentatively identified

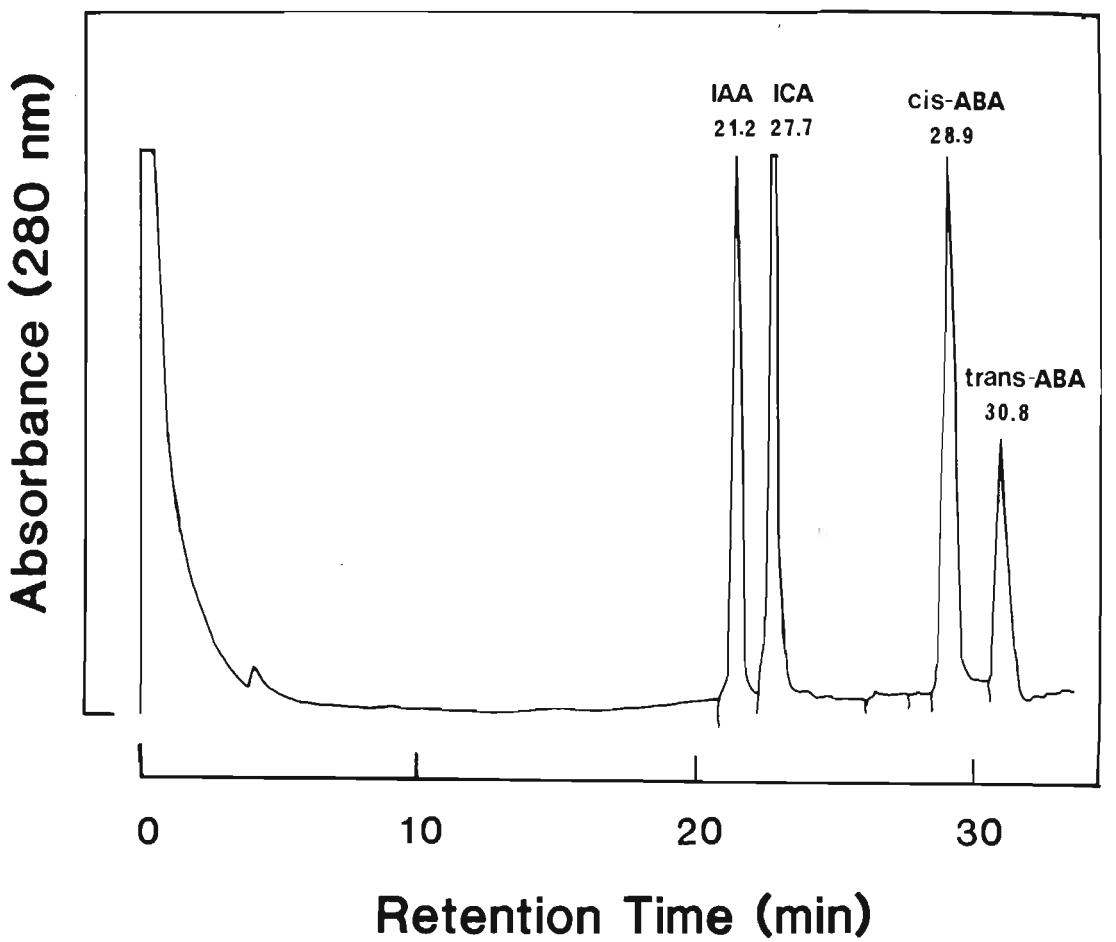

Figure 2.15 The UV absorbance at 280 nm obtained upon the HPLC separation of auxin and abscisic acid standards using a Varian 5 000 HPLC fitted with a Hypersil ODS column and a Varian variable wavelength monitor.The solvents used as eluants were a mix of of 0.2 M acetic acid and methanol, the combination of which altered during the 45 minute running time. (Try = tryptophan, IAcet = indole-3-acetaldehyde, IAA = indole-3-acetic acid, ICA = indole-3-carboxylic acid, IAAld = indole-3-acetaldehyde, ABA = abscisic acid).

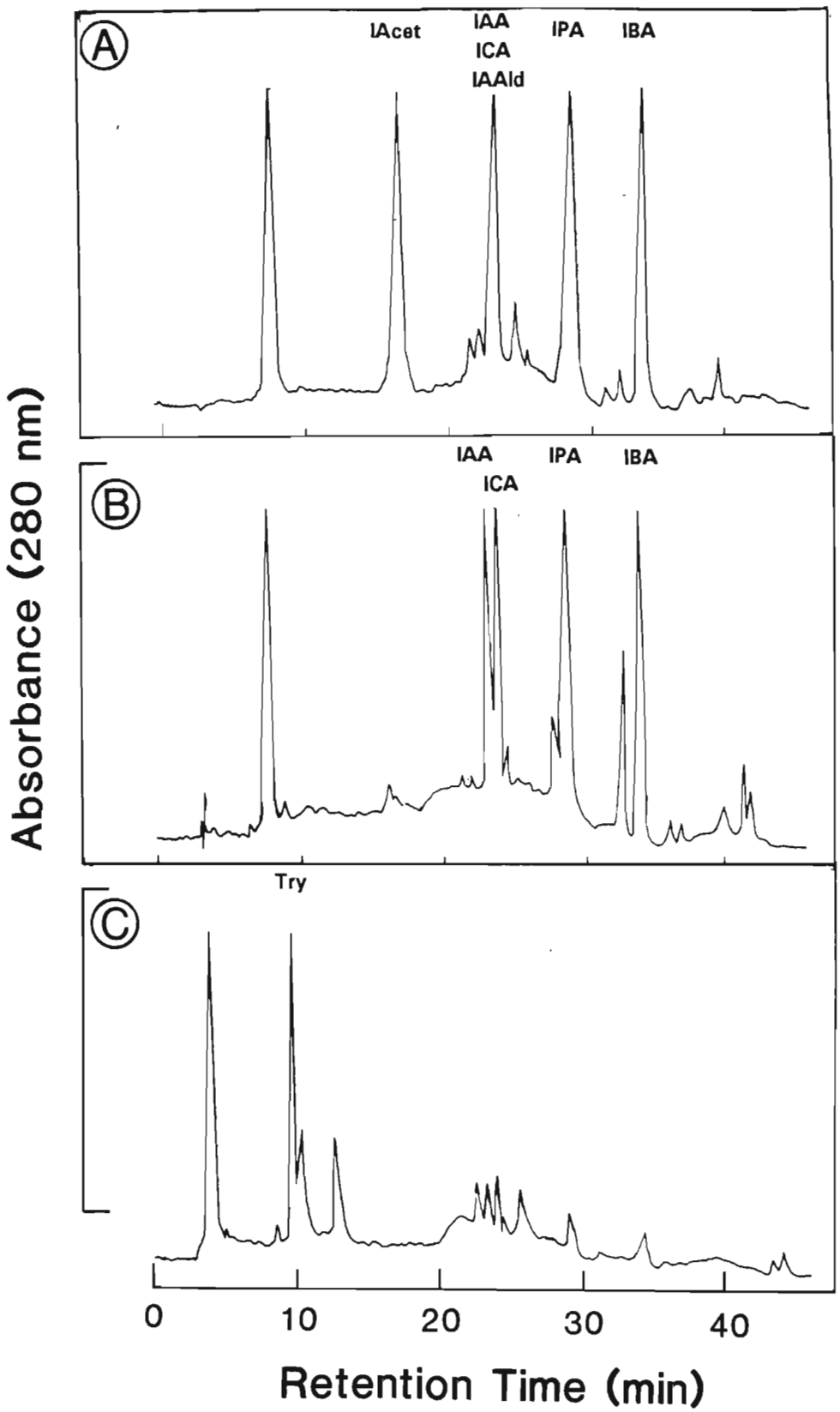

Figure 2.16 The HPLC separation of auxin standards following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C) as outlined by SANDBERG, CROZIER & ERNSTSEN (1987).

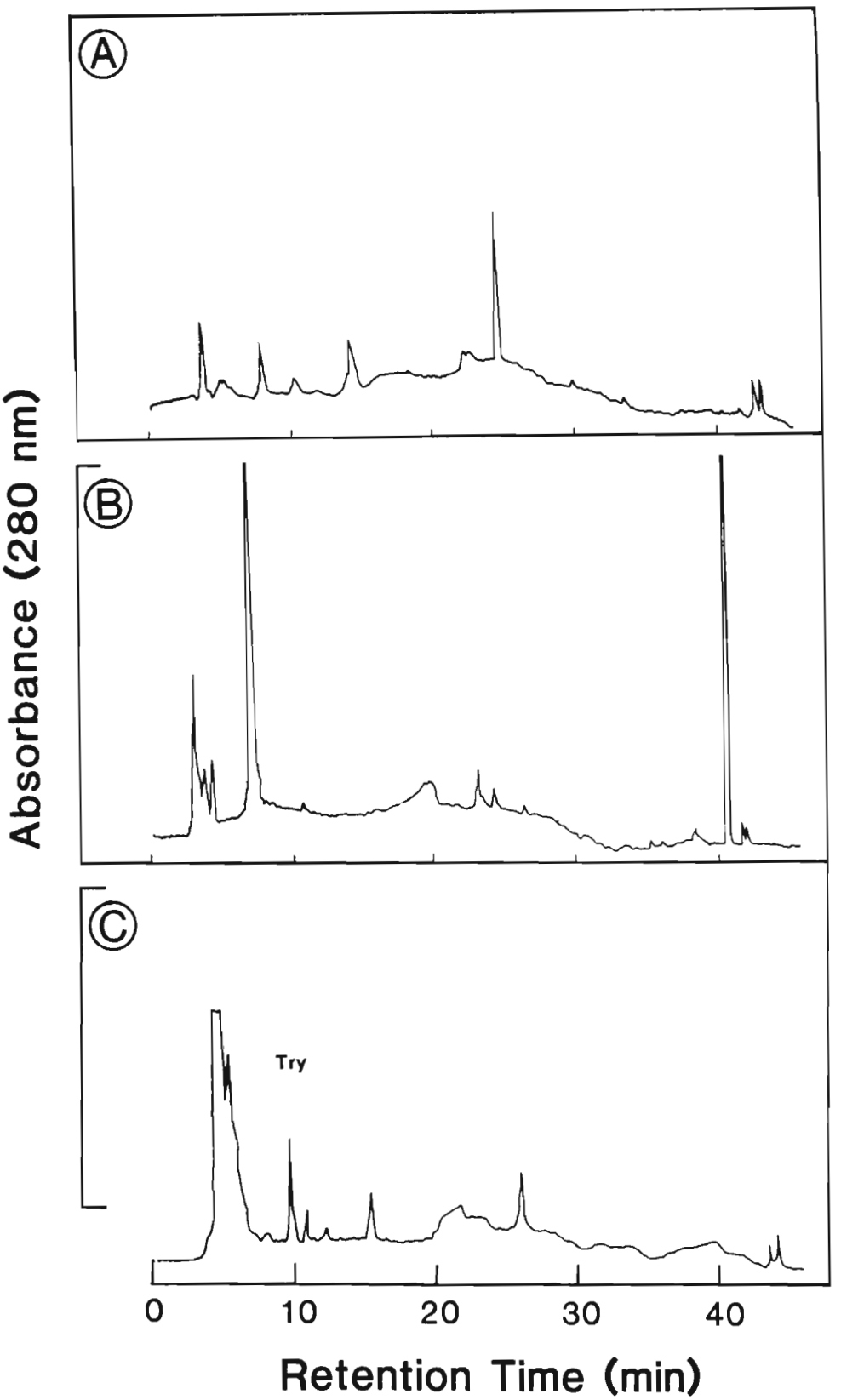

Figure 2.17 The HPLC separation of *E. maxima* holdfast material following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

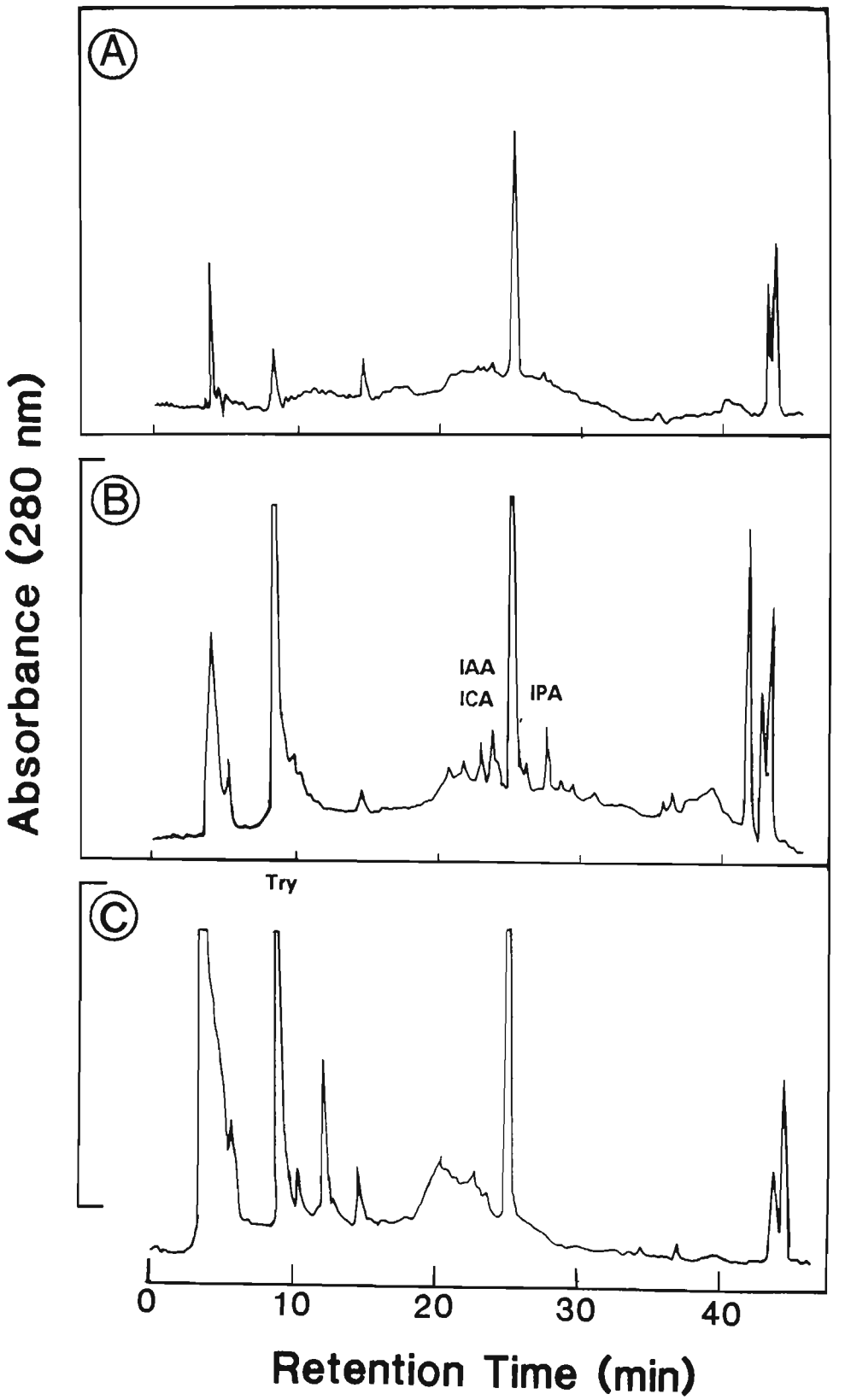

Figure 2.18 The HPLC separation of *E. maxima* stipe material following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

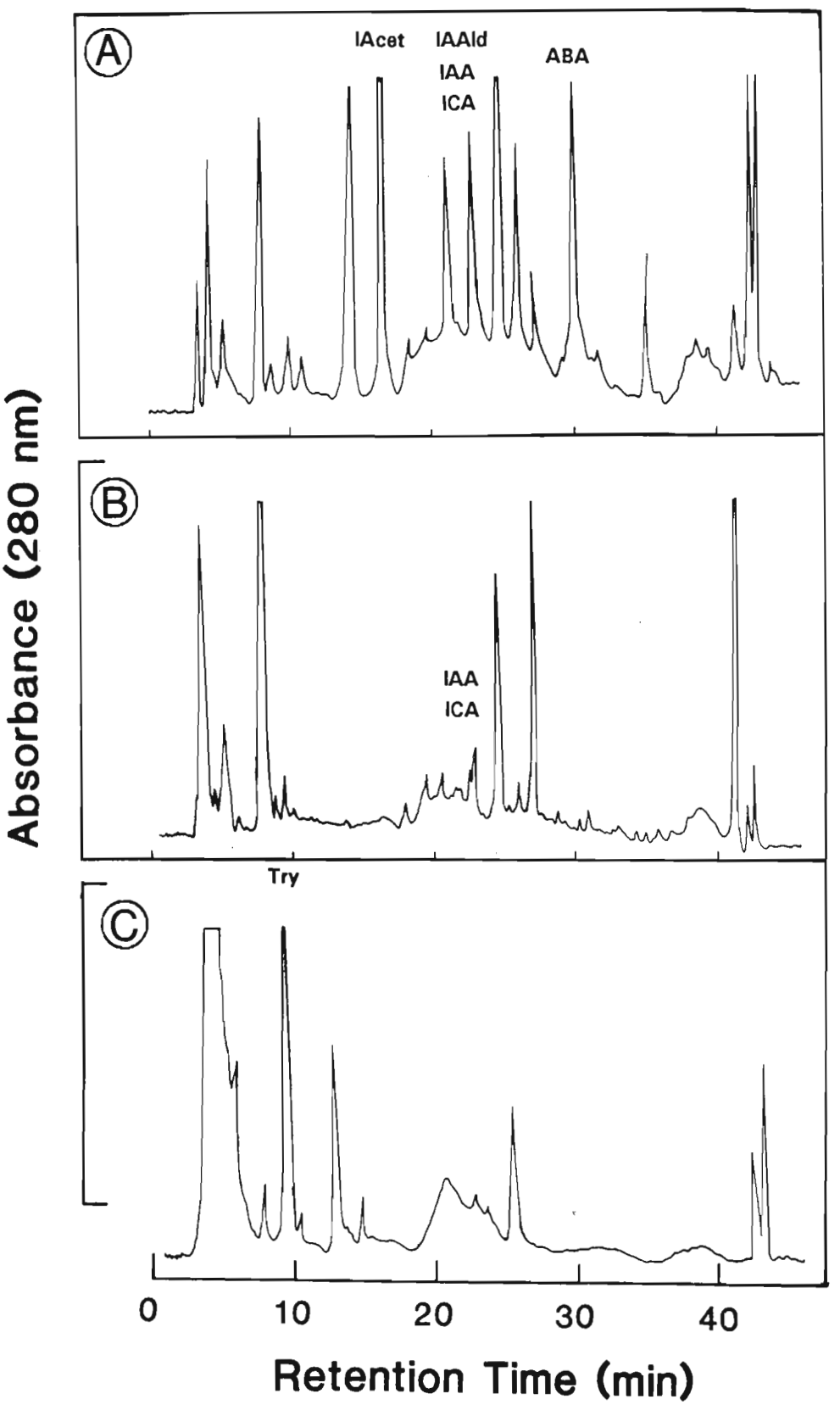

Figure 2.19 The HPLC separation of *E. maxima* lamina material following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

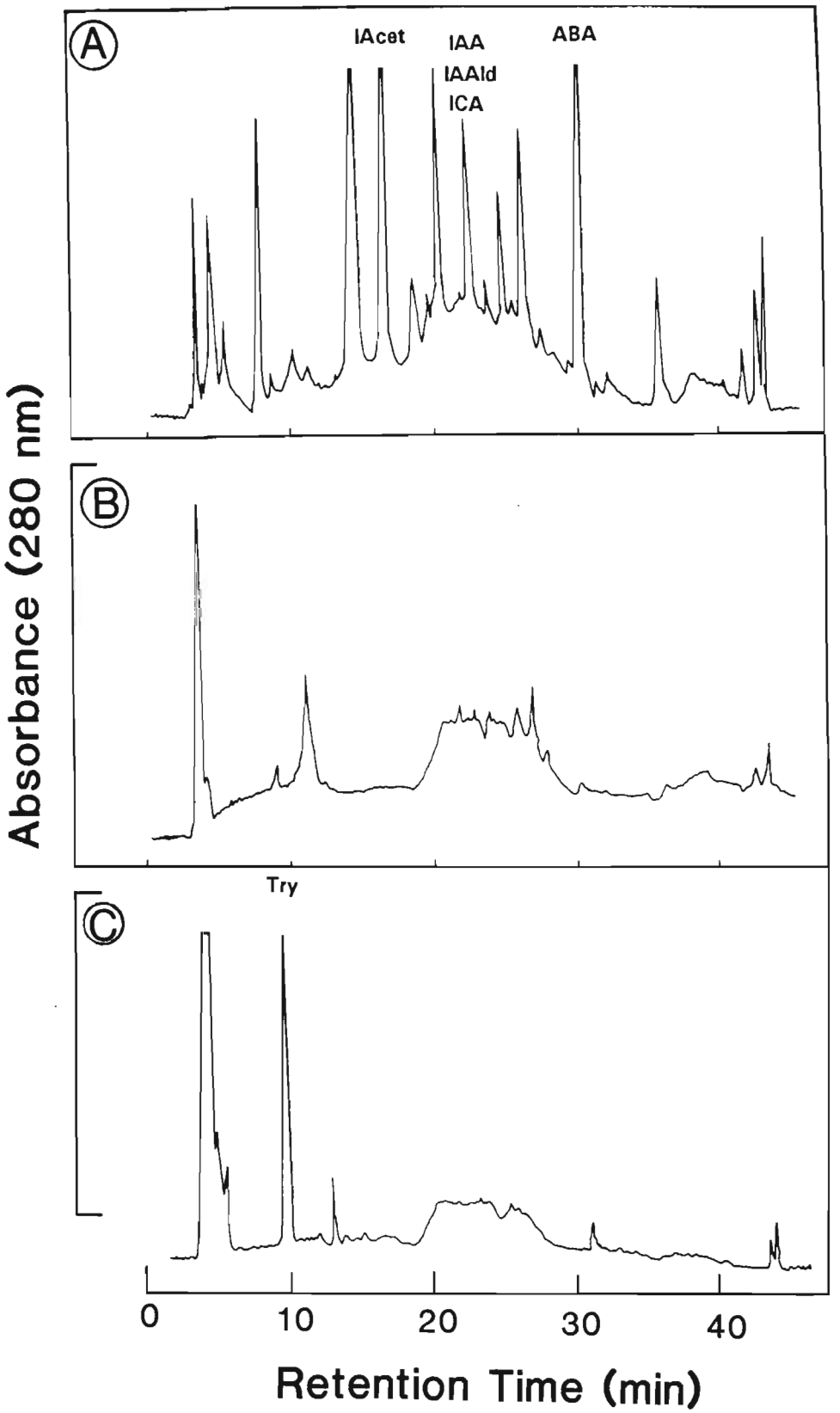

Figure 2.20 The HPLC separation of SWC following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

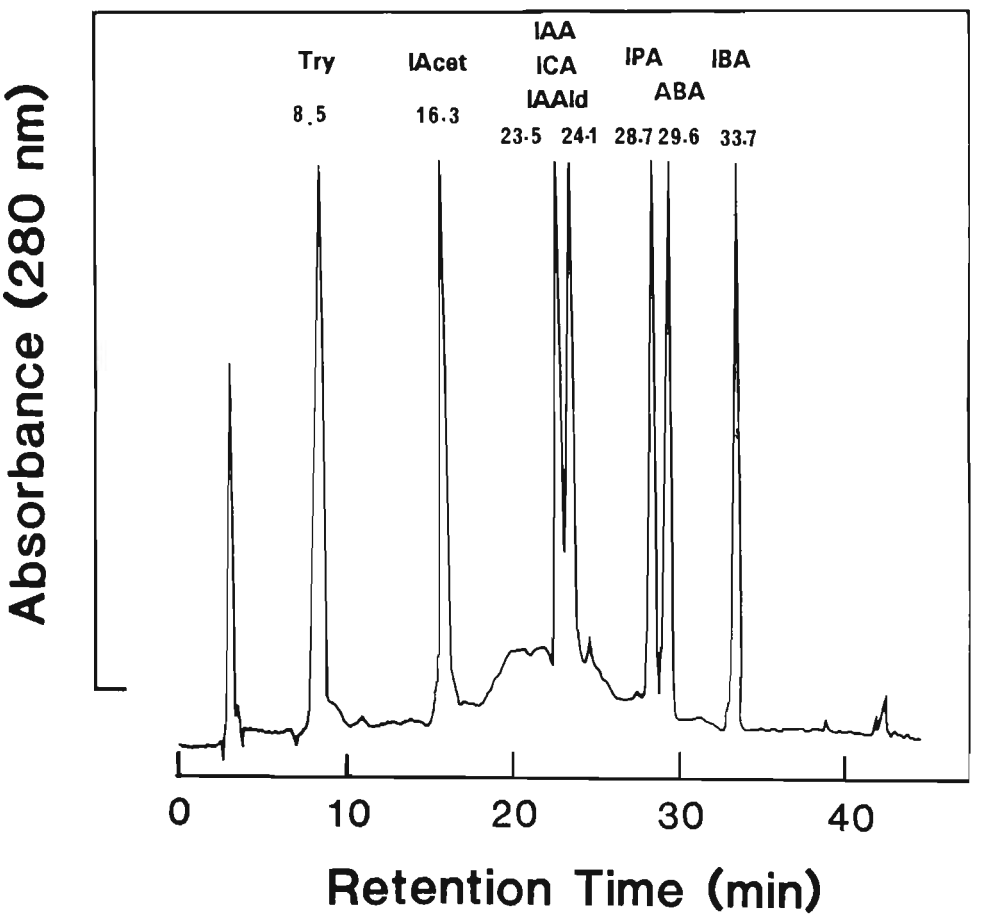

Figure 2.21 **The GLC separation of** authentic indole-3-acetic acid (IAA), indole - carboxylic acid (ICA) and abscisic acid (ABA) using a Varian 3 700 gas chromatograph fitted with an OV 17 column. After 2 minutes at 140°C, the column temperature was increased at a rate of 3°C minute^{-1} to 265°C and held for 5 minutes.

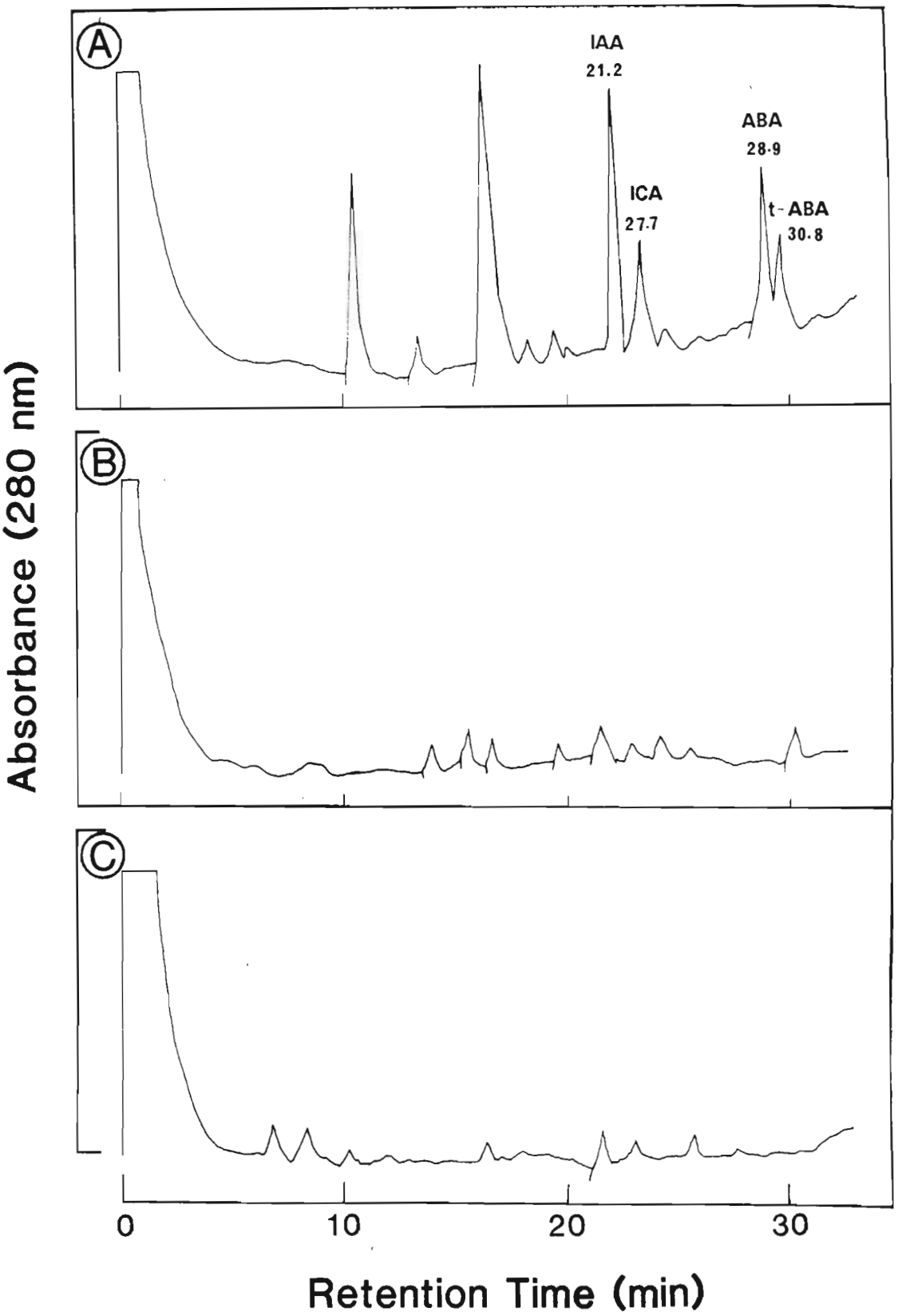

Figure 2.22 The GLC separation of *E. maxima* holdfast material following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

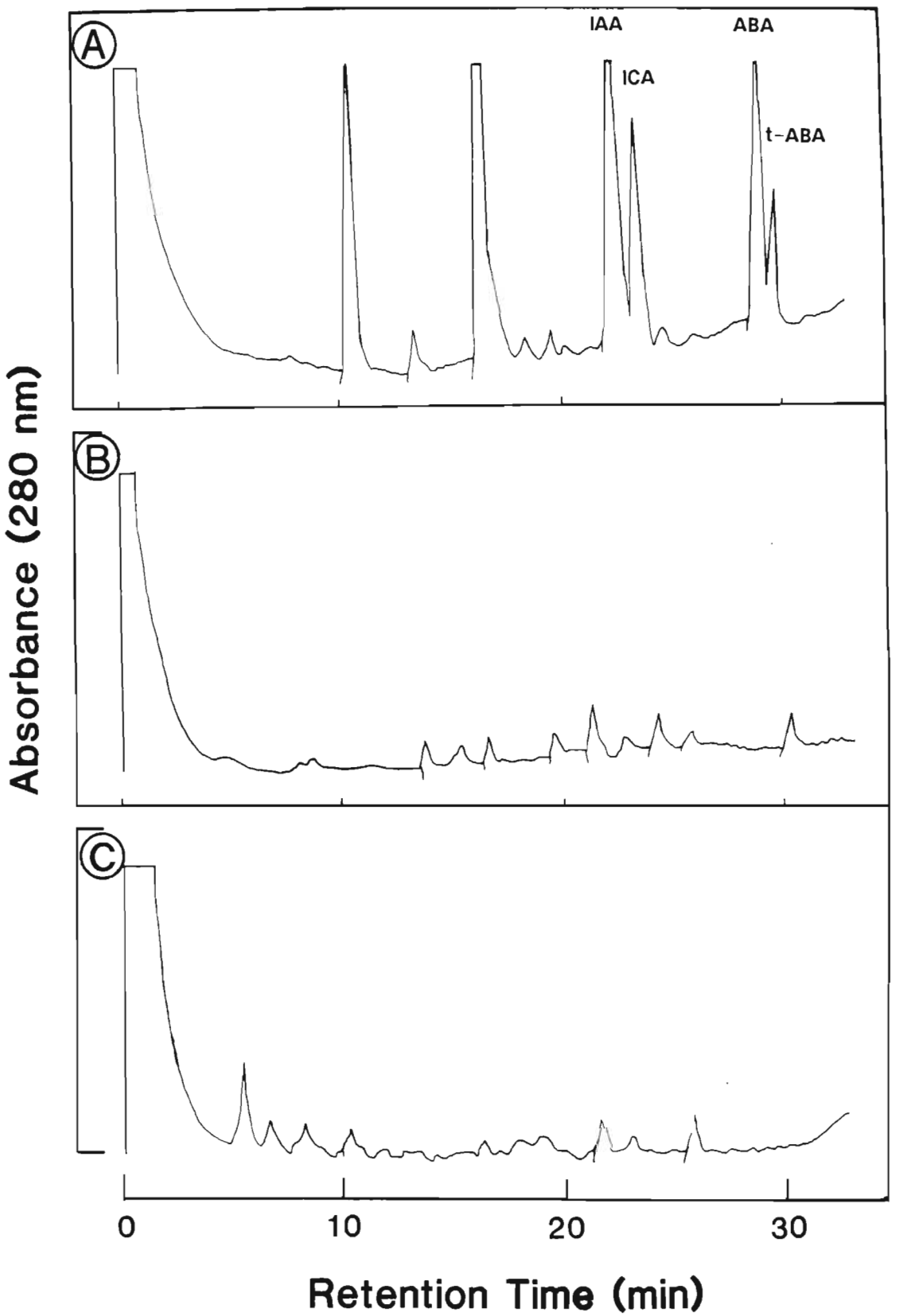

Figure 2.23 The GLC separation of *E. maxima* stipe material following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

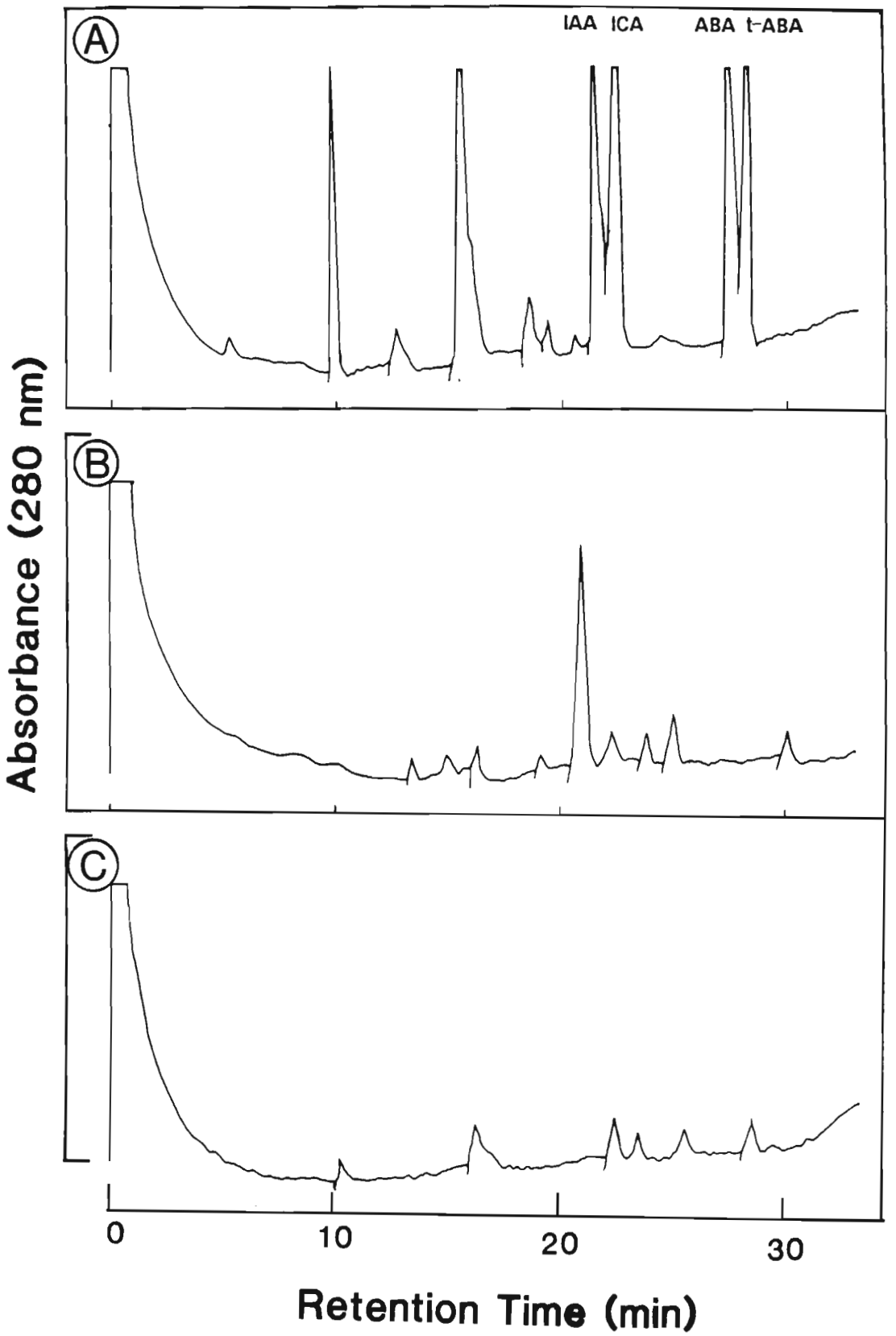

Figure 2.24 The GLC separation of *E. maxima* lamina material following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

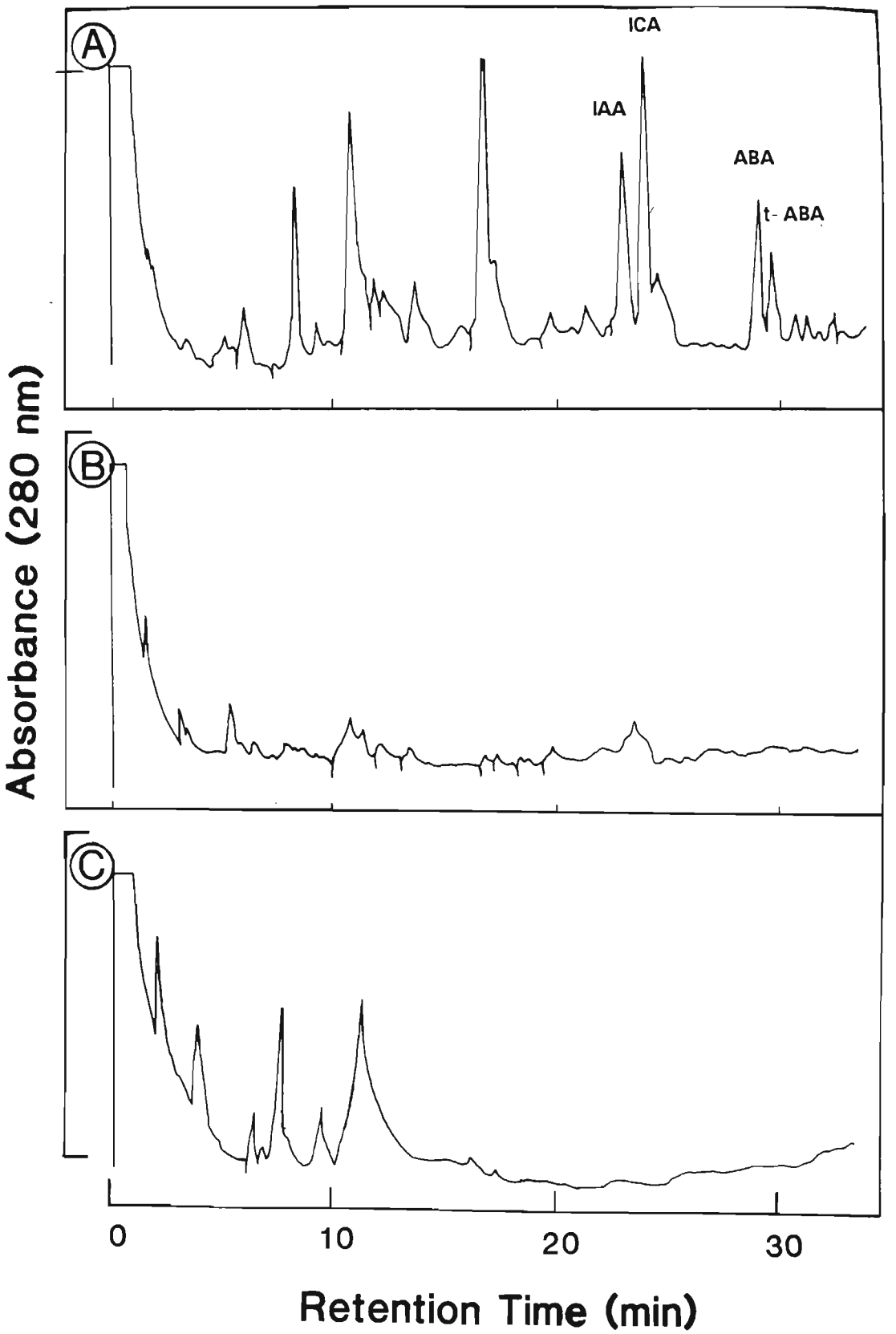

Figure 2.25 The GLC separation of SWC following solvent partitioning for neutral indoles (A), acidic indoles (B) and indole conjugates (C).

by co-chromatography in all neutral fractions, and, to a much lesser degree in the 'acid indole' fractions. Similarly, evidence for the possible presence of *cis*- and *trans*-ABA in the SWC was also obtained by co-chromatography in these fractions.

2.6 Discussion

Although the estimates of hormone content of seaweed extracts has been shown to vary considerably, a common feature in all investigations was that most bioassay systems responded positively to seaweed application. Using these recognised test systems, the SWC was shown to exhibit gibberellin-, abscisic acid- and auxin-like activity.

Whereas auxins and abscisic acid have been identified in seaweed products, the isolation and identification of gibberellins has yet to be established. The instability of this group of hormones may explain their lack of plant growth regulatory activity since gibberellins are thought to rapidly break down during the manufacture, extraction and storage of seaweed products (WILLIAMS, BRAIN, BLUNDEN, WILDGOOSE & JEWERS, 1976).

Although slight gibberellin-like activity was detected in the SWC, the magnitude of the response was too small to significantly affect plant growth. While a positive biological response may be indicative of a gibberellin-like compound, constituents other than gibberellins are known to occur in seaweeds that mimic gibberellin activity (GOPALA, 1984). One such compound is the terpenoid, α-tocopherol which is a major component of the E group of vitamins in seaweeds (JENSEN, 1969). Anti-gibberellin activity has also been noted in a fraction of kelp (*Ecklonia radiata* (C. Ag.) J. Ag.) extract (JENNINGS, 1969b). This activity was demonstrated in maize plants previously treated with gibberellin and is thought to be an algal counterpart of abscisic acid.

An important regulatory role of gibberellins is in the breaking of seed dormancy and promotion of germination. Reports (SUZUKI & TAKAHASHI, 1968) show that exogenous concentrations of 100 mg ℓ^{-1} can be instrumental in this process. While the SWC contained considerably lower levels of gibberellin-like activity, their involvement cannot be ignored. Promotion of seed germination following seaweed application has been reported for table sugar beet (WILCZEK & NG, 1982) and creeping red fescue grass (BUTTON & NOYES, 1964). Although the mode of seaweed action in breaking seed dormancy is not known, it has been postulated that cytokinins rather than gibberellins may be involved (WILCZEK & NG, 1982). This assumption was probably made on evidence of cytokinin content alone.

The lettuce hypocotyl bioassay indicated that inhibitory compounds are present in SWC. A compound known to inhibit plant growth is abscisic acid (ABA). ABA is thought to inhibit growth stimulated by either auxins, gibberellins or cytokinins. This compound has been positively identified in many seaweeds and several seaweed preparations. KINGMAN & MOORE (1982) reported very high levels of ABA (20 000 μg per gram of dried extract) in a commercial seaweed extract of *Ascophyllum nodosum*. BOYER & DOUGHERTY (1988) found the ABA content in a similar commercial preparation to be much lower than the levels recorded by KINGMAN & MOORE (1982) but comparable to that in freshly collected algae. They noted that these levels of ABA were comparable with those generally observed for turgid leaves of higher plants (BOYER & ZEEVAART, 1982; ZEEVAART, 1980).

The inhibitory responses of seaweed noted in the lettuce hypocotyl inhibition bioassay were of a far greater magnitude than the highest level of authentic ABA standard used. It is unlikely that these inhibitory response were elicited by ABA as the treatment showing the greatest inhibitory activity was a discard fraction. This suggests the occurrence in the SWC of inhibitors other than ABA. Several inhibitory compounds have been recorded in seaweed extracts (BLUNDEN, CHALLEN & WOODS, 1968). BLUNDEN, CHALLEN & WOODS (1968) noted that certain amino-acids, laminarin, carrageanan and sodium alginate, all of which are found in seaweeds, can inhibit plant growth. As growth inhibition is generally associated with high levels of

seaweed application (AITKEN & SENN, 1965) it has been suggested that these effects may be due to salt toxicity (BRINER, RICHARDS & BELCHER, 1979). As only very dilute levels of SWC fractions were tested in the ABA bioassay, the reduced hypocotyl growth was more likely to have been caused by specific growth inhibitors than through a high salt content.

The most pronounced biological activity in the SWC was detected as auxin-like growth responses, and in particular as the initiation of roots on mung bean cuttings. Auxins are known to occur in many marine algae and recent research has confirmed their presence in seaweed products. SANDERSON & JAMESON (1986) were the first to unequivocally identify IAA by GC-MS in a freshly made commercial liquid seaweed preparation. Using GLC techniques KINGMAN & MOORE (1982) estimated the content of IAA in a commercial *Ascophyllum* product to be about 50 000 μg per gram of dried extract. While WILLIAMS, BRAIN, BLUNDEN, WILDGOOSE & JEWERS (1976) regard the activity of auxins in seaweeds as being low, the work presented in this chapter suggests otherwise.

The presence of auxin-like compounds in the SWC was confirmed by the positive response in all the bioassays tested. Recent evidence indicates that even dried seaweed meals may contain considerable auxin activity. These auxins are thought to be preserved in their natural state bound to protein-like substances and liberated by hydrolysis (NELSON, 1985). Some of this auxin activity was exhibited by substances lacking an indolic nature (SUMERA & CAJIPE, 1981). Non-indole auxins, such as phenylacetic acid or phenylalanine, may therefore be responsible for some of the observed auxin-like growth responses following seaweed treatment. To determine the presence of indoles and related compounds in *E. maxima* and SWC, the seaweed samples were extracted for auxins, and tentatively identified on the basis of co-chromatography with authentic standards.

The main pathways of IAA biosynthesis proceed from tryptophan via indole-3-acetaldehyde (IAAald) (SANDBERG, CROZIER & ERNSTSEN, 1987). Tryptophan was tentatively identified in the aqueous indole conjugate fraction by co-chromatography

with an authentic sample. Although IAAald was tentatively identified in the 'neutral indole' fraction of *E. maxima* and SWC, compounds in acidic fractions co-chromatographing with IAA were only detected in minor quantities.

The tentative presence of indoles in *E. maxima* was confirmed by both HPLC and GLC separation techniques. HPLC analysis indicated the presence of compounds having similar absorbance and elution times to many auxins. GLC analysis confirmed these results. Using these techniques it was not possible to unequivocally identify these compounds. However, the similarity of the elution times of the different peaks suggests that certain components in seaweed may be closely related to auxins.

In the HPLC analysis, tryptophan and indole-3-acetamide were detected in the 'indole conjugate' and 'neutral indole' fractions respectively. Because these compounds were unable to be derivatized (absence of a carboxyl group) they could not be detected by GLC. The occurrence of compounds in the 'neutral indole' fraction with similar retention times to IAA, ICA and ABA suggest their possible presence in the SWC. However, indole-3-ethanol (IEt), indole-3-methanol (IM) and indole-3-acetamide (IAAald) would also partition to this fraction.

When tentatively identifying plant growth regulators (PGRs) in plant tissue, several factors can affect the final analysis, lending uncertainty to the validity of the results. One such factor is the 'instability' of the compound. The age of plant material; initial sample size; efficiency of the separation technique; and, structural similarities between closely related compounds may all influence the final determination of PGR's. The importance of these factors is emphasised by the degree of variation that has often been recorded by different research groups when doing similar analyses.

Many indoles, including IAA, are fragile compounds that undergo non-enzymatic oxidation in the presence of light, oxygen or peroxides. IM breaks down rapidly below pH 6.5 (SUNDBERG, SANDBERG & JENSEN 1985) as does IAAld and indole pyruvic acid (IPyA), yielding a number of products including IAA (SWEETSER & SWARTZFAGER, 1978; HEMBERG & TILLBERG, 1980). Extensive non-enzymatic conversion of tryptophan

to an IAA-like substance has also been reported (EPSTEIN, COHEN & BANDURSKI, 1980). Extensive handling and the age of the seaweed samples may have caused the decomposition of many endogenous indoles, complicating the detection of these compounds.

The identification of auxins and ABA in seaweeds was therefore subject to some uncertainty. However, compounds showing ABA-and auxin-like biological activity were shown to be present in *Ecklonia maxima* and the SWC.

CHAPTER 3

THE EFFECT OF SEAWEED CONCENTRATE ON POTATO (*SOLANUM TUBEROSUM* L.) SHOOTS CULTURED UNDER *IN VITRO* CONDITIONS.

3.1 Introduction

Tissue culture and the related technique of meristem culture, for reasons of speed and convenience, is being used increasingly for the commercial propagation of many food and ornamental plants. Tissue culture is also a powerful tool for the plant breeder, enabling the preservation of valuable germ plasm under laboratory conditions until it is required in the breeding programme. The importance of meristem culture lies in its wide use in the production and propagation of virus-free clones of many plant species. The utilization of seaweed products in this area of research has yet to be studied. As seaweed application is known to elicit many beneficial growth responses when applied to plants (METTING, ZIMMERMAN, CROUCH & VAN STADEN, 1990), it is possible that these products may prove useful in *in vitro* propagation systems.

The development of an *in vitro* system that is sensitive to seaweed products may prove an effective bioassay for measuring biological activity associated with seaweed application.

In this study, SWC was applied to an *in vitro* system in an attempt to establish its effect on the growth of *in vitro* plants. This chapter describes the use of apical tip meristems and nodal cuttings, from *in vitro* grown potato shoot cultures as a means of testing the seaweed preparation for growth regulatory activity.

3.2 Materials and Methods

3.2.1 Establishment of in vitro potato cultures

Tubers of seed potatoes (*Solanum tuberosum* L. cv. van der Plank) were planted in pots containing moistened autoclaved soil and held inside a closed, dark polypropylene drum (100 cm tall) for 2 to 3 weeks to induce etiolated shoots. Shoots 60 cm in length were used to provide nodal cuttings and apical tips for the initiation of aseptic cultures. Explants were surface sterilised in 1.0% sodium hypochlorite for 12 minutes and then rinsed three times in sterile distilled water.

(i) Establishment of in vitro potato cultures using nodal cuttings as explants (Figure 3.1).

Nodal cuttings were transferred aseptically to culture tubes (100 mm x 25 mm) containing MURASHIGE and SKOOG (1962) (MS) salts mixture modified according to ESTRADA, TOVAR & DODDS (1986) (Figure 3.1a). The media were supplemented with 0.4 mg ℓ^{-1} thiamine HCl, 2.0 mg ℓ^{-1} Ca-pantothenic acid, 0.4 mg ℓ^{-1} gibberellic acid, 0.5 mg ℓ^{-1} benzyladenine, 0.01 mg ℓ^{-1} naphthaleneacetic acid, 2% sucrose and 100 mg ℓ^{-1} inositol. Prior to autoclaving, the pH of the media was adjusted to 5.8 and it was solidified with 0.8% agar.

(ii) Establishment of in vitro potato cultures using apical tip meristems as explants (Figure 3.2).

Sterile apical shoots (5 to 10 mm long) were transferred to a Petri dish containing moistened Whatman Nº1 filter paper and the terminal 0.1 to 0.5 mm of the tip microsurgically removed. The meristematic tips were placed on filter paper bridges

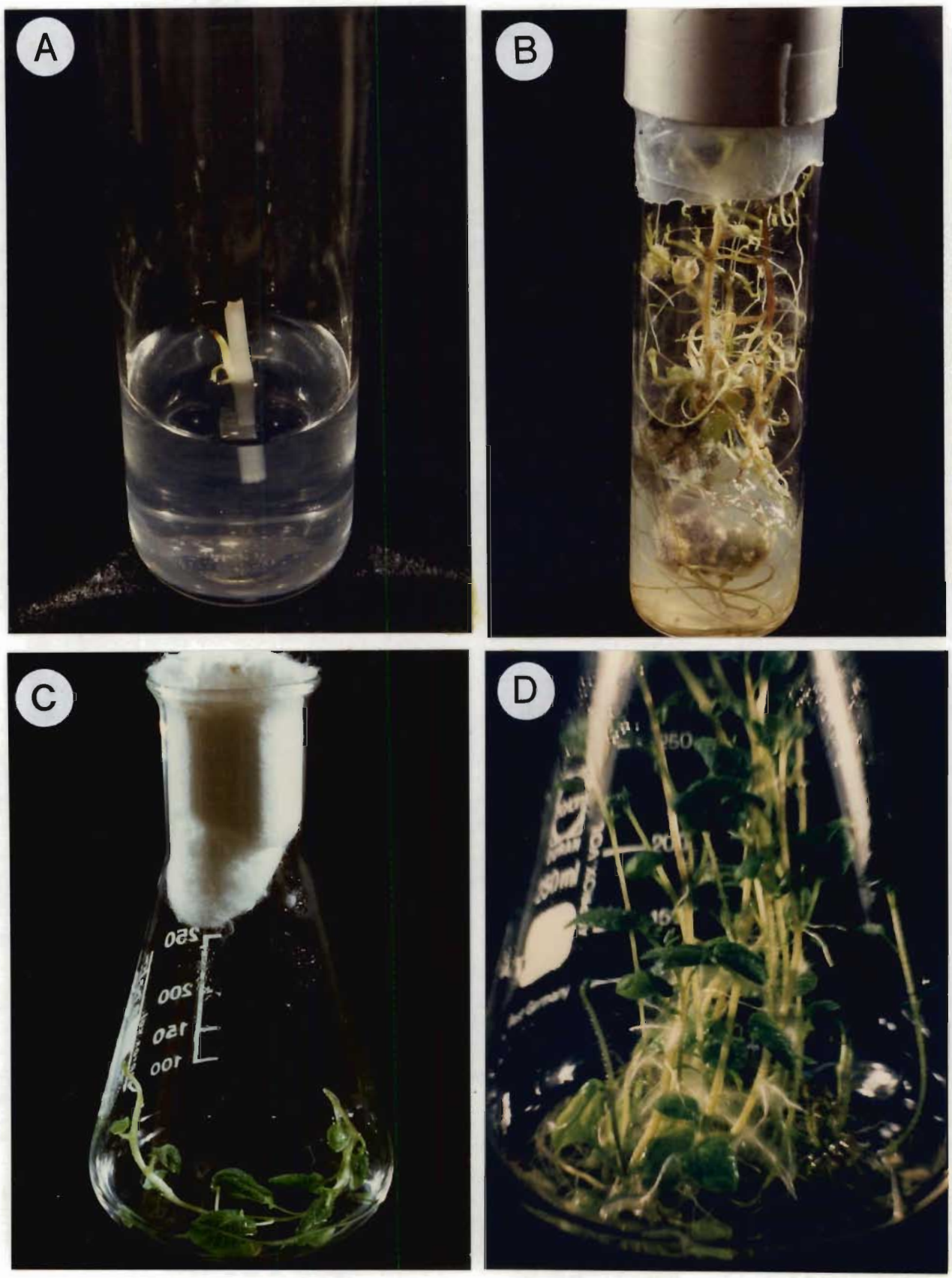

Figure 3.1 Establishment of potato shoot cultures from nodal explants. (A = nodal explant on agar, B = new shoots after 3 weeks, C = single shoot in liquid media, D = multiplication of shoots).

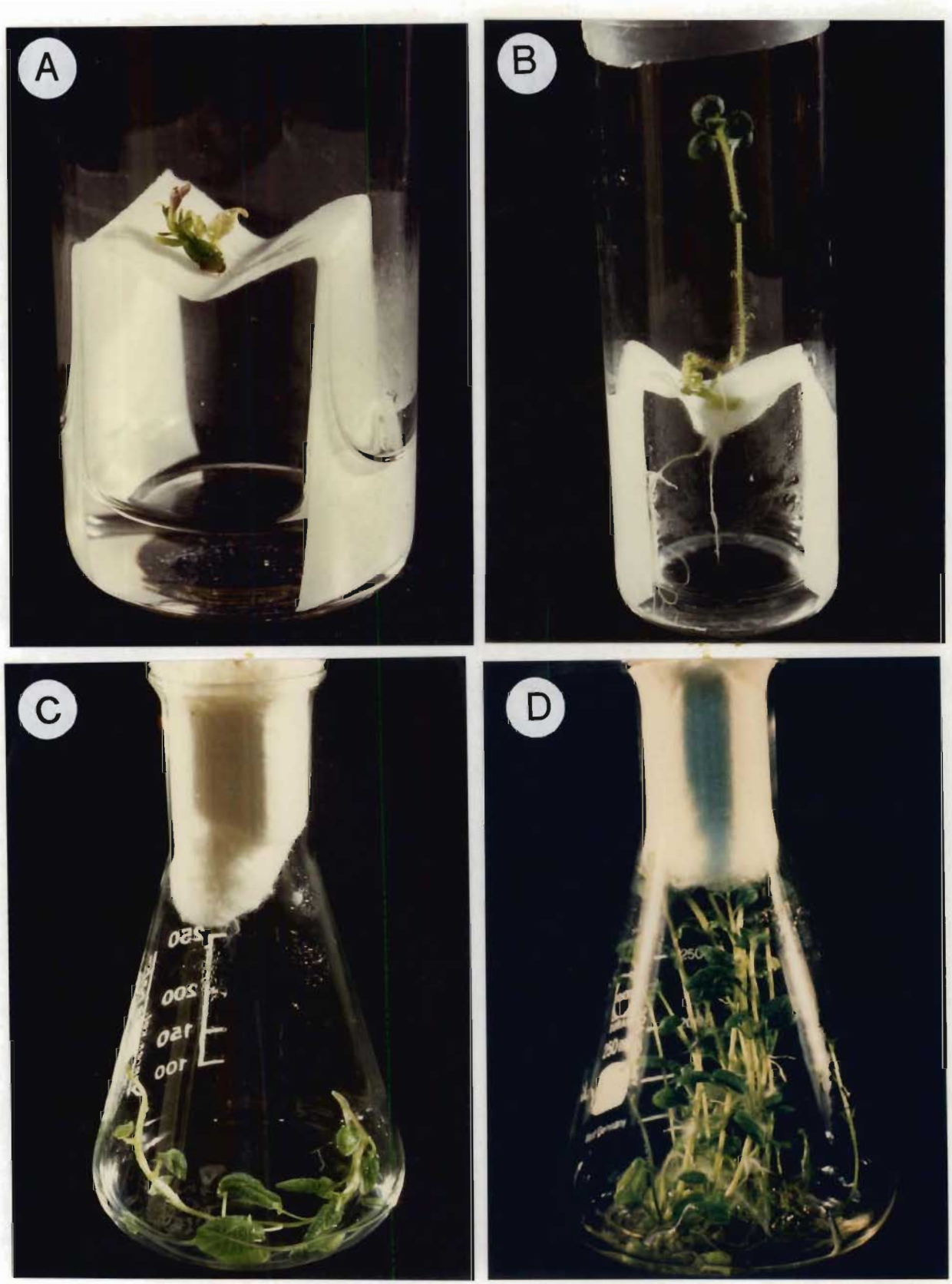

Figure 3.2 Establishment of potato shoot cultures from apical meristems. (A = apical meristem on filter paper bridge, B = new shoot after 3 weeks, C = single shoot in liquid media, D = multiplication of shoots).

in culture tubes containing three mℓ of liquid MS basal media supplemented with 2.0 mg ℓ^{-1} each of IAA and kinetin, and 20 g ℓ^{-1} sucrose (Figure 3.2a).

Cultures were maintained in a growth room at 25 ± 2°C with a 16h light:8h dark cycle. Illumination was supplied by cool white fluorescent tubes with a light intensity of 27 μ mole m^{-2} s^{-1}. After 4 to 6 weeks, the explants produced shoots which were excised and transferred onto the liquid culture medium of ESTRADA, TOVAR & DODDS (1986) (Figures 3.1 & 3.2 b and c). Repeated subculture of axillary shoots (every 7-10 days) produced large numbers of nodal cuttings for experimentation (Figures 3.1 & 3.2 d).

3.3 The effect of SWC on the growth of *in vitro* potato shoots.

The SWC was added to the basic establishment media at concentrations of 0.2% and 0.4%. Five mℓ of each treatment solution was decanted into 24 x 200 mm culture tubes. Each treatment consisted of 20 replicates. The tubes were autoclaved at 120°C and 1 bar for 20 min. Single sterile nodal cuttings (10 mm long) were transferred to the tubes and placed on a rotary shaker at 25 rpm. After 12 and then 16 days in culture, the lengths of the axillary shoots were measured *in situ* by placing a linear scale along the length of the culture tube. On day 20 the length and number of shoots were recorded. Culture tubes were then removed from the shaker, incubated in the dark at 26°C and tuber formation recorded after five weeks.

3.4 Results

Each nodal cutting rapidly gave rise to at least one new shoot (Figure 3.3). SWC applied at a concentration of 0.2% significantly increased the length of the main

Figure 3.3 Effect of SWC on potato shoot growth after 3 weeks in culture.

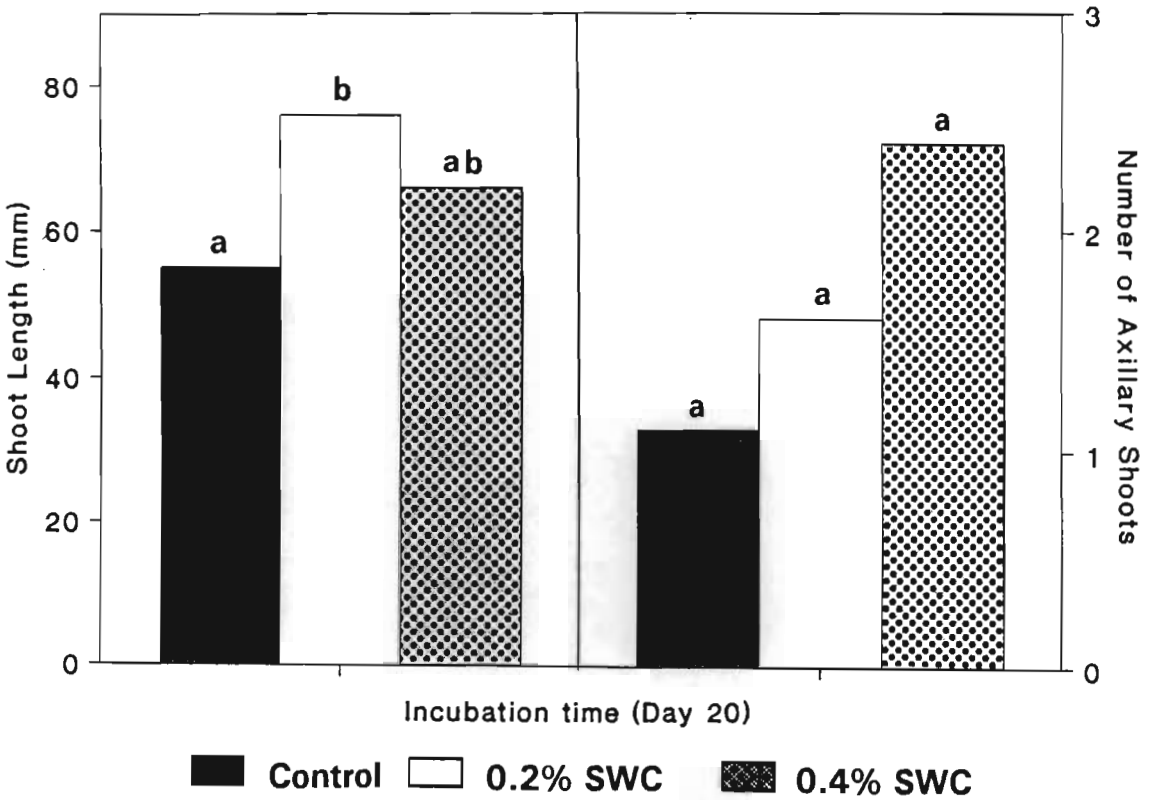

Figure 3.4 Effect of SWC on the growth of potato shoots cultured *in vitro*. Bars with the same letter on top are not significantly different using a multiple range test.

Table 3.1 The effect of SWC on shoot growth of *in vitro* potato cultures.

Treatments	Shoot Length	Percentage Increase
Control	55	
0.20% SWC	78	24.5
0.40% SWC	65	18.2

axillary shoot over that of the control (Figure 3.4, Table 3.1). A higher concentration of 0.4% SWC resulted in the development of two to three lateral shoots from the original nodal cutting. This effect was not observed at the lower level of application. Tuberization only occurred in cultures treated with 0.4% SWC (Figure 3.5).

3.5 Discussion

The results indicate that SWC has the ability to enhance the growth of *in vitro* plantlets. The addition of seaweed products to culture media may thus be beneficial for the growth of explants in *in vitro* cultures. Although enhanced plant growth through the application of SWC to *in vivo* grown plants is well documented (METTING, ZIMMERMAN, CROUCH & VAN STADEN, 1990), there is no record of its utilization in *in vitro* plant propagation systems. This study indicates that SWC not only has an effect on shoot elongation but may also effect apical dominance and bring about tuberization. This suggests that there are probably several active constituents in the seaweed extract acting at any one time. As the media used in this study contained all the essential nutrients required for normal plant growth, it is unlikely that the effect of SWC was due to mineral elements alone. The marked physiological responses of nodal explants to seaweed treatment noted in this study may be due to plant growth regulators. Plant growth regulators are known to effect apical dominance (MARTIN, 1987) and tuberization (KODA & OKAZAWA, 1983; SAUNDERS, 1986). Identification of these compounds in commercial seaweed preparations is not well documented. Although

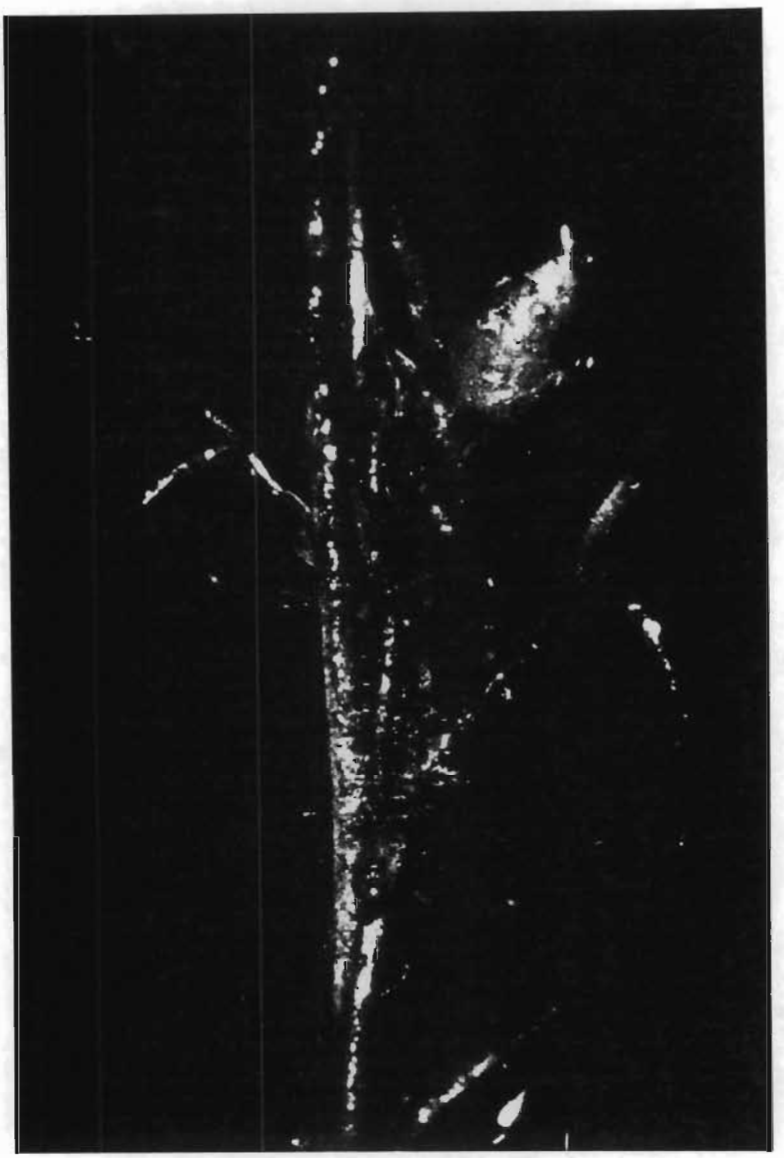

Figure 3.5 **Tuber formation on potato shoots treated with 0.4% SWC after five weeks in *in vitro* culture.**

auxins (AUGIER, 1976a; SANDERSON & JAMESON, 1986), gibberellins (AUGIER, 1976b; WILDGOOSE, BLUNDEN & JEWERS, 1978), cytokinins (MOONEY & VAN STADEN, 1987) and ABA (KINGMAN & MOORE, 1982) are known to occur in seaweeds, only cytokinins have been extensively studied in commercial seaweed concentrates (TAY, MACLEOD, PALNI & LETHAM, 1985; TAY, PALNI & MACLEOD, 1987).

To effectively study plant growth responses to SWC, there is a need for the development of new bioassay systems. Potato explants may serve as an excellent

tool to this end as growth responses were detectable after only three weeks in culture. The advantage of this assay is that all growth parameters can be controlled. Furthermore, shoot extension, lateral bud development and tuberization can be measured in a single bioassay system. This may increase the scope of application over bioassays measuring only a single parameter.

CHAPTER 4

THE EFFECT OF SEAWEED CONCENTRATE ON THE GROWTH OF EXCISED TOMATO ROOTS CULTURED *IN VITRO*.

4.1 Introduction

The beneficial effects of seaweed products on the growth and yield of terrestrial plants is well documented (Table 1.1). However, the effect of SWC on isolated plant organs cultured *in vitro* has received little attention.

BRAIN, CHALOPIN, TURNER, BLUNDEN & WILDGOOSE (1973) were the first to incorporate seaweed extract into an *in vitro* system. They demonstrated a promotion of growth of carrot explants and *Atropa belladonna* in a cytokinin-free medium containing the seaweed extract. FINNIE & VAN STADEN (1985) investigated the effect of SWC on excised tomato root cultures and recorded a significant increase in both root extension and root elongation. Recently, DE WAELE, McDONALD & DE WAELE (1988) found that the application of seaweed to excised maize roots cultured *in vitro*, significantly suppressed the reproduction of the maize nematode *Pratylenchus zeae* Graham.

Many studies report significant increases in root growth after seaweed application (FINNIE & VAN STADEN, 1985; METTING, ZIMMERMAN, CROUCH & VAN STADEN, 1990). An improved root system ultimately increases plant yield. At present it is not known whether the increased root biomass is a result of better soil conditions, improved plant nutrition or a physiological response incurred through growth substances.

To learn more about the mode of action of applied seaweed products, isolated tomato roots were cultured *in vitro* and the effect of SWC on their growth assessed critically. The application of SWC to isolated roots cultured on agar had several

advantages over SWC applied to intact plants grown *in vivo*. First, this system eliminates the effect of SWC on soil structure; second, the effect of the SWC on shoot growth is removed; and third, mineral nutrition effects of the SWC are negated by the abundance of nutrients supplied in the media. An improvement in root growth might suggest the action of endogenous hormones or growth substances in the seaweed.

This study utilized two *in vitro* systems. In the first, sterile tomato roots were cultured in a liquid culture medium. In the second the roots were grown on agar. The latter system enabled roots to be inoculated with nematode egg masses and for nematoxic effects of the SWC to be examined.

4.2 Materials and Methods

4.2.1 Establishment of excised tomato roots in an aseptic liquid culture medium.

Tomato (*Lycopersicon esculentum* Mill.) seeds were surface-sterilized with concentrated commercial bleach (3.5% sodium hypochlorite) for five minutes, washed in sterile water and then germinated on 0.4% water agar at 25°C. After eight days a sterile root from a single seedling was excised and transferred to a 50 cm^3 flask that contained 5 cm^3 half-strength MILLER'S medium (1965) (Table 4.1) from which all hormones were omitted. The medium contained 2% sucrose as a carbon source and the pH was adjusted to 5.8. The culture was maintained on a rotary shaker (120 rpm) at 25°C. Root material was subcultured every 10 days until a large stock of material was available for experimentation. It has previously been shown that while test-to-test fluctuations cannot be entirely eliminated, and as a result control cultures must be included in all experiments, clonal tomato roots nevertheless remain constant in growth rate over a period of many years (STREET, 1957). In all experiments lateral roots only were used. The apical 12 mm of each root was

Table 4.1 Basal medium for soybean callus bioassay (Adapted from MILLER, 1965).

Stock Solution	Chemical	Stock Solution g ℓ^{-1}	Millilitres of stock solution per litre of medium
Stock 1	KH_2PO_4 KNO_3 NH_4NO_3 $Ca(NO_3)_2.4H_2O$ $MgSO_4.7H_2O$ KCl $MnSO_4.4H_2O$	3.0 10.0 10.0 5.0 0.715 0.65 0.14	100
Stock 2	NaFeEDTA $ZnSO_4.7H_2O$ H_3BO_3 KI $Cu(NO_3)_2.3H_2O$ $(NH_4)_6Mo_7O_2.4H_2O$	1.32 0.38 0.16 0.08 0.035 0.01	10
Stock 3	*Myo*-inositol Nicotinic acid Pyridoxine HCl Thiamine HCl	10.0 0.2 0.08 0.08	10
Additional	Sucrose		20 g ℓ^{-1}

removed and these then transferred to the various test solutions. Twenty replicates were used for each experiment. All cultures were gently agitated on a rotary shaker as this is said to improve root growth (SAID & MURASHIGE, 1979).

4.2.2 Establishment of isolated tomato roots on agar and the subsequent inoculation of the cultures with nematodes.

Production of stock cultures of aseptic tomato roots grown in vitro.

Lateral roots from the aseptic clonal stock culture (described above) were used in this investigation. The apical 12 mm of each root was removed and transferred to Petri-dishes containing SKOOG, TSUI and WHITE medium (ORION, WERGIN & ENDO, 1980) solidified with 0.8% agar. Cultures were maintained in the dark at 25°C. Root material was subcultured every six weeks.

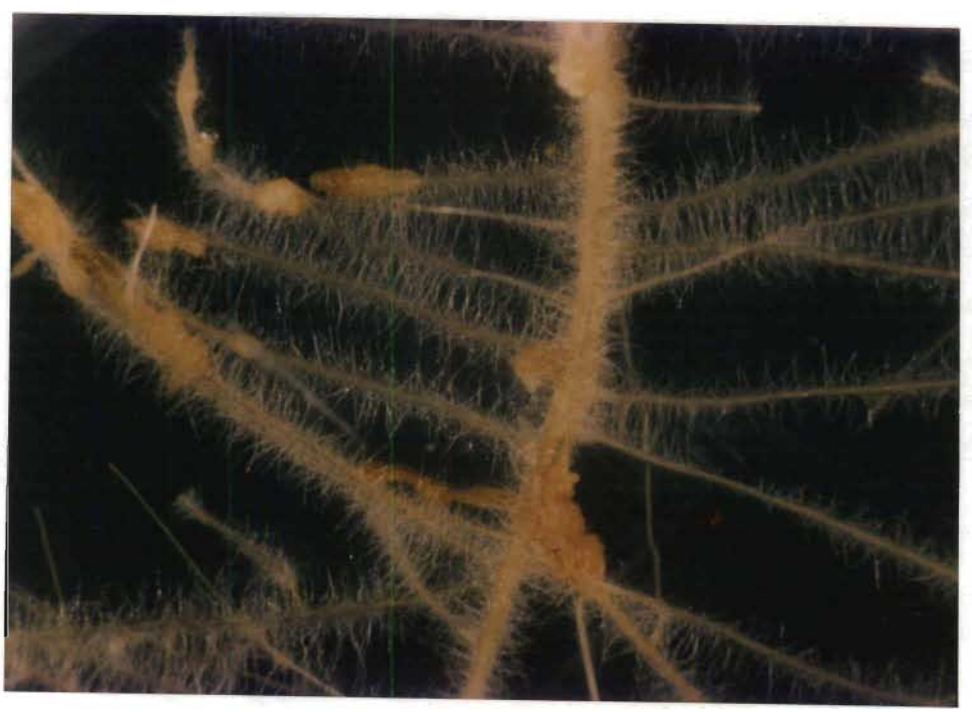

Figure 4.1 ***In vitro*** development of ***Meloidogyne incognita*** galls on excised tomato roots.

Sterilization and inoculation of nematode egg masses to root cultures.

The species of nematode used in this study was *Meloidogyne incognita* (Kofoid et White) Chitwood. Egg masses removed from heavily infested tomato roots, were surface sterilized with Hibitane or mercuric chloride and hydrogen peroxide (Table 4.2) and then washed four times in autoclaved distilled water. The egg masses were then transferred to 3-week-old *in vitro* roots and the inoculated cultures incubated in the dark at 25°C for gall development. Sterile egg masses from these cultures were then used to inoculate all experimental material (Figure 4.1). Depending on the condition of the material a high degree of bacterial contamination was sometimes recorded two or three weeks after the initial sterilization of the egg masses.

4.3 Effect of SWC on the growth of tomato roots cultured *in vitro*.

SWC is usually applied to crops and thus most growth effects have been explained in relation to the functioning of the whole plant. It was hoped that a better understanding of seaweed action might be formulated after examining the effect of seaweed on isolated plant organs. In Chapter 3, the effect of SWC on the growth of potato explants cultured *in vitro*, showed that SWC stimulated shoot growth and induced tuberization. This preliminary research indicated the presence of heat stable components in SWC that are beneficial to the growth and development of shoots. The following report on the growth of excised tomato roots, examined whether or not stimulatory components in SWC enhance root as well as shoot growth.

Table 4.2 **Outline of sterilants used to decontaminate nematode egg masses for *in vitro* inoculation of aseptic tomato roots.**

Sterilants	%	Time mins	Sterilization success (%)
Mercuric chloride ($HgCl_2$) + Hydrogen peroxide	0.1 3.0	3 20	30
Mercuric chloride ($HgCl_2$) + Hydrogen peroxide (H_2O_2) +	0.1 3.0	3 28	15
Hibitane	0.5	20	38

4.3.1 Experimental Procedure and Results

Clonal *in vitro* grown tomato (*Lycopersicon esculentum* Mill. *cv*. Moneymaker) roots were used as experimental material. Various dilutions of SWC, filtered SWC and residual cell-wall material from the manufacturers were applied to sterile tomato roots (Table 4.3). All cultures were allowed to grow for 14 days whereafter the roots were removed and the length of the main axis and the fresh mass of each root recorded. Results were statistically analyzed using one-way anova (where $P<0.01$) and a multiple range test.

SWC had a significant effect on root growth (Figure 4.2 and Figure 4.3). Application of SWC significantly increased root length and root fresh weight. Separating the extract into a soluble liquid and insoluble particulate phase indicated that growth promoting substances were contained in the soluble phase. The particulate matter alone invariably inhibited root growth. Application of SWC by-product material resulted in significantly larger roots when administered at low concentrations, suggesting that this waste material still has the potential to be utilized as a plant additive.

Table 4.3 **Outline of treatments used to assess the effect of SWC on the growth of tomato roots cultured *in vitro*.**

	TREATMENTS
Control	Half strength MILLER'S medium
0.2% SWC	Untreated seaweed concentrate
0.2% SWC	SWC filtered through a 0.4 μm filter
0.2% SWC	SWC particulate phase after filtration
0.4% SWC	Untreated seaweed concentrate
0.4% SWC	SWC filtered through a 0.4 μm filter
0.4% SWC	SWC substrate after filtration
0.04% Residue	By product from manufacturing process. Mainly cell wall fragments. Tested to determine if debris contains growth regulatory substances.

4.3.2 Discussion

The ability of commercial seaweed products to improve root growth is well documented (BLUNDEN & WILDGOOSE, 1977). Enhanced root growth through seaweed application has often been explained in terms of the organic components present in seaweeds. Organic material improves soil texture by improving water retention and soil aeration (QUASTEL & WEBLEY, 1947). MILTON (1964) suggested that degraded complexes of fucoidin, alginates and similar substances present in seaweeds enhance soil aggregate and crumb structure making conditions more suitable for root growth. Because the roots in this study were cultured *in vitro*, improved soil conditions cannot explain enhanced growth. A component of the SWC must therefore be responsible for stimulated growth. As minute quantities of the extract were found to elicit a response, it is probable that growth hormones are involved. FINNIE & VAN STADEN (1985) suggested that cytokinins may be the active factor. On fractionating a SWC, they determined that more than one active fraction improved the growth of *in vitro* roots. Fractions that co-chromatographed with zeatin and ribosylzeatin

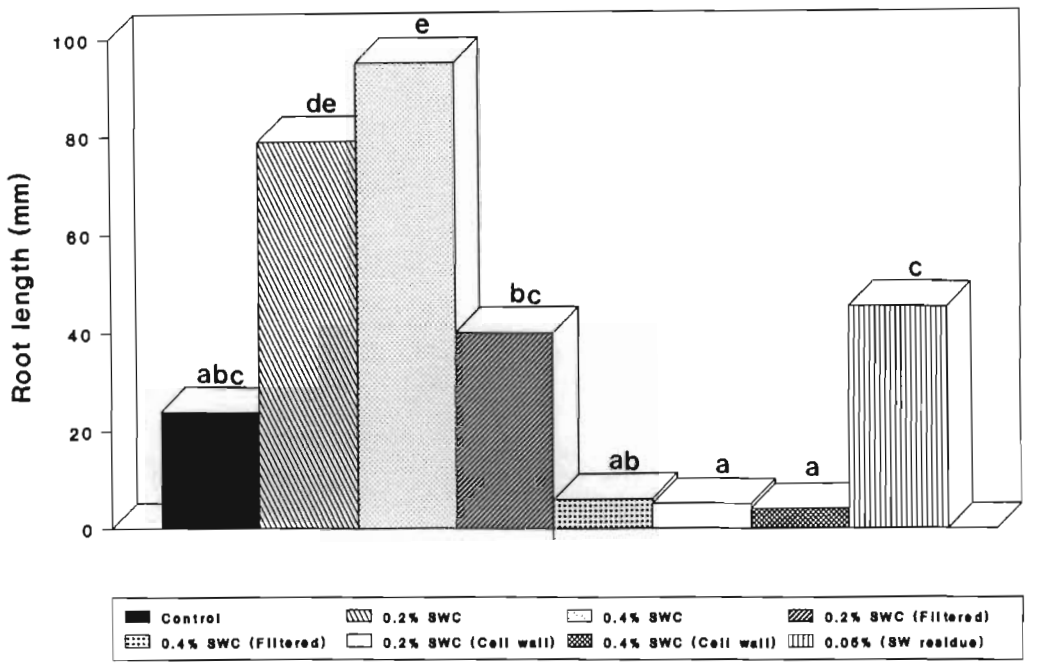

Figure 4.2 The effect of SWC on the length of *in vitro* cultured tomato roots. Bars with the same letter are not significantly different.

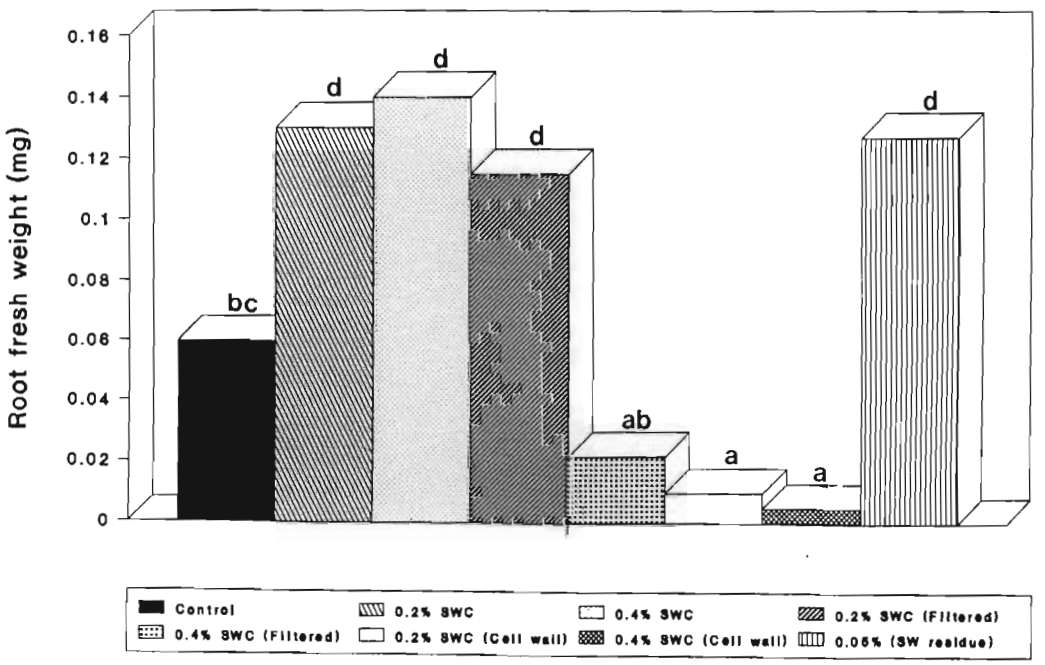

Figure 4.3 The effect of SWC on the fresh weight of *in vitro* cultured tomato roots. Bars with the same letter are not significantly different.

promoted lateral root emergence. It was also found that high concentrations of both SWC and zeatin inhibited root development while low concentrations promoted growth.

There is some doubt about auxins role in stimulating root growth, although some authors feel that it is involved in root development (SCOTT, 1972). THIMANN (1937) proposed that roots have a low threshold of auxin activity and that their endogenous level is above the optimum for growth. Root elongation may result from acropetal transport of auxin to the region of elongation. This transport of auxin from root base to root tip may also stimulate the production of lateral roots provided that a root inhibitor is not present (THIMANN, 1937). SWCs are known to contain both cytokinins (TAY, MACLEOD, PALNI & LETHAM, 1985; TAY, PALNI & MACLEOD, 1987) and auxins (KINGMAN & MOORE, 1982; SANDERSON & JAMESON, 1986). Enhanced root growth may possibly be explained by the presence of these hormones, depending on their relative concentrations in the different treatments.

The removal of particular matter from the SWC by microfiltration resulted in a promotory response from the filtrate and a highly inhibitory response from the particulate matter. Phenolic compounds and mannitol are known to stimulate root development (JACKSON, 1965; POAPST & DUNKEE, 1967). *Ascophyllum nodosum* and other brown algae commonly used in commercial seaweed preparations are known to contain considerable quantities of these compounds (BOOTH, 1969). The presence of inhibitory compounds in seaweed extracts is also well documented (BLUNDEN, CHALLEN & WOODS, 1968). BLUNDEN, CHALLEN & WOODS (1968) noted that amino-acids, laminarin, carrageanan and sodium alginate all of which are constituents of seaweed are inhibitory to plant growth. It is possible that one or more of these stimulatory or inhibitory compounds were responsible for the growth patterns noted in this study. Removal of the particulate matter from the SWC did not result in the filtrate increasing root growth over and above that of the seaweed treatments. This suggests that compounds other than inhibitors are possibly present in the particulate phase that act synergistically with a component(s) in the filtrate to enhance root

growth. Reduced root development may also have arisen from toxic levels of substances that might otherwise have promoted or had no effect on root growth.

4.4 Effect of SWC on the nematode infestation of resistant and susceptible varieties of *in vitro* grown tomato roots.

To gain a better understanding of how seaweed products influence root-knot nematode infestation, a system was developed that isolated roots from the soil and permitted normal host-parasite development in an artificial *in vitro* environment.

This system, involving the inoculation of aseptic nematode egg masses to *in vitro* tomato roots has a number of advantages over an *in vivo* system:

(i) neither micro-organisms nor soil properties interfere with root growth;

(ii) effects of seaweed treatment on whole plants are removed (for instance increased plant quality and vigour);

(iii) chemicals can be precisely added to or deleted from the media (these chemicals could include different plant growth regulators such as cytokinins, auxins and possibly even ethylene;

(iv) the system enables strict control of parameters such as light, temperature, nutrients, and seaweed application;

(v) the system enables easy monitoring of the stages of nematode and gall development; and

(vi) measurement of gall and egg mass numbers is possible without the risk of contaminating the sterile cultures.

Prior to establishing this system a number of difficulties had to be overcome, namely:

(i) reducing the variability within a root culture;
(ii) reducing the amount of contamination within each treatment;
(iii) inoculating a consistent amount of eggs to each petri dish;
(iv) keeping cultures healthy for the duration of the parasites life-cycle; and
(v) providing enough replicates of each treatment to allow for statistical analysis.

In this study the effect of SWC on the infestation and reproduction of nematode populations in excised root cultures was examined. Two varieties of tomato: nematode-resistant (*cv* M1) and nematode-susceptible (*cv* Rana), were used in the investigation.

4.4.1 Experimental Procedure and Results

Twenty replicates each of 0.2% and 0.4% SWC, added to the culture medium, was used to test for nematoxic properties in the SWC. A single sterile egg mass was placed 2 cm away from the growing root tip and the degree of nematode infestation calculated as either the mean number of galls or mean number of egg masses per Petri-dish after four weeks in culture.

Considerably fewer galls were found on the roots of the resistant variety of tomato. Seaweed treatment had no significant effect on gall number but significantly increased the number of egg masses (Figure 4.4 and Figure 4.5). In the susceptible variety, SWC applied at a concentration of 0.4% significantly reduced the number of galls and egg masses (Figure 4.4 and Figure 4.5). Two way analysis of variance indicated a significant interaction between the SWC and reduced incidence of nematode infestation in the roots. It appears therefore that SWC may be inducing resistance in nematode susceptible tomatoes and possibly breaking resistance in

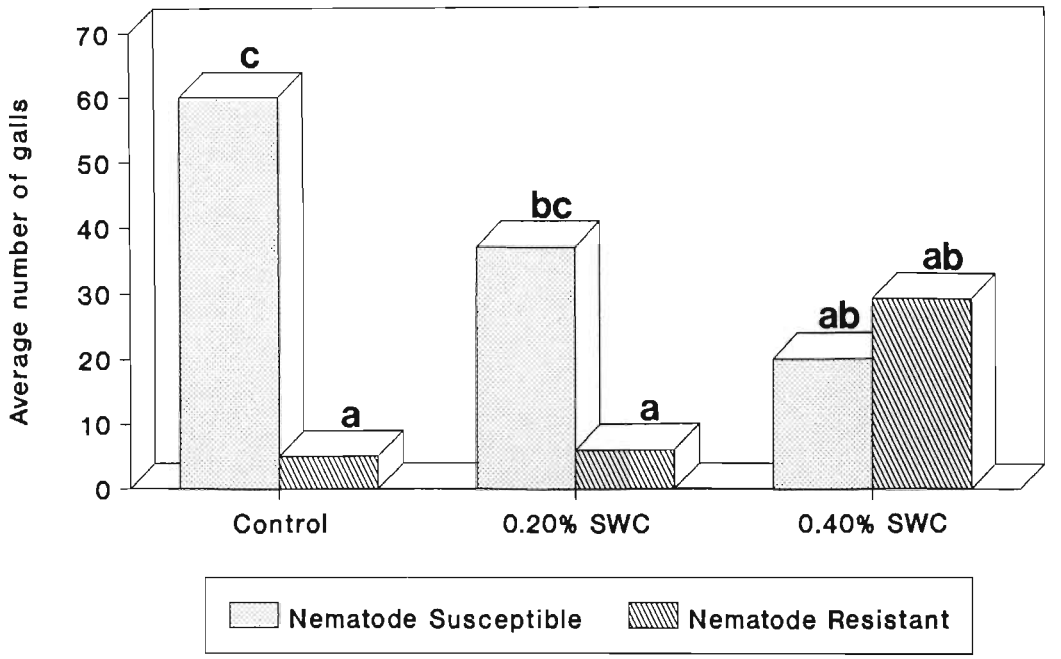

Figure 4.4 The effect of SWC on the number of galls on *in vitro* cultured tomato roots. Bars with the same letter are not significantly different.

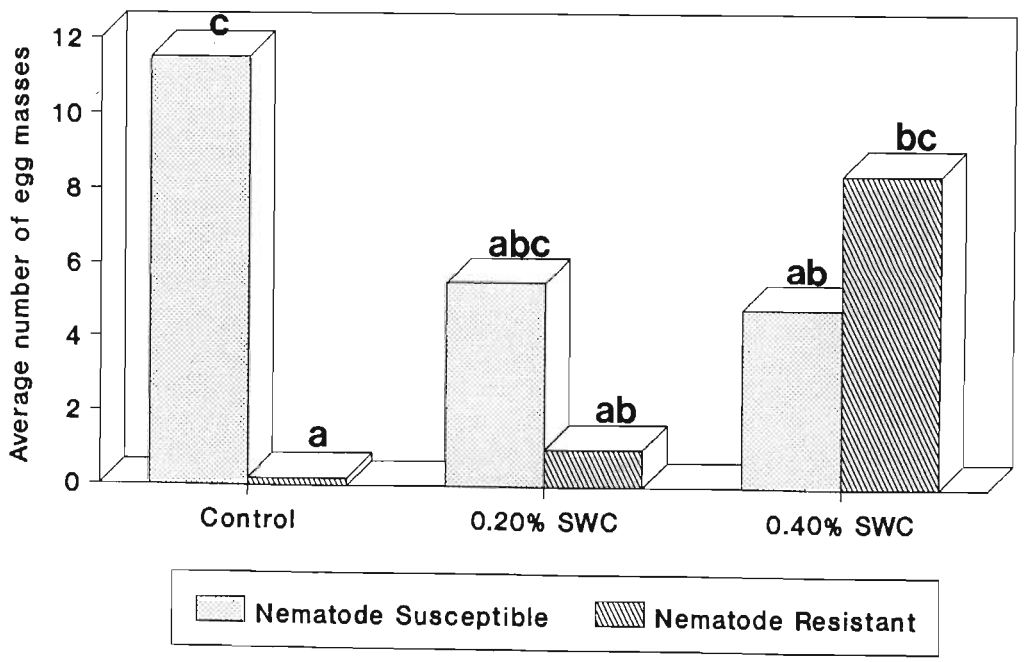

Figure 4.5 The effect of SWC on the number of egg masses on *in vitro* cultured tomato roots. Bars with the same letter are not significantly different.

plants has an adverse effect on resistant mechanisms, as indicated by these results, then it may possibly be economically unfeasible to apply the concentrate to these plants.

4.5 Discussion

Nematode-resistance in plants concerns two concepts: plant tolerance of nematode damage and nematode reproduction. Resistant plants are viewed as those that are generally tolerant of nematode damage and that limit nematode reproduction, whereas susceptible plants are often damaged by nematodes and facilitate nematode reproduction (KAPLAN & DAVIS, 1987). A plant is resistant to RKN's when the giant cells or "feeding cells" fail to develop. As a result, the RKN starves to death. A susceptible host is one that is unable to prevent the formation of these cells. In this study the resistant variety had significantly fewer galls than the susceptible variety. Incorporation of SWC into the medium however, reversed this effect. These results suggest that the seaweed is possibly affecting the host-parasite interaction at the cellular level. KAPLAN & KEEN (1980) reported that incompatibility to nematodes is generally expressed after infection and that active mechanisms of resistance involve compounds produced postinfectionally, rather than preformed constitutive plant products. If the SWC contained a nematoxic component, then a reduction in galling would have been expected in both varieties of tomato. These results therefore imply that the SWC is not affecting the eggs or larva prior to the entry of the nematodes into the root.

The host-parasitic interaction is a complex developmental system with genes from both sources interacting. Failure of the association may occur at any stage: - from initial parasitism of the first cell to maturation of the adult nematode. Failure of the compatible response may result from synthesis of metabolic inhibitors, death of the feeding site, or the failure of host tissue to keep pace with nutritive demands of the nematode. All of these are in one way or another, resistant responses (LEWIS, 1987).

When compounds invoke reactions similar to those in genetically resistant plants they are said to have 'induced resistance' (ORION & PILOWKSI, 1984). It is possible that certain constituents in SWC induce resistance in susceptible plants by adversely affecting the host-parasite interaction - possibly at the feeding site.

The formation of giant cells or feeding cells is associated with nuclear division, cell enlargement, thickening of cell walls (DROPKIN, 1969; PAULSON & WEBSTER, 1970), and increased DNA, RNA and protein synthesis (ENDO, 1971a, 1971b). The cells surrounding the giant cells divide and enlarge and so contribute to gall formation. Since endogenous plant growth regulators affect cell division and enlargement, and stimulate DNA, RNA and protein synthesis (WIGHTMAN & SETTERFIELD, 1968), it is possible that these compounds are involved in giant cell or gall formation. SAWHNEY & WEBSTER (1979) conjectured that they may affect host susceptibility or resistance to nematodes. The general interpretation is that high levels of PGR's e.g. IAA, NAA, kinetin (SAWHNEY & WEBSTER, 1975; CUTLER & KRUSBERG, 1968; KOCHBA & SAMISH, 1972), or compounds that induce or are associated with growth hormone synthesis, e.g. ethylene (ORION & MINZ, 1969), or compounds that serve as precursors in hormone biosynthesis, e.g. tryptophan (LEWIS & McCLURE, 1975) - favour susceptibility (VEECH, 1981).

Studies show that relatively high levels of auxins (CUTLER & KRUSBERG, 1968), cytokinins, or both are found in the roots of susceptible plants infected with nematodes. Exogenous application of kinetin (DROPKIN, HELGESON & UPPER, 1969), or an auxin and kinetin (KOCHBA & SAMISH, 1971; SAWHNEY & WEBSTER, 1975), encouraged giant cell and gall formation in infected roots of plants resistant to *Meloidogyne* species. SAWHNEY & WEBSTER (1975) noted that although the resistant cultivar produced galls, only a few larvae developed to maturity. This indicated that resistance was not completely broken, suggesting that PGR's are not the only factors that determine the host of tomato to *M. incognita*. SWC applied to root cultures resistant to *Meloidogyne* behaved in a fashion similar to that reported above. It is possible therefore, that high levels of cytokinins and auxins in the SWC may be overriding the resistant mechanisms in the roots. If a combination of auxin and cytokinin in the SWC

increased nematode numbers as recorded for resistant varieties, then susceptible plants should have exhibited an even greater degree of infestation. This was not the case adding support to the idea that compounds other than hormones are involved.

Seaweed application to susceptible roots invariably reduced nematode infestation. Thus, the interaction between the SWC and the nematodes was different for each variety of tomato. This suggests that either compounds other than plant hormones are involved or that the SWC is affecting the root physiology differently for each variety. SAWHNEY & WEBSTER (1975) examined the role of auxin (NAA) and cytokinin (kinetin) in the response of susceptible cultivars of tomato to infection with *M. incognita*. NAA and kinetin in combination increased the susceptibility of the susceptible cultivar. However, when applied separately these hormones inhibited larval penetration and root-knot nematode development (DROPKIN, HELGESON & UPPER, 1969; SAWHNEY & WEBSTER, 1979). The presence of either cytokinin or auxin alone, or a high concentration of only one of these hormones may account for the reduced infestation recorded in this study. While cytokinins have been positively identified in several SWCs ((TAY, MACLEOD, PALNI & LETHAM, 1985; TAY, PALNI & MACLEOD, 1987) and tentatively in Kelpak (FEATONBY-SMITH & VAN STADEN, 1983a; FINNIE & VAN STADEN, 1985), auxin is yet to be positively identified in this commercial preparation. However, several auxin bioassays and HPLC studies in Chapter 2 (sections 2.4 and 2.5) indicated the presence of auxin-like compounds in the SWC. It is therefore reasonable to assume that PGR's or compounds other than these two hormones may be involved.

GLAZER, APELBAUM & ORION (1984, 1985a) and GLAZER, ORION & APELBAUM (1983, 1985) found correlations between ethylene production and nematode infestation in tomato roots. Excised roots infected with *M. javanica* produced ethylene at 2 to 6 times the rate of noninfected roots. Gall growth and ethylene production was accelerated by ethylene production stimulators (1-aminocyclopropane-1-carboxylic acid (ACC), indole acetic acid (IAA) and ethrel), whereas the addition of ethylene inhibitors, aminoethoxyvinylglycine (AVG), aminoxyacetic acid (AOA) and silver thiosulphate (STS), reduced the rate of gall development and ethylene production. NELSON & VAN

STADEN (1985) have identified ACC in a SWC. In view of the observations of GLAZER, APELBAUM & ORION (1985) it is possible that SWC may somehow increase ethylene production in the resistant variety of tomato making the roots more susceptible to nematode infestation.

Compounds other than plant hormones known to play a role in the induction of plant resistance to nematodes include certain nitrogenous compounds, amino acids, and phytoalexins (coumestrol, glyceollin and terpenoids) (VEECH, 1981). Application of biologically active chemicals such as maleic hydrazide, morphactins and cycloheximide (KOCHBA & SAMISH, 1972; ORION & MINZ, 1971; SAWHNEY & WEBSTER, 1979) on RKN infested plants led to the inhibition of normal nematode development. Unfortunately these compounds were also found to have adverse effects on the plant meristem or growth patterns. Recently, ORION, WERGIN & ENDO (1980) demonstrated that a form of resistance could be induced by applying a high concentration of ammonium nitrate to a medium on which *M. incognita* were cultured on excised roots of tomato. In this experiment, coenocytes and nematode development were severely hampered, but root growth not affected. In addition urea, thiourea and hydroxyurea when applied at much lower concentrations to *in vitro* and *in vivo* host plants also resulted in inhibition of nematode development and in poor giant cell formation, again without affecting root growth (GLAZER & ORION, 1984, 1985). The impact of these compounds on RKN development in host plants closely resembled the hypersensitive reaction found in genetically resistant plants.

SWCs may reduce nematode infestation in a similar fashion. Nearly 20 amino acids have been identified in Kelpak. Some of these constituents may mimic the action of the nitrogenous compounds described above by acting at the level of coenocyte formation.

It has also been suggested that in resistant plants, enzymes may affect changes in growth regulators, free bound phenols, amino acid composition, and perhaps induce lignification to limit nematode development (GIEBEL, 1974; ROY, 1981). It is possible that the SWC may induce a resistance in susceptible plants by affecting these enzymes.

The exact mode and mechanisms of nematicidal action of SWCs is still not understood. This chapter proposed some preliminary models of seaweed/nematode interaction. While there was no conclusive evidence for any one model, the possibility of seaweed action at the plant cellular level cannot be dismissed.

In both the test systems studied in this chapter, improved plant growth through seaweed application was noted. In the first system the SWC significantly increased root growth and development. Filtering the extract, separated the SWC into a promotory soluble phase and inhibitory insoluble phase suggesting the presence of both growth promoters and growth inhibitors.

In the second system, the SWC reduced nematode infestation in a susceptible variety of tomato but increased infestation in a resistant variety. Although the constituents responsible for these effects are not known, similar responses have been recorded after the application of certain hormones and other selected compounds. A nematicidal compound was thought not to be involved.

This Chapter was of particular interest because it confirmed that SWC contains components that are beneficial to isolated plant parts. In Chapter 3, SWC was shown to improve the growth of isolated potato shoots cultured *in vitro*. That SWC elicits beneficial growth responses regardless of plant part, suggests the presence in the product of more than one active component.

CHAPTER 5

EVIDENCE FOR A ROOTING FACTOR IN SEAWEED CONCENTRATE

5.1 Introduction

Adventitious root formation is essential to the multiplication of many economically important plants. Most ornamental shrubs, many commercial greenhouse crops, and numerous fruit and forest trees are propagated by cuttings. Because of the wide commercial basis, there is an interest in the development of new compounds concerned with the initiation of roots. Although much of the fundamental biology of adventitious root formation is still poorly understood, basic research has contributed significantly to practical propagation, most notably through the discovery, characterisation, and improvement of auxins (DAVIS, HAISSIG & SANKHLA, 1988).

A remarkable feature of several seaweed studies is that commercial seaweed products significantly increase root growth (FEATONBY-SMITH & VAN STADEN, 1984b; BECKETT & VAN STADEN, 1989). Using root cultures FINNIE & VAN STADEN (1985) showed that seaweed concentrate increased both lateral root development and root extension *in vitro*. Improved root growth following seaweed treatment was also noted in Chapter 4. As cytokinins are generally considered to inhibit rooting (VAN STADEN & HARTY, 1988) it is difficult to attribute the observed increase in root growth to these hormones. There are no studies to date supporting the involvement of seaweed products in the initiation of adventitious roots. Evidence of a rooting factor in Kelpak was first noted during the mung bean root initiation bioassay (Chapter 2.4.1). The number of roots initiated following the application of SWC was found to be three times that of authentic auxin treatments. This finding prompted the following investigation to determine the potential of SWC as a rooting agent. The

isolation of an active rooting factor from SWC could be of immense economic significance.

In this chapter the effect of SWC on the initiation of roots on cuttings of several species of garden plants and on a few hard-to-root species of *Eucalyptus* is examined. The standard mung bean bioassay was used to test for endogenous rooting factors in the SWC and to characterise the properties of the compounds involved. This rooting test was originally developed by HESS (1957) to detect naturally occurring substances that stimulate rooting in the presence of IAA.

5.2 Adventitious Root Formation in Cuttings.

The concept of a compound with the specific ability to initiate the regeneration of roots was first proposed by SACHS (1880a, 1880b). He explained the polar regeneration of roots in terms of a 'rhizome' substance synthesised in the leaves of cuttings and transported basipetally to the region of regeneration. BOUILLENNE & WENT (1933) subsequently used the term 'rhizocaline' to describe such a substance, which was seemingly an acid compound of low molecular weight, was heat-stable and stored in cotyledons and buds (WENT, 1934). Other factors such as nutrients, carbohydrates, phenolics, nitrogenous compounds, vitamins and inorganics are now known to be involved in root initiation and development (JARVIS, 1986). The identification of IAA and other synthetic indoles led to the discovery that auxins induce root initiation (THIMANN & WENT, 1934; ZIMMERMAN & WILCOXON, 1935; COOPER, 1935) and that 'rhizocaline' was physiologically similar to auxin (THIMANN & KOEPFLI, 1935). Recently, root initiation has been postulated to involve the interaction of three factors: an unidentified diphenol, an enzyme (possibly polyphenol oxidase) and auxin (BOUILLENNE & BOUILLENNE-WALRAND, 1955). These three factors are sometimes collectively referred to as the 'rhizocaline complex'.

The rooting response of any cutting is dependent on the age of the stock material from which the cutting is taken; the promotory compound used, its concentration; the duration of the treatment and the time interval between excision of the cutting and commencement of treatment (JARVIS, 1987).

Auxins comprise the only group of chemicals which consistently enhance root formation in so-called easy-to-root cuttings (HAISSIG, 1974). A high level of endogenous auxin has been causally related to the initiation of adventitious root primordia. Frequently, maximum rooting occurs when high levels of auxins are applied immediately, or soon after, cuttings are made (JARVIS, ALI & SHAHEED, 1983; SHIBAOKA, 1971). The concentrations used are just below those which induce symptoms of toxicity (MIDDLETON, 1977; JACKSON & HARNEY, 1970).

Several other observations indirectly implicate auxin in the control of adventitious root formation: (i) young leaves and active buds, which are sources of auxin, enhance rooting in some cuttings. Exogenously applied auxin completely or partially replaces these promotive effects; (ii) auxin is transported preferentially in a basipetal direction, an observation consistent with the polarity of root formation; and (iii) compounds interfering with auxin transport or action, such as triiodobenzoic acid (TIBA), have been found to inhibit root regeneration, while chemicals such as phenolics, which are known to influence auxin metabolism often improved the rooting response of cuttings (JARVIS, 1986).

The influence of leaves on adventitious root formation has been widely interpreted in terms of a supply of both auxin and nutritional factors. BASTIN (1966) and HESS (1969) implicated other specific factors in root initiation which may possibly arise in the leaves, stems or buds. However, the influence of leaves may simply enhance uptake of supplied auxin into the appropriate transport system, thereby ensuring a more efficient delivery to those particular cells which are the potential sites for root formation.

A wide range of chemicals, both natural and synthetic are known to enhance the rooting response of cuttings to applied auxins. The terms 'auxin-synergists' and rooting 'co-factors' have been used to describe some of these chemicals. The latter term implies natural occurrence and has been used frequently with reference to phenolic compounds (HESS, 1962; 1969). Other chemicals that do not necessarily act synergistically with auxin, which may be either promotory or inhibitory in nature, are also known to affect rooting.

VAN RAALTE (1954) demonstrated synergistic effects of indole, phenylacetic acid and phenylbutyric acid on IAA-induced rooting of petioles. Subsequent work (GORTER, 1958; 1969) confirmed the synergism between indoles and IAA but showed application of indole alone to enhance rooting. The chemical nature of such substances suggested that they may act via IAA oxidase, particularly in view of the known effect of indole in diminishing the activity of IAA oxidase (VAN RAALTE, 1955; PILET, 1958).

Applied phenolics are now known to promote adventitious rooting in stem cuttings (HESS, 1964a; 1964b; FERNQVIST, 1966; BASSUK, HUNTER & HOWARD, 1981; MITRA, 1986). 2,5-Dihydrobenzoic acid has been shown to promote rooting in *Tilia americana* L. cuttings (MORSINK & SMITH, 1975), coumarin was effective on cuttings of *Impatiens balsamina* L. (DHAWAN & NANDA, 1982), umbelliferone on *Phaseolus vulgaris* L. cuttings (VAZQUEZ, 1973), and rutin on cuttings of *Euonymus alatus* Sieb (LEE & TUKEY, 1971).

BOUILLENNE & BOUILLENNE-WALRAND (1955) suggested that root initiation is dependent on at least three factors which constitute the 'rhizocaline' complex. They viewed auxin as a non-specific component of this complex and proposed that it accelerates cell mitosis but does not determine the characteristic structure of the root. The other two components were viewed as a orthodiphenolic compound, transported from the leaves; and an enzyme, probably polyphenol oxidase, located in those cells which eventually give rise to root initials.

While the 'rhizocaline' concept is acknowledged to have some credibility, some researchers doubt its validity. JAMES & THURBON (1981) revived the idea, proposed by STONIER, HUDEK, VANDE-STOUWE & YANG (1970), that some phenolics might rather: (i) protect IAA by inhibiting peroxidase (IAA oxidase), and (ii) inhibit oxidation reactions in general, maintaining the cell in a reduced state and perhaps allowing cells to divide. BASSUK, HUNTER & HOWARD (1981) following POAPST & DURKEE (1967) drew attention to the more general promotory role of phenolics and their derivatives in the wound response.

Other factors known to effect adventitious root formation, include: carbohydrates, mineral nutrients, and certain plant growth regulators other than auxin.

KRAUS & KRAYBILL (1918) were the first to propose that a relationship may exist between carbohydrate content and the rooting of cuttings. Since then, many studies have demonstrated that sugars, including sucrose, glucose, fructose, ribose, deoxyribose, myo-inositol and dextrose, enhance the rooting response of cuttings in the presence, or absence, of supplied auxin (FERNQVIST, 1966; MIDDLETON, 1977; JARVIS & BOOTH, 1981). The influence of carbohydrate could be largely to enhance transport of the supplied auxin to the site of regeneration (JARVIS, 1986).

The importance of mineral nutrients in adventitious root development is emphasised by several investigations (FERNQVIST, 1966; HESS, 1969; BATTEN & GOODWIN, 1978). Nutrients thought to be involved, include: nitrogen, zinc, manganese, calcium, magnesium and boron. While nitrogen (HAUN & CORNELL, 1951), zinc (SAMISH & SPIEGEL, 1958) and manganese (REUVENI & RAVIV, 1981) possibly have the most influence on root initiation, boron is essential for root growth and development.

The importance of nitrogen in root initiation is clearly supported by findings of mobilization and fertility studies, and the 'need' for N_2 in protein and nucleic acid synthesis (BLAZICH, 1988). However, the promotive influence of nitrogen in root initiation may also be manifested by the manner in which it influences carbohydrate content and metabolism (HARTMANN & KESTER, 1983). Zinc is required for production

(TSUI, 1948; SALAMI & KENEFICK, 1970) of the auxin precursor tryptophan (GOODWIN & MERCER, 1983), and manganese is thought to act as an activator of IAA oxidase (THOMASZEWKSI & THIMANN, 1966).

Unlike root initiation, in which the importance of various minerals has not been clearly demonstrated, the situation with respect to root growth and development is much clearer. Although phosphorus and nitrogen (HAUN & CORNELL, 1951) and to a lesser extent calcium and magnesium (BLAZICH, WRIGHT & SHAFFER, 1983) are thought to affect root growth, only boron (HEMBERG, 1951) has been shown to be essential to the development of adventitious cuttings. GORTER (1958) suggested that whereas roots were initiated in response to auxin, boron was essential for growth of primordia. MIDDLETON, JARVIS & BOOTH (1978) found that treatment of mung bean cuttings with auxin led to the development of primordia and roots only when boron was applied. Events initiated by auxin therefore only lead to formation of root primordia in the presence of boron. This stimulatory effect of boron has been reported for cuttings of other non-woody plants (ELIASSON, 1978), and also for some woody species (WEISER, 1959; WEISER & BLARNEY, 1960). The obligatory role of boron in root growth and development (HAISSIG, 1986) as well as at the whole plant level (LEWIS, 1980) remains speculative. It has been proposed that boron, by enhancing IAA oxidase activity, has a promotive influence on the oxidative destruction of auxin (JARVIS, ALI & SHAHEED, 1983; JARVIS, YASMIN, ALI & HUNT, 1984). Since the high concentrations of auxin which initiate rooting, would be inhibitory to later root growth (MIDDLETON, 1977), the influence of boron on the regulation of endogenous auxin levels is essential for further root development.

The effect of plant hormones, other than auxins, on adventitious root formation have also been studied. These include cytokinins, gibberellins, ethylene and abscisic acid. The apical or basal application of cytokinins (kinetin or 6-benzyladenine (BA)) to stem cuttings is generally found to inhibit root formation. Such inhibitory effects have been reported for *Phaseolus vulgaris* (HUMPHRIES, 1960), *P. aureus* (FERNQVIST, 1966) and *Pisum sativum* (ERIKSEN, 1974). Despite these inhibitory effects, it appears that some cytokinin may be required for root formation since the addition of small

amounts of cytokinins is necessary for the formation of roots in tissue culture (JARVIS, 1986). Low concentrations of benzyladenine have been shown to promote rooting of sunflower hypocotyl tissue whereas high concentrations were inhibitory (FABIJAN, TAYLOR & REID, 1981). A cytokinin antagonist, 3-methyl-7-(3-methyl-butylamino) pyrazolo [4,3-d] pyrimidine, inhibited root initiation in cuttings of *Coleus* (SKOOG, SCHMITZ, BOCK & HECHT, 1973). It seems likely, therefore, that the requirement for root initiation is for a low cytokinin level and a favourable auxin:cytokinin ratio.

Gibberellins have generally been found to inhibit adventitious root formation in a variety of cuttings (KATO, 1958; HARTUNG, OHL & KUMMER, 1980; FABIJAN, TAYLOR & REID, 1981). This inhibition of root formation is thought to be dependent on the time of application (SMITH & THORPE, 1975; COLEMAN & GREYSON, 1977). Light conditions during stock plant growth may also affect the degree of rooting following gibberellin treatment (BOWN, REEVE & CROZIER, 1975; HANSEN, 1975). The mode of gibberellin action in root formation is still obscure. It has been suggested that the application of gibberellin to the base of cuttings may inhibit cell division at the place of root initiation (REINERT & BESEMER, 1967), induce auxin synthesis (ANDERSON & MUIR, 1969), or affect carbohydrate metabolism (NANDA, PUROHIT & MEHROTRA, 1968; HANSEN, 1976).

In several studies, applied abscisic acid (2.5-30 mg ℓ^{-1}) has been found to promote rooting (HARTUNG, OHL & KUMMER, 1980; RASMUSSEN & ANDERSON, 1980). In other studies, however, ABA has been reported to inhibit or have no effect on rooting. RASMUSSEN & ANDERSON (1980) suggested that, depending upon the experimental protocol almost any rooting response to ABA can be obtained. They reported that factors such as concentration, rooting period length, and stock plant growth conditions all influenced the response of pea (*Pisum sativum* L.) cuttings to ABA. Exogenously applied ABA has generally been found to partially overcome GA_3 induced inhibition of adventitious rooting (COLEMAN & GREYSON, 1976; HARTUNG, OHL & KUMMER, 1980). In addition to opposing gibberellin action, applied ABA may also affect rooting in other ways. When applied in conjunction with auxin treatment, ABA has been shown to promote rooting (BASU, ROY & BOSE, 1970). RASMUSSEN & ANDERSON (1980) found that ABA reduced apical shoot growth on pea cuttings, which might improve rooting by

reducing competition for assimilates normally transported to and metabolised at the cutting base.

While the above factors are all possibly involved in rooting to some extent, the overall mechanisms of adventitious root formation are still not clearly understood.

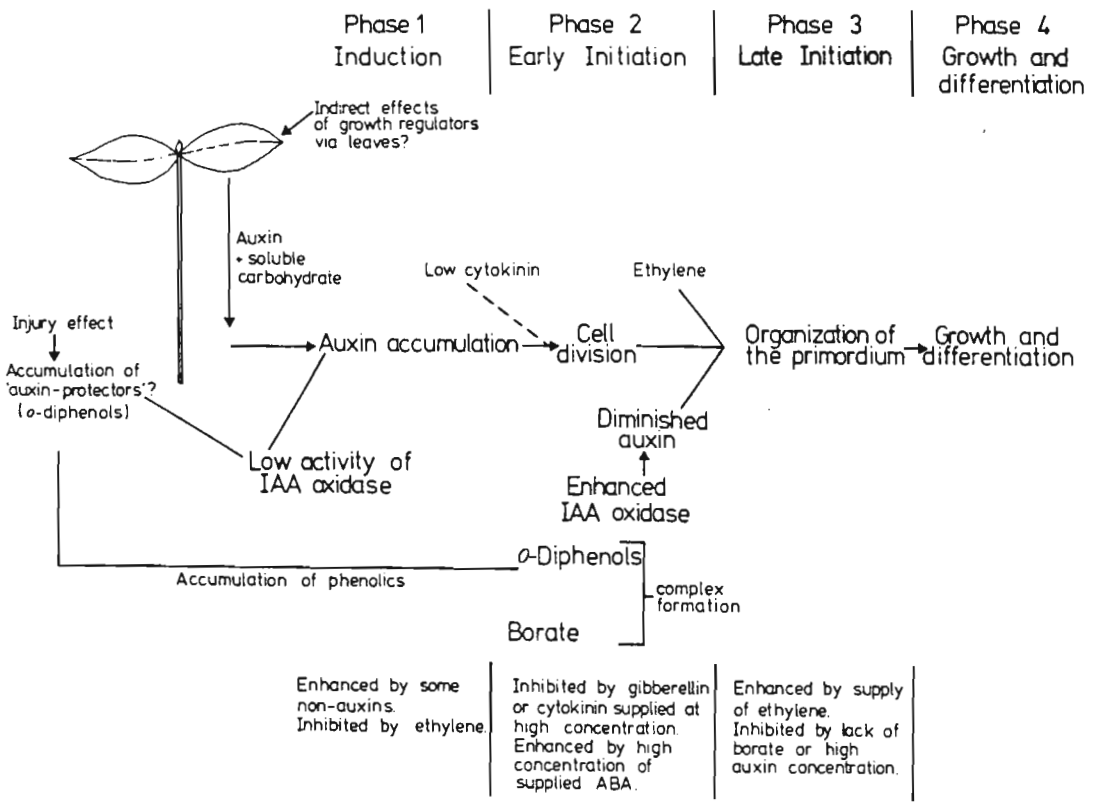

Figure 5.1 **Proposed scheme of events associated with various phases of adventitious root formation (Jarvis, 1986).**

JARVIS (1987) proposed a generalized hypothetical scheme for the formation of adventitious roots on cuttings in which he outlines four interdependent phases in root formation and development (Figure 5.1). The first three phases culminate in the formation of the root primordia and the fourth in the growth and differentiation of the root.

The first phase of the regeneration process, the induction or preparatory phase, is essentially characterised by lack of cell division at the potential sites of root formation. It appears that this phase is induced by chemicals other than auxins (WENT, 1939; SHIBAOKA, 1971). During this phase, basipetally transported auxin accumulates in the region of root formation. Associated with these events is relatively low IAA oxidase/peroxidase activity (CHIBBAR, GURUMURTI & NANDA, 1979; FOONG & BARNES, 1981).

The second, or early initiation phase, is characterised by cell division, which is possibly triggered by auxin accumulation during the first phase. The continued supply of auxin at high concentrations up to, and just beyond the earliest divisions results in the production of large numbers of roots (JARVIS, 1986). There is a need for a constant supply of auxin throughout this phase for both woody (HAISSIG, 1970; 1982) and non-woody cuttings (MOHAMMED & ERIKSEN, 1974; ERIKSEN & MOHAMMED, 1974). The early divisions of this phase are probably dependent on low endogenous cytokinin levels and are inhibited by gibberellins. Excision of the roots removes the source of both cytokinins and gibberellins and this, together with high auxin levels, ensures favourable conditions for the early events of initiation.

The essence of the third phase is a reduction in auxin levels associated with high peroxidase/IAA oxidase activity. BRUNNER (1978) and KEFELI (1978) both observed such a drop in auxin content within the region of root formation during natural regeneration. Primordium formation has been suggested to occur after maximum activity, when basic iso-peroxidases, which destroy auxins, are declining in activity (GASPAR, 1981; MONCOUSIN & GASPAR, 1983).

The IAA oxidase/peroxidase enzyme complex is thought to be controlled by an interaction between borate and phenolics. In the absence of boron, phase one and two proceed but no primordia appear (MIDDLETON, 1977; JARVIS, ALI & SHAHEED, 1983; JARVIS, YASMIN, ALI & HUNT, 1984). It has been suggested that boron may control the effective auxin concentrations and sites of initiation (JARVIS, YASMIN, ALI & HUNT, 1984). Boron has also been shown to enhance the activity of IAA oxidase, the enzyme

responsible for auxin catabolism (PARISH, 1968). While high levels of auxin are necessary for root initiation, low levels are essential for organisation and growth of the primordia.

Interestingly, borate readily complexes with certain phenolic compounds containing *cis*-hydroxyl groups (eg. *o*-diphenols). As *o*-diphenols are known to inhibit IAA oxidase activity (ZENK & MULLER, 1963), the borate required for organisation of the primordium could complex with such phenols thereby enhancing IAA oxidase activity (JARVIS, YASMIN, ALI & HUNT, 1984).

In the final phase of adventitious root development, the induced and initiated primordia grow and differentiate into roots.

5.3 General Material and Methods

5.3.1 Preparation of selected garden plants for root initiation.

Cuttings from *Callistemon citrinus* Skeels; *Evolvulus glomeratus* Nee & Mart; *Vitex agnus-castrus* L.; *Lavandula vera* DC.; *Impatiens auricoma* Baill. and *Dianthus deltoides* L. were used. Twenty cuttings of each species were pulsed for 18 hours in the test solution and placed in a vermiculite:perlite (1:1) medium in speedling trays. The trays were placed in a mist house (mist every 5 min for 2.5 sec, temperature 22-24°C) until the initiation of roots. Depending on the plant and the degree of rooting, either the number of roots or their dry weights were used to express the rooting response. All data, unless otherwise stated, was analyzed by a one way analysis of variance.

5.3.2 Preparation of Eucalyptus cuttings for root initiation

Propagation of *Eucalyptus* by cuttings is widely practised in agricultural and forestry sectors. Although the propagation technique is fairly standard, several precautions were taken to ensure high rates of cutting survival.

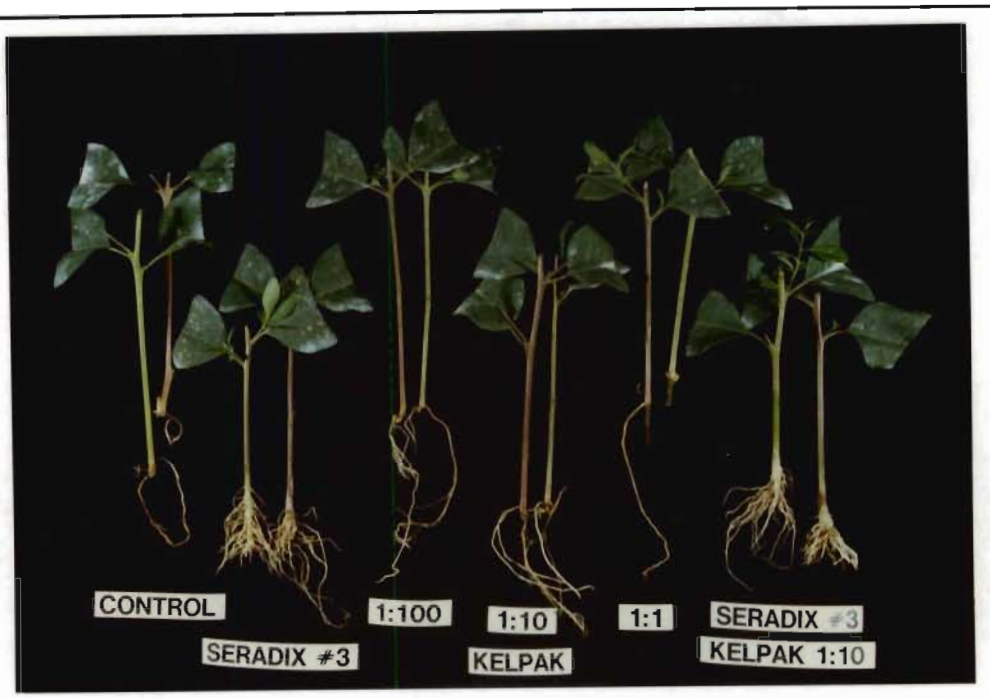

Figure 5.2 **The effect of SWC and a commercial rooting powder (Seradix) on the rooting of *Eucalyptus* cuttings.**

Young shoots from the primary axes of hedge coppices were harvested as close to the tree stumps as possible and placed in buckets half-filled with water. Two-leaf cuttings were prepared from the middle and basal regions of each shoot. The stem diameter varied between 3 to 5 mm and the length was standardised at 10 cm. Both the upper leaves were trimmed to approximately half the original areas and any developing axillary shoots removed (Figure 5.2). Trimmed cuttings were placed in Benlate (0.5 g ℓ^{-1}) for ten minutes and seaweed concentrate (3 cm depth) for 24 hours. After this pulse treatment the cuttings were inserted to a depth of 20-30 mm into holes prepared in a vermiculite:perlite (1:1) medium in UNIGRO (patented) trays. The propagation medium was moistened with Kaptan fungicide (captab, 1 g ℓ^{-1} active ingredient) prior to placement of the cuttings. Trays were placed in a

glasshouse under intermittent mist irrigation, the frequency of which was controlled automatically by an 'electronic leaf' solenoid-valve system. Mist was set to ensure that cuttings did not suffer water stress as indicated by leaf wilt and desiccation. The mean air temperature was 24°C and the bed temperature 22°C.

Cuttings were inspected every third day and leaves which had abscised, as well as cuttings which had begun to show basal rot, were removed. A spray of Bravo (chlorothalonil, 1.5 mg ℓ^{-1} active ingredient) and Dithane M45 (mancozeb, 1.6 mg ℓ^{-1} active ingredient) were administered alternately twice a week to reduce the degree of infection. The trays were rotated weekly to reduce variation between treatments. At the end of the trial, cuttings were gently removed from the trays and the vermiculite washed off the roots. Roots were counted, removed from the stems with forceps and dried in an oven at 50°C for 24 hours before being weighed.

Differences between proportional data for variables such as 'percentage rooting' were compared for significance by calculating the chi square value. The variable 'root number per cutting' consisted of data which was not normally distributed, since many cuttings produced only 1 or 2 roots and few produced many roots. Such data was analyzed by the Kruskal-Wallis non-parametric one-way analysis of variance test. Data of the variable 'root mass per cutting' was transformed to the natural logarithm value before application of the one-way analysis of variance test.

5.3.3 Mung bean bioassay

The standard procedure of HESS (1961a) as outlined in Chapter 2.2.3 was used in this study.

5.3.4 Extraction techniques

Five hundred mℓ of SWC was extracted in 80% methanol (AR grade) for eight hours at 10°C. The methanol fraction was separated from the cellular debris by filtration through Whatman Nº42 filter paper and the filtrate reduced to dryness under vacuum at 35°C. The residue was then resuspended in 100 mℓ phosphate buffer (pH 8.0) and partitioned for auxins according to the method described by SANDBERG, CROZIER & ERNSTSEN (1987) as outlined in Chapter 2 (Figure 2.2).

5.4 The Effect of SWC on the Rooting of Selected Cuttings.

5.4.1 Experimental Procedure and Results

Following the procedure outlined above (Section 5.3.1), cuttings were treated with 10% SWC and maintained in a mist house until root initiation. Many roots formed on *Lavandula* and *Impatiens* cuttings making it difficult to separate and count them thus only their dry weights were recorded. In a separate experiment with *Dianthus*, the rooting effect of SWC was compared to a commercially available rooting powder (Seradix).

When applied to different species of garden plants, 10% SWC increased rooting in every case (Figures 5.3 to 5.11). This was true with respect to both root number and mean root dry weight (Figure 5.3). The percentage increase over the control, while significant in the majority of instances, did however, vary considerably (Figure 5.4). Although the SWC initiated many roots on *Vitex* cuttings (Figure 5.5), after 20 days there was no difference between treated cuttings and those of the controls (Figure 5.6). A 10% dilution of SWC appeared to be slightly toxic to *Lavandula*. This was noticeable as 'tissue necrosis' at the base of the cuttings (Figure 5.8). Roots were initiated above this damaged zone. The only woody species tested, *Callistemon*

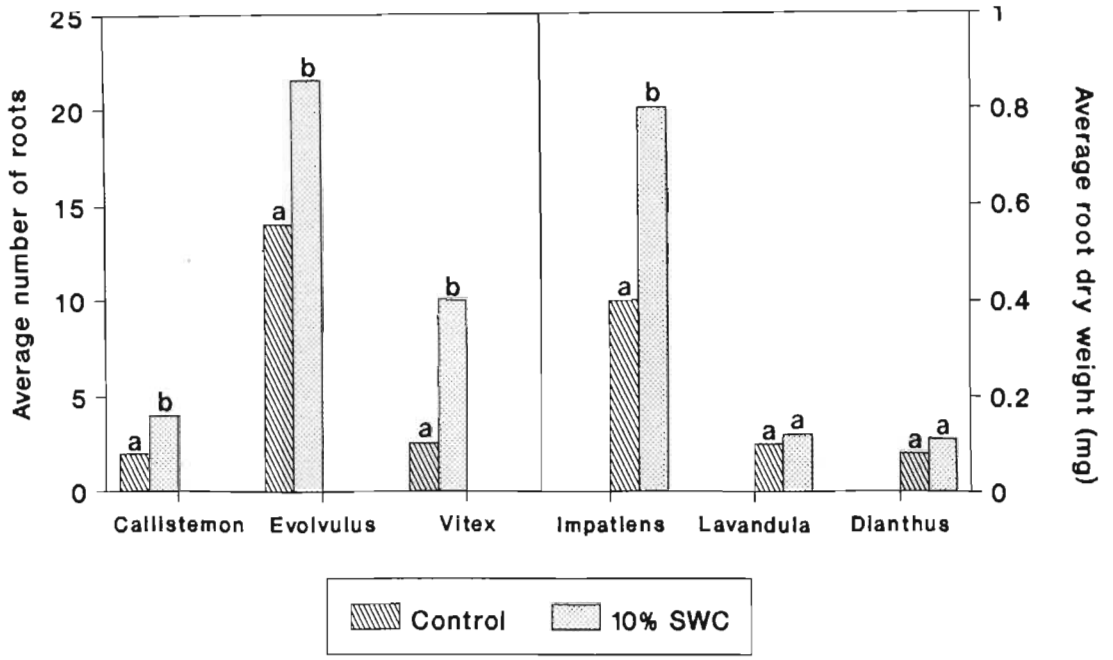

Figure 5.3 Rooting response of cuttings of *Callistemon, Evolvulus, Vitex, Lavandula* and *Impatiens* after an application of 10% SWC. Bars with the same letter are not significantly different.

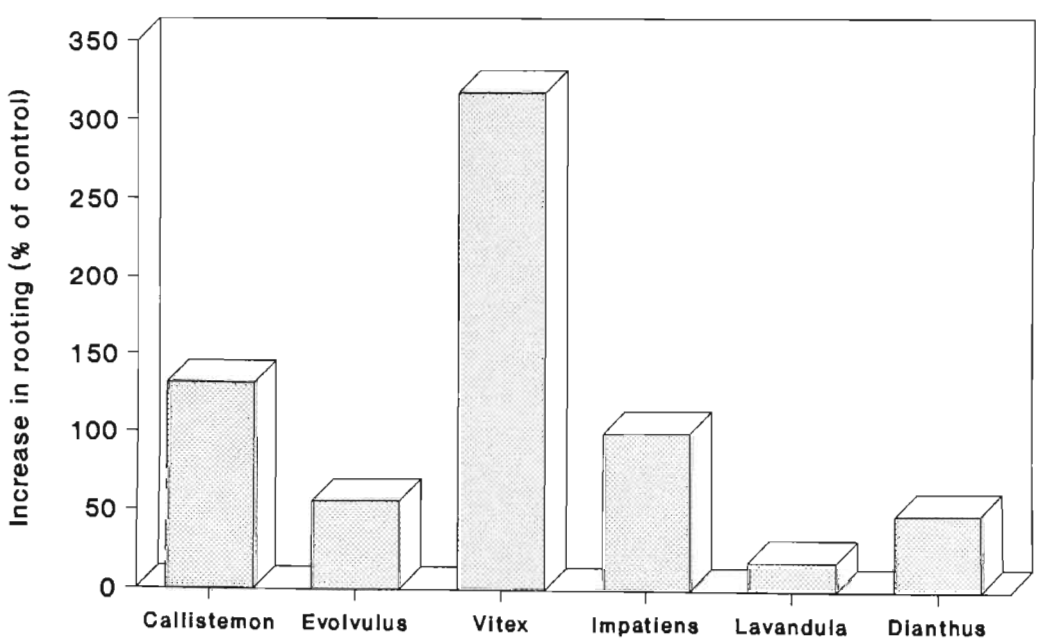

Figure 5.4 The effect of SWC applied as an 18 h pulse on the rooting of six plant species 15 days after being placed in a mist bed.

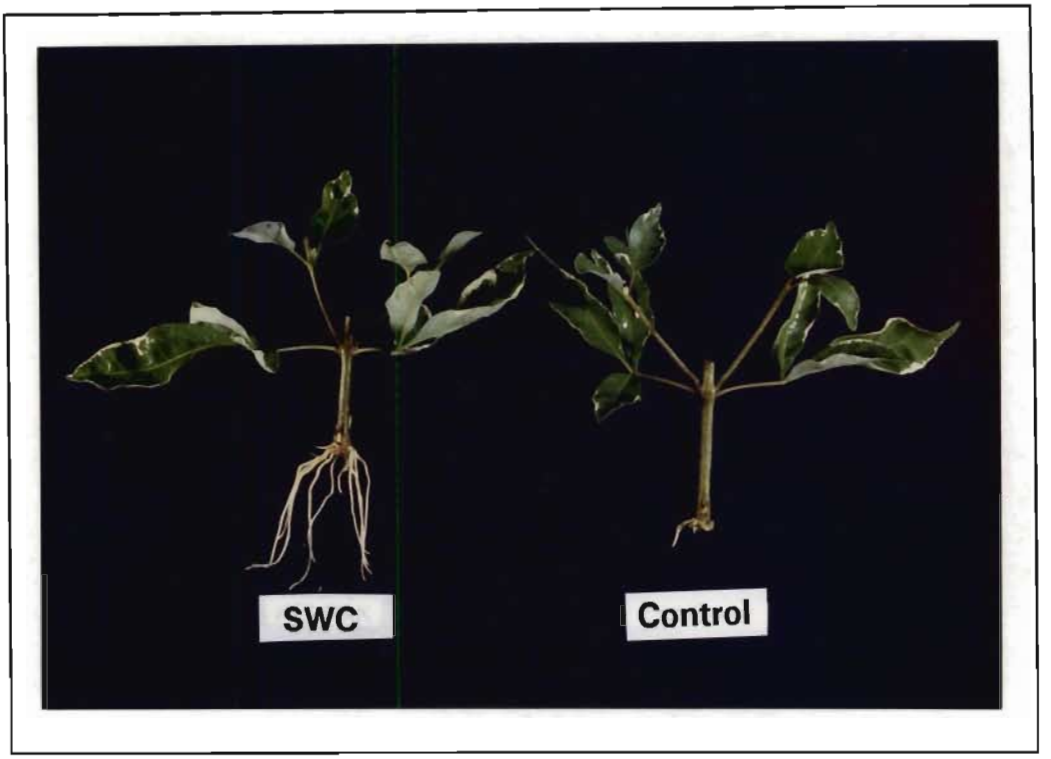

Figure 5.5 Adventitious rooting on *Vitex* cuttings 10 days after the application of 10% SWC as an 18 hour pulse.

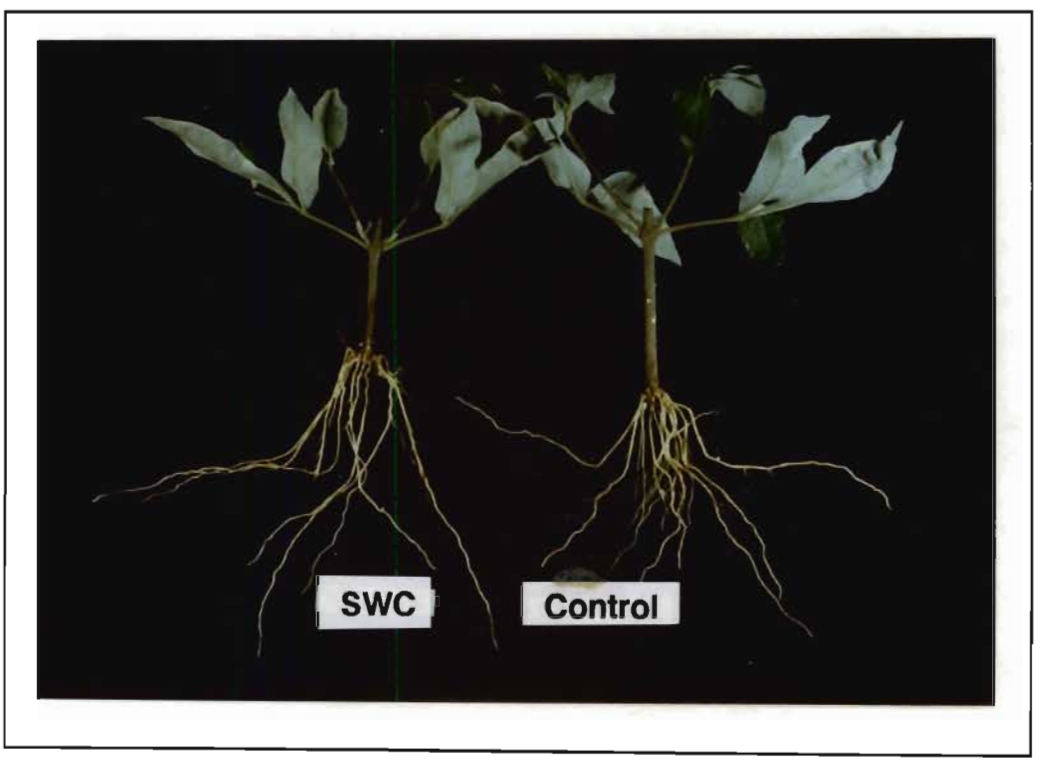

Figure 5.6 Adventitious rooting on *Vitex* cuttings 20 days after the application of 10% SWC as an 18 hour pulse.

Figure 5.7 Effect of an 18 hour pulse of 10% SWC on the rooting of *Lavandula* cuttings.

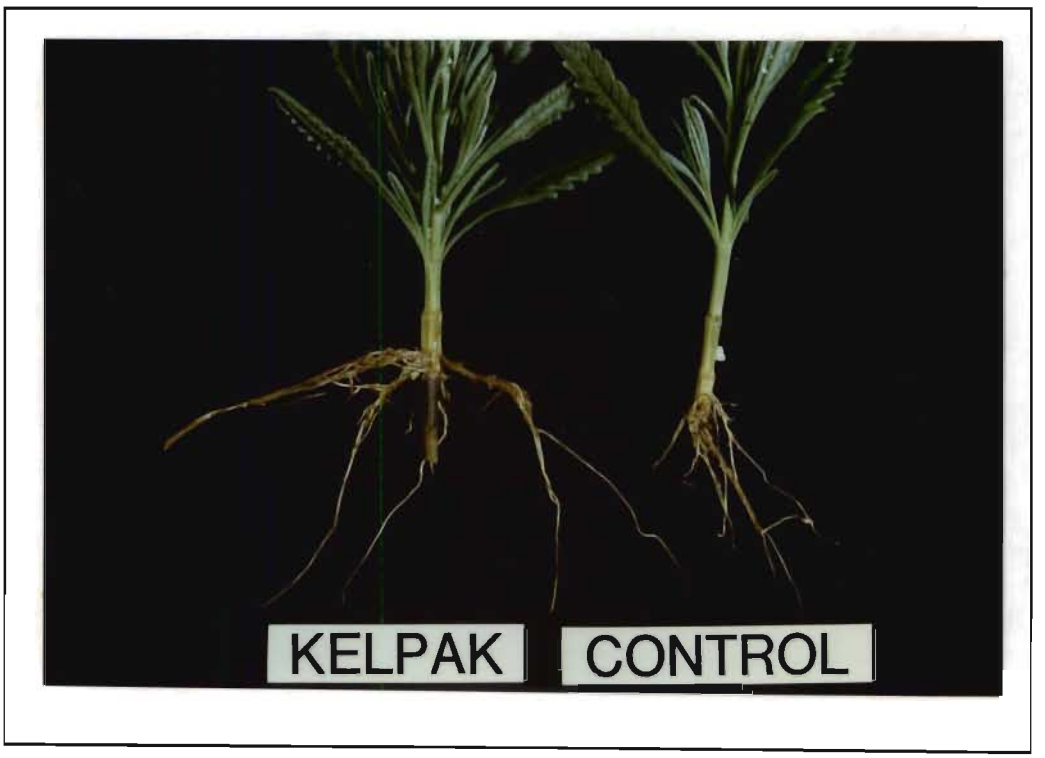

Figure 5.8 Necrosis at the base of *Lavandula* cuttings after an 18 hour pulse of 10% SWC.

Figure 5.9 Adventitious rooting on *Callistemon* cuttings 4 weeks after the application of 10% SWC as an 18 hour pulse.

Figure 5.10 Adventitious rooting of *Impatiens* 21 days after an application of 10% SWC to the cuttings as an 18 hour pulse. The extent of root growth allowed only dry weight to be measured.

Figure 5.11 Adventitious rooting of *Evolvulus 10 days* after an application of 10% SWC to the cuttings as an 18 hour pulse.

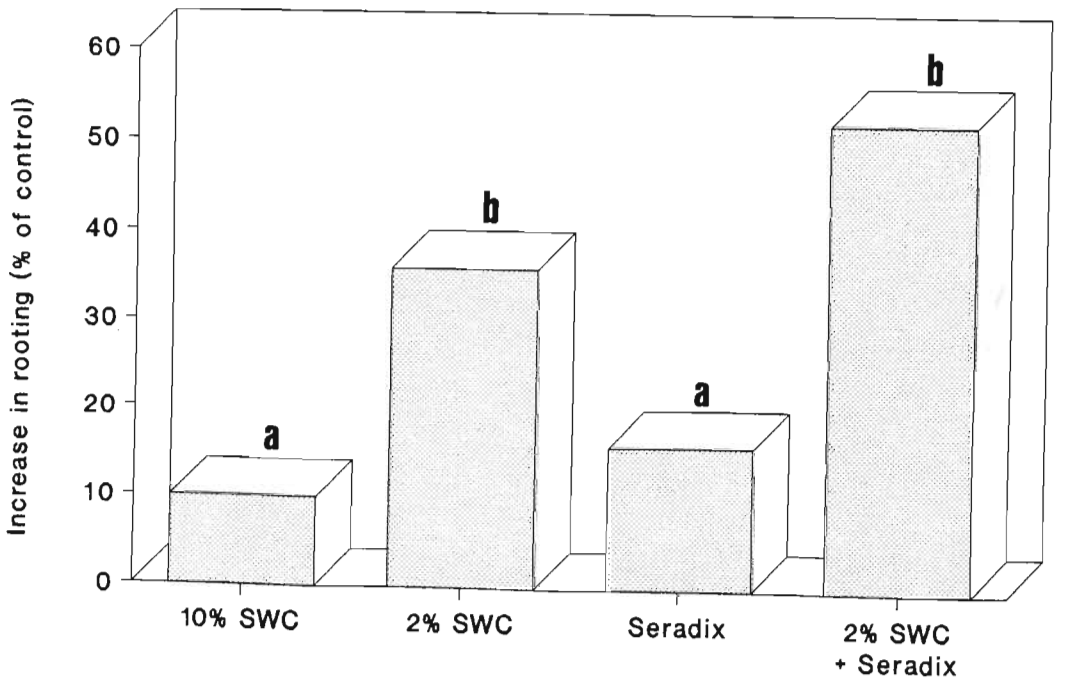

Figure 5.12 Effect of SWC and a commercial rooting powder (Seradix), alone or in combination with SWC, on the rooting of *Dianthus* cuttings. Cuttings received 10% SWC for 18 hours.

took four weeks to root (Figure 5.9) and had significantly more roots than the control cuttings (Figure 5.3). *Impatiens* cuttings rooted very well with SWC (Figure 5.10). The extent of rooting however, made a root count impossible and so the dry weight of the roots was measured.

A 10% dilution of SWC was slightly toxic to *Dianthus* cuttings. Diluting the SWC to 2.0%, increased the mean root dry weight by 37% compared to a 15% increase with Seradix (Figure 5.12). A combination of SWC and Seradix was shown to be cumulative and resulted in an increase of 52% over control values.

5.5 The effect of SWC on the rooting of several easy and difficult-to-root species of Eucalyptus.

When tested on herbaceous garden plants, SWC invariably improved the rooting of cuttings. In the following investigation, the potential of this rooting factor was tested further on several easy and difficult-to-root species of *Eucalyptus*. The importance of rooting *Eucalyptus* lies in the high economic value of this plant. Propagation of *Eucalyptus* is mainly by cuttings and ways of improving the rooting and survival of these cuttings could have a great impact in commercial nurseries.

5.5.1 Experimental Procedure and Results

Several species of *Eucalyptus* were examined including two easy-to-root species (*E. grandis*, Z-14; *E. saligna*, 3-4), a moderately easy-to-root species (*E. maccartheri/grandis* hybrid, MG25) and two clones of a difficult-to-root species (*E. maccartheri*, G3-170; G3-15). SWC was applied alone or in combination with a commercial rooting powder and compared to a distilled water control. After six to eight weeks the cuttings were removed from the trays and the number and dry

weight of the roots recorded. At this time the number of shoots on each cutting was also noted. The overall effect of SWC on the rooting of *Eucalyptus* was calculated by combining the results of each treatment, regardless of species, and expressing the means graphically as indicated in Figures 5.13 to 5.17.

No significant improvement in rooting was noted in any of the *Eucalyptus* species examined at any level of SWC application (Tables 5.1 to 5.3; Figures 5.13 to 5.17). Burning at the base of seaweed treated cuttings suggested that the applied concentrations of SWC were too high or that a shorter pulse time was required (Figure 5.18). A large percentage of cuttings rooted with the commercial rooting powder (Table 5.3; Figure 5.13). These cuttings had the greatest number of roots (Figure 5.14). A combination of Seradix and 10% seaweed concentrate resulted in a small increase in the percentage of cuttings rooted (Figure 5.13). Although the rooting powder was able to overcome the burning effect of the SWC, the number of roots initiated per cutting were always fewer than when Seradix was applied alone (Figure 5.14). Associated with this reduction in root number was a general increase in root size (Figure 5.16). Cuttings treated with SWC alone, only initiated one or two roots (Figure 5.14). These roots were significantly larger than those on non-treated cuttings (Figure 5.15). Cuttings treated with SWC generally had better developed shoots (Figure 5.17).

Table 5.1 **Effect of SWC on measured growth parameters in several *Eucalyptus* species and cultivars. Numbers with the same letter are not significantly different.**

E grandis *Z-14*	Number of roots per cutting	Root mass per cutting (mg)	Average root mass (mg)	Number of shoots per cutting
Control	3.2 a	80.8 a	34.2 b	1.42
Seradix Nº3	24.6 b	165 b	7.8 a	1.66
SWC 1.0%	3.6 a	103 a	35.6 b	1.66
SWC 10%	2.9 a	96.8 a	41.8 b	1.32
SWC 50%	2.7 a	120 ab	48.1 b	1.42
Seradix + SWC 10%	24.0 b	195 b	12.8 a	1.47

E. saligna *-3-4*	Number of roots per cutting	Root mass per cutting (mg)	Average root mass (mg)	Number of shoots per cutting
Control	1.3 a	79.5 a	66.6 b	1.54
Seradix Nº3	3.3 b	166 b	52.8 a	1.76
SWC 1.0%	1.0 a	54.3 a	54.3 b	1.92
SWC 10%	1.0 a	134 a	134 b	1.86
SWC 50%	-	-	-	1.67
Seradix + SWC 10%	2.0 b	136 b	72.2 a	1.76

E. mac/ grandis MG25	Number of roots per cutting	Root mass per cutting (mg)	Average root mass (mg)	Number of shoots per cutting
Control	2.6 a	138 abc	64 ab	1.27
Seradix Nº3	10.1 b	271 bcd	38 a	1.12
SWC 1.0%	1.5 a	113 a	86 b	0.82
SWC 10%	1.6 a	172 a	124 ab	1.33
SWC 50%	1.3 a	117 ab	83 ab	1.05
Seradix + SWC 10%	5.6 b	288 d	97 b	0.97

Table 5.2 **Effect of SWC on measured growth parameters in several *Eucalyptus* species and cultivars. Numbers followed by the same letter are not significantly different.**

E. maccartheri G3-170	Number of roots per cutting	Root mass per cutting (mg)	Average root mass (mg)	Number of shoots per cutting
Control	-	-	-	1.33
Seradix Nº3	5.0 b	180 a	51 a	0.69
SWC 1.0%	1.0 a	286 a	286 b	1.36
SWC 10%	-	-	-	1.47
SWC 50%	2.0 a	272 a	136 ab	1.33
Seradix + SWC 10%	4.4 b	228 a	69 a	0.47

E. maccartheri G3-15	Number of roots per cutting	Root mass per cutting (mg)	Average root mass (mg)	Number of shoots per cutting
Control	1.8 a	249 b	172 bc	0.67
Seradix Nº3	4.8 b	188 ab	64 a	0.35
SWC 1.0%	1.6 a	211 a	124 ab	0.53
SWC 10%	1.3 a	160 a	132 ab	0.57
SWC 50%	1.0 a	274 ab	274 c	0.57
Seradix + SWC 10%	4.1 b	157 b	80 a	0.46

COMBINATION OF ALL SPECIES	Number of roots per cutting	Root mass per cutting (mg)	Average root mass (mg)	Number of shoots per cutting
Control	2.2 a	137 a	84.2 c	1.25
Seradix Nº3	9.8 b	194 d	42.4 a	1.11
SWC 1.0%	1.9 a	153 ab	117 b	1.27
SWC 10%	1.7 a	141 a	108 bc	1.31
SWC 50%	1.8 a	171 cd	135 d	1.21
Seradix + SWC 10%	8.0 b	201 d	66.3 b	1.03

Table 5.3 **Effect of SWC and a commercial rooting powder (Seradix) on the percentage of *Eucalyptus* cuttings rooted.**

	TREATMENTS					
		SWC/Seradix (Ser)				
	Control	Ser	SWC 1.0%	SWC 10%	SWC 50%	Ser + SWC 10%
E. grandis Z-14	60.7	96.4	64.3	53.6	10.7	100
E. mac/grandis	39.3	89.3	21.4	35.7	10.7	92.9
E. saligna-4	14.3	64.3	10.7	10.7	3.6	53.6
E. mac G3-15	46.4	71.4	35.7	10.7	10.7	71.4
E. mac G3-170	0	42.9	3.6	0	3.6	50.0

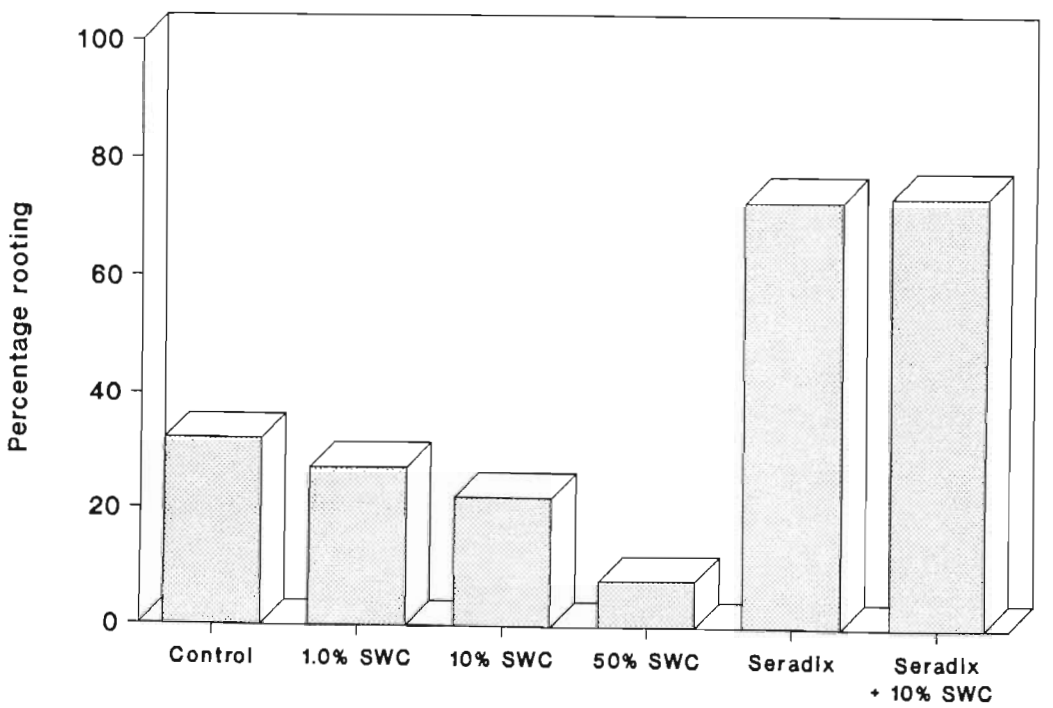

Figure 5.13 **Effect of SWC on the percentage of *Eucalyptus* cuttings rooted. Values represent averaged response for all cultivars and species tested. Bars with the same letter are not significantly different.**

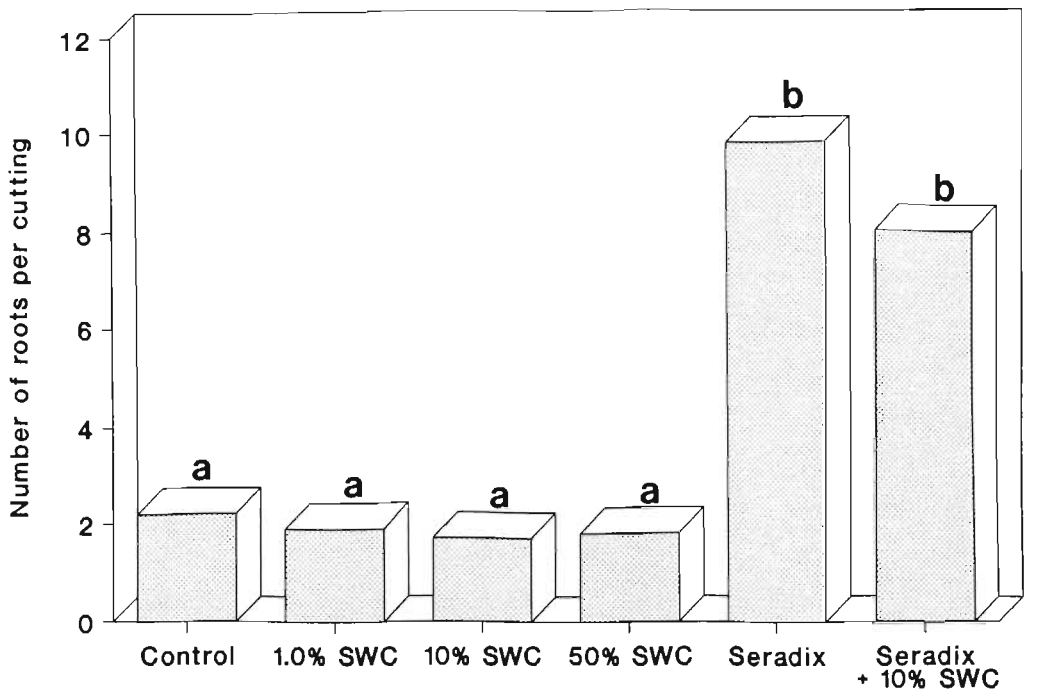

Figure 5.14 Effect of SWC on the initiation of roots on *Eucalyptus* cuttings. Values represent averaged response for all cultivars and species tested.

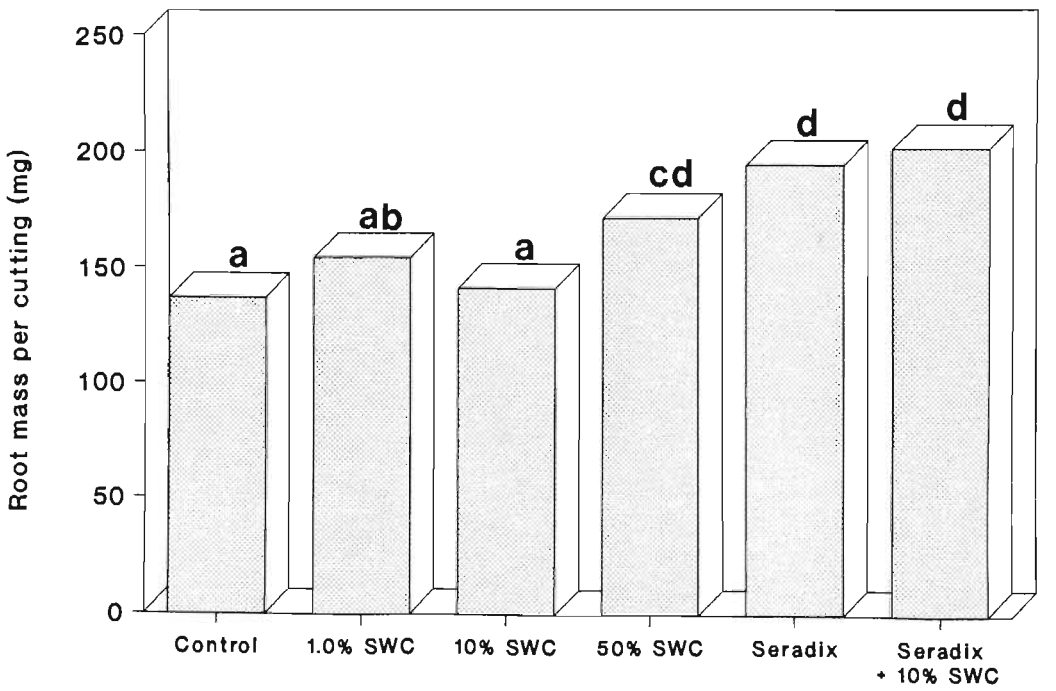

Figure 5.15 Effect of SWC on the root mass per cutting of *Eucalyptus* cuttings. Values represent averaged response for all cultivars and species tested.

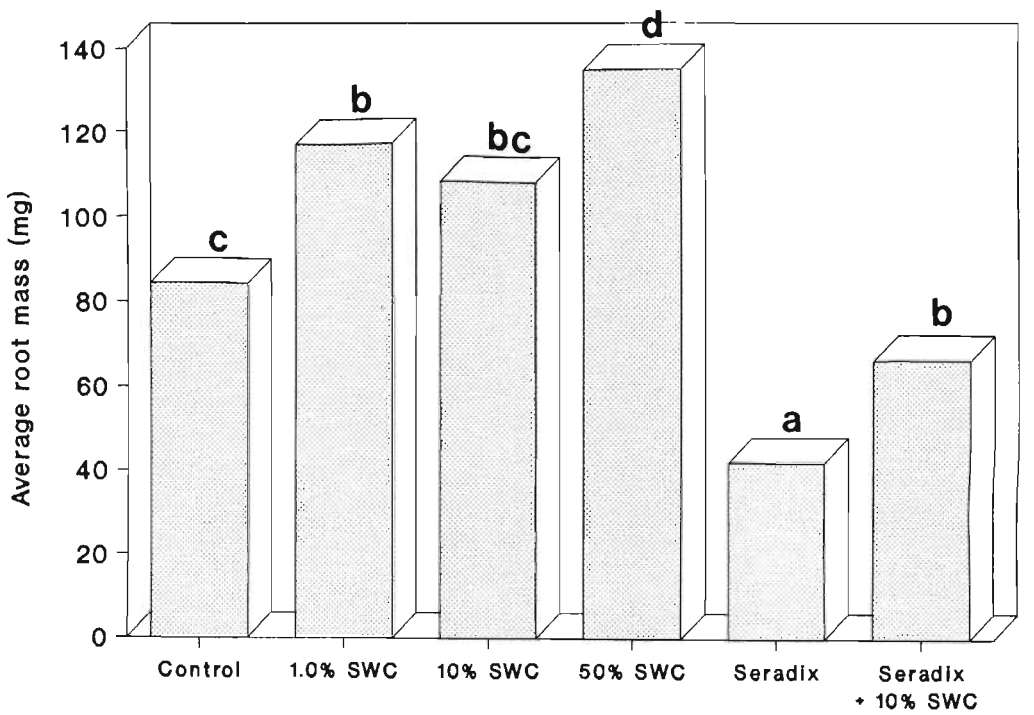

Figure 5.16 **Effect of SWC on the weight of individual roots of *Eucalyptus* cuttings. Values represent averaged response for all cultivars and species tested.**

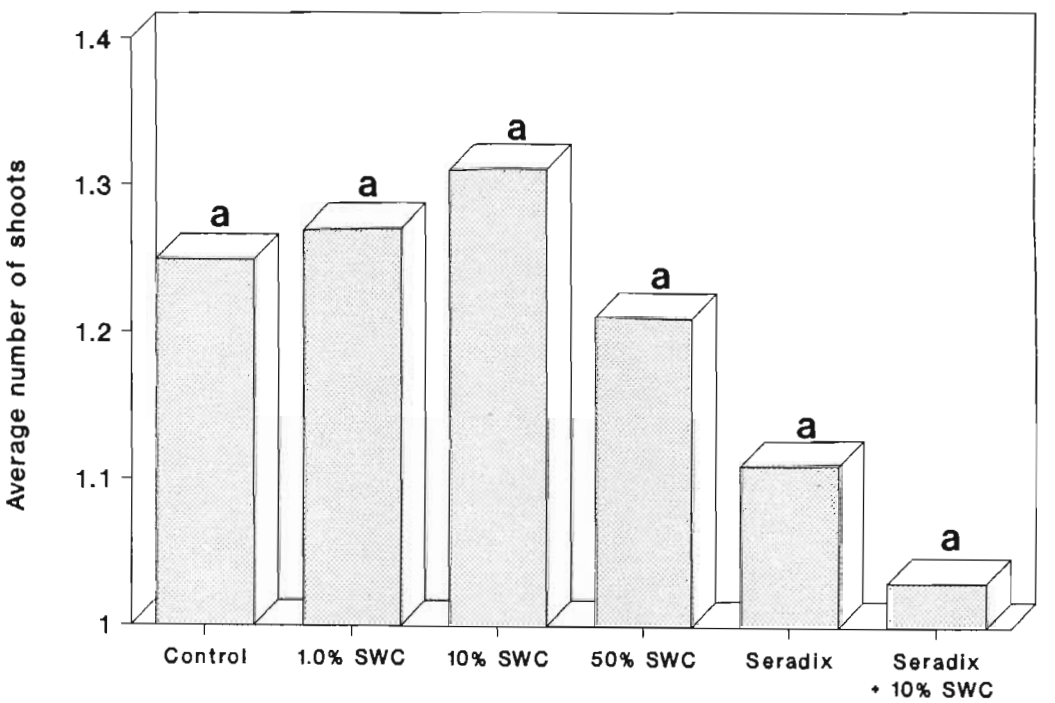

Figure 5.17 **Effect of SWC on the development of shoots on *Eucalyptus* cuttings. Values represent averaged response for all cultivars and species tested.**

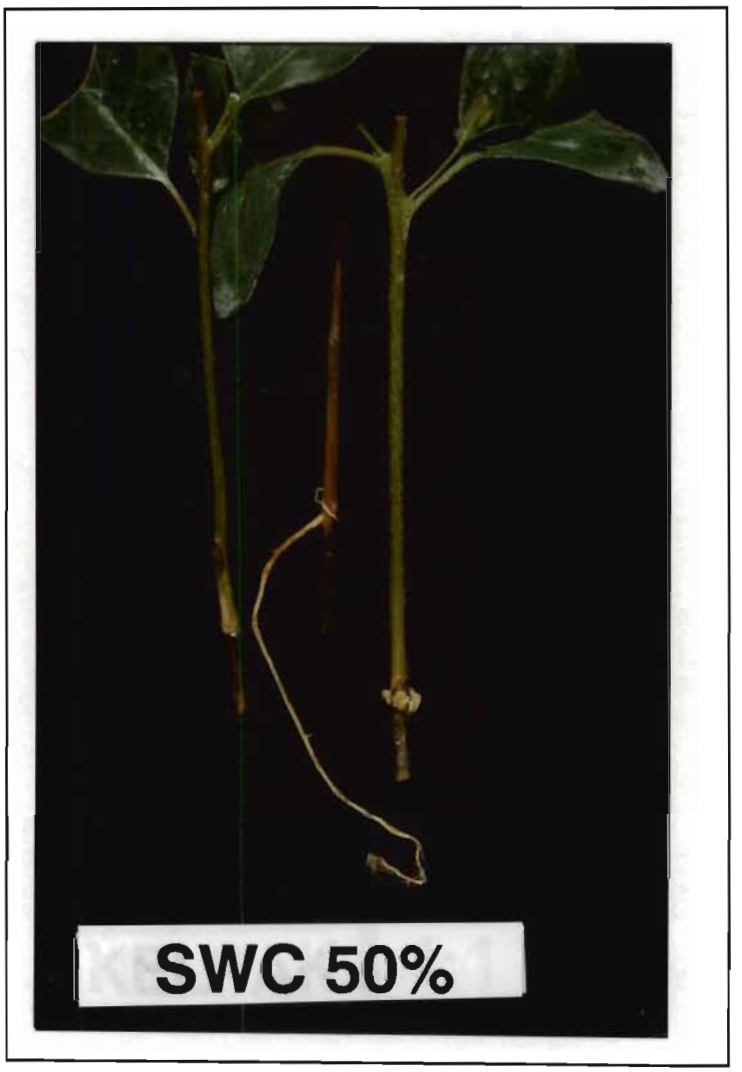

Figure 5.18 Burning at the base of *Eucalyptus* cuttings after a 24 hour pulse of 10% SWC.

5.6 The Mung Bean Bioassay as a means of Determining Various Aspects of SWC Application to Rooting.

The mung bean system has proven to be an effective means of detecting rooting factor(s) by bioassay (HESS, 1961a, 1961b; FADL & HARTMANN, 1967; HEUSER & HESS, 1972), and was used in the present study to test SWC and extracted fractions for root promotory substances. This rooting test was originally developed by HESS (1957) to detect naturally occurring substances that stimulate rooting in the presence of IAA. Using this system HESS (1962, 1964a) detected four rooting co-factors from English Ivy, chrysanthemum (*Chrysanthemum* spp.), and hibiscus (*Hibiscus rosa-sinensis* L.).

The mung bean rooting bioassay is suitable for detecting physiological activity and monitoring the purification of root promotory substances. It is also the only rooting bioassay that can provide the considerable degree of replication for statistically satisfactory results (HEUSER, 1988). With this assay, the timing and location of cell division activity is known, which is important in understanding the multitude of factors involved in a complex process such as rooting (HEUSER, 1988).

In the first part of this investigation, the mung bean bioassay was used to determine the nature of the rooting factor in the SWC. In later studies, the SWC was partitioned in solvents to extract for auxin-like compounds and active fractions further purified by high performance liquid chromatography.

5.6.1 Results of studies to determine various aspects of SWC application to rooting in the mung bean bioassay.

Concentration effect

A dilution series of SWC was applied to the mung bean system to determine which concentration most effectively promotes rooting. In Chapter 2, preliminary tests indicated that a 10% dilution of SWC promoted rooting. In the following experiment,

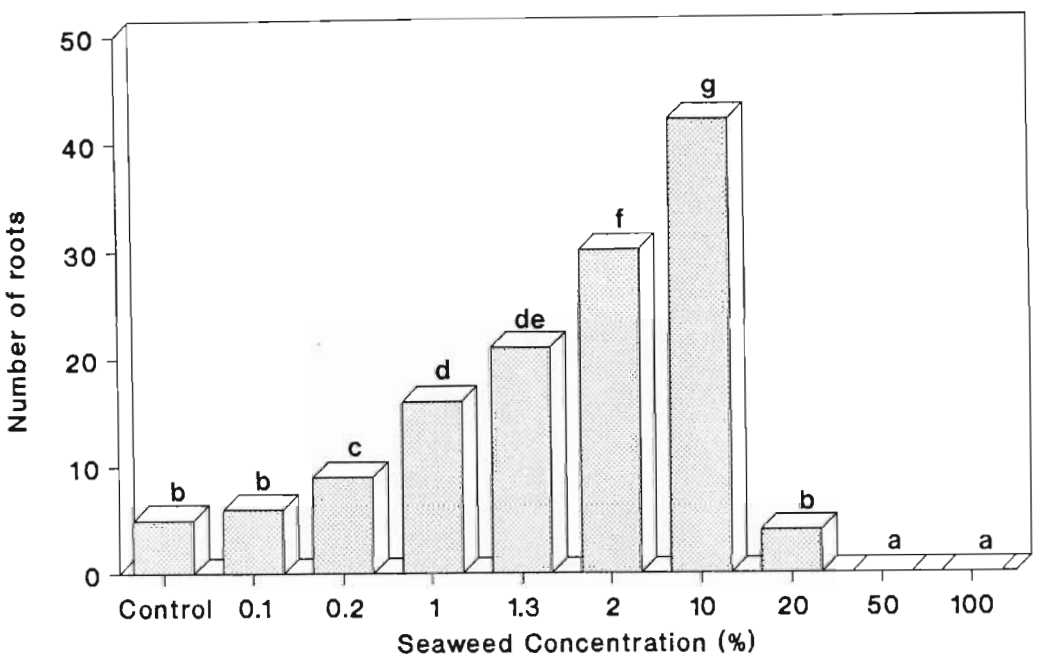

Figure 5.19 **The effect of various concentrations of SWC on the rooting of mung bean cuttings 8 days after an 8 hour pulse treatment. Bars with the same letter are not significantly different.**

the range of SWC dilutions were extended to determine the efficacy of the SWC in promoting rooting. An excellent rooting response was obtained with SWC (Figure 5.19 & 5.20). Concentrations above 10% had a detrimental effect on rooting. However, a dilution as low as 0.2% still gave a better response than the control. Associated with this seaweed application was marked shoot development (Figure 5.21).

Effect of pulse depth:

Mung bean cuttings were immersed in 10% SWC at various depths for 18 hours to ascertain the nature of the rooting factor. The factor(s) for rooting was transported up the stem of the cutting as illustrated in Figure 5.22. Irrespective of whether only the bottom centimetre of the mung bean cuttings were immersed in 10% SWC, or cuttings immersed to a depth of six centimetres (Figure 5.22a), rooting responses were equally good. The height to which adventitious roots developed on the cuttings was not affected by the depth of immersion during treatment (Figure 5.22b).

Effect of pulse time:

Mung bean cuttings were immersed in 10% seaweed concentrate over a range of pulse times to determine the time required for the SWC to elicit a rooting response. Cuttings pulsed with 10% SWC for intervals ranging between one minute and five hours did not improve root initiation (Figure 5.23). A pulse time of 18 h significantly increased the number of roots initiated on cuttings.

Heat stability of SWC.

The heat stability of the rooting factor(s) was tested by autoclaving the SWC at 1 bar and 120°C for 20 minutes prior to use. The rooting factor(s) was not lost by

Figure 5.20 The effect of an 8 hour pulse with 10% SWC on the rooting of mung bean cuttings as observed 8 days after treatment.

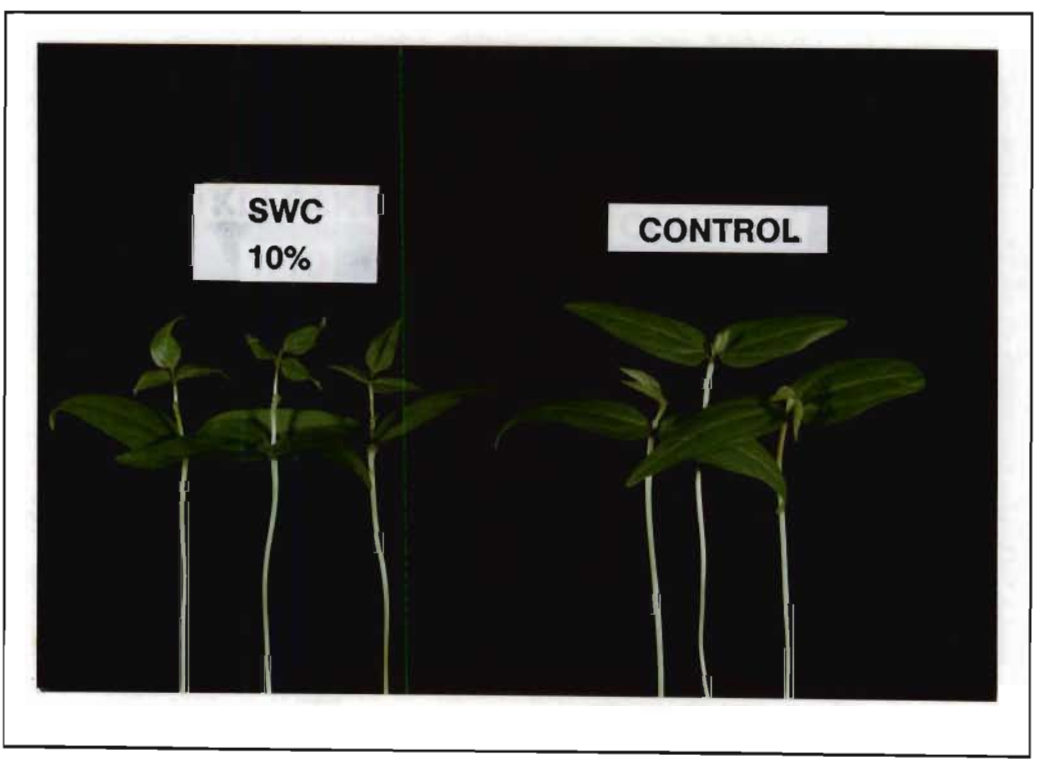

Figure 5.21 The effect of 10% SWC on the development of shoots on mung bean cuttings 8 days after an 8 hour pulse treatment.

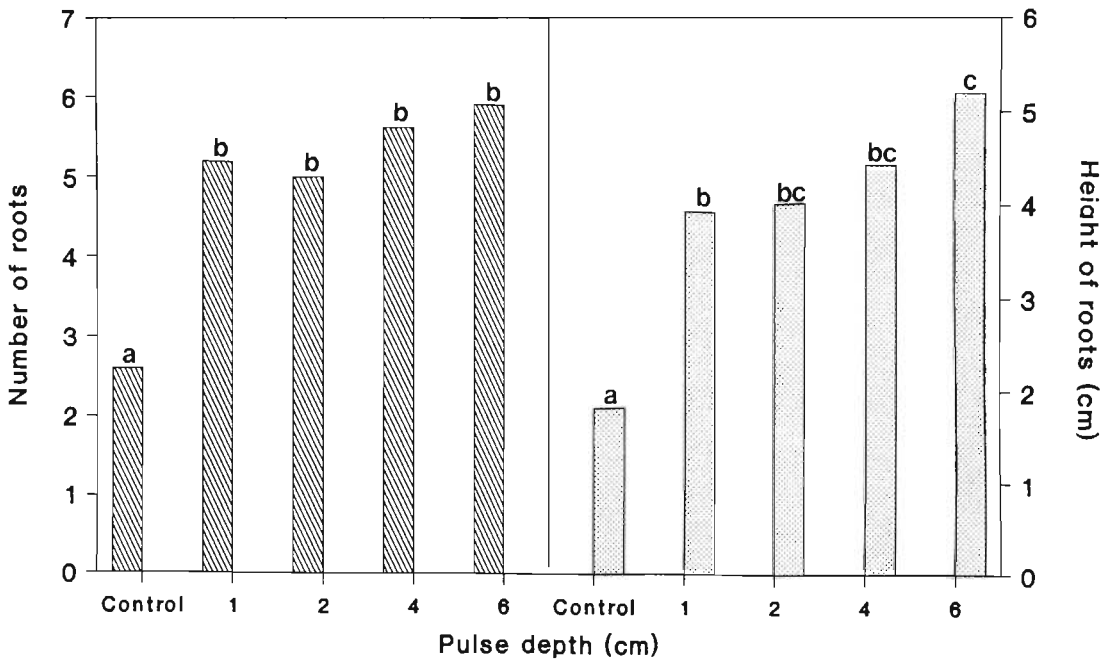

Figure 5.22 The effect of immersion depth of cutting ends on the number of roots (A) and the height to which they developed on the cuttings (B). All cuttings were immersed for 8 hours in 10% SWC.

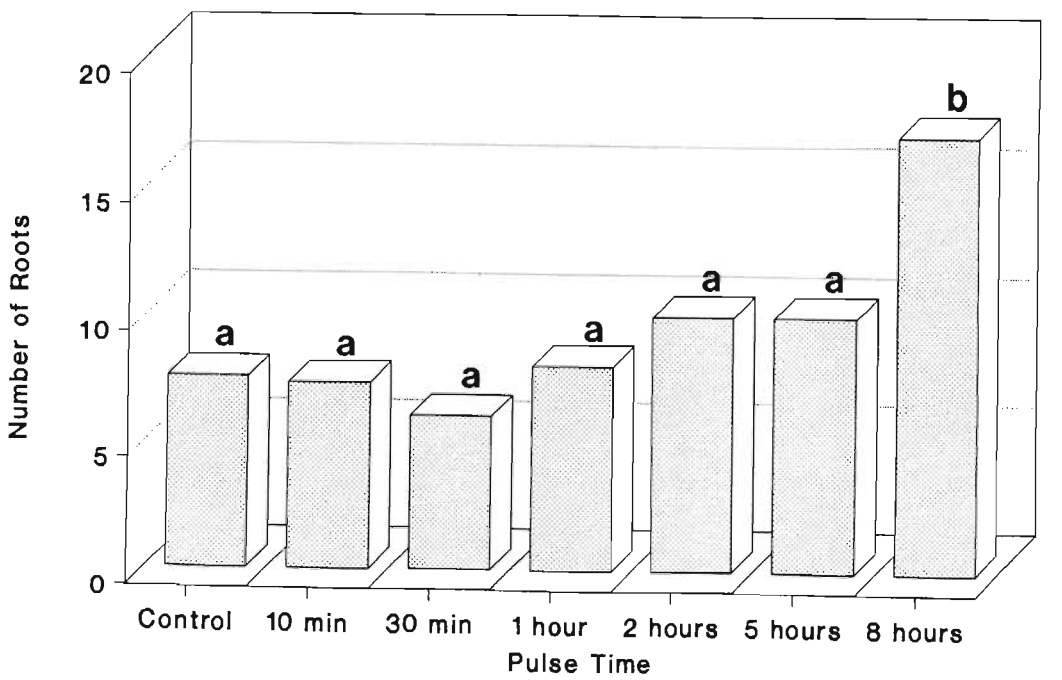

Figure 5.23 The effect of pulse time on the number of roots that developed on mung bean cuttings. All cuttings were immersed in 10% SWC. Bars with the same letter are not significantly different.

autoclaving the SWC (Figure 5.24). At some concentrations autoclaved treatments were slightly better than unheated SWC.

Effect of combining SWC with authentic IAA:

In the original mung bean bioassay developed by HESS (1957), a small amount of auxin was added to the test solutions to test for rooting co-factors which elicit a response only in the presence of IAA. Small amounts of authentic auxins were thus added to 10% SWC to test for the presence of such co-factors in seaweed concentrate. The addition of IAA to the SWC test solutions did not improve the degree of root initiation (Figure 5.25). Many of the combined treatments proved to be detrimental to rooting.

5.7 Isolation and purification of the rooting factor in SWC

Several separation and purification techniques were examined to determine the most effective means of isolating the rooting factor(s) from the SWC. While rooting responses were initially noted with paper and thin-layer chromatographic separation systems (results not shown), the inconsistency of these results discouraged their further use. The most effective means of isolating the rooting factor(s) was by solvent partitioning for auxins followed by further purification by HPLC. At each stage of separation, fractions were tested for rooting activity with the mung bean system. The isolation of the rooting factor into a single HPLC fraction increased the chance of positively identifying the compound.

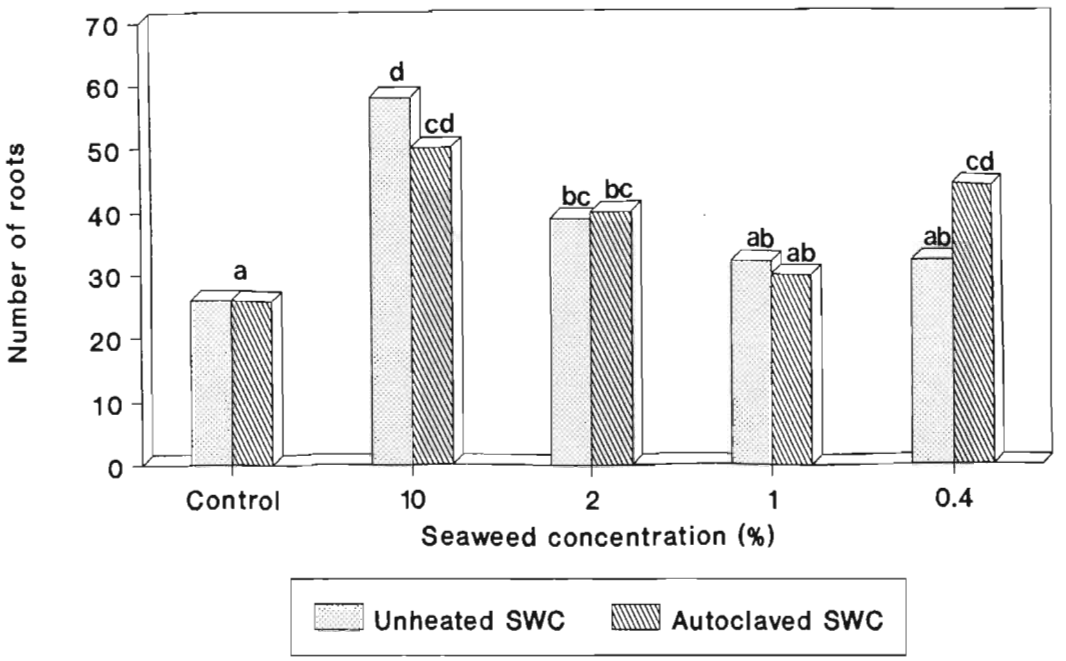

Figure 5.24 The effect of autoclaved SWC on root formation. Cuttings received a 10% solution of SWC for eight hours. Bars with the same letter are not significantly different.

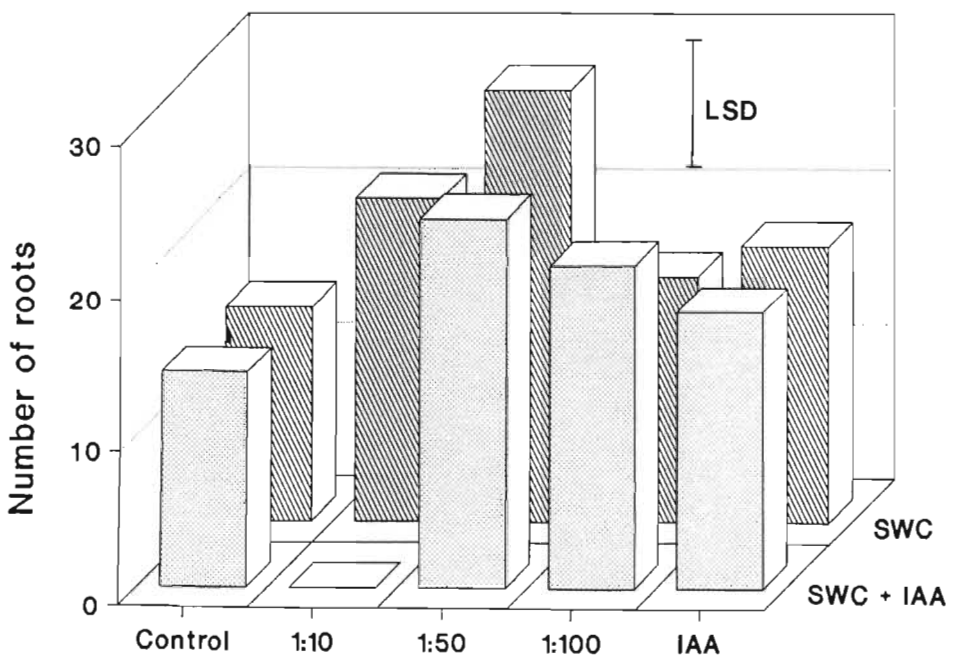

Figure 5.25 The effect of pulsing mung bean cuttings in a combination of 10% SWC and auxin (IAA) for 8 hours.

5.7.1 Experimental Procedure and Results

It is well documented that auxins, and in particular IAA, are involved in root initiation. The SWC was thus extracted for auxins as described earlier (Section 2.21; Figure 2.2), and the resulting fractions tested extensively for rooting activity with the mung bean bioassay.

Only the basic 'neutral indole' fraction showed any marked rooting activity (Figure 5.26). This result was substantiated by repeating the experiment three times. A slight rooting response in the indole conjugate fraction suggested the presence of more than one promotory factor.

In an attempt to isolate the active constituent, 100 $\mu\ell$ of the 'neutral indole' fraction was subjected to purification by HPLC. The sample run was repeated five times and eluates from each run collected in twenty-three fractions (one fraction per two minutes). These fractions were then assayed for root initiating activity (Figure 5.27).

The 15/16 and 17/18 minute HPLC fractions gave the best rooting response. A significant rooting response was also detected in the 37 to 40 minute fractions. Repeating the experiment gave similar results with the exception that in the second bioassay, the 23/24 minute fraction also showed favourable rooting.

5.8 Tentative identification of the rooting factor in SWC

In Chapter 2, the auxins in Kelpak seaweed concentrate were tentatively identified on the basis of co-chromatography with authentic standards. Whether or not the rooting factor in the SWC is an auxin or a related compound has, however, still to be established. As auxin, and in particular IAA, is known to be involved in root initiation, the tentative identification of this and similar compounds in the active

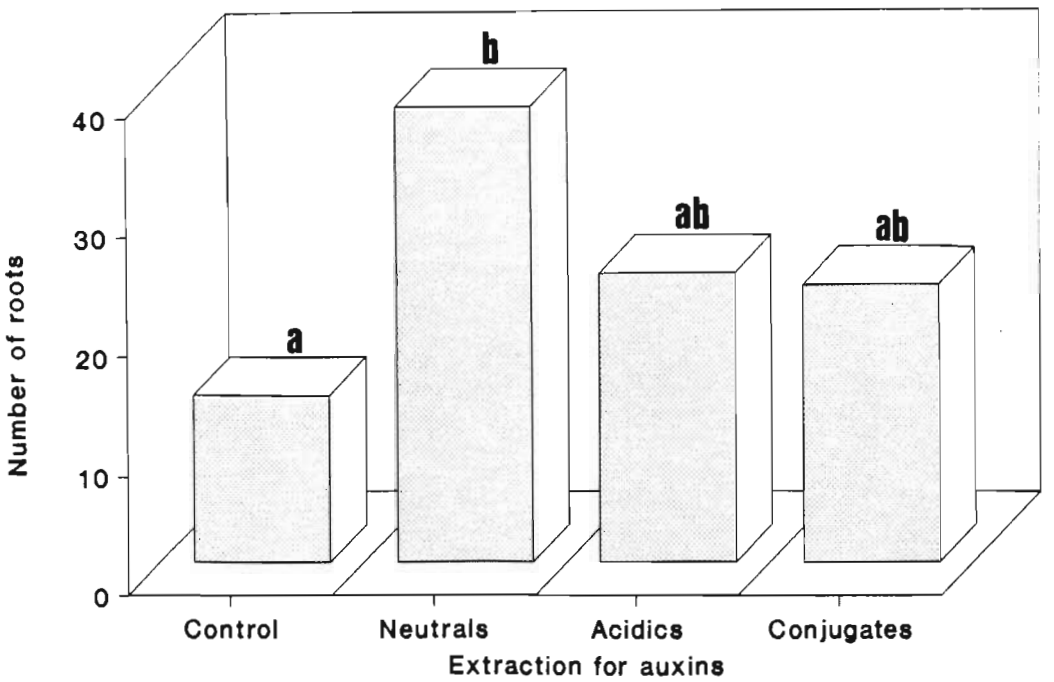

Figure 5.26 The effect of solvent partitioned SWC (SANDBERG, CROZIER & ERNSTSEN, 1987) fractions, applied as an 8 hour pulse, on the rooting of mung bean cuttings.

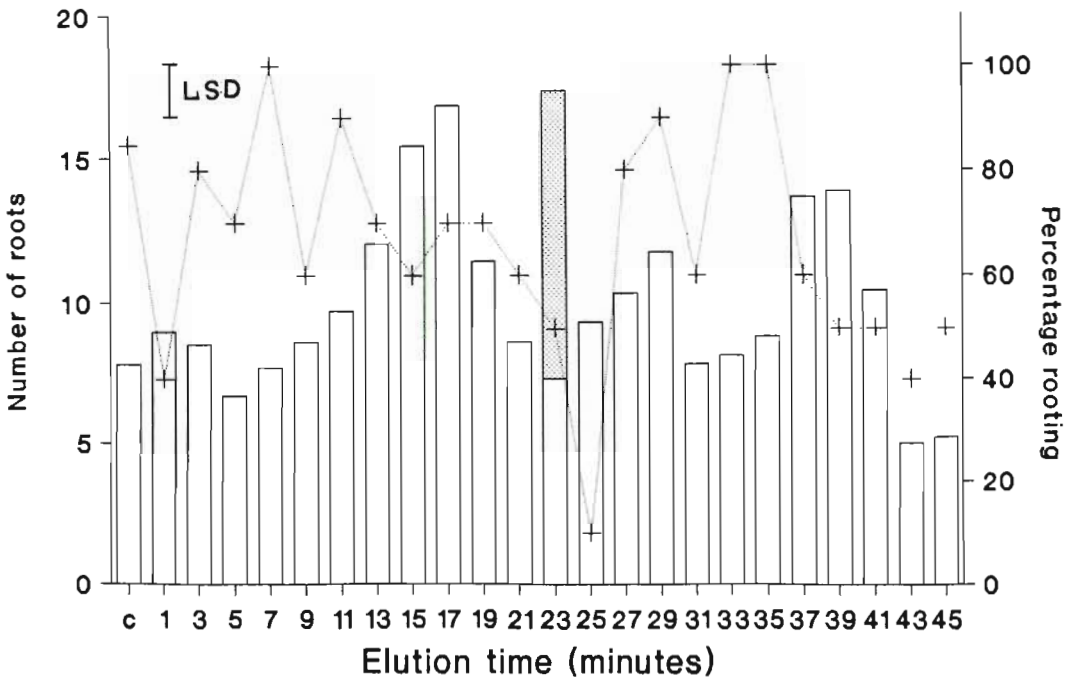

Figure 5.27 Separation of the 'neutral indole' fraction by HPLC. HPLC fractions were applied to the cuttings and the root number recorded (bar). Dotted line represents the percentage of mung bean hypocotyls rooted.

fractions was examined.

Separation of the 'neutral indole' fraction by HPLC isolated three fractions with rooting activity. These active fractions were tentatively identified by co-chromatography with authentic auxin standards.

5.8.1 Results

The only compound detected between 15 and 18 minutes co-eluted with indole-3-acetamide at a retention time of 16.5 minutes (Figure 5.26 & 2.16) As no authentic auxin standards were retained after 34 minutes (Figure 2.15), the tentative identification of the rooting factor present in the 39 minute fraction was not possible. The only other fraction showing rooting activity (23/24 minutes) had a similar retention time to IAA, ICA and IAAld (Figure 2.20).

5.9 Discussion

The mung bean rooting bioassay has been used extensively for the detection of root promotory compounds in plant extracts (HESS, 1961a; FADL & HARTMANN, 1967; HEUSER & HESS, 1972). It is well-established that both endogenous and synthetic auxins stimulate rooting (JACKSON & HARNEY, 1970; HARTMANN & KESTER, 1975). In Chapter 2, a linear rooting response was obtained when a dilution series of IBA was tested using the mung bean rooting bioassay. In this present study, the response obtained with a dilution series of SWC was also linear in the dilution range from 0.2 to 10%. All higher concentrations were inhibitory. This type of response is common with hormones - being promotory at low concentrations and inhibitory at high concentrations. At present it is not known whether the observed rooting response is due to IAA which does occur in marine algae (AUGIER, 1976a, 1976b; KINGMAN & MOORE, 1982). It is clear that SWC prepared from the brown alga *Ecklonia maxima*

does contain rapidly transportable, heat stable compounds that stimulate rooting on mung bean cuttings and on cuttings of other plant species.

The improvement in rooting with 10% SWC, expressed as a percentage of the controls, ranged from 18% for *L. vera* to 317% for *V. agnus-castrus*. This varying degree of efficiency suggests that the optimum concentration for each species needs to be determined prior to routine use. Similar dilutions of SWC were found to be of limited benefit to the rooting of *Eucalyptus* cuttings. Although the application of SWC did not enhance root initiation, results showed that where roots were formed, they were better. Necrosis at the base of the cuttings implied that the concentration needed to be made more dilute, and/or the pulse time shortened. These results thus suggest that a less toxic application of SWC might possibly improve rooting by strengthening and promoting root growth and development. Administering SWC in conjunction with a rooting powder could thus prove invaluable to the survival and establishment of *Eucalyptus* cuttings.

Attempts were made to isolate and identify the active substance(s). Preliminary results suggest that the active compound is possibly indole-3-acetamide, a neutral indole. Evidence of slight activity in the fraction co-eluting with IAA suggested that IAA may also be present in the SWC. As more than one rooting factor was noted (Figure 5.27), the final rooting response is probably the sum of several promotive factors.

Although it is well-established that auxins promote adventitious rooting (HESS, 1964a; 1965), studies which found the response of cuttings to applied auxins to be variable, suggested that compounds other than auxin are required for root formation (WENT, 1938; THIMANN & DELISLE, 1939). Earlier, HESS (1964a, 1964b) defined compounds which acted synergistically with auxin as co-factors. An important aspect of the mung bean rooting bioassay has been its reported insensitivity to IAA except in the presence of such co-factors (HESS 1961a, 1961b). This test system therefore represents a method for detecting substances, other than IAA, which may be necessary for root initiation (HESS, 1961b).

The SWC was able to effectively promote root initiation without the addition of IAA. Pulsing the cuttings, first in SWC and then in IAA, invariably resulted in poor or inhibited rooting. This suggests that small amounts of IAA may be present in the SWC and that the additional IAA resulted in levels of auxin toxic to the plant tissue.

Compounds in SWC, other than auxins, that may affect root initiation and development include some of the other known plant growth regulators (eg. cytokinins and ABA); mineral nutrients such as boron (0.224 mg per cubic decimeter) nitrogen, zinc, calcium, and manganese; and certain carbohydrates (eg. laminarin and alginate) (Table 1.2). Although the involvement of these constituents were not investigated in the present study, their possible involvement cannot be ignored.

It has been demonstrated in the mung bean bioassay that rooting can be promoted by injury caused by high concentrations of various solutes (SOEKARJO, 1965). Recently, it was suggested that a physical wound might propagate a signal which could induce a change in the metabolism of affected cells (WILSON, 1989). The perturbed cells could then become amenable to root initiation. A solute that is mildly injurious at one concentration may therefore be lethal at another. Thus a non-physiological solute could be classified as either a promoter or inhibitor of rooting depending on the concentration of the treatment solution. This serves to indicate the inherent dangers of using bioassay evidence alone to infer the presence of plant growth regulators.

The present results are in keeping with the wounding hypothesis since the SWC promoted rooting in the concentration range of 10% and 0.2%, but was lethal at 50 percent.

This chapter has demonstrated that application of SWC at the correct dilution can result in the initiation and subsequent development of large numbers of roots. This promotion in root growth is not limited to adventitious root development but also includes enhanced lateral root formation (FINNIE & VAN STADEN, 1985). An overall improvement in root growth will undoubtedly aid cuttings at transplanting and

subsequent establishment in *in vivo* conditions. The fact that the unequivocal identification of the active compound(s) and their mode of action is still not fully understood, does not therefore preclude the use of the SWC in stimulating rooting.

CHAPTER 6

THE EFFECT OF SEAWEED CONCENTRATE ON THE GROWTH OF NEMATODE-INFESTED TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) PLANTS.

6.1 Introduction

Root-knot nematodes (RKN) (*Meloidogyne spp.*) are one of the most destructive of plant parasites in tropical and sub-tropical climates (SASSER, 1979). These nematodes inflict severe damage on a wide range of economically important plants by parasitizing their roots. The resultant formation of galls and damage to the vascular system affects the growth of the plant. The most important species associated with tomato are *Meloidogyne incognita, M. arenaria* and *M. hapla*. In South Africa, *M. incognita* and *M. javanica* are the two species of economic importance. The economic impact caused by RKN's amounts to millions of Rands damage annually due to reduction both in quality and quantity of crop yields, and to escalating costs of nematicidal treatment.

Chlorosis and premature leaf drop are usually the first obvious signs of large-scale infections by nematodes. Infected plants are stunted, wilt in dry conditions that a healthy plant withstands, and often show symptoms of nutrient deficiency. Probably the most important result of infection with *Meloidogyne* is the increased plant susceptibility to attack by other organisms such as bacteria and fungi (DROPKIN, 1980).

The consequences of these plant parasitic nematodes on crops is therefore based largely on their ability to incite damage to plant tissue, or to act in complexes with other organisms to cause losses in quantity and often quality of crop yields (JOHNSON & FASSULIOTRE, 1984).

Means of controlling or abating these pests include chemical treatment such as the use of pre-plant soil fumigation; post- plant low phytotoxic nematicides; and, agrotechnical measures such as elaborate crop rotation schemes, introduction of genetically resistant varieties, and to a lesser extent biological control methods. In spite of these efforts, nematodes are still a problem of growing concern. Crop rotation, is impractical in many cases because of the polyphagous nature of the RKN. Nematode-resistant varieties of crops are available only in a limited number of plants and the resistance is frequently overcome by new races of nematodes. Biological control of RKN's as well as of other plant parasitic nematodes is still in the experimental stage.

While some of the most efficient alkyl-halogenated fumigants have been recently banned due to environmental and health hazards, other available non-volatile nematicides have proven to be financially impractical in terms of large scale farming.

The development of a natural bio-degradable product that reduces nematode damage to plants would be of both economic and environmental significance. Seaweeds and seaweed products are known to affect nematode infestation in a variety of crops (Table 1.1). DARRAH & HALL (1976) recorded that seaweed meal applied to Kentucky blue-grass and perennial ryegrass resulted in marked reductions of *Paratylenchus* (pin nematode) and *Pratylenchus* (lesion nematode) populations. TARJAN (1977) found that seaweed applied either as a foliar application or directly to the soil, increased plant weight and decreased nematode infestation in *Citrus medica* L. seedlings 17 weeks after application. It was found, however, that seaweed application to old, established trees had no effect on yield or nematode infestation. There would also appear to be some effect on reducing nematode infestation in lawn grass (*Cynodon dactylon* (L.) Pers.) after kelp treatment (TARJAN & FREDERICH, 1983). FEATONBY-SMITH & VAN STADEN (1983b) treated nematode-infested tomato plants with a SWC and found that application as a soil flush reduced nematode numbers in the plant roots. Recent work by DE WAELE, McDONALD & DE WAELE (1988) demonstrated that a SWC significantly suppressed the reproduction of *Pratylenchus zeae* on excised maize roots by 47-63%

during an *in vitro* experiment. In a greenhouse experiment, reproduction of *P. zeae* was not affected by the seaweed preparation.

In Chapter 4, SWC was found to significantly reduce nematode infestation on excised tomato roots cultured *in vitro.* These results prompted the following study which examined the potential of SWC as a means for controlling root-knot nematode populations in greenhouse tomatoes. The method and time of seaweed application, and the dilution of the SWC on the growth of nematode-infested tomato plants was investigated. Various aspects of growth, including shoot and root development, flower set, and fruit production were measured.

In the final stage of this chapter, the SWC was filtered, ashed, and separated into different Rf zones by paper chromatography. These fractions were then applied to the plants in order to learn more about the active components responsible for improved plant growth and reduced nematode-infestation.

6.2 Materials and Methods

6.2.1 Site of trials

Experiments were carried out under natural lighting and temperatures in either a greenhouse tunnel or under 60% shade cloth.

6.2.2 Seedlings

Seeds of *Lycopersicon esculentum* Mill. (cv. Rana, Karina, or Moneymaker) were germinated in bark in speedling trays and treated with Copper-oxy-chloride and Benlate as a preventative against damping-off or blight. Seedlings of approximately

10 cm were transplanted into 125 mm pots (450 cm^3) containing a sandy soil which was either heavily infested with *Meloidogyne incognita* or later infected with nematode eggs. The tomato plants were subsequently treated with SWC as indicated (see individual experiments). The experimental plants were watered regularly with tap water and were not fertilized during the course of the trials.

6.2.3 Chromatographic Techniques

Paper chromatography

The SWC was extracted as outlined in Chapter 2.2.1. The extract was strip loaded, in a 1 cm strip, onto sheets of Whatman Nº1 chromatography paper. The chromatograms were then developed with *iso*-propanol:25 per cent ammonium hydroxide:water (10:1:1 v/v) (P:A:W) in a descending manner for 10 hours or until the solvent front was approximately 30 cm from the origin. The chromatograms were then dried at 30°C for 24 hours. The chromatograms were subsequently divided into ten equal Rf zones and stored at -20°C until needed.

6.2.4 Fresh and dry weight determination

All mass requirements were done using digital balances sensitive to 0.01 or 0.001 grammes depending on the requirement.

For fresh mass determination, the material was harvested and weighed with a minimum of delay. The pots were carefully emptied under water to keep the roots intact, and the roots cleaned by gently rubbing them in running water. Plants were then washed in distilled water and blotted dry with paper towelling. Roots and shoots were then separated and weighed.

For dry weight determination, material was oven dried in a ventilated oven at 80°C to constant weight and allowed to cool before weighing.

6.2.5 Leaf area determination

Leaf area was determined electronically using a Li-Cor area meter and expressed in cubic centimetres (cm^3).

6.2.6 Root length

Root length was determined electronically using a Comair root length scanner and expressed in metres (m).

6.2.7 Nematode counts

To obtain a standard galling index for infested roots, the roots were spread out in a large glass Petri-dish (15 mm diameter) and the total number of galls per root counted. Counting was aided by a grid marked onto the bottom of the dish. Using root length and fresh weight data, nematode infestation was expressed as the number of galls per gram fresh weight or as the number of galls per metre of root.

6.3 The effect of SWC on the establishment of tomato seedlings in nematode infested soil.

In this trial the effect of SWC on the establishment of tomato seedlings transplanted directly into soil heavily infested with nematodes was examined. As the first seaweed application was at transplanting, the presence of a nematoxic component in the SWC may affect the nematodes prior to their penetration into the tomato roots.

6.3.1 Experimental Procedure and Results

Six-week-old tomato seedlings were transplanted into pots (125 mm) containing two different media. The first medium was a sifted soil/bark that had been treated with methyl bromide to kill any resident nematode populations. The second was the same soil/bark mix heavily infested with root-knot nematodes. 0.2%, 0.4% and 1.0% SWC was applied to the seedlings as a soil drench, and 0.4% SWC as a foliar spray, every seven days. Each treatment consisted of 10 replicates. The plants were harvested after six weeks. Shoot and root fresh and dry weights, leaf surface area, flower number, root length and an index of nematode infestation were recorded. Results were statistically analysed using one-way anova ($P<0.05$) and a multiple range test.

SWC when applied as a soil drench to nematode-free soil invariably increased all plant growth parameters (Figures 6.1 to 6.4; Table 6.1). Foliar application had nc significant effect on growth (Figure 6.1). Plants treated with 0.4% and 1.0% SWC were always significantly larger than control plants regardless of the parameter measured (Table 6.1).

Similar results were obtained from plants grown in nematode infested soil (Figure 6.5; Table 6.2). Foliar application of SWC did not improve plant growth (Figure 6.5). When applied as a soil drench, the SWC resulted in significantly larger plants. 1.0% SWC significantly increased all other plant growth parameters (Table 6.2). SWC above

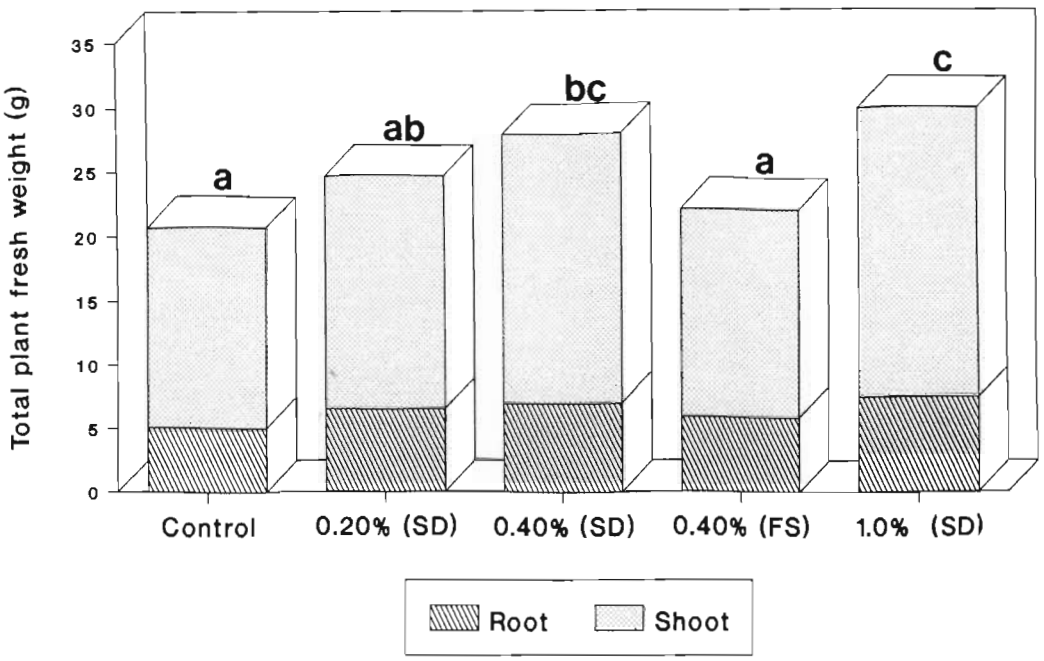

Figure 6.1 The effect of SWC on the total fresh weight of 8-week-old tomato plants. (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

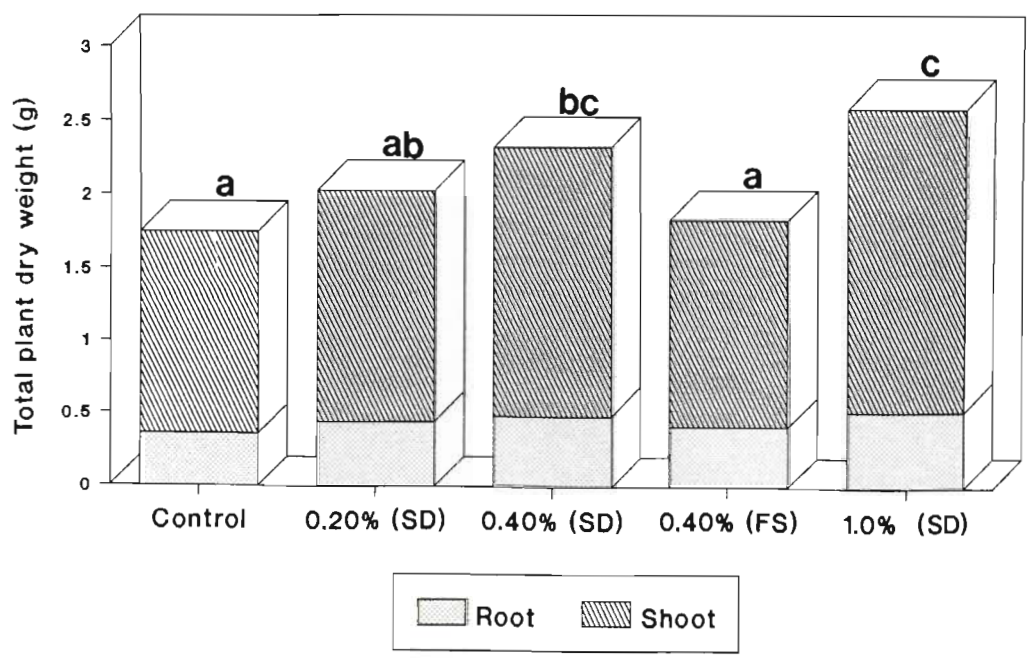

Figure 6.2 The effect of SWC on the total dry weight of 8-week-old tomato plants. (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

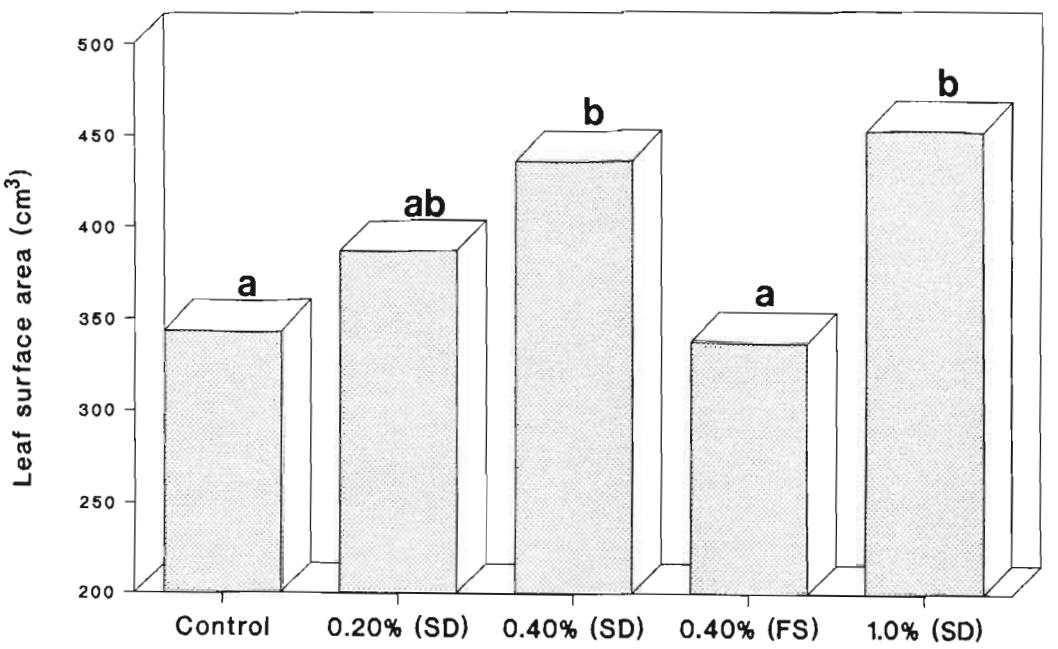

Figure 6.3 The effect of SWC on the leaf surface area of 8-week-old tomato plants (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

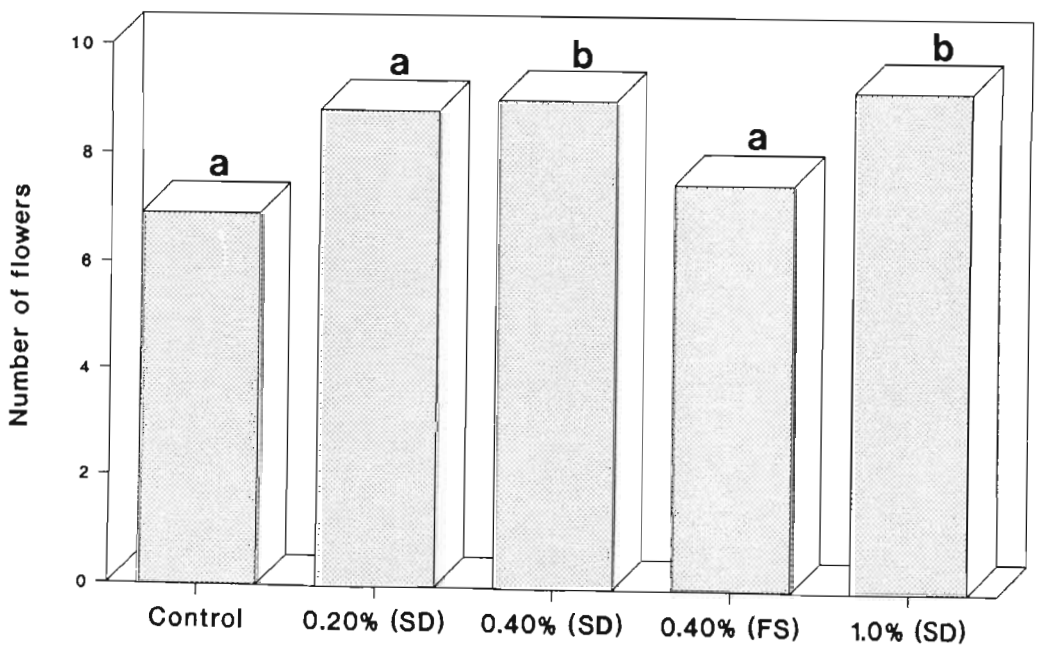

Figure 6.4 The effect of SWC on the number of flowers on 8-week-old tomato plants (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

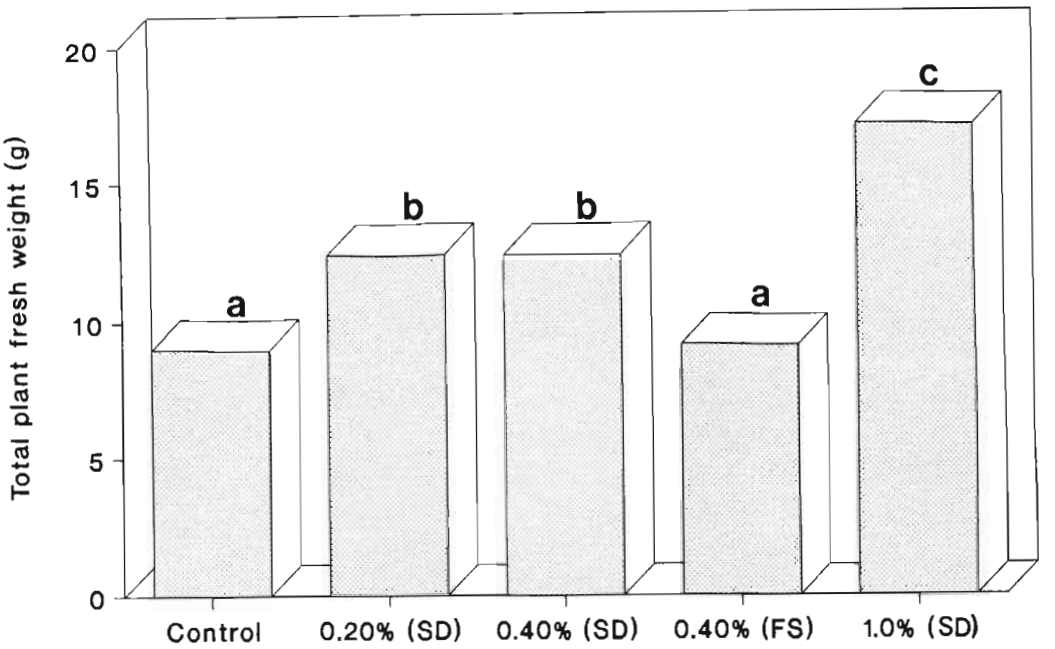

Figure 6.5 The effect of SWC on the total fresh weight of 8-week-old nematode-infested tomato plants (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

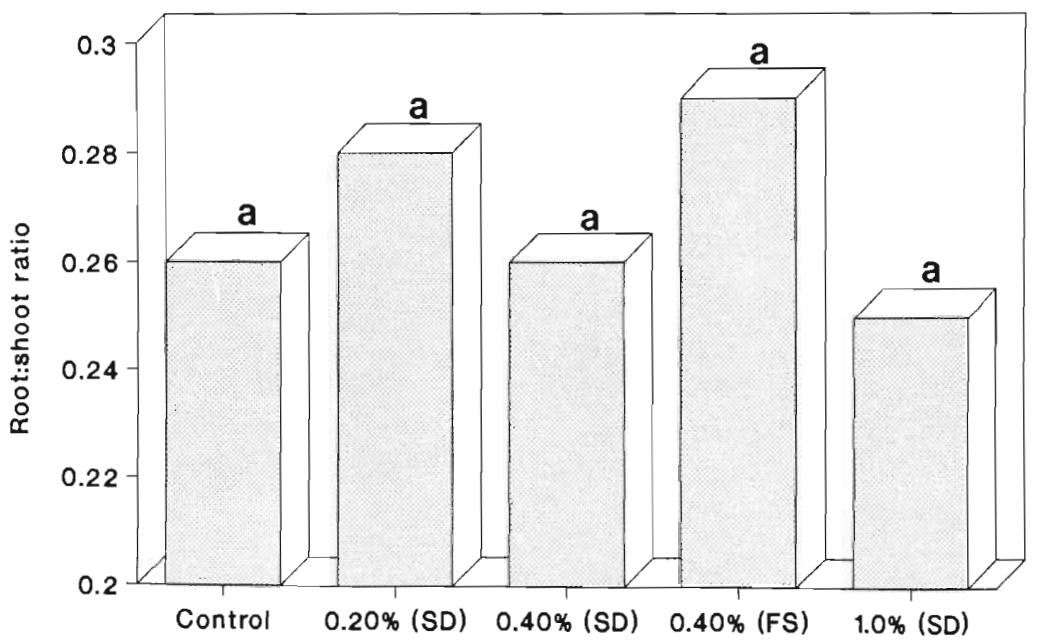

Figure 6.6 The root:shoot ratio of 8-week-old nematode-infested tomato plants following SWC application (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

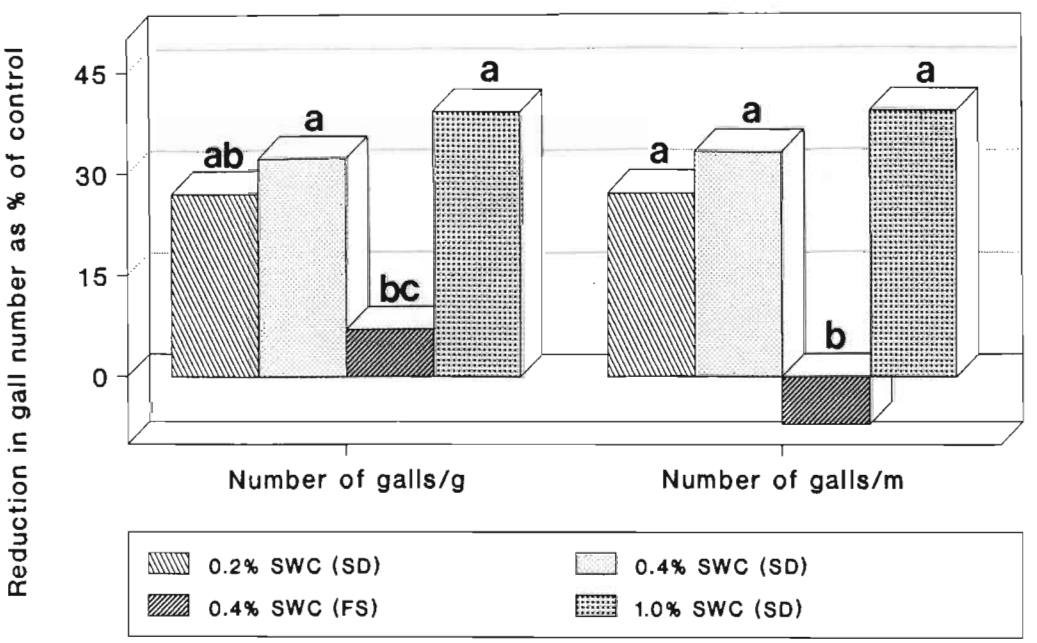

Figure 6.7 The effect of SWC on the degree of nematode infestation on roots of 8-week-old tomato plants (SD = soil drench; FS = foliar spray). Values expressed as the percentage of galls relative to the control.

Table 6.1 **Effect of seaweed concentrate on nematode-free tomato seedlings. Treatments with the same letter are not significantly different.**

	TREATMENTS					
	Water SD	SWC 0.20% SD	SWC 0.40% SD	SWC 0.40% FS	SWC 1.0% SD	SIG LEVEL
Shoot fresh wt. (g)	a	ab	bc	a	c	***
Shoot dry weight (g)	a	ab	bc	a	c	***
Root fresh weight (g)	a	abc	bc	ab	c	**
Root dry weight (g)	a	ab	b	ab	b	**
Total fresh wt. (g)	a	ab	bc	a	c	***
Total dry weight (g)	a	ab	bc	a	c	***
Leaf area (cm^2)	a	ab	b	a	b	***
Root length (m)	a	ab	b	ab	b	**
Number of flowers	a	a	b	a	b	**
R:S ratio fresh	a	a	a	a	a	ns
R:S ratio dry	a	a	a	a	a	ns
Moisture content in shoots (%)	a	a	a	a	a	ns
Moisture content in roots (%)	a	a	a	a	a	ns

(* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$, ns = not significant)

a 0.2% dilution, applied to the soil, favoured shoot growth, while 0.4% foliar applied SWC and 0.2% SWC applied to the soil favoured root growth (Figure 6.6). The higher root:shoot ratio noted after the application of foliar applied SWC was possibly a result of an increased degree of nematode infestation in these plants (Figure 6.7). Nematode-infected plants were always smaller than those grown in nematode-free soil. The application of 1.0% SWC was found to be instrumental in overcoming these adverse effects.

Table 6.2 **Effect of seaweed concentrate on nematode-infested tomato seedlings. Treatments with the same letter are not significantly different.**

	TREATMENTS					
	Water SD	SWC 0.20% SD	SWC 0.40% SD	SWC 0.40% FS	SWC 1.0% SD	SIG LEVEL
Shoot fresh wt. (g)	a	a	a	a	b	***
Shoot dry weight (g)	a	a	a	a	b	***
Root fresh weight (g)	a	ab	ab	a	b	**
Root dry weight (g)	a	a	ab	a	b	**
Total fresh wt. (g)	a	b	b	a	c	***
Total dry weight (g)	a	b	b	a	c	***
Leaf area (cm^3)	a	a	a	a	b	***
Root length (m)	ab	ab	ab	a	c	***
R:S ratio fresh	a	a	a	a	a	ns
R:S ratio dry	a	a	a	a	a	ns
Moisture content in shoots (%)	a	a	a	a	a	ns
Moisture content in roots (%)	a	a	a	a	a	ns
Reduction in Nº of galls/m root	b	a	a	b	a	***
Reduction in Nº of galls/gram root fresh weight	c	ab	a	bc	a	***

(* = P<0.05, ** = P<0.01, *** = P<0.001, ns = not significant)

Application of the SWC to the soil reduced the degree of nematode infestation at all the applied concentrations (Figure 6.7). Significantly fewer galls were noted in roots treated with higher concentrations of extract. A concentration of 1.0% almost halved the degree of nematode infestation. SWC applied as a foliar spray had no effect on root nematode populations.

In conclusion, these results indicate that SWC applied as a foliar spray did not improve plant growth whereas application to the soil as a drench significantly increased all growth parameters and reduced the degree of nematode infestation in the roots.

6.4 The effect of SWC on the yield of nematode-infested greenhouse tomatoes.

This trial examined the effect of SWC on the production of harvestable tomato fruit. Differences between foliar and soil application of the seaweed were examined and preliminary effects of the concentrate on nematode infestation investigated.

6.4.1 Experimental Procedure and Results

Table 6.3 **Outline of treatments used to assess the effect of foliar and soil applications of SWC on the growth of *Lycopersicon esculentum*.**

	Nematode Infected	Nematode free
SWC applied as a Foliar spray	0.20%	0.20%
	0.40%	0.40%
	Control	Control
SWC applied as a Soil drench	0.20%	0.20%
	0.40%	0.40%
	Control	Control

Each treatment comprised 20 pots arranged in a randomised block design (Table 6.3). After 14 days selected treatments were inoculated with about 5000 nematode eggs per pot. The first SWC foliar spray and soil drench were applied at transplanting.

Subsequent applications were made every 14 days. Plants receiving a foliar spray were sprayed to run off (20 mℓ of solution per plant). One hundred mℓs of SWC was applied as a soil drench to plants in that treatment. Ripe fruit were harvested and the date, fresh weight and diameter recorded. To determine the effect of SWC on the rate of fruit production, each treatment within a block was given a numerical ranking between one and twelve depending on when it produced its first ripe fruit. Results were expressed as the total number of ripe fruit at each numerical position calculated as a percentage of the total number of fruiting plants.

At the termination of the trial, shoot and root fresh and dry mass, shoot/root ratio, moisture content, and the number of remaining flowers were determined. Confidence limits (where $P<0.05$) for all data were calculated after performing an analysis of variance.

The application of SWC slightly increased plant biomass (Figures 6.8). A reduction in shoot dry weight was noted for nematode-infected plants when SWC was applied as a soil drench (Figure 6.9).

SWC applied as a soil drench increased root growth of nematode-free plants but resulted in a reduced mass in nematode infested roots. (Figure 6.8). This suggests that the reduced mass in infested-roots may have been a result of a decrease in nematode galling. This trend in root growth was not as obvious with foliar applied SWC. Plants treated with 0.4% foliar applied SWC produced the heaviest roots. As the roots of nematode-free plants, sprayed with SWC, were not significantly larger than those of the controls, an increase in root weight was probably the result of increased nematode infestation.

The SWC stimulated early fruit ripening and production. Nearly 60% of all the first fruit harvested were from plants treated with 0.2% SWC (Figure 6.10). 0.4% SWC treated plants accounted for over 50% of the second fruit to reach maturity. Control plants accounted for the majority of later fruit. This trend suggests that SWC may stimulate flower initiation and/or increase the rate of fruit ripening. It must however,

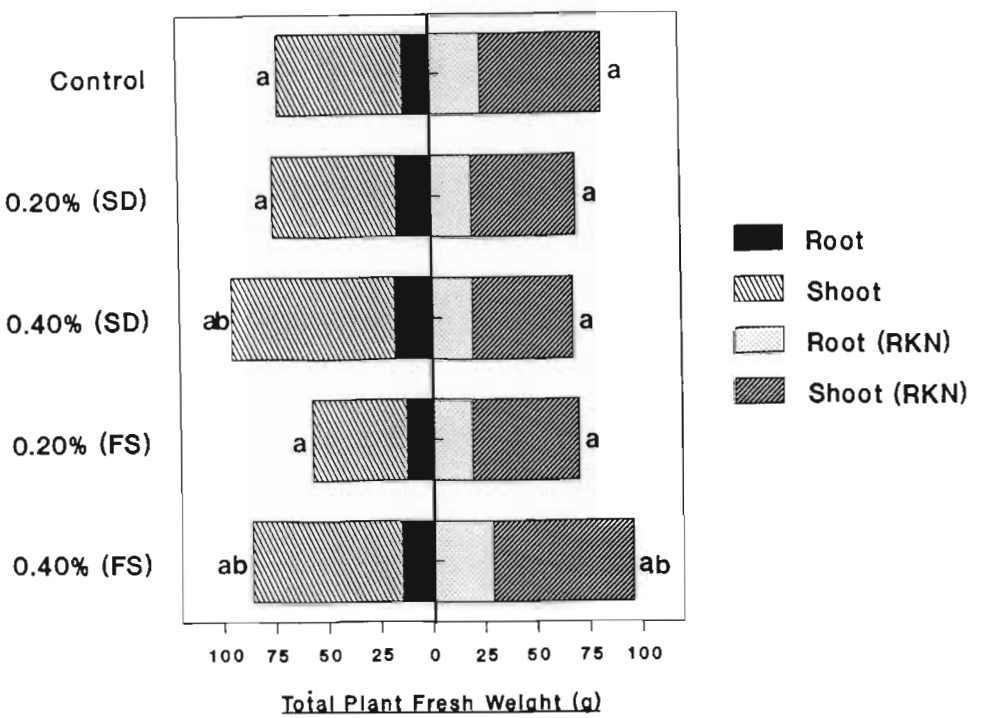

Figure 6.8 The effect of SWC on the total fresh weight of nematode-free and nematode-infested tomato plants (SD = soil drench, FS = foliar spray). Bars with the same letter are not significantly different.

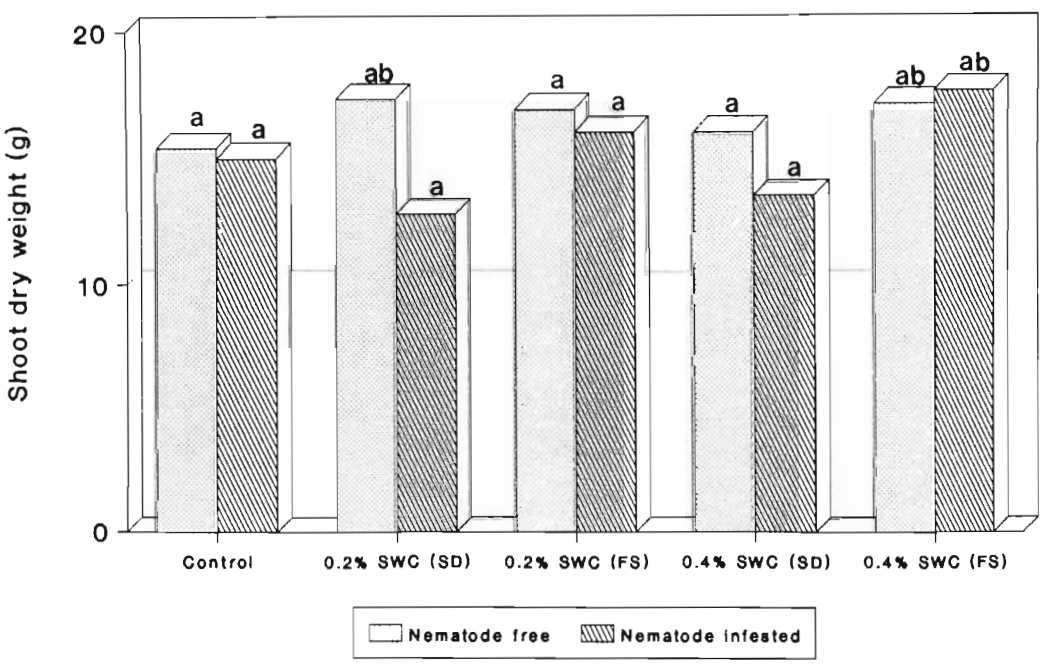

Figure 6.9 The effect of SWC on shoot dry weight of nematode-free or nematode-infested plants (SD = soil drench, FS = foliar spray). Bars with the same letter are not significantly different.

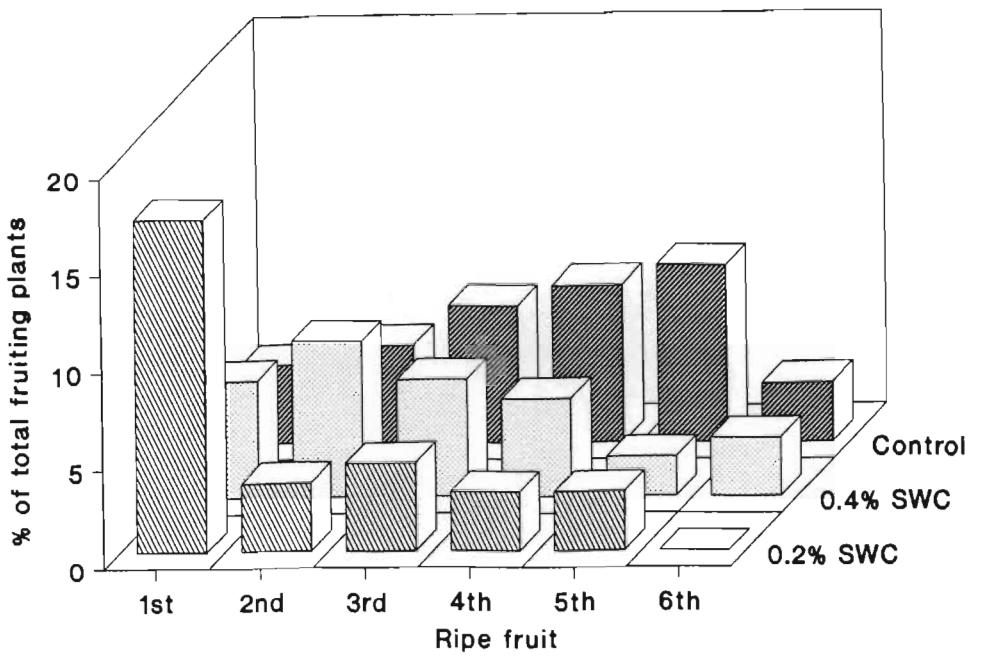

Figure 6.10 The effect of foliar applied SWC on the time of fruit ripening on tomato plants.

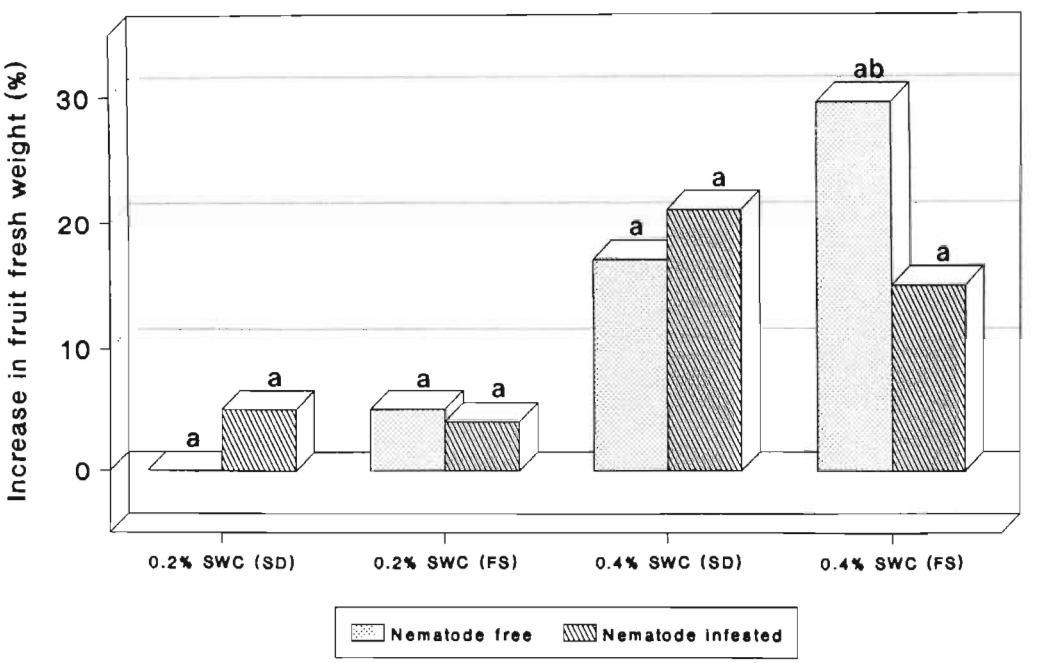

Figure 6.11 The effect of SWC, expressed as a percentage of the control, on the average weight of the first ripe fruit. (SD = soil drench, FS = foliar spray).

be borne in mind that larger, better-developed plants would naturally flower first. Early flowering following seaweed treatment may thus be a direct result of the overall physiological status of the plant. Statistical analysis showed no significant difference in the average size of the first fruit for SWC-treated plants (Figure 6.11). However, when applied as either a root drench or foliar spray, SWC invariably increased the fresh weight of these fruit. An increase in fresh weight of almost 30%, and diameter of 10%, was recorded in plants treated with a foliar application of 0.4% SWC (Figures 6.11 & 6.12). This was almost double the increase recorded for plants receiving the same dilution of SWC as a soil drench.

Total fruit production was not significantly increased by SWC application (Figures 6.13 and 6.15). When compared to controls, SWC-treated plants showed a 17% increase in fruit size but only about a 10% increase in the number of harvested fruit (Figure 6.15). This was evident for plants sprayed with 0.4% SWC. A lower seaweed dilution (0.2%), however, improved the average fruit weight of all harvested fruit by over 10% (Figure 6.14).

A flower count at the termination of the trial indicated that nematode-free plants had the greatest number of remaining flowers (Figure 6.16). Plants that had received 0.4% SWC applied as a foliar spray had over 70% more flowers than non-treated plants. This treatment was also shown to improve fruit yield by about 30%. This suggests that seaweed treatment possibly improves the fruiting potential of the plant.

These preliminary results indicate that SWC, by improving plant growth and development, appears to aid fruit set and production. While a soil drench was found to be effective in increasing plant biomass, foliar application promoted early fruit set and enhanced total fruit yield.

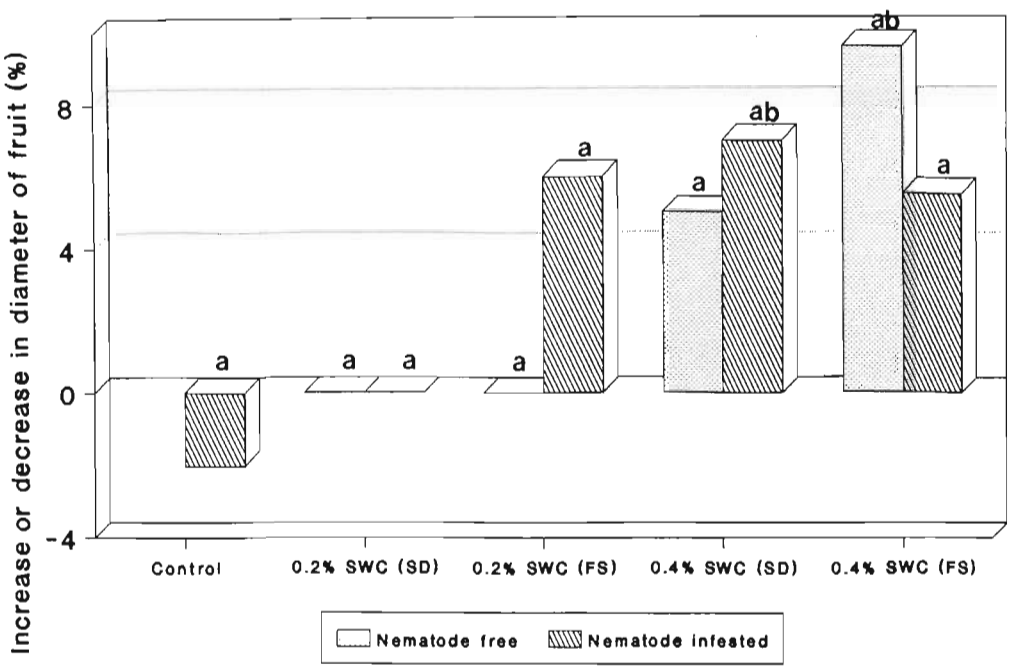

Figure 6.12 The effect of SWC, expressed as a percentage of the control, on the diameter of the first ripe fruit. (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

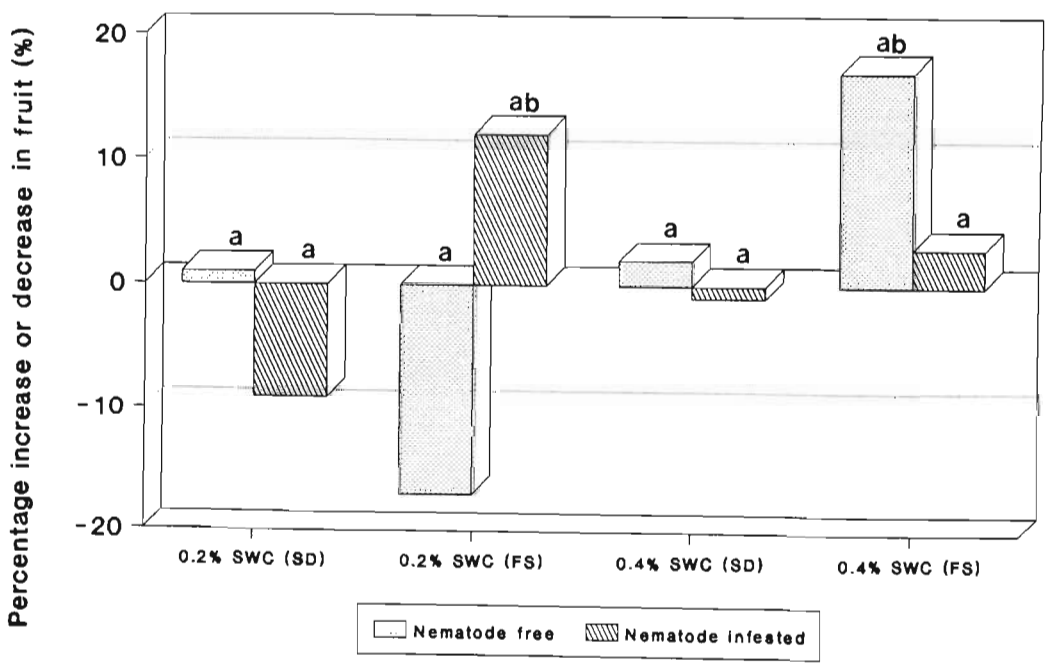

Figure 6.13 The effect of SWC, expressed as a percentage of the control, on the total fresh weight of tomato fruit (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

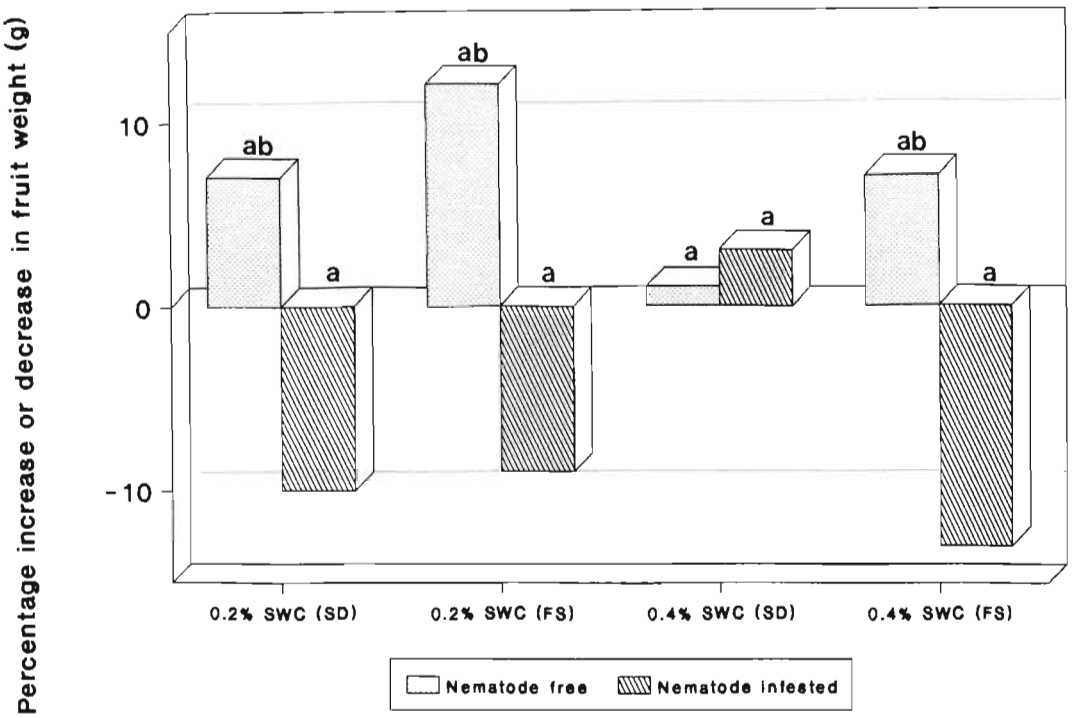

Figure 6.14 The effect of SWC, expressed as a percentage of the control, on average fruit fresh weight. (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

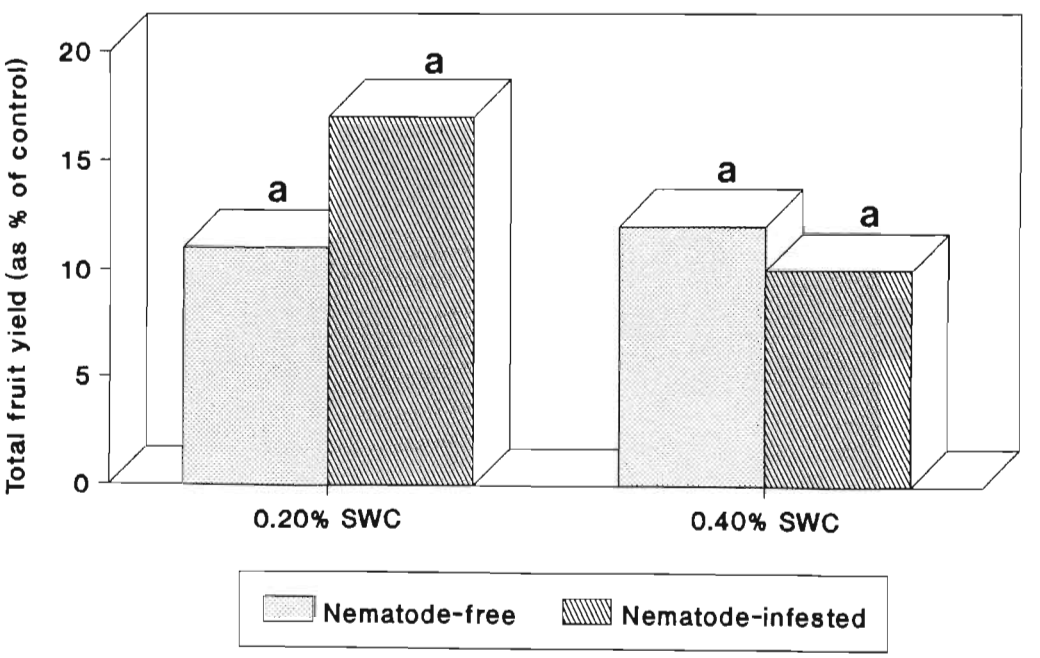

Figure 6.15 The effect of SWC, expressed as a percentage of the control, on final tomato yield. Bars with the same letter are not significantly different.

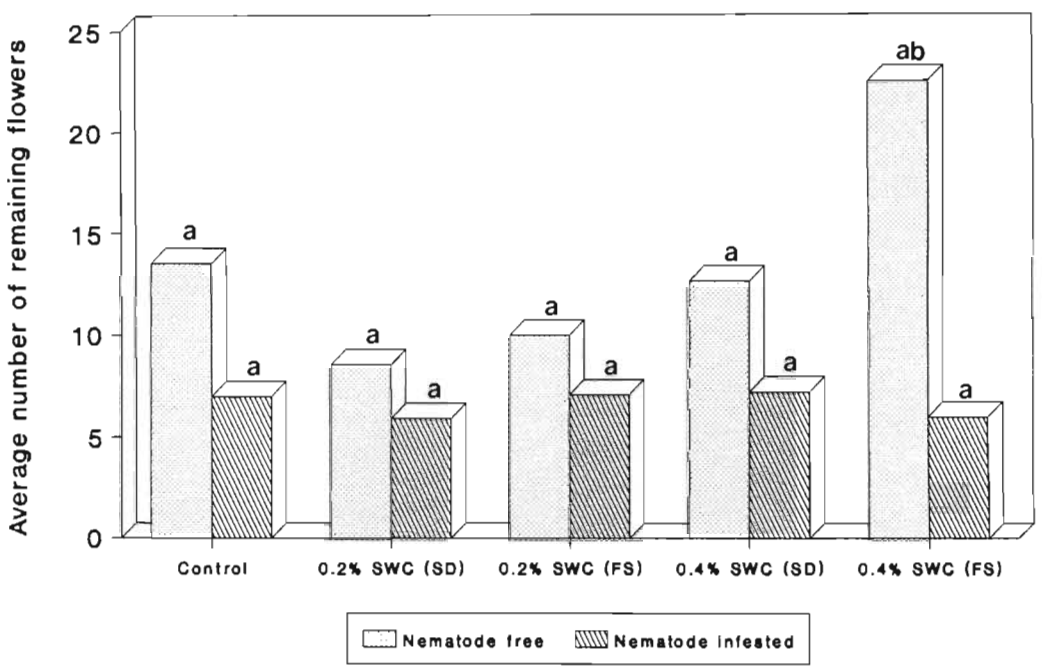

Figure 6.16 **The effect of SWC on the number of flowers present on the tomato plants at the end of the trial (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.**

6.5 The effect of ashed, filtered and extracted SWC on the degree of nematode infestation in tomato seedlings.

This trial examined whether an organic component in the SWC is responsible for improved plant growth. Filtration of the SWC determined whether both the particulate matter (cell-wall debris) and the filtrate (aqueous phase) are required to elicit a nematicidal response. The SWC was also extracted in methanol to release constituents still bound to the cell debris. The effect of this extract on nematode infestation was investigated.

6.5.1 Experimental Procedure and Results

Six-week-old tomato seedlings (var. Karina) were subjected to weekly applications of the treatments outlined in Table 6.4. After seven weeks the plants were harvested and the effect of each treatment on nematode infestation was recorded.

Filtration of SWC

The SWC was first filtered through Whatman Nº 1 filter paper to remove large debris and cell material and then through Whatman Nº 41 to remove small particulate matter. The aqueous filtrate and particulate phase were collected.

Ashed SWC

Ten mℓ of liquid Kelpak were reduced to dryness at 100°C in a ventilated oven. The dry SWC was then ashed in a muffle furnace for 2 hours at 450°C. On cooling, 1.0 M HCl was added. The ashed extract was made up to 1 ℓ with distilled water and the pH adjusted to 5.6 before application.

Table 6.4 **Outline of treatments used to assess the effect of filtered, ashed, extracted and acid-hydrolysed SWC on the growth of nematode-infested tomato seedlings.**

Treatment	Description
Control (With RKN's)	Water
Control (No RKN's)	Water
1.0% SWC	Soil drench
1.0% SWC	Foliar spray
1.0% SWC	Filtered SWC as soil drench
1.0% SWC	Particulate phase
1.0% SWC	Ashed SWC
1.0% SWC	Methanol extracted SWC
1.0% SWC	Acid hydrolysed SWC

Methanol extraction

Ten mℓ of SWC were extracted in 150 mℓ methyl alcohol for 12 hours at 10°C and then centrifuged at 3000 rpm for 12 minutes. The methanol supernatant was reduced to dryness under vacuum at 35°C. The methanol fraction and precipitate were then resuspended in 1 ℓ water.

Acid hydrolysis

Ten mℓ SWC were added to 20 mℓ 1N HCl and heated in a water bath at 95°C for two hours. The hydrolysed precipitate was then made up to 1 ℓ water and the pH adjusted to 5.6.

6.5.2 Results

As previous studies have shown that SWC improves plant growth, the results outlined below only report on the effect of the different treatments on the degree of nematode infestation. Nematode infestation was expressed as either the number of galls per gram (Figure 6.17) or as the number of galls per unit length of root (Figure 6.18). The significance of these results is summarised in Table 6.5 below.

The application of 1.0% SWC to the soil resulted in a highly significant reduction in nematode infestation (± 65%). This nematoxic effect was lost if the SWC was ashed. Applying the SWC as a foliar spray increased the degree of infestation by over 50%. This increase in infestation after foliar application of SWC was also observed in previous experiments. Filtered SWC, applied as a soil drench, resulted in almost a 100% reduction in nematode infestation. Application of an 80% methanol extract of SWC did not significantly improve the nematicidal effect over and above that of the 1.0% SWC treatment.

6.6 The effect of fractionated SWC on the growth of nematode-infested tomato seedlings.

In this trial the SWC was separated into ten fractions by paper chromatography and applied to nematode-infested tomato seedlings.

6.6.1 Experimental Procedure and Results

Six-week-old tomato seedlings were planted in a nematode-free sand:soil (1:1) mix. After 10 days two heavily infested tomato root systems were homogenised in 6500 mℓ water and 50 mℓ of this nematode inoculate applied to each plant. Ten plants

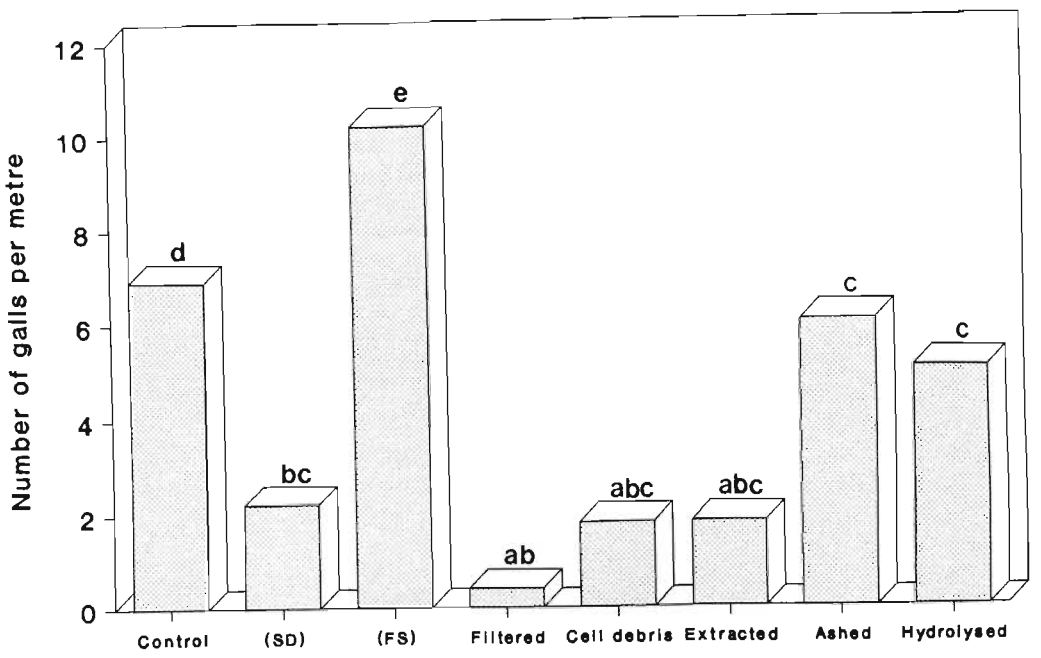

Figure 6.17 The effect of filtered, ashed and methanol extracted SWC on the degree of nematode infestation (number of galls per unit weight) of 8-week-old tomato plants (SD = soil drench; FS = foliar spray).

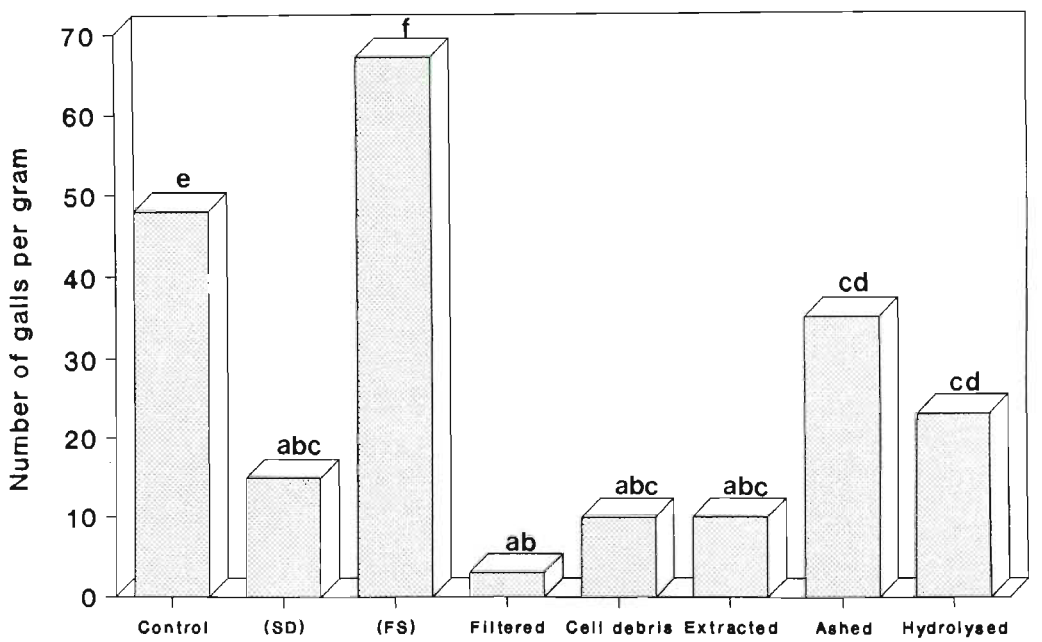

Figure 6.18 The effect of filtered, ashed and methanol extracted SWC on the degree of nematode infestation (number of galls per unit length) of 8-week-old nematode-infested tomato plants.

Table 6.5 **Effect of filtered, ashed, methanol extracted and acid-hydrolysed SWC (1.0%) on the degree of nematode infestation. Numbers with the same letter are not significantly different.**

TREATMENTS	GALLS/M	GALLS/G	COMMENTS
Control	6.9 d	47.2 e	Control plants generally smaller than SWC treated plants.
Soil drench	2.6 bc	16.5 abc	Significant decrease in RKN infestation
Foliar spray	10.6 e	68.7 f	Significant increase in RKN infestation
Filtrate (as soil drench)	0.4 ab	2.4 ab	Almost 100% reduction in nematodes
Particulate matter (cell wall debris)	1.7 abc	12.6 abc	Significantly fewer nematodes - not as effective as above
Ashed SWC	6.3 c	36.2 cd	Nematicidal effects of SWC lost. Suggests organic fraction responsible.
Methanol extract	1.7 abc	11.2 abc	Significant reduction in nematodes
Acid hydrolysed SWC	5.1 c	24.6 cd	Activity of SWC not improved by hydrolysis

were used for each treatment. Each plant received 50 mℓ of test solution every 10 days.

Three hundred and fifty mℓ of SWC was extracted in 1400 mℓ methanol for 12 hours at 10°C. The methanoic extract was filtered, reduced to dryness under vacuum at 35°C and the residue resuspended in 50 mℓ 100% methanol. This extract was streaked onto ten paper chromatograms as described earlier (Section 6.2.5). Each chromatogram represented three applications of 12 treatments (Table 6.6). The Rf strips were eluted overnight in 1ℓ of water and 50 mℓ applied as a soil drench to each plant.

After eight weeks the plants were harvested and shoot and root fresh and dry weights, leaf surface area, moisture content, and the degree of nematode infestation recorded. Nematode infestation was expressed as the number of galls per unit

Table 6.6 **Outline of treatments used to assess the effect of SWC, separated into 10 Rf zones by paper chromatography and applied to tomato seedlings.**

Nº	TREATMENT	Nº	TREATMENT
1	Rf 0	9	Rf 8
2	Rf 1	10	Rf 9
3	Rf 2	11	Rf 10
4	Rf 3	12	Combination Rf0-Rf10
5	Rf 4	13	1.0% SWC
6	Rf 5	14	Control + RKN
7	Rf 6	15	Control - RKN
8	Rf 7		

weight or unit length of root. Results were statistically analysed using one-way anova ($P<0.05$) and a multiple range test.

SWC (1.0%) invariably resulted in a significant increase in plant growth (Figure 6.19). The application of different Rf fractions to the plants gave variable results (Figures 6.19 to 6.21). The baseline residue fraction (Rf 0, Treatment 1) had no significant effect on plant growth. The fraction from Rf 1 (Treatment 2) significantly increased total plant fresh weight and root growth (Figures 6.19 & Table 6.7). This promotion in growth was similar to that elicited by 1.0% SWC. The fraction from Rf 4 (Treatment 5) resulted in a significant increase in root fresh weight and root/shoot ratio (Figures 6.19 & 6.21). The fractions from Rfs 6 & 8 (Treatments 7 & 9) generally inhibited shoot and root growth (Figures 6.19 & 6.20). A combination of all the Rf fractions gave similar results to 1.0% SWC (Figure 6.19).

The effect of separated SWC on RKN infestation indicated that certain fractions had marked nematicidal properties. Rfs 1, 4 & 9 (Treatments 2, 5 & 10) significantly reduced the degree of nematode infestation in the roots by 44, 41 and 29% respectively (Figures 6.22). An application of 1.0% SWC resulted in only an 18.5% reduction in the average number of galls per unit root fresh weight.

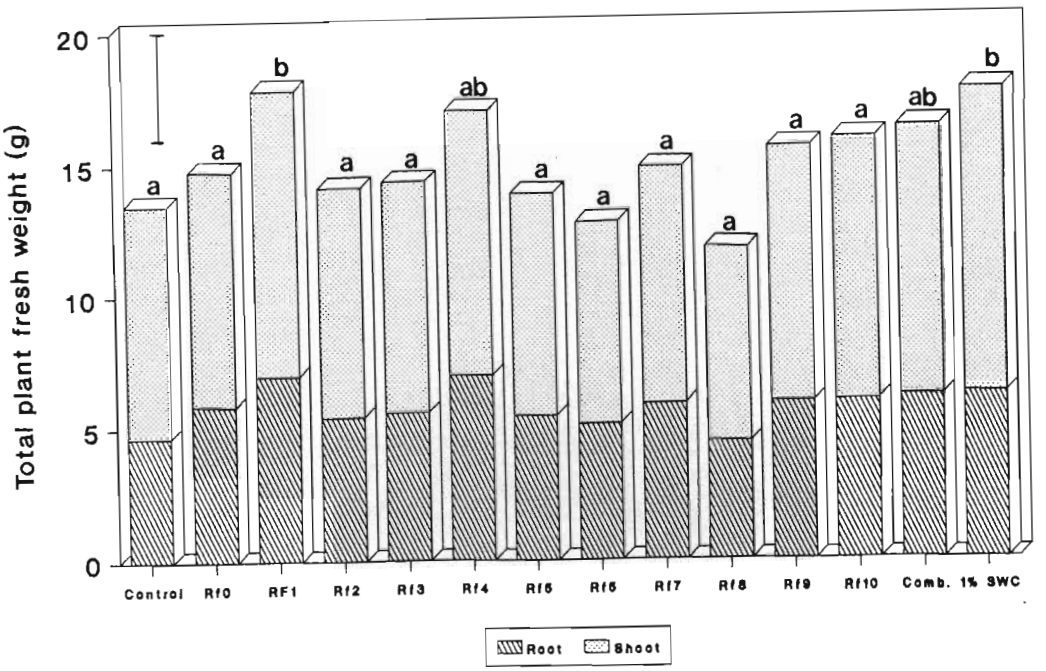

Figure 6.19 The effect of SWC, separated into 10 Rf zones by paper chromatography, on the total fresh weight of 8-week-old nematode-infested tomato plants (SD = soil drench; FS = foliar spray).

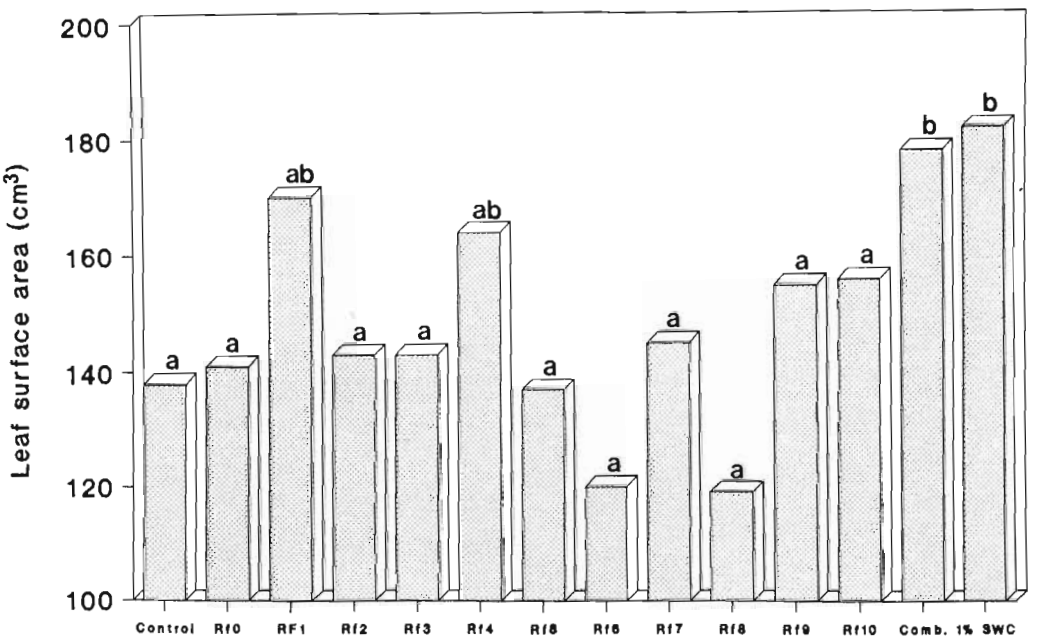

Figure 6.20 The effect of SWC, separated into 10 Rf zones by paper chromatography, on the leaf surface area of 8-week-old nematode-infested tomato plants (SD = soil drench; FS = foliar spray).

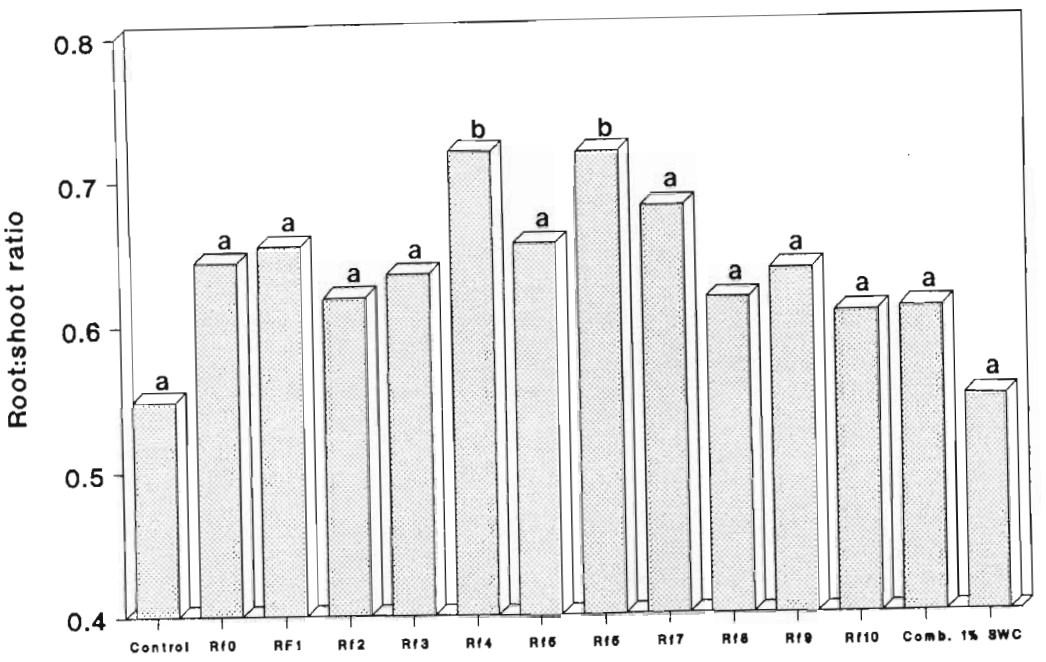

Figure 6.21 The effect of separated SWC on the root:shoot ratio of 8-week-old nematode-infested tomato plants (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

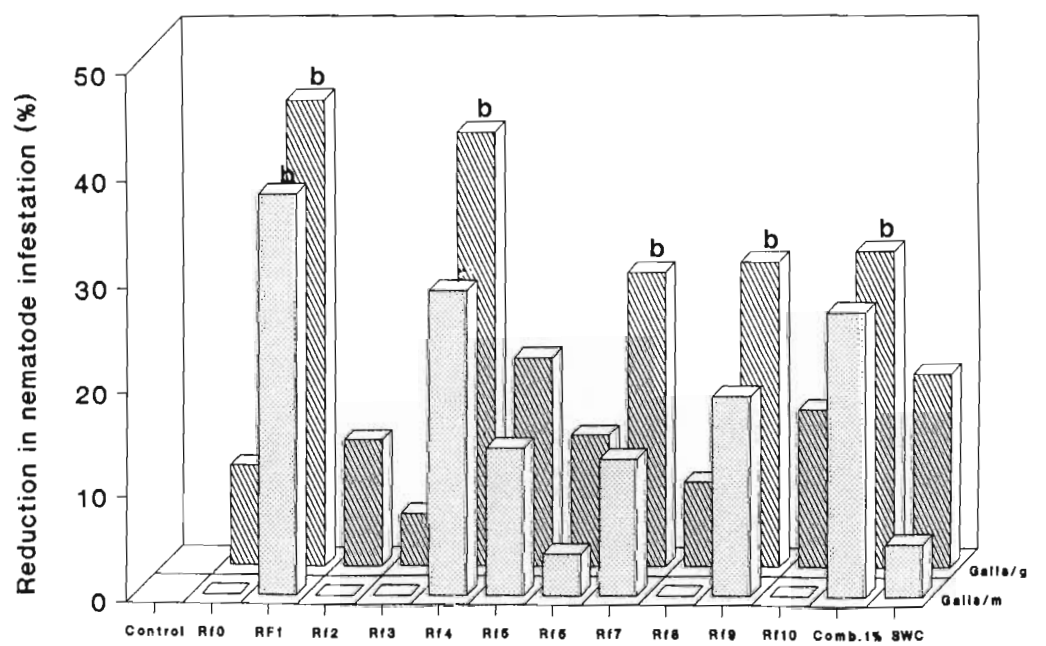

Figure 6.22 The effect of SWC, separated into 10 Rf zones by paper chromatography, on the reduction in nematode infestation of 8-week-old tomato plants (SD = soil drench; FS = foliar spray).

Table 6.7 **The effect of different Rf zones on plant growth as determined by one-way anova. (* = LSD P > 0.05)**

	Treatments												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Shoot Fresh Weight (g)													*
Shoot dry Weight (g)							-		-				
Root Fresh Weight (g)		*			*							*	*
Total Fresh Weight (g)		*											*
Root Length (m)		*											
Leaf Surface Area (cm^3)												*	*
Root: Shoot Ratio					*		*						
Moisture content (%)			*	*					*		*		*
Galls per gram		*			*			*		*		*	
Galls per metre		*											
Ave Nº galls		*								*			

It was interesting to note that treatments having little effect on plant growth often contained significantly more water than control plants (Figure 6.23). This suggests that these treatments may possibly be promoting cell size at the expense of cell wall development.

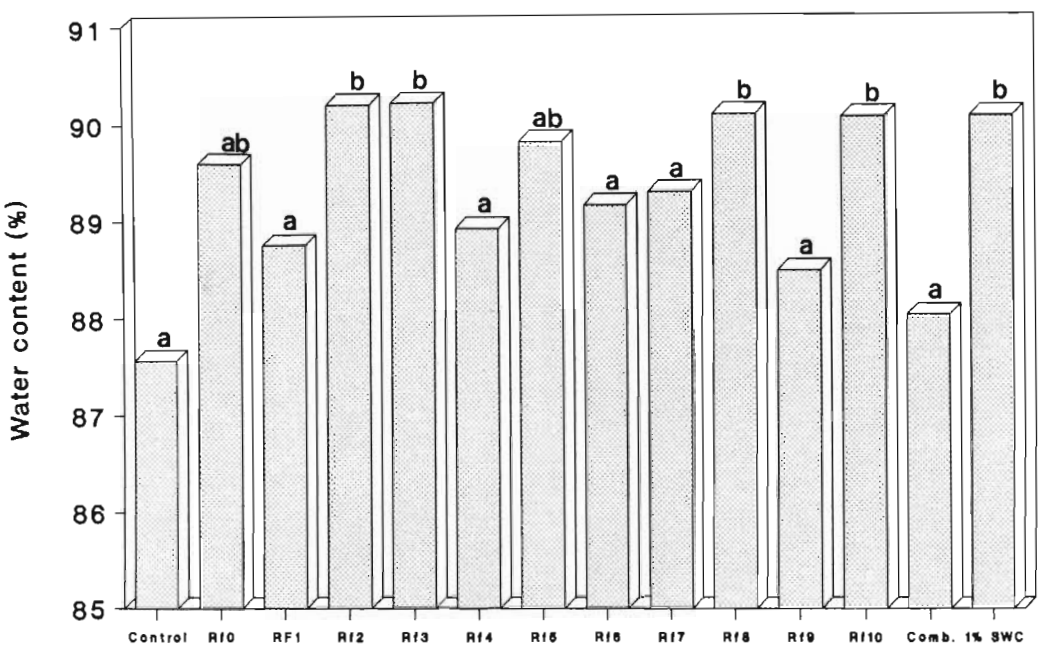

Figure 6.23 The effect of SWC on the moisture content of 8-week-old nematode-infested tomato plants (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different.

6.7 Discussion

In the tomato seedling-establishment studies, SWC applied to the soil invariably enhanced several aspects of plant growth. The use of SWC in reducing transplant shock of vegetables and ornamentals has been examined by ALDWORTH & VAN STADEN (1987). They found that the application of SWC to cabbage and marigold seedlings at transplanting significantly improved seedling growth. In the case of the marigolds, flowering was greatly accelerated. It was suggested that the SWC aided in the establishment of these plants by increasing their root size and vigour.

Studies have shown that up to 95% of mineral nutrients applied to plants in a foliar spray may be absorbed by the foliage. With soil application only about 50% of the nutrients are thought to be available for plant growth (BYFORD-JONES, 1988). The amounts of nutrients required through foliar spraying are thus much less than when the same nutrients are applied to the soil. Foliar applied nutrients are also rapidly absorbed through the plant foliage with up to 70% of available nutrients being absorbed within the first 30 minutes of spraying (BYFORD-JONES, 1988). It was therefore surprising to find that although SWC applied to the soil increased all growth parameters, the same concentration applied as a foliar spray did not improve plant growth. Foliar application is thought to be most effective when roots are unable to absorb nutrients for reasons such as infertile soil, a high degree of soil fixation, losses from leaching, lack of soil moisture and an injured or diseased root system (BYFORD-JONES, 1988). The apparent ineffectiveness of foliar applied SWC therefore suggests that nutrients were never limiting to the roots. This implies that the nematode-infestation never reached a stage whereby the parasites adversely affected nutrient and moisture uptake by the roots.

Treatment with SWC increased both the root:shoot ratio and accumulation of plant biomass. This suggests a two-fold action of SWC: (i) to stimulate root growth at the expense of shoot growth, and (ii) to increase the overall photosynthetic accumulation efficiency of the plant.

Certain constituents in SWC's are thought to be instrumental in increasing root growth (Chapter 4). Phenolic compounds and mannitol have been shown to stimulate root development (JACKSON, 1965; POAPST & DUNKEE, 1967; BOOTH, 1969). MILTON (1964) postulated that the degraded complexes of fucoidin, alginates and similar compounds present in SWC retain very strong polar groups and therefore enhance soil aggregate and crumb structure, which in turn makes conditions more suitable for root growth. *In vitro* studies in Chapter 4 suggest that factors other than improved soil conditions are involved. It was concluded from these root growth experiments that plant growth regulators may be present in the SWC which act to stimulate root development. FEATONBY-SMITH & VAN STADEN (1984) showed that the significant increase in root growth resulting from the application of SWC was reflected in the plant dry weight as well as in an elevation in the cytokinin content in the roots. Plant growth regulatory studies (Chapter 2), and root initiation studies (Chapter 5), demonstrated the presence in SWC of compounds that elicited marked auxin-like growth responses. The presence of endogenous plant hormones in the SWC may thus aid in the growth and development of the roots.

Improved root growth would result in more efficient water and nutrient utilization by the plants (WIDDOWSON, YEATES & HEALY, 1973). This could help explain the overall beneficial growth of those plants treated with SWC (FEATONBY-SMITH, 1984).

The results from the seedling establishment trials indicate that the beneficial effect of SWC on tomato yield is due partly to stimulation of certain growth parameters during the early stages of plant development, and later, to better redistribution of assimilates during the reproductive stages of growth.

In most crops, yield is dependent on the percentage of flowers that actually mature. The 8-week-old tomato seedlings treated with SWC invariably had more flowers than non-treated plants. If flower initiation and development is directly related to the physiological age of the plant, then the SWC may promote flowering solely by encouraging vigorous plant growth. However, many plants subjected to environmental stress are known to shift towards reproductive growth as a survival

strategy. As plants receiving SWC showed no signs of stress, it may be assumed that early flower set is elicited either by improved plant growth or possibly by some endogenous component in the SWC.

There are many references in the literature to the effect of organic compounds on flower set. Although the active components in SWC have not been positively identified, early flowering may result from endogenous plant growth regulators in the SWC. Cytokinins (MAHESHWARI & VENKATARAMAN, 1966), auxins (CLARK & KERNS, 1942; BANDURSKI & NONHEBEL, 1984), gibberellins (CLELAND & BRIGGS, 1969; EVANS, 1971; PHARIS, ROSS, WAMPLE & OWENS, 1976) and ethylene (CHACKO, KOHLI, DORE SWAMY & RANDHAWA, 1976) have been shown to affect flowering.

Associated with this early flower set, was a high percentage of SWC treated plants that produced early fruit. Nearly 60% of all the first fruit picked, and over 50% of all the second, were from plants treated with 0.2% and 0.4% SWC respectively. The majority of fruit on control plants were found to ripen after three or four fruit had already been harvested off SWC treated plants. This trend in fruit ripening may be of economic importance as crops which ripen earlier often fetch better market prices.

Increased income may also arise from larger crop yields of a superior quality. SWC increased the final tomato yield by nearly 20% and improved the average size of the fruit by over 10%. A foliar application of SWC just prior to flowering appeared to stimulate fruit development. BOOTH (1969) reported that the uptake of foliar applied nutrients was enhanced by urea, sugars and the bicarbonate ion. Sugars, are known to be present in varying amounts in seaweeds (Chapter 1.4). These compounds are rapidly absorbed through the leaves and appear to be particularly beneficial to plants after flower set when there is a sudden migration of sugars from the leaf to the rapidly growing fruit.

The reasons why SWC elicited yield increases is however, thought to be primarily related to the hormonal content, and particularly cytokinin content of the products (BRAIN, CHALOPIN, TURNER, BLUNDEN & WILDGOOSE, 1973; BLUNDEN, 1977; FEATONBY-SMITH &

VAN STADEN, 1983a, 1983b). It is well documented that cytokinins are involved in nutrient mobilization in vegetative plant organs (MOTHES & ENGELBRECHT, 1961; TURREY & PATRICK, 1979; GERSANI & KENDE, 1982). The presence of high levels of cytokinins in reproductive organs (LETHAM, 1973; DAVEY & VAN STADEN, 1978; SUMMONS, ENTSCH, LETHAM, GOLLNOW & MACLEOD, 1980) is thought to be associated with this mobilization process (WAREING & SETH, 1967). Fruits, or newly developing and morphologically changing organs, have the potential to act as stronger sinks for cytokinins (VARGA & BRUINSMA, 1974). This would mean that at certain stages of plant development the distribution of endogenous cytokinins, apparently synthesised in the roots, could be monopolised by a specific organ, thus creating preferential transport within the developing plant (HUTTON & VAN STADEN, 1984). NOODEN & LEOPOLD (1978) found that during fruit development the mobilization centre for photosynthate shifts away from the roots, stem and young leaves towards the developing fruit.

FEATONBY-SMITH & VAN STADEN (1984a) found that at the fruiting stage, tomato and bean (*Phaseolus vulgaris* L.) fruits treated with SWC contained higher cytokinin levels than those of control plants. This suggests that a component of the SWC is either increasing the translocation of cytokinins from the roots (DAVEY & VAN STADEN, 1978; VONK, 1979), or increasing the production of cytokinins within the fruit themselves (HAHN, DE ZACHS & KENDE, 1974). These high concentrations of cytokinins may thus be instrumental in increasing plant yield by creating strong physiological sinks capable of competing with the remainder of the plant for nutrients (LUCKWILL, 1977). SWC treated plants have also been shown to contain higher levels of cytokinins in the roots (FEATONBY-SMITH, 1984). These plants may therefore have a greater potential source of cytokinins for transport to the maturing fruit.

According to BRAIN, CHALOPIN, TURNER, BLUNDEN & WILDGOOSE (1973), the cytokinin content of SWC is probably lost when applied to the soil. They suggested that as cytokinins are readily absorbed through leaf surfaces, foliar application may prove an effective means of supplying the plant with exogenous cytokinin. NAITO, TSUJI & HATAKEYAMA (1978), found that RNA, protein and chlorophyll, fresh and dry weights, and leaf surface area increased when bean leaves were treated with the synthetic

cytokinin, benzyladenine. Endogenous levels of cytokinins in SWC are thought to improve plant growth in a similar fashion (FEATONBY-SMITH & VAN STADEN, 1984a).

The observed levels of cytokinin found in SWC do not, however, appear to be sufficiently high to suggest that these are the only compounds responsible for the observed beneficial responses of seaweed products on plants, especially in view of the high dilutions of SWC utilised in practice (TAY, MACLEOD, PALNI & LETHAM, 1985; TAY, PALNI & MACLEOD, 1987). The wide range of physiological responses elicited by SWCs also suggests that growth regulators, other than cytokinins, are involved. In Chapter 2, distinct auxin-like growth responses were detected using recognised bioassay systems. The role of auxin in plant growth and development has been attributed mainly to its cell extension properties (EVANS, 1973) and also to its ability to enhance RNA and protein synthesis (KEY, 1969). Recent evidence however, suggests that auxins influence flower initiation (BANDURSKI & NONHEBEL, 1984) and fruit maturation (LEOPOLD & KRIEDEMANN, 1975). Thus, this group of plant growth regulators may also be instrumental in improving plant yield by affecting these growth processes.

Infestation of tomato plants with RKN ultimately reduced plant growth and the rate of flower development. The application of SWC to the soil overcame these adverse effects partly by reducing the degree of nematodes infecting the roots and partly by improving root vigour. The same dilution applied as a foliar spray did not reduce nematode-infestation and, in some instances, was shown to even increase the severity of nematode attack. While the significance of these findings is unclear, the above results suggest that some component in the SWC is inducing a degree of host/parasite incompatibility. In Chapter 4, it was postulated that this interaction may take place within the root and that a direct nematicidal component in the SWC is probably not affecting the larva prior to penetration of the root. It was also established in Chapter 4, that incompatibility can be induced at any stage of the parasites life cycle - from the emergence of the larva from the eggs to the mature reproductive adults. Failure of the compatible response may result from synthesis of metabolic inhibitors in the root, death of the feeding site, or the failure of the host

tissue to keep pace with nutritive demands of the nematode (LEWIS, 1987).

Numerous studies have implicated hormones, and in particular cytokinins and auxins, in nematode infestation and development in the roots of susceptible hosts (DROPKIN, HELGESON & UPPER, 1969; KOCHBA & SAMISH, 1971; SAWHNEY & WEBSTER, 1975). BRUESKE & BERGESON (1972) found that the infestation of roots with *M. incognita* resulted in decreased cytokinin levels in the root exudate of tomatoes. FEATONBY-SMITH & VAN STADEN (1983b) suggested that the resulting decrease in cytokinin translocation to the shoots may be responsible for the reduced shoot growth associated with nematode infestation. The application of cytokinins found in SWC were thought to possibly overcome this imbalance. Auxins and cytokinins applied alone to tomato roots susceptible to RKNs resulted in a reduction in the degree of infestation (SAWHNEY & WEBSTER, 1975). The presence of these compounds in SWC may have a similar effect on nematode infestation and development.

The nematoxic effect of the SWC was lost if the material was ashed, indicating that the incompatibility effect is associated with an organic fraction rather than inorganic components. Other studies examining the effect of ashed SWC noted similar losses in beneficial plant growth responses. FINNIE & VAN STADEN (1985) found that SWC stimulated the growth of *in vitro* cultured tomato roots, an effect that was lost if the concentrate was ashed. SWC applied to wheat was noted by BECKETT & VAN STADEN (1990b) to be more effective than the same dilution ashed to remove organic molecules. The only record of growth stimulation following the application of ashed SWC to plants was by BLUNDEN, CHALLEN & WOODS (1968) who found that ashed SWC increased the wet and dry weights of mustard, leeks and onions. In this instance improved growth may have been a result of a mineral nutrient effect.

Filtering the SWC to remove particular matter (cell wall debris) invariably improved plant growth further and resulted in a highly significant ($P < 0.01$) reduction in nematode infestation. The application of the particulate phase to the plants was ineffective in stimulating growth. Similar results were reported in Chapter 4, where the filtrate fraction significantly enhanced root growth and the particulate fraction

was found to be inhibitory to root development. The removal, by filtration, of possible growth inhibitory components from the SWC may thus prove beneficial to the effectiveness of seaweed products in improving plant growth and reducing nematode infestation.

Extracting the SWC in methanol, to include any beneficial constituents still bound to the cell wall material, did not improve the growth of the plants. However, chromatographic separation of the SWC into ten Rf zones indicated more than one fraction with growth promotory properties. Associated with this improved growth was a reduction in nematode infestation. This suggests: (i) that there is more than one active constituent in the SWC, and (ii) that certain of the components which improves plant growth may also be responsible for induced host/parasite incompatibility.

It can be concluded from the above and similar studies that the reaction obtained from the application of SWC to plants is dependent on how and when the SWC is applied. SWC applied as a soil drench was shown to aid in the establishment of seedlings and also reduce the degree of nematode infestation in the roots. Foliar applied SWC was found to improve fruit production and development. This suggests that SWC applied as a soil drench in conjunction with a series of foliar sprays may possibly elicit the best results. Fractionation studies indicated that possibly more than one factor is responsible for enhanced plant growth. Ashing the SWC determined that these factors are of an organic nature.

CHAPTER 7

GENERAL CONCLUSION

Considerable attention is being focused on the development of new biodegradable products that enhance plant growth and improve yield characteristics. As seaweeds are one of the last remaining natural resources as yet unexploited by man, it is hardly surprising that current research is being directed at learning more about the agricultural potential of this resource. The aim of this project was to assess some of the growth regulatory properties that have been attributed to commercial seaweed concentrates. Apart from examining the occurrence of growth hormones in SWC, the potential of SWC in tissue culture systems, as a rooting agent and in reducing nematode infestation was also investigated. It is hoped that the results of this study will aid in the understanding of the role of SWC in plant growth and development.

A review of the literature indicated that seaweed, in one form or the another, has been applied to over 80 different species of plant in carefully controlled experiments. An examination of these studies revealed that there are several schools of thought pertaining to the mode of action of seaweeds on plant growth. Early research attributed improved plant growth to either soil conditioning properties of the seaweed or to an enhancement of nutrient uptake by the plants. While certain constituents of seaweed meals possibly improve soil structure, these same constituents cannot explain the beneficial growth responses resulting from dilute amounts of SWC administered to plants as either a soil drench or foliar spray.

The reaction obtained with the foliar application of SWC has, in the past, been ascribed to the presence of trace elements. However, if the concentration at which the products are applied is taken into account, it is clear that the amount of mineral elements in the spray mixture is too low to have any significant growth effect. Recent research has shown the occurrence, in seaweed products, of certain plant

hormones. At present the observed beneficial effects are attributed to these constituents. Since the effect of plant hormones in the plant usually depends on the balance between two or more of these substances, and these ratios change continuously from one growth stage to another, it could be expected that the effect of a seaweed product will depend on the type of crop, its growth stage, and the composition and concentration of the seaweed product (DE VILLIERS, KOTZE & JOUBERT, 1983).

Many of the growth effects elicited by SWCs were similar in nature to those which might be expected to be produced by cytokinin activity. This led to the study of cytokinins in SWC receiving considerable attention, and auxins and other plant growth regulators being investigated to a far lesser extent. Using recognised bioassays, abscisic acid-, gibberellin- and auxin-like compounds were detected in the SWC. As auxins were of particular interest in this study, the SWC and *E. maxima* material were purified by reversed phase HPLC and auxin-like compounds tentatively identified by co-chromatography with authentic standards. This chromatographic system revealed the presence of relatively large amounts of compounds co-eluting with tryptophan and indole-3-acetamide, and to a lesser extent compounds co-eluting with indole-3-carboxylic acid, indole-3-acetic acid, indole-3-acetaldehyde and abscisic acid. Although these compounds were not unequivocally identified by GC-MS, HPLC and bioassay results strongly suggested their presence in SWC.

While several bioassays have been used to determine the presence of growth regulators in SWC, there is a need for the development of new systems that can effectively measure plant growth responses to SWC. The *in vitro* potato system outlined in Chapter 3 may serve as an excellent tool to this end as growth responses were detectable after only three weeks in culture. The advantage of this assay was that all growth parameters could be controlled. Furthermore, shoot extension, lateral bud development and tuberization were all measurable in a single bioassay system. SWC treated potato nodal explants produced significantly larger shoots. This assay therefore indicated that increased plant growth is not only due to an improved root system, but also to the stimulation of shoot growth.

One of the most pronounced effects following SWC application was an enhanced root growth. Studies examining the effect of SWC on the growth of excised tomato roots cultured *in vitro* confirmed that SWC contains compounds stimulatory to root development. Filtering the SWC revealed that these stimulatory compounds were located in the aqueous filtrate, and that compounds of an inhibitory nature are present in the particulate phase. Whether these responses are elicited by known plant hormones, still needs to be determined. FINNIE & VAN STADEN (1985) found that the enhanced root growth was associated with increased root extension and lateral root formation. They attributed this improved growth to a low cytokinin content in the SWC.

SWC applied as a pulse treatment to cuttings indicated marked root initiating activity. Auxins have been shown to be essential to the initiation of roots, it is therefore highly probable that this group of plant growth regulators are present in SWC. Auxins, as well as cytokinins, may therefore be involved in the regulation and control of SWC enhanced root development. Indeed, it was found that HPLC fractions co-eluting with indole-3-acetamide, indole-3-acetic acid, indole-3-carboxylic acid and indole-3-acetaldehyde enhanced adventitious root formation on mung bean cuttings.

The final stage of this study examined the effect of SWC on the growth of nematode-infested tomato plants. The SWC aided in seedling establishment, stimulated early flowering, improved yield parameters, and reduced the degree of nematode infestation.

By reducing transplant shock, seaweed treated seedlings grew quicker than control plants. Although this has often been ascribed to an improved root system, *in vitro* potato studies (Chapter 3) demonstrated that SWC is also effective in stimulating shoot growth and development.

Income from a crop can be raised in three basic ways: yields may be increased; quality may be improved; or the produce may ripen when market prices are higher. Early flowering following SWC application has been noted for *Capsicum annuum*

(Sweet peppers), *Euphorbia pulcherrima* (Poinsettia) (SENN & KINGMAN, 1978), and *Tagetes patula* (Marigold) (ALDWORTH & VAN STADEN, 1987). In the tomato trials, SWC treated plants were the first to flower. Associated with early flower set was early fruit ripening and increased yield. Although yield quality was not tested in terms of fruit firmness, sugar content or shelf-life, the effect of SWC on these parameters is well documented (Chapter 1.6.6).

Improved plant yield following seaweed application has been attributed to the cytokinin content of the SWCs (FEATONBY-SMITH, 1984). Higher levels of cytokinins in fruit act as stronger sinks for photosynthates. This mobilization of nutrients thus enhances the rate of fruit development and maturation. SWC may affect this process in two ways by: (i) aiding in the translocation of cytokinins from the roots to the developing fruit or, (ii) supplying a supplementary source of cytokinins to the fruit.

Studies on the effect of SWC on nematode infestation found that SWC was capable of inducing a resistance in tomato plants to RKNs. Fractionation studies revealed that more than one component was able to reduce nematode infestation. The fractions containing these nematoxic components were also found to stimulate plant growth. While these results suggest that the reduction in nematode infestation is possibly responsible for the enhanced growth, other studies indicated that fractionated SWC is able to improve plant growth regardless of nematode infestation. Although SWC proved effective in reducing the degree of RKN infestation in the roots, these products could never be classified as nematicides as there was never complete eradication of the parasites.

The results obtained in the above studies clearly indicate that certain constituents in SWC elicit beneficial growth when applied to plants or isolated plant organs. However, even after more than fifty years of research the exact mechanism and mode of seaweed action is still not fully understood. Although it is doubtful that the heightened responses obtained from the use of SWC can be attributed to mineral nutrient constituents of the products, it has yet to be demonstrated that synergistic effects between the mineral nutrient elements and growth regulatory substances do

not exist (TEMPLE & BOMKE, 1989). Recent investigations documenting synergistic effects on plant growth to foliar applications of various elements with phytohormones add support to this view (MARSCHNER, 1982; MENGEL & KIRKBY, 1982; NEWMAN & NOODEN, 1983). Indeed, it is probable that the beneficial responses from SWC application are due to the additive effect of several promotory factors in the SWC acting at any one time.

This study was initiated to answer some of the unsolved questions connected with seaweed research. However, in attempting to answer these, many more were generated. The experiments outlined in this study indicate that future work should be restricted to several specific areas of research. Of prime importance is the need to isolate and identify the root initiating factor in SWC. The development of a new rooting compound that, in many instances shows a better rooting response than authentic auxins, could be economically important in many agricultural and horticultural sectors. Other areas of research that still require further work are: the unequivocal determination of auxins, gibberellic acid and abscisic acid in SWC; the study of the factor in SWC that initiates early flower set; and the isolation and identification of the nematoxic component in SWC. The tomato trials also indicated growth differences when SWC was applied to the roots as a soil drench or to the leaves as a foliar spray. Results suggest that as a soil drench, SWC may be particulary beneficial in reducing transplant shock and aiding in seedling establishment, whereas as a foliar spray, prior to flowering, the SWC enhances fruit set and maturation. No research has studied the effect of combining drip irrigation with foliar sprays of SWC. This is possibly another area that still requires extensive research under both greenhouse and field conditions.

As more information about the composition, biological activity, and nutrient content of various seaweed formulations becomes available, these products will probably be used to a greater extent in large-scale agriculture. Their relevance in agriculture today is emphasised by their relative cheapness and because they are a natural biodegradable product.

REFERENCES

ABETZ, P. 1980. Seaweed extracts: Have they a place in Australian agriculture or horticulture? *The Journal of the Australian Institute of Agricultural Science* **46**: 23-29.

ABETZ, P. and YOUNG, C.L. 1983. The effect of seaweed extract sprays derived from *Ascophyllum nodosum* on lettuce and cauliflower crops. *Botanica Marina* **26**: 487-492.

AITKEN, J.B. 1964. The effect of seaweed extract and humic acids on the oxygen uptake of *Citrus sinensis* seedlings grown in nutrient medium deficient culture. Thesis, Clemson College, South Carolina. U.S.A.

AITKEN, J.B. and SENN, T.L. 1965. Seaweed products as a fertilizer and soil conditioner for horticultural crops. *Botanica Marina* **8**: 144-148.

ALDWORTH, S.J. and VAN STADEN, J. 1987. The effect of seaweed concentrate on seedling transplants. *South African Journal of Botany* **53**: 187-189.

ANDERSEN, A.S. and MUIR, R.N. 1969. Gibberellin induced changes in diffusible auxins from savoy cabbage. *Physiologia Plantarum* **22**: 354-363.

AUGIER, H. 1972. Contribution à l'étude biochimique et physiologique des substances de croissance chez les algues. These Doct d'Etat, Marseille.

AUGIER, H. 1974a. Les phytohormones des algues. I. Etude biochimique. *Annales des Sciences Naturelles Botanique et Biologie Vegetale* **15**: 1-64.

AUGIER, H. 1974b. Les phytohormones des algues. II. Etude physiologique. *Annales des Sciences Naturelles Botanique et Biologie Vegetale* **15**: 119-180.

AUGIER, H. 1976a. Les hormones des algues. Etat actuel des connaisances. I. Recherche et tentatives d'identification des auxines. *Botanica Marina* **19**: 127-143.

AUGIER, H. 1976b. Les hormones des algues. Etat actuel des connaisances. II. Recherche et tentatives d'identification des gibberellines, des cytokinines et

de diverses autre substances de nature hormonales. *Botanica Marina* **19**: 245-254.

AUGIER, H. and HARADA, H. 1972. Présence d'hormones de type cytokinine dans le thalle des algues marines. *Comptes Rendus des Seances de l'Academie des Sciences (Paris)* **275**: 1765-1768.

AUGIER, H. and HARADA, H. 1973. Contribution à l'étude des cytokinines endogenes des algues. *Tethys* **5**: 81-93.

BAKKEN, T.J. and BOE, A.A. 1982. Two bioassay techniques for determining abscisic acid concentrations. *Journal of the American Society for Horticultural Science* **107**: 109-112.

BANDURSKI, R.S. and NONHEBEL, H.M. 1984. Auxins. In: Wilkins, M.B. (ed.), *Advanced Plant Physiology*. Pitman Publishing Ltd., (London). pp. 1-20.

BASSUK, N.L., HUNTER, L.D. and HOWARD, B.H. 1981. The apparent involvement of polyphenol oxidase and phloridzin in the production of apple rooting co-factors. *Journal of Horticultural Science* **56**: 313-322.

BASTIN, M. 1966. Metabolisme auxinique et rhizogenese: III. Interactions entre l'auxine et quelques substance phenoliques présentes dans les boutures. In: les Phytohormones et l'organogenese, pp. 123-140. Congres etcolloques de l'Université de liège.

BASU, R.N., ROY, B.N. and BOSE, T.K. 1970. Interaction of abscisic acid and auxins in rooting of cuttings. *Plant and Cell Physiology* 11: 681-684.

BATTEN, D.J. and GOODWIN, P.B. 1978. Phytohormones and the induction of adventitious roots. In: Letham D.S. (ed.), *Phytohormones and Related Compounds: A Comprehensive Treatise*. Vol **2**. Elsever/North Holland, Amsterdam. pp. 137-173. ISBN 0-444-800549.

BECKETT, R.P. and VAN STADEN, J. 1988. The effect of Kelpak seaweed extract on the uptake of foliar applied zinc by lemons (*Citrus lemon*). *South African Journal of Science* **84**: 775.

BECKETT, R.P. and VAN STADEN, J. 1989. The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. *Plant and Soil* 116: 29-36.

BECKETT, R.P. and VAN STADEN, J. 1990a. The effect of seaweed concentrate on the uptake of foliar-applied Cu, Mn and Zn by tomato seedlings. *South African Journal of Botany* **56**: 389-392.

BECKETT, R.P. and VAN STADEN, J. 1990b. The effect of seaweed concentrate on the yield of nutrient stressed wheat. *Botanica Marina* **33**: 147-152.

BELEAU, M.H., HEIDELBAUGH, N.D. and VAN DYKE, D. 1975. Open ocean farming of kelp for conversion to animal and human foods. *Food Technology* **29**: 27-30,45.

BENTLY, J.A. 1960. Plant hormones in Marine Phytoplankton, Zooplankton and Sea Water. *Journal of the Marine Biological Society of the United Kingdom* **39**: 433-444.

BENTLY-MOWAT, J.A. and REID, S.M. 1968. Investigation of the radish leaf bioassay for kinetins and demonstration of kinin-like substances in algae. *Annals of Botany* **32**: 23-32.

BIDDINGTON, N.L. and DEARMAN, A.S. 1982/1983. The involvement of the root apex and cytokinins in the control of lateral root emergence in lettuce seedlings. *Plant Growth Regulator* **1**: 183-193.

BLACK, W.A.P. 1955. Seaweeds and their constituents in food for man and animals. *Chemistry and Industry* (London) **51**: 1640-1645.

BLAU, K. and KING, G. 1977. Handbook of derivatives for chromatography. Heyden & Son, London. 576 pp.

BLAZICH, F.A. 1988. Mineral Nutrition and adventitious rooting. In: Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.), *Adventitious Root Formation in Cuttings*. Advances in Plant Science Series. Vol **II**. Dioscorides Press, Portland. pp. 61-69.

BLAZICH, F.A., WRIGHT, R.D. and SCHAFFER, H.E. 1983. Mineral nutrient status of 'Convexa' holly cuttings during intermittent mist propogation as influenced by exogenous auxin application. *Journal of the American Society for Horticultural Science* **108**: 425-429.

BLUNDEN, G. 1972. The effects of aqueous seaweed extract as a fertilizer additive. *Proceedings of the International Seaweed Symposium* 7: 584-589.

BLUNDEN, G. 1977. Cytokinin activity of seaweed extracts. In: Faulker, D.L. and Fenical, W.H. (eds.), *Marine Natural Products Chemistry*. Plenum Publishing Corporation, New York.

BLUNDEN, G. and GORDON, S.M. 1986. Betaines and their sulphonio analogues in marine algae. In: Round, F.E. and Chapman, D.J. (eds.), *Progress in Phycological Research* **4**. Biopress Ltd, Bristol.

BLUNDEN, G. and WILDGOOSE, P.B. 1977. The effects of aqueous seaweed extract and kinetin on potato yields. *Journal of the Science of Food and Agriculture* **28**: 121-125.

BLUNDEN, G., CHALLEN, S.B. and WOODS, D.L. 1968. Seaweed extracts as fertilizers. *Journal of the Science of Food and Agriculture* **19**: 289-293.

BLUNDEN, G., CRIPPS, A.L., GORDON, S.M., MASON, T.G. and TURNER, C.H. 1986. The characterisation and quantificative estimation of betaines in commercial seaweed extracts. *Botanica Marina* **29**: 155-160.

BLUNDEN, G., EL BAROUNI, M.M., GORDON, S.M., McLEAN, W.F.H. and ROGERS, D.J. 1981. Extraction, purification and characterisation of Dragendorff-positive compounds from some British marine algae. *Botanica Marina* **24**: 451-456.

BLUNDEN, G., JONES, E.M. and PASSAM, H.C. 1978. Effects of post-harvest treatment of fruit and vegetables with cytokinin-active seaweed extracts and kinetin solutions. *Botanica Marina* **21**: 237-240.

BLUNDEN, G., ROGERS, D.J. and BARWELL, C.J. 1984. Biologically active compounds from the British marine algae. In: Krogsgaard-Larsen, C.P., Brogger, S., Christensen, S. and Kofod, H. (eds.), *Natural Products and Drug Development*. Alfred Benzon Symposium, Vol **20** Munksgaard, Copenhagen. pp. 179-190.

BLUNDEN, G., WILDGOOSE, P.B. and NICHOLSON, F.E. 1979. The effects of aqueous seaweed extract on sugar beet. *Botanica Marina* **22**: 539-541.

BLUNDEN, G., WILDGOOSE, P.B. and NICHOLSON, F.E. 1981. The effects of aqueous seaweed extract on sugar beet. *Proceedings of the International Seaweed Symposium* **8**: 667-672.

BLUNDEN, G. and WOODS, D.L. 1969. Effects of carbohydrates in seaweed fertilizers. *Proceedings of the International Seaweed Symposium* **6**: 647-653.

BOKIL, K.K., MEHTA, V.C. and DATAR, D.S. 1972. Seaweeds as manure. III. Field manurial trials on *Pennisetum typhoids* S.H. (Pearl millet) and *Arachis hypogaea* (Groundnuts). *Botanica Marina* **15**: 148-150.

BOOTH, C.O. 1964a. Seaweed has possibilities apart from its fertilizer use. *Grower* **62**: 442-443.

BOOTH, E. 1964b. Trace elements and seaweed. *Proceedings of the International Seaweed Symposium* **4**: 385-392.

BOOTH, E. 1965. The manurial value of seaweeds. *Botanica Marina* **8**: 138-145.

BOOTH, E. 1966. Some properties of seaweed manures. *Proceedings of the International Seaweed Symposium* **5**: 349-357.

BOOTH, E. 1969. The manufacture and properties of liquid seaweed extracts. *Proceedings of the International Seaweed Symposium* **6**: 655-662.

BOOTH, E. 1973. Appendix D: Recent research results. In: Stephenson, W.A. (ed.), *Seaweed in Agriculture and Horticulture*. pp. 216-226.

BOOTH, E. 1974. Some factors affecting seaweed fertilizers. *Proceedings of the International Seaweed Symposium* **8**: 661-666.

BOUILLENNE, R. and BOUILLENNE-WALRAND, M. 1955. Auxines et Bouturage. *Proceedings of the Fourteenth International Horticultural Congress* 1: 231-238.

BOUILLENNE, R. and WENT. F. 1933. Recherches experimentales sur la neoformation des racines dans les plantules et les boutures des plantes superieures. *Annales du Jardin botanique de Buitenzorg*, **43**: 25-202.

BOWN, A.W., REEVE, D.R. and CROZIER, A. 1975. The effect of light on the gibberellin metabolism and growth of *Phaseolus vulgaris* seedlings. *Planta* **126**: 83-91.

BOYER, G.L. and DOUGHERTY, S.S. 1988. Identification of abscisic acid in the seaweed *Ascophyllum nodosum*. *Phytochemistry* **27**: 1521-1522.

BOYER, G.L. and ZEEVART, J.A.D. 1982. Isolation and quantification of ß-D-Glucopyranosul Abscisate from leaves of *Xanthium* and Spinach. *Plant Physiology* **70**: 227.

BRAID, G.H. 1978. Effects of algal extract on soil microflora. Honours thesis. University of Tasmania.

BRAIN, K.R., CHALOPIN, M.C., TURNER, T.D., BLUNDEN, G. and WILDGOOSE, P.B. 1973. Cytokinin activity of commercial aqueous seaweed extract. *Plant Science Letters* **1**: 241-245.

BRINER, G.P., RICHARDS, D. and BELCHER, R.S. 1979. Seaweed concentrate: Growth regulator or fertilizer? *New Zealand Journal of Agriculture* **138**: 20.

BRUESKE, C.H. and BERGESON, G.B. 1972. Investigations of growth hormones in xylem exudates and root tissue of tomato infected with root-knot nematodes. *Journal of Experimental Botany* **23**: 14-22.

BRUINSMA, J. 1982. Plant growth regulators in field crops. In: Mclaren, J.S. (ed.), *Chemical Manipulation of Crop Growth and Development*. Butterworth Scientific, London. pp. 3-11. ISBN 0-4 08-10767-7.

BRUNNER, H. 1978. Influence of various growth substances and metabolic inhibitors on root regenerating tissue of *Phaseolus vulgaris* L. Changes in the contents of growth substances and in peroxide and IAA oxidase activities. *Zeitschrift für Pflanzenphysiologie* **88**: 13-23.

BUTTON, E.F. and NOYES, C.F. 1964. Effect of seaweed extract upon the emergence and survival of seedlings of creeping red fescue. *Agronomy Journal* **60**: 324-326.

BYFORD-JONES, C. 1988. Seaweed - Stock and crop booster. *Farmers Weekly* **June 3**: 32-33.

CAIOZZI, M., PEIRANO, P., RAUCH, E. and ZUNINO, H. 1968. Effect of seaweed on the levels of available phosphorus and nitrogen in a calcareous soil. *Agronomy Journal* **60**: 324-326.

CASTILLO, N.O. 1966. Effects of the brown algae, *Macrocystis integrifolia* in increasing iron availability of a calcareous soil. *Anales de la Facultad de Quimica Farmacia, University of Chile* **18**: 120-126.

CHACKO, E.K., KOHLI, R.R., DORE SWANY, R. and RANDHAWA, G.S. 1976. Growth regulators and flowering in juvenile mango *(Mangifera indica* L.) seedlings. *Acta Horticulturae* **56**: 173-181.

CHALLEN, S.B. and HEMMINGWAY, J.C. 1966. Growth of higher plants in response to feeding with seaweed extracts. *Proceedings of the International Seaweed Symposium* **5**: 359-367.

CHAPMAN, D.J. 1970. Seaweeds and their uses. Methuen & Co. Ltd, London.

CHAPMAN, J.F. and CHAPMAN, D.J. 1980. Seaweeds and their uses. Third Edition, Chapman & Hall Ltd., London ISBN 0-412-15740-3.

CHIBBAR, R.N., GURUMURTI, K. and NANDA, K.K. 1979. Changes in IAA oxidase activity in rooting hypocotyl cuttings of *Phaseolus mungo* L. *Experientia* **35**: 202-203.

CLARK, H.E. and KERNS, K.R. 1942. Control of flowering with phytohormones. *Science* **95**: 536-537.

CLELAND, C.F. and BRIGGS, W.R. 1969. Gibberellin and CCC effects on flowering and growth in the long-day plant *Lemma gibba*. *Plant Physiology* **44**: 503-507.

COLEMAN, W.K. and GREYSON, R.I. 1976. Root regeneration from leaf cuttings of *Lycospersicon esculentum* Mill.: Application of the leaf plastochron index and responses to exogeneous gibberellic acid. *Journal of Experimental Botany* **27**: 1339-1350.

COLEMAN, W.K. and GREYSON, R.I. 1977. Promotion of root initiation by gibberellic acid in leaf disks of tomato (*Lycopersicon esculentum*) cultured *in vitro*. *New Phytologist* **78**: 47-54.

COOPER, W.C. 1935. Hormones in relation to root formation on stem cuttings. *Plant Physiology* **10**: 789-794.

COULSON, C.B. 1953. Amino acids of marine algae. *Chemistry and Industry*. pp. 971-972.

CROUCH, I.J. and VAN STADEN, J. 1990a. Evidence for rooting factors in a seaweed concentrate prepared from *Ecklonia maxima*. *Journal of Plant Physiology*. In Press.

CROUCH, I.J. and VAN STADEN, J. 1990b. The effect of seaweed concentrate on nematode-infested tomato plants. *Plant Physiology* (Suppl.) **93**: 149.

CROUCH, I.J., BECKETT, R.P. and VAN STADEN, J. 1990. Effect of seaweed concentrate on the growth and mineral nutrition of nutrient stressed lettuce. *Journal of Applied Phycology* **2**: 269-272.

CUTLER, H. and KRUSBERG, L. 1968. Plant growth regulators in *Ditylenchus dipsaci*, *Ditylenchus triformis* and host tissues. *Plant and Cell Physiology* (Tokyo) **9**: 479-497.

DARRAH, C.H. and HALL, J.R. 1976. Twin shields *Fusarium* nematode study. Economic Development of Administration, United states Department of Commerce.

DAVEY, J.E. and VAN STADEN, J. 1978. Cytokinin activity in *Lupinus albus*. III. Distribution in fruits. *Physiologia Plantarum* **43**: 87-93.

DAVIS, T.D., HAISSIG, B.E. and SANKHLA, N. 1988. Adventitious root formation in cuttings. *Advances in Plant Sciences Series*, Vol **2**, Dioscorides Press, Portland, 315 pp.

DEKKER, J. 1963. Effect of cytokinin on powdery mildew. *Nature* **197**: 1027-1028.

DE VILLIERS, J., KOTZE, W.A.G. and JOUBERT, M. 1983. Effect of seaweed foliar sprays on fruit quality and mineral nutrition. *The Deciduous Fruit Grower* **33**: 97-101.

DE VOS, N.M., DILZ, K. and BRUINSMA, J. 1967. Effects of 2-chloroethyl-trimethylammonium chloride (CCC) on yield and lodging of wheat. *Netherlands Journal of Agricultural Science* **15**: 50-62.

DE WAELE, D., McDONALD, A.H. and DE WAELE, E. 1988. Influence of seaweed concentrate on the reproduction of *Pratylenchus zeae* (Nematoda) on maize. *Nematalogia* **34**: 71-77.

DHAWAN, R.S. and NANDA, K.K. 1982. Stimulation of root formation on *Impatiens balsamina* L. cuttings by coumarin and the associated biochemical changes. *Biologia Plantarum* **24**: 177-182.

DRIGGERS, B.F. and MARUCCI, P.E. 1964. Observation of the effect of seaweed extracts on peaches and strawberries. *Horticultural News* **45**: 4-15.

DROPKIN, V.H. 1969. Cellular responses of plants to nematode infections. *Annual Review of Phytopathology* 7: 101-122.

DROPKIN, V.H. 1980. *Introduction to Plant Nematology*. John Wiley and Sons, New York.

DROPKIN, V.H., HELGESON, J.P. and UPPER, C.D. 1969. The hypersensitivity reaction of tomatoes resistant to *Meloidogyne incognita* reversal by cytokinins. *Journal of Nematology* **1**: 55-61.

EHRESSMANN, D.W., DEIG, E.F., HATCH, M.T., DISALVO, L.H. and VEDROS, N.A. 1977. Antiviral substances from California marine algae. *Journal of Phycology* **13**: 37-40.

EHRESSMANN, D.W., DEIG, E.F. and HATCH, M.T. 1979. Anti-viral properties of algal polysaccharides and related compounds. In: Hoppe, H.A., Levring, T. and Tanaka, Y. (eds.), *Marine algae in pharmaceutical science*. Walter der Gruyter & Co., Berlin. pp. 109-115.

ELIASSON, L. 1978. Effects of nutrients and light on growth and root formation in *Pisum sativum* cuttings. *Physiologia Plantarum* **43**: 13-18.

ENDO, B.Y. 1971a. Synthesis of nucleic acid at infection sites of soybean roots by *Heterodera glycines*. *Phytopathology* **61**: 395-399.

ENDO, B.Y. 1971b. Nematode induced syncytia (giant cells). Host parasitic relationships of Herodeidae. In: Zuckerman, B.W., Mai, W.F. and Rohde, R.A. (eds.), *Plant Parasitic Nematodes*, **2**. Academic Press, New York. pp. 91-117.

EPSTEIN, E., COHEN, J. and BANDURSKI, R.S. 1980. Concentration and metabolic turnover of indoles in germinating kernels of *Zea mays* L. *Plant Physiology* **80**: 256-258.

ERASMUS, D.J., NELSON, W.R. and VAN STADEN, J. 1982. Combined use of a selective herbicide and seaweed concentrate. *South African Journal of Science* **78**: 423-424.

ERIKSEN, E.N. 1974. Root formation in pea cuttings. III. The influence of cytokinins at different developmental stages. *Physiologia Plantarum* **30**: 163-167.

ERIKSEN, E.N. and MOHAMMED, D.S. 1974. Root formation in pea cuttings. II. The influence of indole-3-acetic acid at different developmental stages. *Physiologia Plantarum* **30**: 158-162.

ESTRADA, R., TOVAR, P. and DODDS, J.H. 1986. Induction of *in vitro* tubers in a wide range of potato genotypes. *Plant Cell, Tissue and Organ Culture* **7**: 3-10.

EVANS, L.T. 1971. Flower induction and the florigen concept. *Annual Review of Plant Physiology* **22**: 365-394.

EVANS, M.L. 1973. Rapid stimulation of plant cell elongation by hormonal and non-hormonal factors. *Bioscience* **23**: 711-719.

FABIJAN, D., TAYLOR, J.S. and REID, D.M. 1981. Adventitious rooting in hypocotyls of sunflower (*Helianthus annus*) seedlings. II. Action of gibberellins, cytokinins, auxins and ethylene. *Physiologia Plantarum* **53**: 589-597.

FADL, M.S. and HARTMANN, H.T. 1967. Isolation, purification and characterization of an endogenous root promoting factor obtained from the basal sections of pear hardwood cuttings. *Plant Physiology* **42**: 541-549.

FEATONBY-SMITH, B.C. 1984. Cytokinins in Ecklonia maxima and the effect of seaweed concentrate on plant growth. Ph.D. Thesis. Department of Botany, University of Natal, Pietermaritzburg.

FEATONBY-SMITH, B.C. and VAN STADEN, J. 1983a. The effect of seaweed concentrate and fertilizer on the growth of *Beta vulgaris*. *Zeitschrift für Pflanzenphysiologie* **112**: 155-162.

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 Horticulturae* **20**: 137-146.

FEATONBY-SMITH, B.C. and VAN STADEN, J. 1984a. The effect of seaweed concentrate and fertilizer on growth and the endogenous cytokinin content of *Phaseolus vulgaris*. *South African Journal of Botany* **3**: 375-379.

FEATONBY-SMITH, B.C. and VAN STADEN, J. 1984b. Identification and seasonal variation of endogenous cytokinins in *Ecklonia maxima* (Osbeck) Papenf. *Botanica Marina* **27**: 524-531.

FEATONBY-SMITH, B.C. and VAN STADEN, J. 1987a. Effect of seaweed concentrate on yield and seed quality of *Arachis hypogaea*. *South African Journal of Botany* **53**: 190-193.

FEATONBY-SMITH, B.C. and VAN STADEN, J. 1987b. Effects of seaweed concentrate on grain yield in barley. *South African Journal of Botany* **52**: 125-128.

FENICAL, W. 1982. Investigation of benthic algae as a resource for new pharmaceuticals and agricultural chemicals. Proceedings Joint United States - China Phycological Symposium, Qingdao, People's Republic of China, November 1981.

FERNQVIST, I. 1966. Studies on factors in adventitious root formation. *Lantbruskhög skolans Annaler* **32**: 109-244.

FINNIE, J.F. and VAN STADEN, J. 1985. The effect of seaweed concentrate and applied hormones on *in vitro* cultured tomato roots. *Journal of Plant Physiology* **120**: 215-310.

FOONG, T.W. and BARNES, M.F. 1981. Rooting 'cofactors' in Rhododendron: The fractionation and activity of components from an easy-to-root variety. *Biochemie und Physiologie der Pflanzen* **176**: 507-523.

FOWDEN, L. 1962. Amino acids and proteins. In: Lewin, R.A. (ed.), *Physiology and Biochemistry of the Algae*. Academic Press, New York and London. pp. 189-209.

FRANCKI, R.I.B. 1960a. Manurial value of seaweeds: I. Effects of *Pachymenia himantophora* and *Durvillea antartica* meals on plant growth. *Plant and Soil* **12**: 297-310.

FRANCKI, R.I.B. 1960b. Studies in manurial value of seaweeds: II. Effects of *Pachymenia himantophora* and *Durvillea antartica* on the immobilization of nitrogen. *Plant and Soil* **12**: 311-323.

FRANCKI, R.I.B. 1964. Studies in manurial value of seaweeds. II. Effect of *Pachymenia himantophora* on manganese release and physical properties of soils. *Plant and Soil* **20**: 65-73.

GASPAR, T.H. 1981. Rooting and flowering, two antagonistic phenomena from a hormonal point of view. In: Jeffcoat, B. (ed.), *Aspects and Prospects of Plant Growth Regulation*. British Plant Growth Regulator Group, Wantage. pp. 39-49.

GERSANI, M. and KENDE, H. 1982. Studies on cytokinin- stimulated translocation in isolated bean leaves. *Journal of Plant Growth and Regulation* 1: 161-171.

GIEBEL, J. 1974. Biochemical mechanisms of plant resistance to nematodes: A review. *Journal of Nematology* **6**: 175-184.

GLAZER, I., APELBAUM, A. and ORION, D. 1984. Reversal of nematode induced growth retardation in tomato plants by inhibition of ethylene action. *Journal of the American Society for Horticultural Science* **109**: 886-889.

GLAZER, I., APELBAUM, A. and ORION, D. 1985. Effect of inhibitors and stimulators of ethylene production on gall development in *M. javanica*-infected tomato roots. *Journal of Nematology* **17**: 145-149.

GLAZER, I. and ORION, D. 1984. Influence of urea, hydroxyurea and thiourea on *Meloidogyne javanica* and infected excised tomato roots in culture. *Journal of Nematology* **16**: 125-130.

GLAZER, I. and ORION, D. 1985. An induced resistance effect of hydroxyurea on plants infected by *Meloidogyne javanica*. *Journal of Nematology* **17**: 21-24.

GLAZER, I., ORION, D. and APELBAUM, A. 1983. Interrelationships between ethylene production, gall formation, and root-knot nematode development in tomato plants infected with *M. javanica*. *Journal of Nematology* **15**: 539-544.

GLAZER, I., ORION, D. and APELBAUM, A. 1985. Ethylene production by *Meloidogyne* spp-infected plants. *Journal of Nematology* **17**: 61-63.

GOODWIN, T.N. and MERCER, E.I. 1983. *Introduction to Plant Biochemistry*. Second Edition. Pergamon Press, New York U.S.A. pp. 574-576.

GOPALA, R.P. 1984. Gibberellin-like behaviour of α-tocopherol in green gram *Vigna radiata*. *Geobios* **11**: 21-25.

GORTER, C.J. 1958. Synergism of indole and indole-3-acetic acid in the root production of *Phaseolus* cuttings. *Physiologia Plantarum* **11**: 1-9.

GORTER, C.J. 1969. Auxin synergists in the rooting of cuttings. *Physiologia Plantarum* **22**: 497-502.

GUPTA, A.B. and SHUKLA, A.C. 1969. Effect of algal extracts of *Phormidium* species on growth and development of rice seedlings. *Hydrobiologia* **34**: 77-84.

GÜVEN, K.C., GÜLER, E. and YÜCEL, Y. 1976. Vitamin B^{12} content of *Gelidium capillaceum* Kütz. *Botanica Marina* **19**: 395-396.

HAGER, A., MENZEL, H. and KRAUSS, A. 1971. Versuche und hyopothese zur primarwirkung des auxins beim streckungswaschstum. *Planta* **100**: 74-75.

HAHN, H., DE ZACKS, R. and KENDE, A. 1974. Cytokinin formation in pea seeds. *Naturwissenchaften* **61**: 170-171.

HAISSIG, B.E. 1970. Influence of indole-3-acetic acid on adventitious root primordia of brittle willow. *Planta* **95**: 27-35.

HAISSIG, B.E. 1974. Influences of auxins and auxin synergists on adventitious root primordium initiation and development. *New Zealand Journal of Forestry Science* **2**: 311-323.

HAISSIG, B.E. 1982. Carbohydrate and amino acid concentrations during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. *Forest Science*. **28**: 813-821.

HAISSIG, B.E. 1986. Metabolic processes in adventitious rooting of cuttings. In: Jackson, M.B. (ed.), *New Root Formation in Plants and Cuttings*. Martinus Nijhoff Publishers, Dordrecht. pp. 150-152.

HANSEN, J. 1975. Light dependent promotion and inhibition of adventitious root formation by gibberellic acid. *Planta* **123**: 203-205.

HANSEN, J. 1976. Adventitious root formation induced by gibberellic acid and regulated by the irradiance to the stock plants. *Physiologia Plantarum* **36**: 77-81.

HARTMANN, H.T. and KESTER, D.E. 1975. *Plant propagation: Principles and Practices*. Prentice Hall, Englewood Cliffs.

HARTMANN, H.T. and KESTER, D.E. 1983. *Plant Propagation: Principles and Practices*. Fourth Edition. Prentice Hall, Englewood Cliffs, U.S.A. pp. 234-297.

HARTUNG, W., OHL, B. and KUMMER, V. 1980. Abscisic acid and the rooting of runner bean cuttings. *Zeitschrift für Pflanzenphysiologie* **98**: 95-103.

HAUN, J.R. and CORNELL, D.E. 1951. Rooting response of geranium (*Pelargonium horturum*, Bailey var. Ricard) cuttings as influenced by nitrogen, phosphorus, and potassium nutrition of the stock plant. *Proceedings of the American Society for Horticultural Science* **58**: 317-323.

HEDDEN, P. 1987. Gibberellins. In: Rivier, L. and Crozier, A. (eds.), *Principles and Practice of Plant Hormone Analysis*. Vol **21**. Academic Press, London.

HEMBERG, T. 1951. Rooting experiments with hypocotyls of *Phaseolus vulgaris* L. *Physiologia Plantarum* **4**: 358-369.

HEMBERG, T. and TILLBERG, E. 1980. The influence of extraction procedure on yield of indole-3-acetic acid in plant extracts. *Physiologia Plantarum* **50**: 176-180.

HESS, C.E. 1957. A physiological analysis of rooting in cuttings of juvenile and mature *Hedera helix* L. Ph.D. Thesis, Cornell University.

HESS, C.E. 1961a. The mung bean bioassay for the detection of root promotory substances. *Plant Physiology* (suppl.) **36**: XXI.

HESS, C.E. 1961b. The physiology of root initiation in easy-and-difficult-to-root cuttings. *Hormolog* **3**: 3-6.

HESS, C.E. 1962. A Physiological analysis of root initiation in easy and difficult to root cuttings. *Sixteenth International Horticultural Congress* 4: 375-387.

HESS, C.E. 1964a. Characterisation of the rooting co-factors extracted from *Hedera helix* L. and *Hibiscus rosa-sinensis* L. *Proceedings of the Sixteenth International Horticultural Congress* 4: 382-388.

HESS, C.E. 1964b. Naturally occurring substances which stimulate root initiation. *Colloques Internationaux du Centre National de la Recherche Scientifique* (Paris) **123**: 517-527.

HESS, C.E. 1965. Rooting co-factors, identification and functions. *International Plant Propagators Society Combined Proceedings* **15**: 181-186.

HESS, C.E. 1969. International external factors regulating root initiation. In: Whittington, W.J. (ed.), *Root Growth*. Butterworths, London. pp. 42-64.

HEUSER, C.W. 1988. Bioassay, Immunoassay and verification of adventitious root promoting substances. In: Davis, A.T.D., Haissig, B.E. and Sankhla, N. (eds.), *Adventitious Root Formation in Cuttings*. Advances in Plant Science Series, Vol **2**, Dioscorides Press, Portland.

HEUSER, C.W. and HESS, C.E. 1972. Isolation of three lipid root-initiating substances from juvenile *Hedera helix* shoot tissue. *Journal of the American Society for Horticultural Science* **97**: 571-574.

HOFMAN, P.J., FEATONBY-SMITH, B.C. and VAN STADEN, J. 1986. The development of ELISA and IRA for cytokinin estimation and their application to a study of lunar periodicity in *Ecklonia maxima* (Osbeck) Papenf. *Journal of Plant Physiology* **122**: 455-466.

HOPPE, H.A. and LEVRING, T. 1982. *Marine algae in pharmaceutical science*. Vol 2. Walter de Gruyter, Berlin - New York. p. 309.

HUMPHRIES, E.C. 1960. Inhibition of root development on petioles and hypocotyls of dwarf bean (*Phaseolus vulgaris*) by kinetin. *Physiologia Plantarum* **12**: 659-663.

HUNDIN, H. and ERICSON, L.E. 1956. The occurrence of vitamins in marine algae. *Proceedings of the International Seaweed Symposium* **2**: 39-43.

HUSSIAN, A. and BONEY, A.D. 1969. Isolation of kinin-like substances from *Laminaria digitata*. *Nature* **223**: 504-505.

HUSSIAN, A. and BONEY, A.D. 1973. Hydrophylic growth inhibitors from *Laminaria* and *Ascophyllum*. *New Phytologist* **72**: 403-410.

HUTTON, M.J. and VAN STADEN, J. 1984. Transport and metabolism of labelled zeatin applied to the stems of *Phaseolus vulgaris* at different stages of development. *Zeitschrift für Pflanzenphysiologie* **114**: 331-339.

HUVÉ, H. and PELLIGRINI, M. 1969. Contribution à l'étude chimique de quelques espèces du genre *Laurencia* (Céramailes, Rhodemélacées). *Proceedings of the International Seaweed Symposium* **6**:483-492.

JACKSON, M.B. 1986. *New root formation in plants and cuttings*. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

JACKSON, M.B. and HARNEY, P.M. 1970. Rooting co-factors, indoleacetic acid, and adventitious root initiation in mung bean cuttings (*Phaseolus aureas*). *Canadian Journal of Botany* **48**: 943-947.

JACKSON, W.T. 1965. Mannitol-induced stimulation of elongation of root hairs of *Agrostis alba* L. *Physiologia Plantarum* **18**: 24-30.

JAMES, D.J. and THURBON, I.J. 1981. Phenolic compounds and other factors controlling rhizogenesis *in vitro* in the apple rootstocks M9 and M26. *Zeitschrift für Pflanzenphysiologie* **105**: 11-20.

JARVIS, B.C. 1986. Endogeneous control of adventitious rooting in non-woody cuttings. In: Jackson, M.B. (ed.), *New Root Formation in Plants and Cuttings*. Martinus Nijhoff Publishers, Dordrecht. pp. 191-222.

JARVIS, B.C. and BOOTH, A.B. 1981. Influence of indole-butyric acid, boron, myo-inositol, vitamin D_2 and seedling age on adventitious root development in cuttings of *Phaseolus aureus*. *Physiologia Plantarum* **53**: 213-218.

JARVIS, B.C., ALI, A.H.N. and SHAHEED, A.I. 1983. Auxin and boron in relation to the rooting response and ageing of mung bean cuttings. *The New Phytologist* **95**: 509-518.

JARVIS, B.C., YASMIN, S., ALI, A.H.N. and HUNT, R. 1984. The interaction between auxin and boron in adventitious root development. *The New Phytologist* **97**: 197-204.

JENNINGS, R.C. 1968. Gibberellins as endogenous growth regulators in green and brown algae. *Planta* **80**: 34-42.

JENNINGS, R.C. 1969a. Cytokinins as endogenous growth regulators in the algae *Ecklonia* (Phaeophyta) and *Hypnea* (Rhodophyta). *Australian Journal of Biological Science* **22**: 621-627.

JENNINGS, R.C. 1969b. Gibberellin antagonism by material from a brown alga. *The New Phytologist* **68**: 683-688.

JENNINGS, R.C. and McCOMB, A.J. 1967. Gibberellins in the red alga *Hypnea musciformis* (WULF.) LAMOUR. *Nature* (London) **215**: 872-873.

JENSEN, A. 1969. Tocopherol content of seaweed and seaweed meal. I. Analytical methods and ditribution of tocopherols in benthic algae. *Journal of the Science of Food and Agriculture* **20**: 449-453.

JENSEN, A. 1971. The nutritive value of seaweed meal for domestic animals. *Proceedings of the International Seaweed Symposium* 7: 7-14.

JOHNSON, A.W. and FASSULIOTRE, G. 1984. Nematode parasites of vegetable crops. In: Nickle, W. (ed.), *Plant and Insect Nematodes*. Marcel Dekker, Inc. New York.

KANAZAWA, A. 1963. Vitamins in algae. *Bulletin of the Japanese Society of Scientific Fisheries* **29**: 713-731.

KANNAN, S. 1986. Foliar absorption and transport of inorganic nutrients. *Critical Reviews in Plant Sciences* **4**: 341-373.

KAPLAN, D.T. and DAVIS, E.L. 1987. Mechanisms of plant incompatibility with nematodes. In: Veech, J.A. and Dickson, D.W. (eds.), *Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists*. Society of Nematologists, Inc. U.S.A.

KAPLAN, D.T. and KEEN, N.T. 1980. Mechanisms conferring plant incompatibility to nematodes. *Revue de Nematologie* **3**: 123-134.

KATO, J. 1958. Studies on the physiological effect of gibberellin. II. On the interaction of gibberellin with auxins and growth inhibitors. *Physiologia Plantarum* **11**: 10-15.

KATO, J., PURVES, W.K. and PHINNEY, B.O. 1962. Gibberellin-like substances in plants. *Nature* (London) **196**: 687-688.

KEFELI, V.I. 1978. Principles of analysis of phytohormones and natural growth inhibitors. In: Junk, W. (ed.), *Natural Plant Growth Inhibitors and Phytohormones*. Dordrecht.

KENTZER, T.R., SYNAK, R., BURKIEWICZ, K. and BANAS, A. 1980. Cytokinin-like activity in sea water and *Fucus vesiculosis* L. *Biologia Plantarum* **22**: 218-225.

KETRING, C.L. and SCHUBERT, A.M. 1981. Reproduction of peanuts treated with a cytokinin-containing preparation. *Agronomy Journal* **73**: 350-352.

KEY, J.L. 1969. Hormones and nucleic acid metabolism. *Annual Review of Plant Physiology* **20**: 449-474.

KHALEAFA, A.F., KHARBOUSH, M.A.M., METWALLI, A., MOSHEN, A.F. and SERWI, A. 1975. Antibiotic (fungicidal) action from extracts of some seaweeds. *Botanica Marina* **18**: 163-165.

KINGMAN, A.R. and MOORE, J. 1982. Isolation, purification and quantification of several growth regulating substances in *Ascophyllum nodosum* (Phaeophyta). *Botanica Marina* **25**: 149-153.

KOCHBA, J. and SAMISH, R.M. 1971. Effects of kinetin and 1-naphthylacetic acid on root-knot nematodes in resistant and susceptible peach rootstocks. *Journal of the American Society for Horticultural Science* **96**: 458-461.

KOCHBA, J. and SAMISH, R.M. 1972. Levels of endogenous cytokinins and auxins in roots of nematode resistant and susceptible peach stocks. *Journal of the American Society for Horticultural Science* **97**: 115-119.

KODA, Y. and OKAZAWA, Y. 1983. Influences of environmental, hormonal and nutritional factors on potato tuberization *in vitro*. *Japanese Journal of Crop Science* **52**: 582-591.

KOTZE, W.A.G. and JOUBERT, M. 1980. Influence of foliar spraying with seaweed products on the growth and mineral nutrition of rye and cabbage. *Elsenburg Joernaal* **4**: 17-20.

KRAUS, E.J. and KRAYBILL, H.R. 1918. Vegetation and reproduction with special reference to the tomato. *Oregon Agricultural College Experimental Station Bulletin* p. 149.

KURAISHI, S. and OKUMURA, F.S. 1956. Effects of kinetin on leaf growth. *Botanical Magazine Tokyo* **69**: 817-818.

LEE, C.I. and TUKEY, H.B. 1971. Induction of root promoting substances in *Euonymous alatus* 'compactus' by intermittent mist. *Journal of the American Society for Horticultural Science* **96**: 731-736.

LEE, Y.H., MOK, M.C., MOK, D.W.S., GRIFFIN, D.A. and SHAW,G. 1985. Cytokinin metabolism in *Phaseolus* embryos. *Plant Physiology* 77: 635-641.

LEOPOLD, A.C. and KRIEDEMANN, P.E. 1975. Plant growth and development. McGraw-Hill, Inc. U.S.A.

LETHAM, D.S. 1973. Cytokinins from *Zea mays*. *Phytochemistry* **12**: 2445-2455.

LETHAM, D.S. 1978. Cytokinins. In: Letham, D.S., Goodwin, P.B. and Higgins, T.J. (eds.), *Phytohormones and Related Compounds: A Comprehensive Treatise*. I. Elsevier/Holland, Amsterdam. ISBN 0-444-80053-0. pp. 205-251.

LEWIS, D.H. 1980. Boron, lignification and the origin of vascular plants - a unified hypothesis. *The New Phytologist* **84**: 209-229.

LEWIS, S.A. 1987. Nematode - plant compatability. In: Veech, J.A. and Dickson, D.W. (eds.), *Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists*. Society of Nematologists, Inc. U.S.A.

LEWIS, S.A. and McCLURE, M.A. 1975. Free amino acids in roots of infected cotton seedlings resistant and susceptible to *Meloidogyne incognito*. *Journal of Nematology* **7**: 10-15.

LUCKWILL, L.C. 1977. Growth regulators in flowering fruit development. In: Plimmer, J.R. (ed.), *Pesticide Chemistry in the Twentieth Century. A.C.S. Symposium Series* **37**:293-304.

LYNN, L.B. 1972. The chelating properties of seaweed extract *Ascophyllum nodosum* vs. *Macrocystis perifera* on the mineral nutrition of sweet peppers, *Capsicum annuum*. M.Sc. Thesis, Clemson University, Clemson.

MAHESHWARI, S.C. and VENKATARAMAN, R. 1966. Induction of flowering in duckweed by a new kinin zeatin. *Planta* **70**: 304-306.

MARSHNER, H. 1982. Effects of mineral nutrition on phytohormone balance in plants. In: Scaife, A. (ed.), Plant Nutrition. *Proceedings of the International Plant Nutrition Colloquium* Vol I, Commonwealth Agricultural Bureau. pp. 354-359.

MARTIN, G.C. 1987. Apical dominance. *HortScience* **22**: 824-833.

MENGEL, K., and KIRKBY, E.A. 1982. Principles of Plant Nutrition. Third Edition. Interntional Potash Institute, Switzerland.

METTING, B., RAYBURN, W.R. and REYNAUD, P.A. 1988. Algae and agriculture. In: Lenbi, C.A. and Waaland, R.A. (eds.), *Algae and Human Affairs*. The Cambridge University Press, Cambridge.

METTING, B., ZIMMERMAN, W.J., CROUCH, I. and VAN STADEN, J. 1990. Agronomic uses of seaweed and microalgae. In: I. Akatsuka (ed.), *Introduction to Applied Phycology*. SPB Academic Publishing bv., The Hague, Netherlands. pp. 269-307.

MIDDLETON, W. 1977. Root development in cuttings of *Phaseolus aureus* Roxb. Ph.D. Thesis, University of Sheffield, U.K.

MIDDLETON, W., JARVIS, B.C. and BOOTH, A. 1978. The boron requirement for root development in stem cuttings of *Phaseolus aureus* Roxb. *New Phytologist* **81**: 287-297.

MILLER, C.O. 1963. Kinetin and kinetin-like compounds. In: Linskens, H.F. and Tracy, M.V. (eds.), *Modern Methods of Plant Analysis*. Springer-Verlag, Berlin **6**: 194-202.

MILLER, C.O. 1965. Evidence for the natural occurrence of zeatin and derivatives: Compounds from maize which promote cell division. *Proceedings of the National Academy of Sciences of the United States of America* **54**: 1052-1058.

MILLER, C.O. 1968. Naturally occurring cytokinins. In: Linkskens, H.F. and Setterfield, G. (ed.), *Biometry and Physiology of Plant Growth Substances*. Runge Press, Ottawa. pp. 33-45.

MILTON, R. 1964. Liquid Seaweed as a fertilzer. *Proceedings of the International Symposium* **4**: 428-431.

MITCHELL, R. 1963. Addition of fungal cell-wall components to soil for biological disease control. *Phytopathology* **53**: 1068-1071.

MITRA, S.K. 1986. Auxin synergists in the rooting of cuttings of some tropical fruit trees. *Horticultural Science* **21**: 111.

MOHAMMED, S. and ERIKSEN, E.N. 1974. Root formation in pea cuttings. IV. Further studies on the influence of indole-3-acetic acid at different developmental stages. *Physiologia Plantarum* **32**: 94-96.

MONCOUSIN, C. and GASPAR, T. 1983. Peroxidase as a marker for rooting improvement of *Cynara scolymus* L. cultured *in vitro*. *Biochemie und Physiologie der Pflanzen* **178**: 263-271.

MOONEY, P.A. 1983. Cytokinins in *Sargassum heterophyllum* under natural and *in vitro* conditions. M.Sc. Thesis. Department of Botany, University of Natal, Pietermaritzburg.

MOONEY, P.A. and VAN STADEN, J. 1984a. Lunar periodicity of the levels of cytokinins in *Sargassum heterophyllum* (Phaeophyceae). *Botanica Marina* **27**: 467-472.

MOONEY, P.A. and VAN STADEN, J. 1984b. Seasonal changes in the levels of endogenous cytokinins in *Sargassum heterophyllum* (Phaeophyceae). *Botanica Marina* **27**: 437-442.

MOONEY, P.A. and VAN STADEN, J. 1985. Effect of seaweed concentrate on the growth of wheat under conditions of water stress. *South African Journal of Science* **81**: 632-633.

MOONEY, P.A. and VAN STADEN, J. 1986. Algae and cytokinins. *Journal of Plant Physiology* **123**: 1-21.

MOONEY, P.A. and VAN STADEN, J. 1987. Tentative identification of cytokinins in *Sargassum heterophyllum* (Phaeophyceae). *Botanica Marina* **30**: 323-325.

MORSINK, W.A.G. and SMITH, V.G. 1975. The effect of some monohydroxybenzoic and dihydroxybenzoic acids as auxin synergists on rooting softwood cuttings of basswood (*Tilia americana* L.) under mist. *Canadian Journal of Forest Research* **5**: 500-502.

MOTHES, K. and ENGELBRECHT, L. 1961. Kinetin-induced directed transport of substances in excised leaves in the dark. *Phytochemistry* 1: 58-62.

MOWAT, J.A. 1963. Gibberellin-like substances in algae. *Nature* **200**: 453-455.

MOWAT, J.A. 1964. Auxins and gibberellins in marine algae. *Proceedings of the International Seaweed Symposium* **4**: 352-359.

MOWAT, J.A. 1965. A survey of results on the occurrence of auxins and gibberellins in algae. *Botanica Marina* **8**: 149-155.

MUNDA, M. and GUBENSEK, F. 1975. The amino-acid composition of some common marine algae from Iceland. *Botanica Marina* **19**: 85-92.

MURAKAMI, Y. 1968. A new rice seedling test for gibberellins, microdrop method, and its use for testing extracts of rice and morning glory. *Botanical Magazine*, (Tokyo) **81**: 33-43.

MURASHIGE, T. and SKOOG, F. 1962. A revised medium for the growth and bioassay with tobacco callus tissue culture. *Physiologia Plantarum* **15**: 473-497.

MYKLESTAD, S. 1964. Experiments with seaweed as supplemental fertilizer. *Proceedings of the International Seaweed Symposium* **4**: 432-438.

MYKLESTAD, S. 1968. Ion-exchange properties of brown algae. I. Determination of rate mechanism for calcium - hydrogen ion - exchange for particles from *Laminaria hyperbora* and *Laminaria digitata*. *Journal of Applied Chemistry* **18**: 30-36.

MYKLESTAD, S. 1979. Heavy metal exchange by *Ascophyllum nodosum* (Phaeophyceae) plants *in situ*. *Proceedings of the International Seaweed Symposium* **9**: 143-151.

NAITO, K., TSUJI, H. and HATAKEYAMA, I. 1978. Effects of benzyladenine on DNA, RNA, protein and chlorophyll contents in intact bean leaves: Differential responses to benzyladenine according to plant age. *Physiologia Plantarum* **43**: 367-371.

NANDA, K.K., PUROHIT, A.N. and MEHROTA, K. 1968. Effects of sucrose, auxins and gibberellic acid on rooting of stem segments of *Populus nigra* under varying light conditions. *Plant and Cell Physiology* **9**: 735-743.

NELSON, W.R. 1985. The effect of seaweed concentrate on the growth of wheat. M.Sc. Thesis. Department of Botany, University of Natal, Pietermaritzburg.

NELSON, W.R., and VAN STADEN, J. 1984a. The effect of seaweed concentrate on the growth of nutrient-stressed, greenhouse cucumbers. *HortScience* **19**: 81-82.

NELSON, W.R., and VAN STADEN, J. 1984b. The effect of seaweed concentrate on wheat culms. *Journal of Plant Physiology* **115**: 433-437.

NELSON, W.R., and VAN STADEN, J. 1985. 1-Aminocyclopropane -1-carboxylic acid in seaweed concentrate. *Botanica Marina* **28**: 415-417.

NELSON, W.R., and VAN STADEN, J. 1986. Effect of seaweed concentrate on the growth of wheat. *South African Journal of Science* **82**: 199-200.

NEUMAN, P.M. and NOODEN, L.D. 1983. Interaction of mineral and cytokinin supply in control of leaf senescence and seed growth in soybean explants. *Journal of Plant Nutrition* **6**: 735-742.

NITSCH, J.P. and NITSCH, C. 1956. Studies on growth of coleoptile and first internode sections. A new, sensitive straight-growth test for auxins. *Plant Physiologia* **31**: 94

NOODEN, L.D. and LEOPOLD, A.C. 1978. Phytohormones and the endogenous regulation of senescence and abscission. In: Letham, D.S., Goodwin, P.B. and Higgins, T.J. (eds.), *Phytohormones and Related Compounds: A Comprehensive Treatise*. I. Elsevier/Holland, Amsterdam. pp. 329-369.

OFFERMANS, C.N. 1968. Effect of brown algae (*Macrocystis intergrifolia*) in increasing iron availability of a calcareous soil. *Chemical Abstracts* **68**: 104216.

OKAMI, Y. 1982. Potential use of marine organisms for antibiotics and enzyme production. *Pure Applied Chemistry* **54**: 1961-1962.

ORION, D. and MINZ, G. 1969. The effect of etherel (2-chlorethane phosphonic acid) on the pathogenecity of the root-knot nematode, *Meloidogyne javanica*. *Nematologica* **15**: 608-614.

ORION, D. and MINZ, G. 1971. The influence of morphactin on the root-knot nematode (*Meloidogyne javanica*) and its galls. *Nematologica* **17**: 107-112.

ORION, D. and PILOWSKI, M. 1984. Excised tomato root culture as a tool for testing root-knot nematode resistance. *Phytoparasitica* **12**: 71-73.

ORION, D., WERGIN, W.P. and ENDO, B.Y. 1980. Inhibition of syncytia formation and root-knot nematode development on cultures of excised tomato roots. *Journal of Nematology* **12**: 196-203.

PAECH, K. TRACEY, M.V. 1955. Modern Methods of Plant Analysis. Vol III. Springer - Verlag (Berlin). 761 pp.

PADMINI SCREENIVASA RAO, P., SCREENIVASA RAO, P. and KARMARKAR, S.M. 1986. Antibacterial substances from brown algae. II. Efficiency of solvents in the evaluation of antibacterial substances from *Sargassum johnstonii* Setchell et Gardner. *Botanica Marina* **29**: 503-507.

PADMINI SCREENIVASA RAO, P., SCREENIVASA RAO, P. and KARMARKAR, S.M. 1988. Antibacterial activity of Indian species of *Sargassum*. *Botanica Marina* **31**: 295-298.

PARISH, R.W. 1968. *In vitro* studies on the relationship between boron and peroxidase. *The New Phytologist* **84**: 209-230.

PAULSON, R.E. and WEBSTER, J.M. 1970. Giant cell formation in tomato roots caused by *Meloidogyne incognita* and *M. Hapha* (nematode) infection. A light and electron microscope study. *Canadian Journal of Botany* **48**: 271-276.

PEDERSEN, M. 1973. Identification of a cytokinin, 6-(3-methyl- 2-butenylamino) purine in sea water and the effects of cytokinins on brown algae. *Physiologia Plantarum* **28**: 101-105.

PEDERSEN, M. and FRIDBORG, G. 1972. Cytokinin-like activity in sea water from *Fucus-Ascophyllum* zone. *Experièntia* **28**: 111-112.

PELLEGRINI, M. 1968. Contribution à l'étude chimique des algues méditerranéennes (Fractions azotées, acides aminés protidiques). Thèse de Spécialité, Aix-Marseille, pp.156.

PELLEGRINI, M. 1969. Contribution à l'étude chimique des algues méditerranéennes. Compositions en acides aminés de *Falkenbergia rufolanosa* (Harvey) Schmitz et d'*Aparagopsis armata* Harvey. *Botanica Marina* **12**: 179-184.

PELLEGRINI, M., PELLEGRINI, L., CHABOT, R., PERCEHAIS, S. and YVIN, J.C. 1987. Effects of a liquid seaweed extract derived from *Ascophyllum nodosum* on the ultrastructure of *Vitis vinifera* leaf tissue. *Botanica Marina* **30**: 437-446.

PESANDO, D. and CARAM, B. 1984. Screening of marine algae from the French Mediterranean Coast for antibacterial and antifungal activity. *Botanica Marina* **27**: 381-386.

PILET, P.E. 1958. Action de l'indole sur la destruction des auxines en relation avec la senescence cellulaire. *Comptes Rendus* **246**: 1896-1958.

PHARIS, R.P., ROSS, G.D., WAMPLE, R.L. and OWENS, J.N. 1976. Promotion of flowering in conifers of the Pinaceae by certain of tthe gibberellins. *Acta Horticulturae* **56**: 155-162.

POAPST, P.A. and DUNKEE, A.B. 1967. Root-differentiating properties of some simple aromatic substances of the apple and pear fruit. *Journal of Horticultural Science* **42**: 429.

POVOLNY, M. 1969a. Investigations on the effectiveness of seaweed extract on yield and quality of pickling cucumbers. *Horticultural Abstract* **64**: 857.

POVOLNY, M. 1969b. Einflus des extraktes von seealgen auf die lagerungsfaehigkeit von aepfeln. *Proceedings of the International Seaweed Symposium* **6**: 703-713.

POVOLNY, M. 1969c. The influence of seaweed extracts on the storability of apples. *Rostlinna Vyroba* **15**: 545-554.

POVOLNY, M. 1971. Effect of sea algae extract on the yield of glasshouse cucumbers. *Rostlinna Vyroba* **17**: 877-888.

POVOLNY, M. 1972. The effect of seaweed extract on ripening and storage capacity of peaches and apricots. *Rostlinna Vyroba* **18**: 703-710.

POVOLNY, M. 1974. The effect of the dipping of peat-pulp pots in the extracts from sea algae on the quality of tomato planting stock. *Sbornik UVTIZ Zahradnictvi (Praha)* **1**: 51-57.

POVOLNY, M. 1976. The effect of an extract from sea algae on the yield, ripening, and storage of tomatoes. *Sbornik UVTIZ Zahradnictvi (Praha)* **3**: 133-144.

POZSAR, B.I., EL HAMMADY, M. and KIRALY, Z. 1967. Cytokinin effect of benzyladenine: Increase of nucleic acid and protein synthesis in bean leaves. *Nature* **214**: 273-274.

QUASTEL, J.H. and WEBLEY, D.M. 1947. The effects of the addition to soil of alginic acid and other forms of organic matter on soil aeration. *Journal of Agricultural Science* **37**: 257-266.

RADLEY, M. 1961. Gibberellin-like substances in plants. *Nature* **191**: 684-685.

RASMUSSEN, S. and ANDERSEN, A.S. 1980. Water stress and root formation in pea cuttings. II. Effect of abscisic acid treatment of cuttings from stock plants grown under two levels of irradiance. *Physiologia Plantarum* **48**: 150-154.

RAUB, M.F., CARDELLINA, J.H. and SCHWEDE, J.G. 1987. The green agal pigment Caulerpin as a plant growth regulator. *Phytochemistry* **26**: 619-620.

REINERT, J. and BESEMER, J. 1967. Gibberrellinsäure, ein Inhibitor Morphogenetischer Pogesse. Wissenschaftliche Zeitschrift der Universitaet Rostock, *Mathematisch-Naturwissenschaftliche* **16**: 599-604.

REUVENI, O. and RAVIV, M. 1981. Importance of leaf retention to rooting of avocado cuttings. *Journal of the American Society for Horticultural Science* **106**:127-130.

RHINEHART, K.L. and SHIELD, L. (1978). Marine-derived antibiotics. In: Weintein, M.J. and Wagman, G.H. (eds.), *Antibiotics: Isolation, Separation and Purification*. Elsevier, Amsterdam. pp. 309-385.

ROUND-TURNER, N.L. 1985. Fertilizer alternatives - Seaweed extracts? *Invermay Farmers' Field Day*. pp. 33-34.

ROY, T.K. 1981. Biochemical aspects of host-parasite relationships in plant parasitic nematodes. *Proceedings of Indian National Science Academy* **47**: 919-936.

SACHS, J. 1880a. Stoff und Form der Pflanzenorgane. I. Arbeiten aus den Botanischen Institut des Wurzberg **2**: 452-488.

SACHS, J. 1880b. Stoff und Form der Pflanzenorgane. II. Arbeiten aus den Botanischen Institut des Wurzberg **4**: 689-718.

SAID, A.G.E. and MURASHIGE, T. 1979. Continuous cultures of tomato and citron roots *in vitro*. *In Vitro* **15**: 543-602.

SAKAI, A. and YOSHIDA, S. 1968. Protective action of various compounds against freezing injury in plant cells. *Teion kazaku, Seibutsu-Hen* **26**: 13-21.

SALAMI, A.U. and KENEFICK, D.G. 1970. Stimulation of growth in zinc-deficient corn seedlings by the addition of tryptophan. *Crop Science* **10**: 291-294.

SAMISH, R.N. and SPIEGEL, P. 1958. The influence of nutrition of the mother vine on the rooting of cuttings. *Ktavim* (Records of the Agricultural Reseach Station, State of Israel) **8**: 93-100.

SANDBERG, G., CROZIER, A. and ERNSTSEN, A. 1987. Indole-3-acetic acid and related compounds. In: Rivier, L. and Crozier, A. (eds.), *Principles and Practice of Plant Hormone Analysis* **2**. Academic Press Ltd. London.

SANDERSON, K.J. and JAMESON, P.E. 1986. The cytokinins in a liquid seaweed extract: Could they be the active ingredients? In: Luckwill, L.C. (ed.), *Fifth International Symposium on Growth Regulators in Fruit Production* 1. *Acta Horticulturae* **179**: 113-116.

SASSER, J. N. 1979. Economic importance of *Meloidogyne* in tropical countries. In: Lamberti, I. and Tayler, C.E. (eds.), *Root-knot Nematodes (Meloidogyne species)*. Academic Press, New York.

SAUNDERS, A.R. 1986. Manipulating potato tuber production and growth with the aid of growth regulators. *Record of Agricultural Research* 34: 39-41.

SAWHNEY, R. and WEBSTER, J.M. 1975. The role of plant growth hormones in determining the resistance of tomato plants to the root-knot nematode *Meloidogyne incognita*. *Nematologica* **21**: 95-103.

SAWHNEY, R. and WEBSTER, J.M. 1979. The influence of some metabolic inhibitors on the response of susceptible/ resistant cultivars of tomato to *Meloidogyne incognita*. *Nematologica* **23**: 86-93.

SCHIEWER, U. 1967. Auxinvorkommen und Auxinstoffwechsel bei mehrzelligen Ostseealgen. I. Zum Vorkommen von Indol-3-Essigsäure. *Planta* **74**: 313-323.

SCHIEWER, U. and LIBBERT, E. 1965. Indoleacetamide - an intermediate in the formation of indole acetic acid from indoleacetonitrile in the alga *Furcellaria*. *Planta* **66**: 377-380.

SCOTT, T.K. 1972. Auxins and roots. *Annual Revue of Plant Physiology* **23**: 235-258.

SCREENIVASA RAO, P. and PAREKH, K.S. 1981. Antibacterial activity of Indian seaweed extracts. *Botanica Marina* **24**: 577-582.

SCREENIVASA RAO, P. and SHELAT, Y.A. 1982. Antibiotic activity of different fractions of extracts from Indian Seaweeds. In: Hoppe, H.A., Levring, T. and Tanaka, Y. (eds.), *Marine Algae in Pharmaceutical Science*. Vol **2**, Walter de Gruyter, Berlin, New York. pp. 93-98.

SENN, T.L. 1987. Seaweed and Plant Growth. Faith Printing Co., Taylor, South Carolina. 166 pp.

SENN, T.L. and KINGMAN, A.R. 1978. Seaweed research in crop production: Economic Development of Administration, United states Department of Commerce, Washington, D.C. pp. i-xx; 1-25.

SENN, T.L. and SKELTON, B.J. 1966. Review of seaweed research 1958-1965. *South Carolina Agricultural Experimental Station, Research serial number* **76**.

SENN, T.L. and SKELTON, B.J. 1969. The effect of Norwegian seaweed on metabolic activity of certain plants. *Proceedings of the International Seaweed Symposium* **6**: 723-730.

SENN, T.L., MARTIN, J.A., CRAWFORD, J.H. and DERTING, C.W. 1961. The effect of Norwegian seaweed (*Ascophyllum nodosum*) on the development and composition of certain horticultural and special crops. *South Carolina Agricultural Experimental Station, Research serial number* **23**.

SHAW, M., BHATTACHARYA, P.K. and QUICK, W.A. 1965. Chlorophyll, protein and nucleic acid levels in detached, senescing wheat leaves. *Canadian Journal of Botany* **43**: 739-746.

SHIBAOKA, H. 1971. Effects of indole-acetic acid, p-chlorophenoxyisobutyric acid and 2-4-6 trichlorophenoxyacetic acid on three phases of rooting *Azukia* cuttings. *Plant and Cell Physiology* **12**: 193-200.

SIMPSON, K. and HAYES, S.F. 1958. The effect of soil conditioners on plant growth and soil structure. *Journal of the Science of Food and Agriculture* **9**: 163-170.

SKELTON, B.J. and SENN, T.L. 1969. Effect of seaweed sprays on quality and shelf-life of peaches. *Proceedings of the International Seaweed Symposium* **6**: 731-735.

SKOOG, F., SCHMITZ, R.Y., BOCK, R.M. and HECHT, S.M. 1973. Cytokinin antagonists: synthesis and physiological effects of 7-substituted 3-methylpyrazolo [4, 3-d] pyrimidines. *Phytochemistry* **12**: 25-37.

SKORYNA, S.C. and TANAKA, Y. 1969. Biological activity of fractionation products of brown algae. *Proceedings of the International Seaweed Symposium* **6**: 737-746.

SLADE, D.A. 1967. New Zealand Fruit Growers Association, Summary of experiments 1966-67.

SMITH, B.D. and CLARKE, G.M. 1967. Phytotoxicity to blackcurrants of sprays containing sulphur. *Annals of Applied Biology* **59**: 101-109.

SMITH, D.R. and THORPE, T.A. 1975. Root intitiation in cuttings of *Pinus radiata* seedlings. II. Growth regulator interactions. *Journal of Experimental Botany* **26**: 193-202.

SMITH, J. 1961. Sea farming. *Farm Quarterly* **16**: 72-73.

SOEKARJO, R. 1965. On the formation of adventitious roots in cuttings of *Coleus* in relation to the effect of indole acetic acid on the epinastic curvature of isolated petioles. *Acta Botanica Neelandica* **14**: 373-400.

STENLID, G. 1982. Cytokinins as inhibitors of root growth. *Physiologia Plantarum* **56**: 500-506.

STEPHENSON, J.W. 1974b. The effects of a seaweed extract on the yield of a variety of field and glasshouse crops. *Proceedings of the International Seaweed Symposium* **8**: 740-745.

STEPHENSON, W.A. 1968. Seaweed in agriculture and horticulture. Faber & Faber, London.

STEPHENSON, W.A. 1974a. Seaweed in agriculture and horticulture. Third Edition. Pauma Valley, California. Bargyla and Glyver Rateaver Conservation Gardening and Farming series C Reprints.

STEPHENSON, W.M. 1966. The effect of hydrolysed seaweed on certain plant pests and diseases. *Proceedings of the International Seaweed Symposium* **5**: 405-415.

STONIER, T., HUDEK, J., VANDE-STOUWE, R. and YANG, H.M. 1970. Studies of auxin protectors. 8. Evidence that auxin protectors act as cellular poisers. *Physiologia Plantarum* **23**: 775-783.

STREET, H.E. 1957. Excised root culture. *Biological Revue* **32**: 117-155.

SUMERA, F.C. and CAJIPE, G.J.B. 1981. Extraction and partial charactisation of auxin-like substances from *Sargassum polycystum* C. Ag. *Botanica Marina* **24**: 157-163.

SUMMONS, R.E., ENTSCH, B. LETHAM, D.S., GOLLNOW, B.I. and MACLEOD, J.K. 1980. Metabolites of zeatin in sweetcorn kernels: purification and identification using HPLC and chemical-ionisation mass spectrometry. *Planta* **147**: 422-434.

SUNDBERG, B., SANDBERG, G. and JENSEN, E. 1985. Identification and quantification of indole-3-methanol in Scots pine (*Pinus Sylvestirs* L.). *Plant Physiology* **77**: 952-955.

SUZUKI, Y., and TAKAHASHI, N. 1968. Effects of after-ripening and gibberellic acid on the thermoinduction of seed germination in *Solanum melongena*. *Plant Cell Physiology* **9**: 653-660.

SWEETSER, P.B. and SWARTZFAGER, D.G. 1978. Indole-3-acetic acid levels of plant tissues as determined by a new high performance liquid chromatographic methanol. *Plant Physiology* **61**: 254-258.

TANAKA, Y., HURLBURT, A., ANGELHOFF, L. and SKORYNA, S. 1971. Application of algal polysaccharides as *in vitro* binders of metal pollutants. *Proceedings of the International Seaweed Symposium* **7**: 602-604.

TARJAN, A.C. 1977. Kelp derivatives for nematode-infected citrus trees. *Journal of Nematology* **9**: 287.

TARJAN, A.C. and FREDERICK, J.J. 1983. Comparative effects of kelp preparations and ethoprop on nematode infested Bermuda grass, *Cynodon dactylon*. *Nematropica* **13**: 55-62.

TAYLOR, I.E.P. and WILKINSON, A.J. 1977. The occurrence of gibberellin-like substances in algae. *Phycologia* **16**: 37-42.

TAY, S.A.B., MACLEOD, J.K., PALNI, L.M.S. and LETHAM, D.S. 1985. Detection of cytokinins in a seaweed extract. *Phytochemistry* **24**: 2611-2614.

TAY, S.A.B., PALNI, L.M.S. and MACLEOD, J.K. 1987. Identification of cytokinin glucosides in a seaweed extract. *Journal of Plant Growth and Regulation* **5**: 133-138.

TEMPLE, W.D. and BOMKE, A.A. 1988. Effects of kelp (*Macrocystis integrifolia*) on soil chemical properties and crop response. *Plant and Soil* **105**: 213-222.

TEMPLE, W.D. and BOMKE, A.A. 1989. Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop yield. *Plant and Soil* **117**: 85-92.

TEMPLE, W.D. and BOMKE, A.A., RADLEY, R.A. and HOLL, F.B. 1989. Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop growth and nitrogen nutrition under varying soil moisture regimes. *Plant and Soil* **117**: 75-83.

TEERI, A.E. and BIEBER. 1958. B-complex vitamins in certain brown and red algae. *Science* **127**: 1500-1501.

THIMANN, K.V. 1937. On the nature of inhibitors caused by auxin. *American Journal of Botany* **24**: 407-412.

THIMANN, K.V. and DELISLE, A.L. 1939. The vegetative propagation of difficult-to-root plants. *Journal of the Arnold Arboretum* **20**: 116-136.

THIMANN, K.V. and KOEPFLI, J.B. 1935. Identity of the growth-promoting and root-forming substances of plants. *Nature* **135**: 101-102.

THIMANN, K.V. and WENT, F.W. 1934. On the chemical nature of the root forming hormone. *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen* **37**: 456-459.

THIVY, F. 1959. Cited In: Bokil, K.K., Mehta, V.C. and Datar, D.S. (1972). *Seaweeds as manure III*. Field manural trials on *Pennisetum typhoids* (Pearl millet) and *Arachis hypogae* (Groundnuts). *Botanica Marina* **15**: 148-150.

THOMASZEWSKI, M. and THIMANN, K.V. 1966. Interaction of phenolic acids, metallic ions, and chelating agents on auxin-induced growth. *Plant Physiology* **41**: 1443-1454.

TISDALE, S.L., NELSON, W.L. and BEATON, J.D. 1985. Soil fertility and fertilizers. Fourth Edition, Macmillan, New York.

TORREY, J.G. 1976. Root hormones and plant growth. *Annual Review of Plant Physiology* **27**: 435-459.

TSUI, C. 1948. The role of zinc in auxin synthesis in the tomato plant. *American Journal of Botany* **35**: 172-179.

TURREY, P.M. and PATRICK, J.W. 1979. Kinetin-promoted transport of assimilates in stems of *Phaseolus vulgaris* L. Localised versus remote site(s) of action. *Planta* **147**: 151-155.

VACCA, D.D. and WALSH, R.A. 1954. The antibacterial activity of an extract obtained from *Ascophyllum nodosum*. *Journal of the American Pharmaceutical Association* **43**: 24-26.

VAN RAALTE, M.H. 1954. On the synergism of indole and indole-3-acetic acid in root production. *Annales Bogarienses* 1: 167.

VAN STADEN, J. and BREEN, C. 1973. Cytokinins in fresh water algal cultures. *Plant Science Letters* **1**: 325-330.

VAN STADEN, J. and COOK, E.L. 1986. Cytokinins and fruit production. In: Luckwill, L.C. (ed.), *Fifth International Symposium on Growth Regulators in Fruit Production: 1. Acta Horticulturae* **179**: 73-82.

VAN STADEN, J. and HARTY, A.R. 1988. Cytokinins and adventitious root formation. In: Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.), *Adventitious Root Formation in Cuttings*. Dioscorides Press, Portland. pp. 185-204.

VAN STADEN, J., OLATOYE, S.T. and HALL, M.A. 1973. Effect of ethylene upon cytokinin levels in seed of *Spergula arvensis*. *Journal of Experimental Botany* **24**: 662-666.

VARGA, A. and BRUINSMA, J. 1974. The growth and ripening of tomato fruits at different levels of endogenous cytokinins. *Journal of Horticultural Science* **49**: 135-142.

VAZQUES, A. 1973. Effect of umbelliferone on rooting in bean cuttings. *Plant Science Letters* **1**: 433-438.

VEECH, J.A. 1981. Plant resistance to nematodes. In: Zuckerman, B.M. and Rohde, R.A. (eds.), *Plant Parasitic Nematodes* **3**. New York Academic Press. pp. 377-403.

VONK, C.R. 1979. Origin of cytokinins transported to the phloem. *Physiologia Plantarum* **46**: 235-240.

WAREING, P.F. and SETH, A.K. 1967. Ageing and senescence in the whole plant. *Symposia of the Society for Experimental Biology* **21**: 543-548.

WEAVER, R.L. and VAN OVERBEEK, J. 1963. Kinins stimulate grape growth. *California Agriculture* **17**: 12.

WEISER, C.J. 1959. Effect of boron on the rooting of *Clematis* cuttings. *Nature* **183**: 559-560.

WEISER, C.J. and BLANEY, L.T. 1960. The effects on the rooting of English Holly cuttings. *Proceedings of the American Society of Horticultural Science* **75**: 704-710.

WENT, F.W. 1934. A test method for rhizocaline, the root-forming substance. *Proceedings Koninklijke Nederlanse Akademie van Wetenschappen* **37**: 445-455.

WENT, F.W. 1938. Specific factors other than auxin affecting growth and root formation. *Plant Physiology* **13**: 55-80.

WENT, F.W. 1939. The dual effect of auxin on root formation. *American Journal of Botany* **26**: 24-29.

WHEELER, A.W. 1973. Endogenous growth substances. *Report of the Rothamsted Experimental Station* **1**: 101-102.

WIDDOWSON, J.P., YEATES, C.W. and HEALY, W.B. 1973. The effect of root-knot nematodes on the utilization of phosphorous by white clover on a yellow-brown loam. *New Zealand Journal of Agricultural Research* **16**: 77-80.

WIGHTMAN, F., SCHNEIDER, E.A. and THIMANN, K.V. 1980. Hormonal factors controlling the initiation and development of lateral roots. II. Effects of exogenous growth factors on lateral root formation in pea roots. *Physiologia Plantarum* **49**: 304-314.

WIGHTMAN, F. and SETTERFIELD, G. 1968. Biochemistry and physiology of plant growth substances. The Runge Press, Ottawa.

WILCZEK, C.A. and NG, T.J. 1982. Promotion of seed germination in table beet by an aqueous seaweed extract. *HortScience* **17**: 629-630.

WILDGOOSE, P.B., BLUNDEN, G. and JEWERS, K. 1978. Seasonal variation in gibberellin activity of some species of Fucaceae and Laminariaceae. *Botanica Marina* **21**: 63-65.

WILLIAMS, D.C., BRAIN, K.R., BLUNDEN, G., WILDGOOSE, P.B. and JEWERS, K. 1976. Plant growth regulatory substances in commercial seaweed extracts. *Proceedings of the International Seaweed Symposium* **8**: 59-63.

WILSON, P.J. 1988. Adventitious rooting in stem cuttings of *Eucalyptus grandis* Hill ex Maid. Ph.D. Thesis. Department of Botany, University of Natal, Pietermaritzburg.

WYN-JONES, R.G. and STOREY, R. (1981). Betaines. In: Paleg, L.G. and Aspinall, D. (eds.), *The Physiology and Biochemistry of Drought Resistance in Plants*. Academic Press, Sydney. pp. 171-204. ISBN 0-12-544380-3.

YAMAMOTO, T. and ISHIBASHI, M. 1972. The content of trace elements in seaweeds. *Proceedings of the International Seaweed Symposium* 7: 511-514.

YAMAMOTO, T., OTSUKA, Y., OKAZAKI, M. and OKAMOTO, K.I. 1979. The distribution of chemical elemnts in algae. In: Hoppe, H.A., Levring, T. and Tanaka, Y. (eds.), *Marine Algae in Pharmaceutical Science*. Walter de Gruyte, Berlin, New York. pp. 569-607.

ZEEVART, J.A.D. 1980. Changes in the levels of abscisic acid and its metabolites in excised leaf blades of *Xanthium strumarium* during and after water stress. *Plant Physiology* **66**: 672.

ZENK, M.H. and MULLER, G. 1963. *In vitro* destruction of exogeneously applied indolyl-3-acetic acid as influenced by naturally occurring phenolic acids. *Nature* **200**: 761-763.