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Identification of Auxins in a Commercial Seaweed Concentrate

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Summary

Compounds active in the mung bean rooting bioassay were isolated from the neutral indole fraction of a commercial seaweed concentrate by high performance liquid chromatography. A gas-chromatographic-mass spectrometric analysis of the most active fractions indicated the presence of the following indoles: indole-3-acetic acid; indole-3-carboxylic acid; N,N-dimethyltryptamine; indole-3-aldehyde; and in addition, *iso*-indole,1,3-dione (N-hydroxyethyl phthalimide). This appears to be the first report of an indole amine and a phthalimide in algae. Attempts are currently being made to determine the efficacy of these compounds individually, and in combination, in the mung bean bioassay.

Key words: Seaweed concentrate; Ecklonia maxima; root formation; indole-3-acetic acid; indole-3-carboxylic acid; N,N-dimethyltryptamine; indole-3-aldehyde; iso-indole,1,3-dione (N-hydroxyethyl phthalimide).

Abbreviations: SWC = seaweed concentrate; IAA = indole-3-acetic acid; ICA = indole-3-carboxylic acid; IAId = indole-3-aldehyde; IAcet = indole-3-acetamide; TRP = tryptophan.

Introduction

It is well-documented that commercial seaweed preparations improve plant growth (Metting et al., 1991). Many of these effects have been attributed to the presence of growth substances, particularly the cytokinins, which are known to occur at relatively high levels in various seaweeds and commercial seaweed preparations (Pederson, 1973; Blunden and Wildgoose, 1977; Featonby-Smith and Van Staden, 1984a; Tay et al., 1985, 1987). The application of SWC to plants has been reported to significantly increase root initiation and growth (Featonby-Smith and Van Staden, 1984b; Beckett and Van Staden, 1989). Since it is well-established that both endogenous and synthetic auxins stimulate rooting (Jackson and Harney, 1970; Hartmann and Kester, 1975), and cytokinins inhibit rooting (Van Staden and Harty, 1988), the possibility exists that the observed rooting response following seaweed application is due to auxins. Kingman and Moore (1982) detected indole-3-acetic acid in the commercial seaweed extract, ‹Maxicrop›, using GLC techniques, a finding recently confirmed by GC-MS identification (Sanderson et al., 1987).

The commercial seaweed concentrate, ‹Kelpak›, was recently shown to increase rooting in the mung bean bioassay in a concentration-dependent manner, and promoted rooting in cuttings of several ornamentals (Crouch and Van Staden, 1991). This paper reports the isolation and identification of a number of indole derivatives from the commercial preparation of the brown alga *Ecklonia maxima* (Osbeck) Papenfuss.

Materials and Methods

The seaweed concentrate used in this study is marketed as ‹Kelpak› and prepared by a cell-burst process from the brown alga *Ecklonia maxima* (Osbeck) Papenfuss (Featonby-Smith and Van Staden, 1983).

One hundred millilitres of SWC was extracted in 80% methanol (AR grade) for 12 h 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 resuspended in 100 mL phosphate buffer (pH 8.0) and solvent partitioned into neutral indoles, acidic indoles and indole conjugates according to the procedure of Sandberg et al. (1987). Each fraction was then reduced to dryness, resuspended in 100 mL distilled water and adjusted to pH 6.5.

Mung bean bioassay

The standard mung bean bioassay as described by Hess (1961) was used in order to test for rooting factors in the partitioned SWC. Seeds of *Vigna mungo* L. were surface sterilized for 20 min in 3.5 % sodium hypochlorite, rinsed, then soaked in tap water for 6 h. The seeds were planted in moist vermiculite in large trays (60 × 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 × 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, used for each treatment. The vials were placed 6 cm apart, in trays and were left at 24 ± 3 °C at a light intensity of 10.2 $\mu mol\ m^{-2} s^{-1}$ 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 numbers of roots formed were recorded.

HPLC separation of the indole fraction

The active indole fraction was evaporated to dryness, resuspended in 500 µL 80 % HPLC methanol and passed through a 0.22 µm Millipore filter prior to analysis. Aliquots (100 µL) were injected onto a Hypersil 25 × 0.4 cm 5 ODS C18 column fitted to a Varian 5000 liquid chromatograph operated at a flow rate of 1.0 $mL\ min^{-1}$. Absorbance was recorded using a Varian variable wave-length monitor at 280 nm fitted with an 8 µL flow cell. The aqueous buffer consisted of 0.2 M acetic acid adjusted to pH 3.5 with triethylamine. A linear gradient of methanol:aqueous buffer (20 : 80 to 80 : 20 over 45 min) was used to effect separation. Fractions of 1.0 mL were collected, air dried, and assayed for rooting activity after being taken up in distilled water.

Packed column gas chromatography

A sample of the neutral indole fraction was taken to dryness, methylated with diazomethane (Blau and King, 1977), and analysed

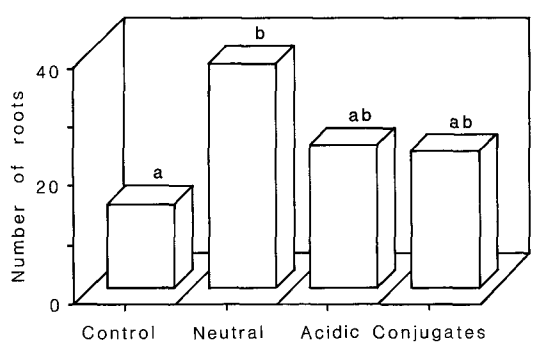

Fig. 1: The effect of solvent-partitioned SWC fractions, applied as an 8 h pulse, on the rooting of mung bean cuttings. Columns with the same letter do not differ significantly at $P < 0.05$.

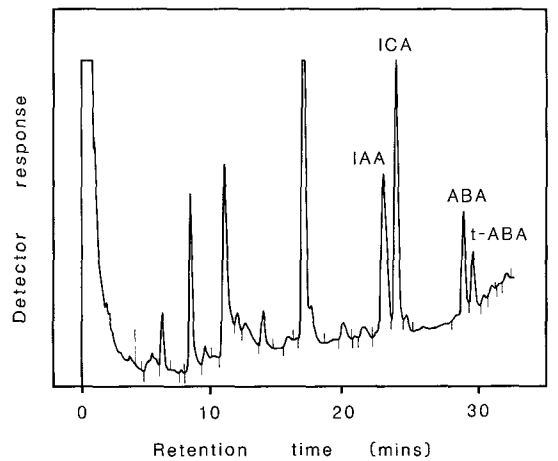

Fig. 2: GLC separation of SWC following solvent partitioning for neutral indoles. Compounds tentatively identified by co-chromatography.

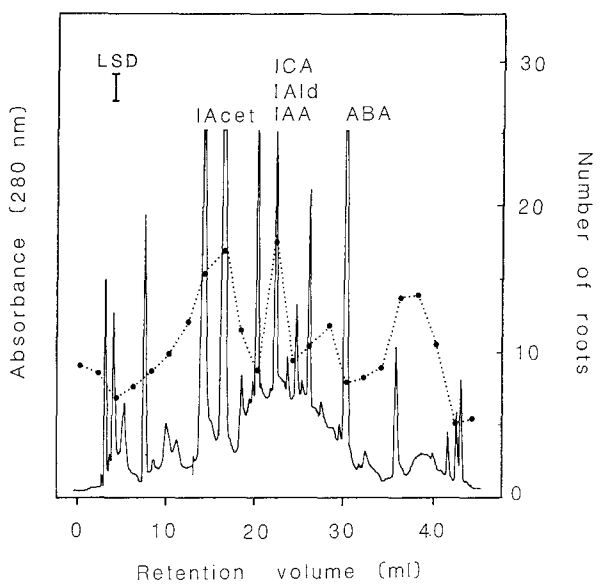

Fig. 3: HPLC separation of SWC following solvent-partitioning for neutral indoles. Compounds identified by co-chromatography indicated. Dotted line indicates root number recorded in a mung bean bioassay. Bar denotes LSD ($P < 0.05$).

on a Varian 3700 GC fitted with glass column (2 m × 6 mm o.d.) packed with OV17 on Chromosorb WHP 80/100 mesh. Oven conditions were: 140 °C for 2 min, ramping at 3 °C min^{-1} to 265 °C with a 5 min hold. Auxins and ABA were tentatively identified on the basis of co-chromatography with methyl esters of standard compounds.

GS-MS analysis

Active HPLC fractions were evaporated to dryness, methylated with diazomethane, and subjected to GC-MS analysis on a Kratos MS80 RFA mass spectrometer interfaced to a Carlo Erba Mega GC. A fused silica OV1 capillary column (25 m × 0.32 mm i.d.) was held at 50 °C for 1 minute, and then ramped to 200 °C at 15 °C · min^{-1},

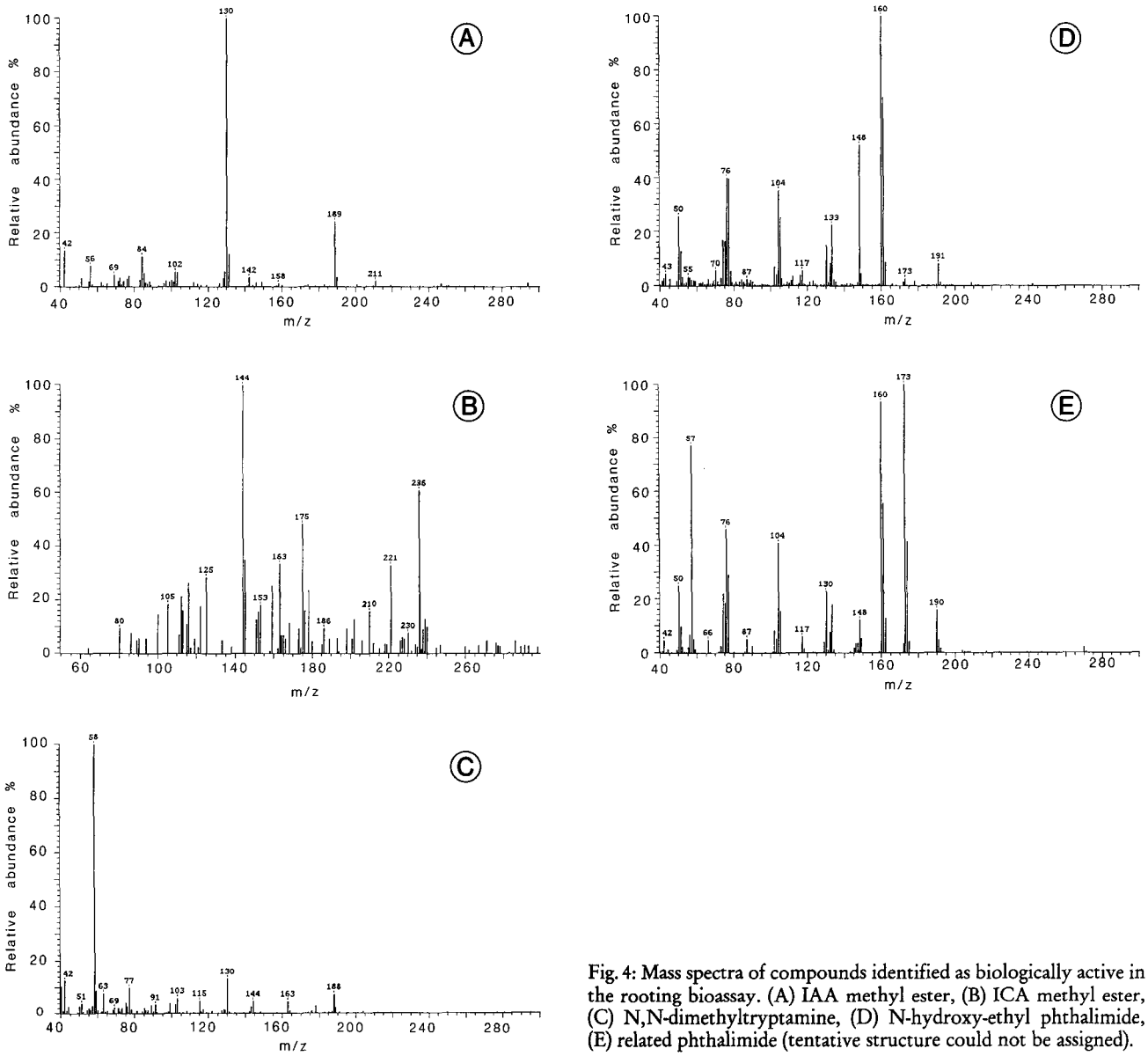

Fig. 4: Mass spectra of compounds identified as biologically active in the rooting bioassay. (A) IAA methyl ester, (B) ICA methyl ester, (C) N,N-dimethyltryptamine, (D) N-hydroxy-ethyl phthalimide, (E) related phthalimide (tentative structure could not be assigned).

and finally to 300 °C at 4 °C · min^{-1}. Spectra were obtained under EI conditions at 70 e.V. and a scanning rate of 1 sec decade^{-1}. Helium carrier flow rate was 2 mL · min^{-1}, the ion source was held at 200 °C. Scans of interest were background subtracted and compared with library spectra of known indoles. A computer algorithm was used to obtain a reliability match for the spectra.

Results

Tentative identification of active constituents

Significant rooting activity was only detected in the neutral fraction of solvent-partitioned SWC (Fig. 1), and was substantiated by repeating the experiment three times. When this fraction was dried, methylated and subjected to packed-column GC, six major peaks were observed (Fig. 2). Some of these co-chromatographed with TRP, IAA methyl ester, ICA methyl ester, and c,tABA. It was observed that IAId (underivatized) and IAA methyl ester co-eluted under the conditions employed here.

When the neutral fraction was subjected to HPLC analysis, several active fractions, which gave significantly higher numbers of roots than the water control, were identified at retention volumes of 15–16, 17–18, 23–24 and 37–40 mL. The fractions collected between 15–16 and 17–18 mL co-chromatographed with IAcet; those between 23–24 mL co-chromatographed with IAA, ICA and IAId; none of the standards available co-eluted between 37 and 40 mL (Fig. 3).

GC-MS analysis

When the fractions most active in the mung bean bioassay were methylated and subjected to GC-MS analysis the fol-

lowing compounds were identified (values in parenthesis are HPLC retention volumes): IAA methyl ester (15–16 mL); ICA methyl ester and N,N-dimethyltryptamine (21–22 and 23–24 mL); IAA methyl ester, IAId, and N,N-dimethyltryptamine (23–24 mL); and N-hydroxyethyl phthalimide and a related phthalimide (36–38 mL; Figs. 4 D and E). Fragmentation patterns (Figs. 4 A–C) were found to be in agreement with those in the literature (Powers, 1968; Agurell et al., 1969; Sandberg et al., 1987).

Discussion

It was demonstrated earlier that SWC exerted marked root-initiating properties in the mung bean bioassay (Crouch and Van Staden, 1991). This suggested the presence in the concentrate of compounds physiologically similar to auxins. Partitioning the SWC into neutral indoles, acidic indoles and indole conjugates resulted in only the neutral indole fraction showing any marked root-initiating activity. HPLC analysis of the acidic indole fraction, showed no evidence of either acidic indoles or ABA (results not presented). In addition, TLC analysis, and staining with Van Urk-Salkowski reagent (Ehmann, 1977), revealed the presence of indoles only in the neutral fraction. GC-MS analysis of the active HPLC fractions indicated the presence of several indolic compounds. In the past, rooting research has been concerned with only a few selected auxins, and in particular IAA (Jackson and Harney, 1970; Hartmann and Kester, 1975). It was significant that rooting activity was associated with the elution of several co-eluting indoles (Fig. 3). It was also noteworthy that the ABA fraction was not inhibitory to rooting. We are unable to exclude the possibility that some of the indole-3-acetic acid present in SWC may be a result of the breakdown of closely-related compounds (Sweetser and Swartzfager, 1978; Hemberg and Tillberg, 1980).

The substituted phthalimide (1-(3-chlorophthalimide)-cyclohexane carboximide; AC-94,377) has been shown to exert growth-regulating activity in bioassays (Suttle and Schreiner, 1982) and when applied to horticultural crops (Los et al., 1980), some of which may be accounted for in terms of gibberellin-like activity (Upadhyaya et al., 1986). Although the hydroxyethyl phthalimide identified in SWC is structurally simpler than the synthetic regulators developed (Los et al., 1980), it may nevertheless have growth regulatory potential. If the compound is not an artifact produced during the processing of the kelp, it may represent the first report of the natural occurrence of this class of compound in plants.

The occurrence and distribution of tryptamines and related compounds in fungi and higher plants has been reviewed (Smith, 1977) but we are unable to find any reports on the presence of dimethyltryptamine in the algae, or of any possible growth regulatory activity. The observation that cucumber seedlings converted [^{14}C]-tryptamine to [^{14}C]-IAA (Sherwin and Purves, 1969) provided a biochemical basis for the earlier observation by Skoog (1937) that tryptamine was active in the *Avena* curvature test. Although one of the proposed routes in IAA biosynthesis is tryptophan → tryptamine → indole-3-acetaldehyde → IAA (Marumi, 1986), this is not considered a likely pathway in tomato shoots or germinating seeds of *Zea mays* (Wightman, 1973; Reinecke and Bandurski, 1987). Further studies are in progress to quantify the levels of indoles present in the SWC and to test the indoles identified, alone or in combination, for root-initiating activity.

Acknowledgements

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References

Agurell, S., B. Holmstedt, and J.-E. Lindgren: Alkaloids in certain species of *Virola* and other South American plants of ethanopharmacologic interest. Acta Chem. Scand. *23*, 903–916 (1969).

Beckett, R. P. and J. van Staden: The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. Plant and Soil *116*, 20–36 (1989).

Blau, K. and G. King: Handbook of derivatives for chromatography. Heyden and Son, London (1977).

Blunden, G. and P. B. Wildgoose: The effect of aqueous seaweed extract and kinetin on potato yield. J. Sci. Food Agric. *28*, 121–125 (1977).

Crouch, I. J. and van Staden: Evidence for rooting factors in a seaweed concentrate prepared from *Ecklonia maxima*. *J. Plant Physiol. 137*, 297–300 (1991).

Ehmann, A.: The Van Urk-Salkowski reagent – a sensitive and specific chromatogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J. Chromatogr. *132*, 267–276.

Featonby-Smith, B. C. and J. van Staden: The effect of seaweed concentrate on the growth of tomato plants in nematode-infested soil. Sci. Hortic. *20*, 137–146 (1983).

– – Identification and seasonal variation of endogenous cytokinins in *Ecklonia maxima* (Osbeck). Papenf. Bot. Mar. *27*, 524–531 (1984 a).

– – The effect of seaweed concentrate and fertilizer on growth and the endogenous cytokinin content of *Phaseolus vulgaris*. S. Afr. J. Bot. *3*, 375–379 (1984 b).

Hartmann, H. T. and D. E. Kester: Plant Propagation: Principles and Practices. Prentice Hall, Englewood Cliffs (1975).

Hemberg, T. and E. Tillberg: The influence of extraction procedure on yield of indole-3-acetic acid in plant extracts. Physiol. Plant. *50*, 176–180 (1980).

Hess, C. E.: The mung bean bioassay for the detection of root promotory substances. Plant Physiol. *36*, XXI (1961).

Jackson, M. B. A. and P. W. Harney: Rooting, co-factors, indoleacetic acid and adventitious root initiation in mung bean cuttings (*Phaseolus vulgaris*). Can. J. Bot. *48*, 943–946 (1970).

Kingman, A. R. and J. Moore: Isolation, purification and quantification of several growth regulating substances in *Ascophyllum nodosum* (Phaeophyta). Bot. Mar. *25*, 149–153 (1982).

Los, M., C. A. Kust, G. Lamb, and R. E. Diehl: Phthalimides as plant growth regulators. HortScience *15*, 22 (1980).

Marumo, S.: Auxins. In: Takahashi, N. (ed.): Chemistry of Plant Hormones. pp. 9–56. CRC Press, Boca Raton, Florida (1986).

Metting, B., W. J. Zimmerman, I. J. Crouch, and J. van Staden: Agronomic uses of seaweed and microalgae. In: Akatsuka, I. (ed.): Introduction to Applied Phycology, pp. 589–627. SPB Academic Publishing, The Hague, Netherlands (1990).

Pederson, M.: Identification of a cytokinin, 6-(3-methyl-2-butenylamino)purine in sea water and the effect of cytokinins on brown algae. Physiol. Plant. *28,* 101–105 (1973).

Powers, J. C.: The mass spectrometry of simple indoles. J. Org. Chem. *33,* 2044–2050 (1968).

Reinecke, D. M. and R. S. Bandurski: Auxin biosynthesis and metabolism. In: Davies, P. J. (ed.): Plant Hormones and Their Role in Plant Growth and Development, pp. 24–42. Martinus Nijhoff, Dordrecht (1987).

Sandberg, G., A. Crozier, and A. Ernstsen: Indole-3-acetic acid and related compounds. In: Rivier, L. and A. Crozier (eds.): Principles and Practise of Plant Hormone Analysis. 2. pp. 169–301. Academic Press, London (1987).

Sanderson, K. J., P. E. Jameson, and J. A. Zabkiewicz: Auxin in a seaweed extract: Identification and quantification of indole-3-acetic acid by gas chromatography-mass spectrometry. J. Plant Physiol. *129,* 363–367 (1987).

Sherwin, J. E. and W. K. Purves: Tryptophan as an auxin precursor in cucumber seedlings. Plant Physiol. *44,* 1303–1309 (1969).

Skoog, F.: A deseeded *Avena* test method for small amount of auxins and auxin precursors. J. Gen. Physiol. *20,* 311–314 (1937).

Smith, T. A.: Tryptamine and related compounds in plants. Phytochem. *16,* 171–175 (1977).

Suttle, J. C. and D. R. Schreiner: The biological activity of AC 94,377 [1-(3-chlorophthalimide)-cyclohexanecarboxamide]. J. Plant Growth Regul. *1,* 139–146 (1982).

Sweetser, P. B. and D. G. Swartzfager: Indole-3-acetic acid levels of plant tissue as determined by a new high performance liquid chromatographic method. Plant Physiol. *61,* 254–258 (1978).

Tay, S. A. B., J. K. MacLeod, L. M. S. Palni, and D. S. Letham: Detection of cytokinins in a seaweed extract. Phytochemistry *24,* 2611–2614 (1985).

Tay, S. A. B., L. M. S. Palni, and J. K. MacLeod: Identification of cytokinin glucosides in a seaweed extract. J. Plant Growth Regul. *5,* 133–138 (1987).

Upadhyaya, M. K., A. Hsiao, and M. E. Bonsor: Differential response of pure lines of *Avena fatua* L. to substituted phthalimides: germination and endosperm-mobilization studies. Ann. Bot. *58,* 455–463 (1986).

Van Staden, J. and A. R. Harty: Cytokinins and adventitious root formation. In: Davis, T. D., B. E. Haissig, and N. Sankhla (eds.): Adventitious Root Formation in Cuttings, pp. 185–204. Dioscorides Press, Portland (1988).

Wightman, F.: Biosynthesis of auxins in tomato shoots. Biochem. Soc. Symp. *38,* 247–275 (1973).