detected in the first and second fractions. This is the characteristic location for synthetic IAA (ref. 8). Relative fluorescence intensity of the solution from the first fraction was 3·4 (equivalent to 2·2 μg of IAA in the 10 ml.); that from the second fraction was 2·0 (equivalent to 1·4 μg of IAA).

There seems to be no doubt that the native auxin in the developing banana fruit is indeed IAA. The extracted auxin was active in the *Avena* curvature test and behaved in a manner identical to synthetic IAA in paper, thin-layer and column chromatography. In addition, the isolated material had the same excitation and fluorescence spectra as IAA and reacted with the classic reagents of Ehrlich and Salkowski. From the fluorometric and biological estimations I conclude that the 300 g of banana fruit tissue contained 18 μg of IAA, a concentration of 0·06 p.p.m.

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Production of Oat Callus and its Susceptibility to a Plant Parasitic Nematode

DICOTYLEDONS grown on White's medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) readily produce callus which is an ideal substrate for plant nematodes[1,2]. Monocotyledons produce callus less readily. Morel and Wetmore[3] initiated callus in species of *Amorphophallus* with carefully balanced vitamins and added naphthaleneacetic acid (NAA), and maintained the callus by the addition of coconut milk. Carew and Schwarting[4] obtained callus from the embryo of rye by adding yeast extract, casein hydrolysate and 2,4-D to the medium. The production of oat and onion callus tissues is described below. The oat callus was used to culture the chrysanthemum nematode, *Aphelenchoides ritzemabosi* (Schwartz).

Hulled oat (*Avena sativa*, variety 'Sun II') seed was surface-sterilized in 20 per cent calcium hypochlorite for 30 min and onion (*Allium cepa*, variety 'Bedfordshire Champion') seed was sterilized in 327 mg/l. ethyl mercury phosphate for 20 min. The germinated seeds were placed aseptically in tubes containing Heller's[4] nutrient agar medium supplemented in various ways (Table 1). Glucose (20 mg/l.) or the same quantity of sucrose was used as the carbon source, and growth substances were added in the following concentrations: 5 mg/l. of 2,4-D; 2 mg/l. of indolyl-3-acetic acid (IAA); 25 mg/l. of NAA; 100 ml./l. of coconut milk; 100 mg/l. of casein hydrolysate; 500 mg/l. of yeast extract; and 1 mg/l. of diaminoethanetetra-acetic acid (EDTA). The tubes were kept at 24° C, half in the light and half in the darkness.

Table 1. EFFECT OF LIGHT AND ADDITIVES ON THE CALLUS FORMATION OF OATS AND ONIONS ON A STERILE MEDIUM

Additives	Oats		Onions	
	Light	Dark	Light	Dark
(1) *S*	−	−	−	−
(2) *G* + coconut milk	−	−	−	−
(3) *S* + casein hydrolysate + yeast	−	−	−	−
(4) *G* + IAA	0	0	−	−
(5) *S* + IAA + NAA	+	−	+	+
(6) *S* + 2,4-D	+	+	+ +	+ +
(7) *S* + 2,4-D + EDTA	+ +	+	+ +	+
(8) *G* + 2,4-D + IAA	+ +	+	+	+

0, No test; −, no response; +, poor callus; + +, good callus; *S*, sucrose; *G*, glucose.

Callus formed only in the presence of IAA, NAA and 2,4-D (Table 1). Within 6 weeks of inoculation, the medium containing 2,4-D, IAA and glucose produced a firm, golden oat callus, which was maintained through several sub-cultures for 3 yr. Onion produced the best callus on the medium containing 2,4-D, EDTA and sucrose, but this wet, white callus grew slowly and survived only 12 months. Callus formed more readily in the light than in the dark.

Oat callus in tubes was inoculated aseptically with *A. ritzemabosi* (30 nematodes/tube), and after 6 weeks the number of nematodes increased fifty-fold. *A. ritzemabosi* is not a recorded parasite of oats, and the fact that oat callus tissue is susceptible parallels the behaviour of rose, apple, potato and red clover callus tissues which also are not hosts of this nematode but give callus that supports its multiplication[2]. When seedlings of resistant oat variety 'Manod' were sprayed with 2,4-D their susceptibility to the stem nematode, *Ditylenchus dipsaci*, increased[5].

The fate of excess 2,4-D in monocotyledons is not completely known[6], but 2,4-D affects oat tissue and dicotyledons similarly in that it releases the natural plant growth substances and increases cell activity, which provide an internal plant environment favouring the multiplication of nematodes.

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Cytokinins in Citrus: Isolation of a Cell Division Factor from Lemon Seeds

STIMULATORS of plant cell division have been demonstrated in several types of fruits[1–3] and seeds[4,5]. Stimulators of cell division have been reported in the fruitlets of apple, plum, peach, pear, quince and pumpkin[1]. Explants from the secondary phloem of carrot roots were used for bioassay. A highly active kinetin-like preparation has been isolated from peas and has been described as containing at least one active purine derivative which is different from kinetin[5]. Cell division factors from apple fruitlets and coconut milk have also been reported[3].

The study of naturally occurring hormones of *Citrus* has revealed the existence in various tissues of auxins[6,7], gibberellins[8,9] and inhibitors[10]. An important group of hormones, namely, cytokinins, was not investigated in *Citrus*. The work reported here represents a preliminary investigation of the cytokinins in lemon seeds.

Ten pounds of lemon seeds (*Citrus limon*, Linn.) were dried in an oven at 60° C and ground up. The powder was extracted with hexane and then with methanol as described previously[11]. The methanol extract was evaporated in a vacuum at 50° C and the residue was dissolved in 200 ml. of distilled water. The *p*H of the water solution was adjusted to 3·0 with hydrochloric acid and the solution was washed four times with ethyl acetate to remove cell division inhibitors[12]. The aqueous phase was used in the bioassay after being adjusted to *p*H 5·6 with sodium hydroxide.

We used tobacco callus tissue in the bioassay and the basal medium was a modification of that described by Murashige and Skoog[13]. The organic constituents of the basal medium were sucrose (30·0 g/l.), myo-inositol

Table 1. CONSTITUENTS OF THE EXPERIMENTAL MEDIA FOR BIOASSAY OF THE CELL DIVISION FACTOR IN LEMON SEEDS

Treatment	Plant extract Concentration (per cent)	Plant extract volume (ml.)	Water (ml.)	Basal medium (ml.)
1	0	0	125	125
2	2	5	120	125
3	4	10	115	125
4	8	20	105	125

(100 mg/l.), and thiamine hydrochloride (0·4 mg/l.). Indolyl-3-acetic acid was added at a concentration of 2·0 mg/l. and the agar concentration was 10·0 g/l. Neither kinetin nor any other organic compound was added. The culture solution was first made up to a volume of 500 ml.; the concentration was adjusted later as indicated in Table 1.

Four different concentrations of the plant extract were examined and each concentration was replicated ten times. Details of the concentrations and volumes used are shown in Table 1. The total volume representing each treatment was 250 ml.; this was divided into ten 25 ml. portions and each portion was placed in a 50 ml. test-tube. The tubes were covered with tight caps and autoclaved for 15 min at 120° C and 15 lb. pressure. Sections of tobacco callus tissue were added to the cooled tubes in aseptic conditions.

The first indication of kinetin-like activity of the seed extract was clearly visible after 3 weeks. After 4 weeks of incubation, the callus tissue sections grown on media fortified with the plant extract were considerably larger than the control sections (Fig. 1). The average weight of the control sections was $0{\cdot}35 \pm 0{\cdot}08$ g, compared with $0{\cdot}60 \pm 0{\cdot}13$ g when 2 per cent of the seed extract was added, $0{\cdot}83 \pm 0{\cdot}19$ g with 4 per cent seed extract, and $0{\cdot}98 \pm 0{\cdot}24$ g with 8 per cent. The activity of these three concentrations cannot be compared at this time with standard concentrations of kinetin because of the possibility that cell division inhibitors might exist in the plant extract. All concentrations of the seed extract, however, allowed cell division activity comparable with a kinetin concentration within the range 10–50 μg/l. This estimate is based on the fact that a number of roots formed on the callus tissue both in 2 per cent and 8 per cent concentrations (Fig. 1).

A fluorometric enzyme assay for purine derivatives, as described by Lewis and Khalifah (unpublished results), was applied to the lemon seed extract to examine the nature of its cell division factor. The reaction between prospective purine derivatives in the plant extract and the enzyme xanthine oxidase was followed fluorometrically

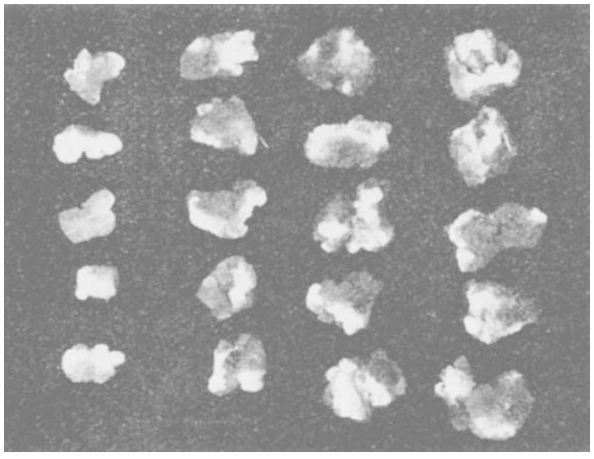

Fig. 1. Sections of tobacco callus tissue after 4 week incubation with media containing 0, 2, 4 and 8 per cent of the lemon seed extract. Note root formation on the second and fifth sections of the 2 per cent concentration and on the second section of the 8 per cent concentration.

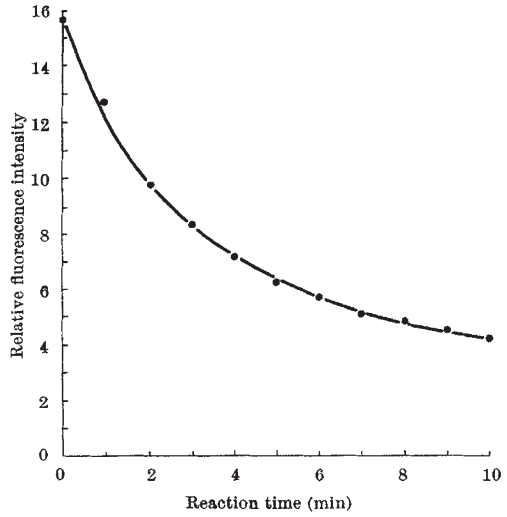

Fig. 2. Changes in the concentration of FAD as indicated by its relative fluorescence intensity. Determinations were made at maximum excitation wavelength of 272 mμ and 550 mμ maximum emission. The reaction medium (3·3 ml.) consisted of 0·2 mg/ml. of FAD; 1 ml. of plant extract; 1 ml. of phosphate buffer at *p*H 5·5, and 0·3 ml. of xanthine oxidase (13·7 μ/ml.)

by the utilization of flavin adenine dinucleotide (FAD), a coenzyme of this reaction. The resulting disappearance of FAD was detected by recording its relative fluorescence intensity (RFI) at maximum excitation wavelength of 272 mμ and maximum emission of 550 mμ (ref. 14) at 1 min intervals, using a spectrophotofluorometer. The reaction took place at 26° C and *p*H 5·5 (phosphate buffer). The plant extract behaved in a manner similar to kinetin with regard to reaction with xanthine oxidase. The results indicated in Fig. 2 strongly suggest that at least one purine derivative exists in the lemon seed extract. The concentration of FAD in the reaction medium dropped sharply and the reaction velocity was steady after 7 min. Most cell division factors are known to contain a purine ring[5,12], and so we assume that the stimulator of cell division in lemon seed possesses the same property.

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