Biochem. J. (1974) **139**, 525–534
Printed in Great Britain

The Relationship of Root-Cap Slimes to Pectins

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(*Received* 29 *October* 1973)

1. The patterns of incorporation of radioactivity from D-[U-^{14}C]glucose into the pectic components of sections of sycamore roots changed so that sections nearer the tip incorporated relatively more label into arabinose and galactose compared with uronic acid. 2. Radioactive maize root-cap slime was prepared and found to contain three water-soluble component polymers which were electrophoretically (i) neutral, (ii) weakly acidic and (iii) strongly acidic at pH6.5. The neutral component was a glucan. The other components, which could be degraded by *trans*-elimination, consisted of an acidic backbone chain composed of galacturonic acid and glucose, attached to which were different proportions of neutral sugars. Arabinose, galactose and fucose, the main neutral sugars of the weakly and strongly acidic materials, were absent from the neutral fraction. 3. Fucose was a major sugar in maize-root slime and in a slime of similar composition synthesized by a maize callus of shoot origin. Only trace amounts were found in sycamore, pea and wheat root tips, and in pectin prepared from maize roots and coleoptiles. A high proportion of fucose is therefore a chemical characteristic of maize slime, and slime synthesis indicated a state of differentiation of the tissue. 4. The similarity between the slime and pectin is discussed; slime is a form of pectin modified in such a way as to provide a hydrated protective coating around the root tip.

The pectic polysaccharides of the cell wall occur in the matrix and their composition varies, although their general pattern is constant. They consist of neutral polymer(s) composed of arabinose and galactose, weakly acidic polymers containing galacturonic acid, galactose and arabinose and strongly acidic polysaccharides consisting mainly of polygalacturonic acid.

The composition of the various components of the pectin changes during the growth of the cell and the components may interchange between the various polysaccharides by transglycosylation. Pectin contributes to the mechanical and physical properties of the wall and, in association with water, any change in the composition of the components and their relationship one to another can extensively alter the texture and strength of the wall (Northcote, 1972). These compounds are thus related to the mechanisms of cell growth and extension.

Root slime of wheat is chemically similar to pectic material of the wall and is formed within the cytoplasm and exported by means of the Golgi apparatus in a similar manner to that of the matrix polysaccharides of the wall (Northcote & Pickett-Heaps, 1966). Many gums are composed of a mixture of neutral and acidic polymers chemically similar to the pectic wall substances (Aspinall & Stephen, 1973). Both these types of polysaccharides have distinctive physicochemical properties (Smith & Montgomery, 1959), which allow them to function as sealing material for wounds or as lubricants for the root as it moves through the soil.

The slime of maize root contains a high concentration of fucose and is a distinctive feature of a particular differentiation state of the outer root-cap cells of this root (Harris & Northcote, 1970). Polysaccharides containing fucose also occur in the slime on the outer surface of some seaweeds (Smith & Montgomery, 1959) and this sugar may partly confer on the polymers their properties as lubricating material.

The pectins, slimes and gums therefore form a family of exported polysaccharides with important physicochemical properties. Their synthesis can vary during the growth and differentiation of the cell (Stoddart & Northcote, 1967) and their composition can in some instances be related to the conformation of the molecules and their function in the wall or as extracellular material (Rees, 1969, 1973).

The purpose of the present work was to investigate the changing pattern of pectin synthesis in the walls of growing plant tissue and to correlate this with the production of extracellular pectin-like material. The slime of maize was separated into its components and the polysaccharides of the cap regions of sycamore and other roots were also investigated.

Methods

Collection of stem fractions of sycamore

The fractions were isolated by stripping the bark from two sycamore tree trunks (diameter approx. 150mm) and scraping the inner surface of the bark

and the outer surface of the bole to yield cambium-rich and differentiating-xylem-rich fractions respectively (Thornber & Northcote, 1961*a*). The samples were freeze-dried and analysed by g.l.c. (Harris & Northcote, 1970).

Sterilization and germination of seedlings

Seeds were collected from an isolated tree of *Acer pseudoplatanus* L. in early November 1972, and were surface-dried and stored in a polythene bag at 4°C. The wings were clipped and the seeds, still retaining their pericarp, soaked in Milton solution (Richardson-Merrell Ltd., London W.1, U.K.) for 2h. Seeds were then removed from their pericarp with the aid of a sterile micro-spatula and transferred to a nutrient-agar (28g/l) (Oxoid Ltd., London E.C.4, U.K.) contained in sterile plastic pots (Sterilin Ltd., Richmond, Surrey, U.K.). From this point onwards the seeds were maintained in darkness. The seeds were shaken from time to time during a period of 2 days so that the whole of their surface came into contact with the surface of the agar. Less than 5% of the original number of seeds showed no contamination. Sterile seeds were soaked in chloramphenicol (10mg/l) (Parke, Davis and Co. Ltd., Hounslow, Middx., U.K.) for 18h, washed, and then a portion of the testa was torn away (Webb & Wareing, 1972). The seeds with damaged testas were thoroughly washed and transferred to a fresh sterile plastic pot which contained a cylinder of Whatman 3MM chromatography paper, so that the seed was sandwiched between the paper and the side of the pot. Sterile water (5ml) was then added, and this was then changed every other day.

The seeds germinated more slowly (90% after 30 days) than those used by Webb & Wareing (1972), probably because they were collected earlier in the year and used more quickly after collection. Sterile seedlings with roots approx. 30mm long were used for the experiments. The stage of unfolding of the cotyledons was not taken into account.

Feeding of radioactive precursor

Roots were fed individually by dipping their tips into a 20μl drop of sterile water containing 2μCi of D-[U-^{14}C]glucose (specific radioactivity 268mCi/mmol) supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Sterile conditions were maintained and manipulations were carried out on a Gallenkamp laminar-flow sterile-air bench. After 2.5h the roots were removed, rinsed quickly in water, and the washings combined with those of the slide on which the drop had been placed. The roots were then washed overnight at 4°C in three to five changes of 50% (v/v) ethanol (10ml each) and these washings were collected separately. Samples of the washings were dried down on paper strips for radioactive counting. Liquid-scintillation counting of these and other strips was carried out as described by Harris & Northcote (1970).

Analysis of roots

After washing each root was cut with a scalpel into sections (Table 1) by placing it on top of a graduated slide on the stage of a dissecting microscope. The sections were stored in a desiccator over P_2O_5 and analysed after hydrolysis for neutral sugars and uronic acids (Harris & Northcote, 1970); the solvent used for separation of the sugars by descending paper chromatography (ethyl acetate – pyridine – water, 8:2:1, by vol.) was designated solvent 1. Water-saturated phenol (100g of phenol, 39g of water: solvent 2) was used for the separation of xylose and fucose. Phenol was subsequently removed from the paper by suspending it vertically in a fume cupboard and washing it free of phenol with chloroform from a wash-bottle. The uronic acids were separated by paper electrophoresis at 4kV, pH3.5 (5ml of pyridine, 50ml of acetic acid made up to 1 litre with water), for 45min. Chromatograms were cut into 40mm×10mm strips and counted for radioactivity directly.

Microscopy and photography

The procedures used have been described (Wright & Northcote, 1972). Photographs of maize callus were taken by using a Nikon Multiphot Photomicrography unit.

Preparation of maize pectin

Maize (*Zea mays* var. Caldera) coleoptiles and maize roots from which the terminal 5mm portion had been removed were extracted directly by refluxing for 3h with 2% (w/v) sodium hexametaphosphate adjusted to pH3.9 with HCl. The solution was filtered and the filtrate dialysed against water for several hours. The filtrate was evaporated under reduced pressure to small volume, digested with a preparation of salivary amylase (Olaitan & Northcote, 1962) for 2h and dialysed again. The material remaining inside the dialysis tubing was adjusted to 80% (v/v) with ethanol and the precipitated pectin collected by filtration. The pectin was redissolved in water and reprecipitated.

Analysis of pectin by g.l.c.

Pectin samples were hydrolysed in 3% (w/w) H_2SO_4 for 1h at 120°C. The hydrolysates were neutralized with $BaCO_3$, evaporated to dryness under vacuum and taken up in water (1ml). NH_3 solution (sp.gr. 0.88) (two drops) was added together

with 10mg of $NaBH_4$. The mixture was left for 2h at room temperature. After this period acetic acid was added until the mixture ceased to evolve H_2. It was then passed down a cation-exchange column [Amberlite IR-120 (H^+ form); BDH Ltd., Poole, Dorset, U.K.] with washing. The material passing through the column was rotary-evaporated to dryness and washed three times with redistilled methanol, being evaporated to dryness after each wash. The prepared alditols were then acetylated in approx. 0.2ml of pyridine–acetic anhydride (1:1, v/v) in a sealed tube at 100°C for 2–3h (Sloneker, 1972). The reaction mixture was cooled in ice, 2–3ml of water were added and the mixture was extracted with 2vol. of methylene chloride (3ml). This solution was evaporated to dryness and taken up in a known volume of methylene chloride.

The column used was glass (1.5m×3.2mm) containing 0.2% polyethylene glycol succinate (LAC-4R-886) and 0.4% 2-cyanoethylsilicone (YE-60) both obtained from Phase Separations Ltd., Deeside Industrial Estate, Queensferry, Flintshire, U.K., and 0.2% polyethylene glycol adipate (BDH) adsorbed on acid-washed Diatomite C (100–120 mesh) supplied by Pye–Unicam Ltd., Cambridge, U.K.

The carrier gas was argon at 60ml/min and the oven temperature was programmed at 155–190°C at 1°C/min.

Preparation and analysis of radioactive slime from maize roots

Seeds of *Zea mays* var. Caldera were germinated under sterile conditions of high humidity (Harris & Northcote, 1970). After 2 days seedlings with their primary root 30mm long were taken and placed in groups of five so that the tips of the longest roots of each seedling were in contact. D-[U-^{14}C]Glucose (10μCi) in 100μl of water was then applied to the tips of the roots.

After 2.5h the roots were removed from the solution and the slime from the outer root cap was wiped directly on to a piece of Whatman no. 1 paper and then electrophoresed at 5kV, pH2.0 (80ml of acetic acid, 20ml of formic acid made up to 1 litre with water) for 40min. Neutral material was eluted by bulk washing of paper strips in water and the eluate was dialysed against five changes of 3 litres of water for 5h. The material not passing through the membrane was rotary-evaporated to dryness, taken up in a small volume of water, and applied to Whatman GF81 glass-fibre paper. Electrophoresis was carried out at 2kV, pH6.5 (100ml of pyridine, 3ml of acetic acid made up to 1 litre with water) for 30min unless otherwise stated. Approx. 60% of the material that remained at the origin at pH2 was eluted with water. Of the remainder some was soluble in 3% (v/v) H_2SO_4 and some in 5% (v/v) KOH, although a residue of 6% of the radioactivity could not be eluted with either solvent.

The distribution of radioactive material on the glass-fibre paper was determined by cutting with a razor blade into strips (40mm×10mm) and counting them for radioactivity by using the procedure used for the paper strips. Subsequent manipulations of various components were carried out after washing the scintillant from the strips with four changes of toluene (approx. 5ml) followed by three changes of benzene (approx. 5ml). Material eluted from zones of the electrophoretogram was hydrolysed and analysed as described (Harris & Northcote, 1970). *trans*-Elimination was accomplished by dissolving the material in 0.1M-sodium phosphate buffer, pH6.8, and heating in a boiling-water bath for 4h (Albersheim, 1959; Barrett & Northcote, 1965). The components of the buffer were removed by paper electrophoresis (pH2.0, 30min, 5kV) before further study.

Isolation of maize callus

Sterile maize coleoptile tissue was placed on a solid nutrient medium (Medium used for S2; Wright & Northcote, 1973). The tissue developed a callus and this has been maintained away from the parent tissue since 1971 on both the initiation medium and PRL 4 medium containing 6mg of 2,4-dichlorophenoxyacetic acid/litre in place of the 1-naphthylacetic acid (Wright & Northcote, 1973). It was cultured in the dark at 26±2°C and subcultured every 5–6 weeks.

Results

Experiments with sycamore tissue

Analysis of stem preparations. The xylem and cambium fractions were freeze-dried, hydrolysed, neutralized and the monosaccharides separated overnight by descending paper chromatography in solvent 1. The area corresponding to xylose/fucose was eluted and the trimethylsilyl ethers were prepared and subjected to g.l.c. (Harris & Northcote, 1970). The ratios of xylose to fucose in these preparations from the xylem and cambium fractions were 20:1 (w/w) and 10:1 (w/w) respectively.

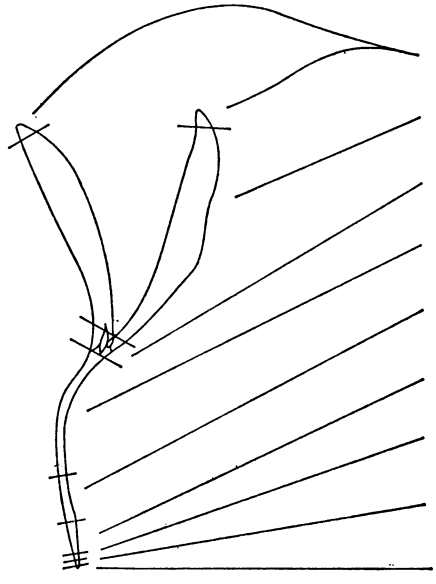
Uptake of label by the sycamore seedlings. Roots (eight) were fed, and the amount of radioactivity in the tissue and washes was determined on three occasions. The roots took up 80–90% of the radioactivity supplied, and of this 35–45% was incorporated into polysaccharide material.

Distribution of label between the sections of the roots. Table 1 shows the way in which the seedlings were sectioned (1–9) for analysis, the total radioactivity found in each fraction (pooled sections of eight

Table 1. *Incorporation of radioactivity into the fractions of sycamore roots*

Seedlings were supplied with D-[U-^{14}C]glucose and cut into the sections 1–9. The pooled material (from eight roots) from corresponding sections was dried and weighed; this pooled material is referred to as fractions 1–9. The dry weight of the material from fraction 1 was standardized at 10 arbitrary units and the other dry weights are expressed relative to this. Material from the fractions was hydrolysed and neutralized and the radioactivity corresponding to a neutral marker on electrophoresis at pH2 was taken as the total radioactivity. Variable section lengths are marked *.

Diagram of seedling



Section number	Length along axis (mm)	Total radioactivity (c.p.m.)	Relative bulk (arbitrary units)	Radioactivity per bulk
9	5	2277	360	6
8	*	258970	5480	47
7	3	2796	272	10
6	*	3033	235	13
5	7	111803	283	395
4	5	1059190	127	8340
3	1	559015	21	26620
2	1	497495	27	18426
1	0.5	307827	10	30783

Table 2. *Distribution of label between the sugars and uronic acids in hydrolysates of sections* 1–4 *of the sycamore roots and the incubation medium*

The roots were fed with radioactive glucose and sectioned as in Fig. 2, hydrolysed and analysed. The values are expressed as the percentage of the total radioactivity in the neutral sugars and uronic acids.

	Radioactivity (%)				
	Incubation	Section			
Sugar	medium	1	2	3	4
Uronic acids	7.8	5.0	15.0	14.5	21.6
Galactose	17.7	18.6	14.2	12.7	12.2
Glucose	55.3	42.9	42.9	45.8	42.4
Mannose	6.6	2.7	3.6	4.1	2.9
Arabinose	7.5	23.2	16.3	15.5	14.6
Xylose	1.9	4.7	4.8	4.5	4.0
Fucose	3.3	1.3	1.4	1.1	0.7
Rhamnose	Not analysed	1.5	1.8	1.8	1.6
Total radioactivity (c.p.m.)	105259	307827	497495	559015	1059190

roots) after hydrolysis, the relative weights of the fractions and the radioactivity incorporated per bulk of tissue.

Distribution of label between the sugars and uronic acids of the sections. Since the amount of incorporation of label was different in the various sections it has been necessary to express the extent of labelling of each component as a percentage of the total label in the neutral sugars and uronic acids of each fraction. These are shown in Table 2 for the incubation medium (incubation medium plus the washings after dialysis against five changes of water for 18h) and the fractions (1–4) of the roots.

Although the galacturonic acid was resolved by electrophoresis at pH3.5 the region corresponding to glucuronic acid usually showed two peaks which may have represented glucuronic acid, methylated uronic acids (Stoddart & Northcote, 1967) or aldobiouronic acids (Fig. 1). The pattern of radioactivity was qualitatively the same in all sections of the roots.

The high incorporation of label into glucose polymers must include a contribution from starch synthesis, which may not be the same in the different sections. The radioactivity in arabinose, galactose and uronic acid (pectin components) is shown in Fig. 2 as a percentage of the total radioactivity in all the sugars and uronic acids except glucose.

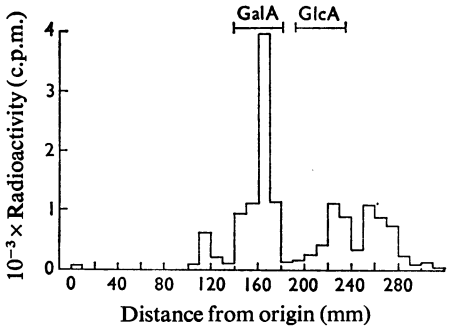


Fig. 1. *Distribution of radioactivity within the uronic acid fraction of sycamore root hydrolysates*

Material from hydrolysates which was neutral at pH 2 and which did not move after 40h descending paper chromatography in Solvent 1 was eluted and subjected to electrophoresis (pH 3.5, 45min, 4kV). The paper was cut into strips and the radioactivity determined. The preparation illustrated was taken from fraction 5. The positions of galacturonic acid and glucuronic acid markers are shown.

Cellular composition of the sections. The tissues which were present in the various root sections were investigated by light microscopy. Fig. 2 shows a diagram of a sycamore root; sections of the root are shown in Plate 1. The essential features are very like those found in maize roots (Juniper & French, 1970). In making the measurements allowance has been made for dehydration shrinkage, and the dimensions shown are those of the tissue in 50% ethanol.

Experiments with maize tissue

Extraction and analysis of root and coleoptile pectin. Samples of pectin prepared from the root (excluding the terminal 5mm) and also from the shoot were hydrolysed and the neutral sugars separated by paper chromatography in solvent 1 (18h) The area corresponding to xylose/fucose was eluted with water. A sample of the eluate was separated by paper chromatography in solvent 2 and spots were identified in the position of fucose after staining by the method of Trevelyan *et al.* (1950). Further samples were dried and separated by g.l.c. both as the alditol acetates, and as the trimethylsilyl ethers.

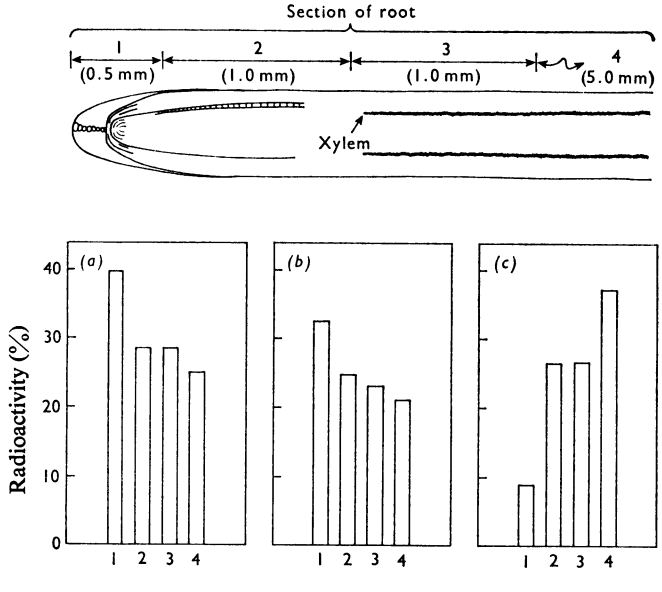


Fig. 2. *Cellular composition of fractions* 1–4 *of the sycamore roots and the extent of incorporation of radioactivity into the pectic components of these fractions from* D-[*U*-^{14}*C*]*glucose*

The diagram was compiled from a study of microscopic preparations of sycamore roots, some of which are illustrated in Plate 1. The histograms show the proportion of radioactivity in arabinose (*a*), galactose (*b*) and the uronic acids (*c*) of the sections, expressed as a percentage of the total radioactivity in all the uronic acids and neutral sugars except glucose.

The proportions of galactose:arabinose:fucose were 90:20:1, by weight and 30:10:1, by weight in the root and shoot pectins respectively.

Investigations on the maize slime. A sample of maize slime was collected on glass-fibre paper and analysed for sulphur content (A. Bernhardt, Mikroanalytisches Laboratorium, Elbach über Engelskirchen, West Germany). None was detected and the slime was therefore not sulphated.

Radioactively labelled slime was prepared and the component polymers were separated by using glass-fibre paper electrophoresis (Barrett & Northcote, 1965; Stoddart & Northcote, 1967). Profiles of the electrophoretograms are shown in Fig. 3(*a–c*). Three components were identified, analogous to those separated from sycamore tissue (Stoddart & Northcote, 1967; Stoddart *et al.*, 1967), although the relative amounts of each component differed between preparations. Electrophoretically, the components could be distinguished as neutral (i), weakly acidic (ii) and strongly acidic (iii). Material from the peaks of the various preparations was analysed and the compositions are shown in Table 3.

The uronic acids of the fractions of the slime were separated by paper electrophoresis at pH3.5; in these analyses the major peak of radioactivity ran slightly behind the marker galacturonic acid ($m_{\text{glucuronic acid}}$ 0.69; compare $m_{\text{glucuronic acid}}$ 0.78 for galacturonic acid). We have investigated this uronic acid and have found it to be an acidic oligosaccharide composed of galacturonic acid and glucose (G.Wright & D. H. Northcote, unpublished work).

The strongly acidic fraction was found in three preparations out of four. Its major radioactive component was the acidic oligosaccharide (56% of the total label), which accounted for its mobility, and arabinose (16%) and galactose (18%) were the other major radioactive sugars.

The weakly acidic component contained relatively less of the radioactive acidic oligosaccharide and correspondingly more radioactive neutral sugars.

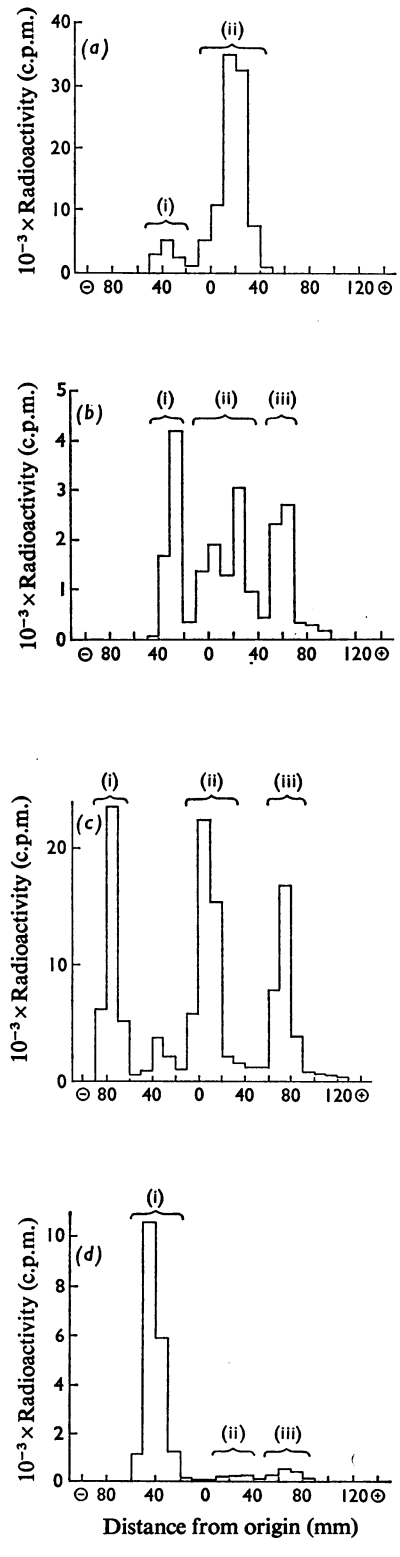
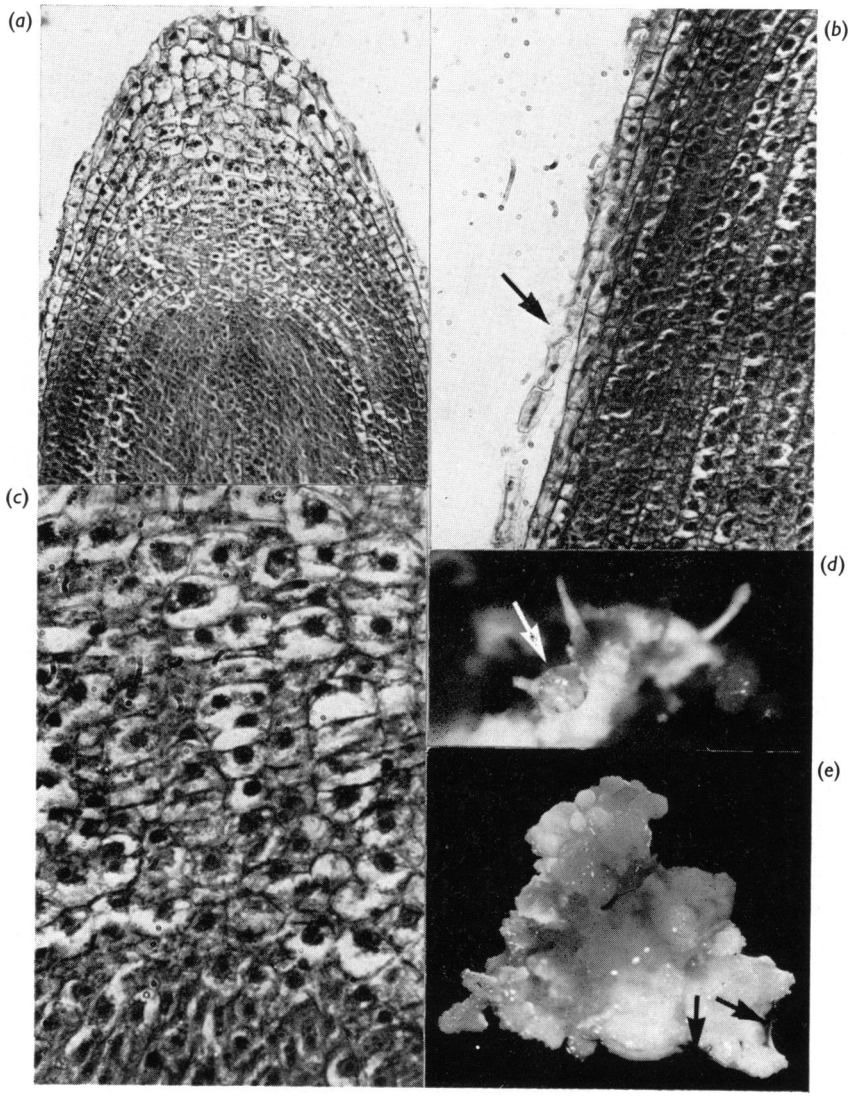


Fig. 3. *Separation of components of radioactively labelled maize slime by glass-fibre paper electrophoresis*

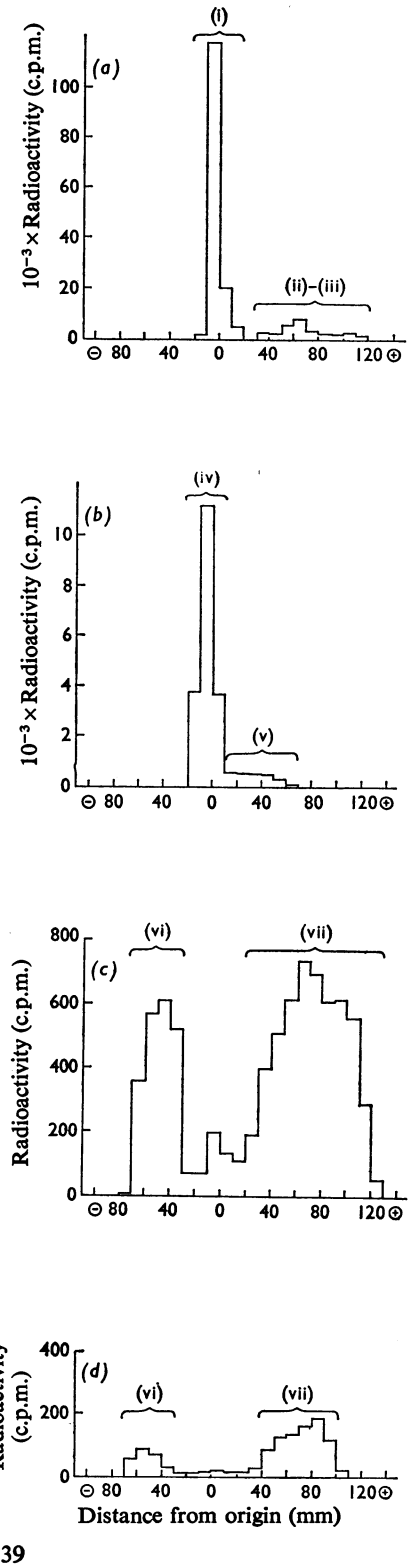
(*a*) Radioactive slime was prepared by dipping maize roots in a solution of D-[U-^{14}C]glucose. The slime was wiped directly from the root-cap on to paper and subjected to electrophoresis at pH2. Neutral material was eluted and dialysed against water. Material remaining inside the bag was decreased in volume and applied to glass-fibre paper for electrophoresis (pH6.5, 30min, 2kV). The paper was cut into strips and counted for radioactivity. (*b*), (*c*) These are profiles of radioactivity from different batches of maize slime, prepared as in (*a*). (*d*) A solution of D-[U-^{14}C]glucose was applied to the underside of a piece of maize callus for 18h. After this period the slime was wiped on to paper and subjected to the procedure described in the caption to (*a*) above.



EXPLANATION OF PLATE 1

Cellular structure of sycamore roots (a–c) and the slime formed by maize callus (d,e)

(*a*) Section through the tip region of a sycamore root, showing the cap cells containing amyloplasts and the outer root-cap cells which form the slime. ×130. (*b*) Section through a sycamore root showing the epidermal cell layers. The outermost root-cap cells can be seen breaking away from the epidermal tissue (arrow). ×160. (*c*) A higher magnification of an area shown in (*a*). This section illustrates the root-cap meristem, from which the cells which synthesize the root-cap slime arise. ×448. (*d*) Roots arising from the surface of maize callus. Globules of slime can be seen (arrow) in association with the roots. ×4.8. (*e*) A view of the underside of a piece of maize callus. The whole area is slimy but this is particularly evident at the edges of the callus (arrows). ×2.4.



In one preparation no radioactive glucose was found in the neutral sugar complement, but most of the radioactivity was present in galactose (33%), arabinose (11%) and fucose (27%); fucose was always present in this weakly acidic fraction. However, glucose was sometimes labelled also and then the other neutral sugars were labelled to a lesser degree.

The neutral component invariably contained only radioactive glucose (85–95%), mannose and a small amount of an unidentified constituent that did not run in solvent 1 and moved slightly (but more than a neutral marker) on electrophoresis at pH3.5.

A separate preparation of radioactive slime was made (Fig. 4*a*) and the neutral (i) and acidic (ii–iii) fractions were isolated separately. A sample from the neutral material was incubated for 18h with a few drops of a preparation of salivary amylase in the presence of toluene. The treated sample and an untreated control were separated by paper chromatography in solvent 1 for 75h; salivary amylase had no effect. The origin material was eluted and digested once more under the same conditions; after paper chromatography in solvent 1 (75h) the profiles of radioactivity were again identical.

The acidic material (ii–iii; Fig. 4*a*) was dissolved in 0.1M-phosphate buffer (pH6.8) and heated in a boiling-water bath for 4h to prepare the *trans*-elimination products (Albersheim, 1959). The phosphate buffer was removed by paper electrophoresis at pH2 and the neutral material re-run on glass-fibre paper at pH6.5 (Fig. 4*b*). The partially separated components (iv) and (v) were eluted and reapplied to glass-fibre paper for separation by electrophoresis for a longer period (40min). A neutral and an acidic component were resolved (Figs 4*c* and 4*d*). The constituents of these components are shown in Table 3.

Studies on maize callus. The callus grew roots on both media, but more on medium PRL 4. Spherical droplets of liquid occurred on the surface of the

Fig. 4. *Preparation of radioactive maize root-cap slime for trans-elimination studies, and the separation of the products of trans-elimination*

(*a*) Maize slime was prepared and its components separated as described in the caption to Fig. 3(*a*) except that glass-fibre paper electrophoresis at pH6.5, 2kV, was carried out for only 15min. (*b*) The acidic material of peaks (ii–iii) of (*a*) was eluted and treated at 100°C in 0.1M-phosphate buffer, pH6.8. The products were subjected to electrophoresis at pH2. Neutral material was transferred to glass-fibre paper for electrophoresis at pH6.5, 2kV for 15min. The paper was cut into strips and counted for radioactivity. (*c*), (*d*) Material representing peaks (iv) and (v) of (*b*) was eluted and re-applied to glass-fibre paper for electrophoresis at pH6.5, 2kV for 40min. Strips were cut from the paper and these were counted for radioactivity (*c*, peak iv; *d*, peak v).

Table 3. *Distribution of radioactivity between the component sugars of polysaccharide material from peaks shown in Figs. 3(a–d) and 4(c,d)*

Radioactive slime was prepared and separated into peaks (i), (ii) and (iii) by using glass-fibre paper electrophoresis. Material (vi) and (vii) was isolated after *trans*-elimination (Figs. 4*c*, 4*d*). Polysaccharide from the peaks was eluted andt he distribution of radioactivity measured as described.

	Radioactivity (%)											
	Neutral component (i) Figs.				Weakly-acidic component (ii) Figs.			Strongly-acidic component (iii) Figs.		Peak (vi) Fig.	Peak (vii) Figs.	
Sugar	3*a*	3*b*	3*c*	3*d*	3*a*	3*b*	3*c*	3*b*	3*c*	4*c*	4*c*	4*d*
Unidentified constituent	4.9	8.2	5.5	4.01	—	19.4	18.7	—	78.6	Trace	4.0	100
Acidic oligosaccharide	—	—			15.0			56.6		35.8	96.0	
Glucuronic acid	—	—			6.6			—		2.7	—	
Galactose	—	—	—	—	33.6	20.9	31.1	17.6	—	22.2	—	—
Glucose	85.8	91.8	88.6	96.0	—	49.4	4.3	0.7	—	5.8	—	—
Mannose	9.3	—	6.0	—	2.1	1.2	4.5	2.8	—	5.9	—	—
Arabinose	—	—	—	—	10.7	4.3	12.4	15.8	23.2	7.4	—	—
Xylose	—	—	—	—	4.4	4.7	6.2	6.5	—	20.2	—	—
Fucose	—	—	—	—	27.2		22.9		—		—	—
Total radioactivity (c.p.m.)	1405	2945	8828	7778	30850	2155	5472	1585	4973	2155	894	4713

callus, often in association with the roots (Plate 1), and the underside of the callus was moist with a mucous material resembling the slime of the maize roots (Plate 1).

The material was collected on glass-fibre paper and analysed, after hydrolysis, by g.l.c. The neutral sugar composition was: galactose, 37.7%; glucose, 16.7%; mannose, 7.5%; arabinose, 6.7%; xylose, 12.8%; fucose, 16.7%.

A piece of the callus was inverted on its agar medium and 50μl of water containing 5μCi of D-[U-^{14}C]glucose placed on the surface that had previously been in contact with the agar. After 18h the mucous material was collected and separated on glass-fibre paper at pH6.5 (Fig. 3*d*).

Experiments on other tissues. Terminal 1mm tips from the primary root of peas were collected, hydrolysed and analysed by g.l.c. Only trace quantities of fucose were found.

Another monocotyledon has also been examined. Sterile wheat seedlings (var. Capelle Desprez) were supplied with radioactive glucose as described for sycamore seedlings and the terminal 1mm root tips collected, hydrolysed and analysed. The results of the analysis of the distribution of the radioactivity between the polysaccharide components were: uronic acids, 12.6%; galactose, 8.3%; glucose, 40.1%; mannose, 1.7%; arabinose, 16.7%; xylose, 19.8% and fucose, 0.8%.

Discussion

Thornber & Northcote (1961*a*,*b*, 1962) analysed the component sugars of xylem and cambial cell walls of sycamore tissue, but the analytical methods they used would not have distinguished fucose from xylose. To examine whether fucose was a component of the pectin and hemicellulose, samples were prepared and analysed by g.l.c. The results indicated that fucose was only a minor component of the fractions.

The amount of radioactivity supplied per sycamore root was the same as that used by Harris & Northcote (1970) for maize, and a similar percentage of the radioactivity was taken up by the tissue. The extent of incorporation of labelled glucose into the different sections of the seedling showed clearly that the precursor was not uniformly available to tissues within the plant. Some of it must have reached the conducting tissue, since the radioactivity was incorporated into the polysaccharides of the cotyledons. It seems likely, however, that the outer tissues of the root incorporated most of the radioactivity into polysaccharides. Roberts & Butt (1967) did not find any localized areas of incorporation of D-[U-^{14}C]-glucose into polymers by using a radioautographical technique applied to maize roots. However, they used excised tips in which the glucose must also have been available through the cut end. Sections more distant from the root tip of sycamore seedlings incorporated relatively less radioactivity into arabinose and galactose and relatively more into the uronic acid fraction. The incorporation into xylose remained approximately constant, so that the xylose/arabinose ratio increased in the sections as they contained progressively more maturing and secondarily thickening tissue (Thornber & Northcote, 1961*a*,*b*; Jeffs & Northcote, 1966).

Pectin from sycamore callus has been shown to consist of a polygalacturonic acid backbone containing some neutral material and a separate arabinogalactan polymer which could possibly act as a precursor for the neutral component attached to the uronic acid backbone (Barrett & Northcote, 1965; Stoddart & Northcote, 1967). The relatively higher proportion of radioactivity incorporated into arabinose and galactose in the sections close to the tip of sycamore roots suggests that larger amounts of the neutral component with respect to the uronic acid component were being synthesized in this region. If this neutral material were also attached to the main backbone of polygalacturonic acid, it would increase the extent of branching of the polymer and hence the degree of hydration (Rubery & Northcote, 1970; Wright & Northcote, 1973). Thus such a modification of the structure of the pectin could alter its physical properties so as to make it suitable to provide a hydrated protective coating around the growing tip of the root. The incorporation of radioactivity into fucose in sycamore roots was small, but increased towards the tip of the root. It is therefore possible that the sycamore root cap lubricates its passage through the soil by synthesizing a relatively very small amount of polysaccharide which itself is rich in fucose; however, this explanation seems less likely than the one given above.

Pectin exported by vesicles derived from the Golgi apparatus is normally retained within the wall by the microfibrillar network of cellulose. Some of the neutral components of the cell wall may, however, be washed out into the medium of the callus tissues cultured in a liquid suspension (Aspinall *et al.*, 1969). Where the microfibrillar component is absent, the entire pectin component may be released into the medium; Hanke & Northcote (1974) found that soya-bean callus protoplasts supplied with uniformly labelled glucose synthesized radioactive pectic substances which could be recovered from the culture medium during the early stages of wall regeneration.

It is well established that the root-cap slime of maize and other tissues is also synthesized in the Golgi apparatus and exported in the same way as pectin (Northcote & Pickett-Heaps, 1966; O'Brien, 1972). Taken in conjunction with the idea that sycamore slime is a modified pectin this suggested that the high proportion of label channelled into fucose from D-[U-^{14}C]glucose by maize roots (Harris & Northcote, 1970; Bowles & Northcote, 1972) represented a changed emphasis in production of the components of normal maize pectin. Pectin was therefore prepared from root and shoot tissue of maize and found to contain a small amount of fucose. Also radioactive fucose has been identified in a hydrolysate of a Golgi fraction isolated from maize coleoptiles supplied with D-[U-^{14}C]glucose (D. J. Bowles & D. H. Northcote, unpublished work). However, the amount of fucose in both cases was small and the presence of a polysaccharide containing a large proportion of fucose is therefore a characteristic of differentiated maize root-cap tissue. Little fucose was found in the tip region of sycamore, pea or wheat roots.

A maize callus of shoot origin was able to form roots and synthesized a mucous substance which was analysed and found to contain a large proportion of fucose; its chemical composition was very similar to slime isolated from maize roots. Fucose is a chemical marker for root-cap slime and it indicates that new synthetic activity has been induced in callus derived from stem during the period of its isolation and growth. This callus would therefore provide very suitable material for the investigation of the control of the induction of the differentiation process which results in slime production.

The extent to which maize slime had the properties of a pectin was investigated by using glass-fibre paper electrophoresis of radioactively labelled material. This allowed us to analyse the very small amounts of slime that were available. The relative proportions of the radioactive sugars in the components of the slime that were separated do not necessarily indicate the chemical composition. The tissue had been exposed to the radioactive glucose for 2.5h and during this length of time it is known that the slime becomes extensively radioactively labelled, so that although the analysis does not reflect the quantitative nature of the components it probably indicates their qualitative composition. This was shown to be a reasonable assumption, since the mobility of the components on the electrophoretograms was related to the relative proportion of radioactive uronic acids and neutral sugars they contained.

An unusual feature of the slime was the presence in the hydrolysate of a large amount of an acidic oligosaccharide composed of galacturonic acid and glucose (K. Wright & D. H. Northcote, unpublished work). In other respects the three components that could be separated and investigated were similar to those identified in sycamore pectin (Stoddart & Northcote, 1967; Barrett & Northcote, 1965). Two components consisted of acidic polymers carrying different amounts of neutral material; this was shown by the *trans*-elimination studies which degraded the acidic components into oligomers some of which contained not only galacturonic acid and glucose but also glucuronic acid and the neutral sugar blocks. The third component was neutral and contained a polymer consisting mainly of glucose; the *trans*-elimination studies showed that although this glucose component could be joined to the uronic acid material there were other neutral sugars present, particularly galactose, arabinose and fucose. These other neutral sugar residues were found only on the main backbone and not as a separate neutral polymer.

Amylase digestion showed that the neutral material was not starch. Buchala & Meier (1973) have reported the isolation and characterization of a glucan present in the water-soluble hemicellulose fraction from mature maize stems. The glucan was resistant to α-amylase and was shown by methylation analysis and periodate oxidation to contain β-(1→3)- and β-(1→4)-links in the ratio 1:2. The properties of the neutral component of the maize slime are such that it could be a similar polymer.

A common feature between the substances produced by root-cap cells of different species is the mucous nature of the slime. This material, which is synthesized by the dictyosomes and exported via the vesicles derived from this organelle, remains extremely hydrated so long as water is available; this suggests that the hypertrophied nature of the dictyosomes of the root-cap cells is due to the physical properties of the polymer that is being synthesized and is absorbing water from the cytoplasm.

In the fucoxyglucan characterized from the medium of suspension-cultured sycamore cells the fucose was assigned to a terminal position (Aspinall *et al.*, 1969) linked (1→3) to the xylose side chains off the glucose backbone. However, Haaland (1972) found the link Glc*p*Aβ1-4L-Fuc in a degradation product of pectin preparation from *Tussilago* leaves, and Aspinall *et al.* (1968) have identified this linkage in lemon-peel pectin. It is noteworthy that when radioactive glucuronic acid was found in the maize slime it was present in the fractions that contained a high proportion of fucose. If the fucose more commonly occurs terminally, the occurrence of GDP-fucose (Harris & Northcote, 1970, Scheme 1) in a membrane-bound reaction mixture synthesizing a polysaccharide might cause the termination of growing side chains and induce other branches to occur. In maize, for instance, this might change the character of the pectin from polyuronic acid with infrequent large neutral blocks, to polyuronic acid with frequent shorter side chains.

We thank Mr. R. M. Davey, Mr. K. J. Goulding and Mrs. M. Wilson for technical and secretarial assistance. K. W. thanks Tate and Lyle Ltd. for a Fellowship, during the tenure of which this work was carried out.

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