

# CYTOLOGY AND CYTOCHEMISTRY OF VASCULAR DISCOLORATION IN CASSAVA ROOTS

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## Introduction

The rapid post-harvest deterioration of cassava (*Manihot esculenta* Crantz) roots has been known from the earliest times, and is a major constraint on the economic exploitation of the crop (Ingram and Humphries, 1972) although there have been conflicting views as to the relative importance of physiological and pathological causative factors. The work of Booth and Coursey (1974), Booth (1976) and Noon and Booth (1977) clarified the previously confused situation and showed that the initial stage of primary deterioration is an endogenous physiological reaction which occurs in the absence of pathogens. Microorganisms are normally only involved later, in the stage of secondary deterioration, and even then are usually generalized saprophytes utilizing already moribund tissues, rather than true pathogens.

It has been suggested that vascular discoloration occurs in response to water stress at sites of root injury (Marriott, Been and Perkins, 1978) and that susceptibility to this stress varies according to the physiological state of the root when harvested and/or injured (Lozano, Cock and Castano, 1978; Marriott, Been and Perkins, 1979). The principal symptom of primary deterioration is blue or brown discoloration of the xylem vessels, often termed vascular streaking (Drummond, 1953; Averre, 1967; Booth, 1976). The effect has been well illustrated at macroscopic level by Montaldo (1973), Booth (1976) and others, while Drummond (1953) illustrates some microscopical observation.

Increased incidence of vascular discoloration as a result of pre-harvest infection by cassava bacterial blight has been reported by Leu (1976) although Lozano and Sequeira (1974) state that roots of infected plants usually remain healthy.

The formation of occlusions in xylem vessels is a well known phenomenon in many plants as a response to pathogenic invasion (Beckman and Zargoogian, 1967; VanderMolen, Beckman and Rodehorst, 1977). Occlusions formed in the absence of pathogens as a stress response have been reported by Lineberger and Steponkus (1976). When rose stems were held in water, granular proteinacious occlusions formed when bacterial contamination occurred, while occlusions of a different type, consisting of carbohydrate material formed under sterile conditions, presumably as a physiological response to wounding.

The formation of black deposits in the xylem vessels of cassava roots undergoing vascular discoloration was reported by Drummond (1953) but does not appear to have received further study. This author gave no information as to the nature of the occluding material, but clearly indicated that bacterial or fungal pathogens were not involved. The present studies were initiated to investigate the nature and origins of the

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occluding material formed in cassava root xylem during the development of vascular discoloration in the absence of any infection. Preliminary accounts of part of this work have already appeared (Rickard, 1979a; Rickard, Marriot and Gahan, 1979).

### Materials and Methods

Cassava roots were air-lifted to London from Jamaica (cv. Yellow Heart) and Ghana (cv. Ankrah) in boxes lined with perforated polythene and containing wet sawdust, and stored at 20°C. The roots were washed in water, cut laterally into sections 50 mm. long and re-washed. Primary deterioration was induced by dehydration (Marriot, et al, 1978) or by placing the sections on perforated plastic shelves in aerated, distilled water to a depth of 10 mm.

During all the microscopical studies, the material under examination was rigorously scrutinized for any staining reactions that would indicate the presence of microbial infection.

Unfixed, frozen sections 10-20  $\mu\text{m}$ . thick were prepared (Gahan, *et al.*, 1967) and histochemical tests conducted on both discolored and on unpigmented material, and on wound surfaces 2 days after cutting. The test methods used were those of Gahan (1965); Pearse (1968); Mace (1963; 1978) and Wong and Preece (1978).

Specimen blocks for scanning electron microscope (SEM) studies were fixed in formalin-aceto-alcohol, dehydrated in acetone and critical-point-dried using carbon dioxide as the transition fluid. The mounted blocks were sputter-coated in gold and examined using a Cambridge Stereoscan SEM 600.

Transmission electron microscope (TEM) specimens were fixed in 1.5 per cent gluteraldehyde on 0.1 M. cacodylate buffer, dehydrated in ethanol and embedded in Spurr's epoxy resin. Sections 1.0-2.0  $\mu\text{m}$ . thick were cut on an ultra-microtome and examined by light microscopy to identify areas for TEM investigation. Ultra-thin sections (60-140 nm.) were stained with lead citrate for TEM observation, and examined with an AEI EM6B instrument.

### Results

Micro-organisms were not detected in any of the material microscopically examined, confirming the reports of Noon and Booth (1977) that microbial infection is not essential to the development of vascular discoloration.

Although in intact tissue the pigmented material in cassava initially appears blue and becomes brown on aging, it is always brown in tissue sections. It occurs in xylem, either as a gel-like material lining the vessel walls and forming connecting strands or as large granular masses filling the vessel lumen.

Adjacent parenchyma cells are observed to contain a dense, granular, pigmented material which appears to enter the xylem via the pit areas (Plate 1). Examination of semi-thin sections of resin-embedded material showed the presence of tyloses in the vessels, apparently originating from adjacent cells and containing cytoplasm and nuclei. The pigmented material was visible as granular masses with no particular structural form as reported by Drummond (1953).

Ultra-thin sections of pigmented vessels observed with TEM (Plate 2) show the presence of a fine-stranded matrix containing particles of varying electron-density. This occluding material is localized close to the vessel wall and often coalesces into large

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masses of variable size and shape; no enclosing membrane is detectable.

Observations under SEM showed that pigmented vessels contain a dense and sometimes granular layer adhering to the vessel walls and obscuring the pits, often accompanied by tylose formation (Plate 3a and b). Tylose formation also occurs in vessels of wounded pieces which do not develop vascular discoloration, the granular layer being absent in these vessels (Plate 4a and b).

Similar results are obtained when pigmented and unpigmented vessels from a single root are compared. All attempts to extract the pigment with aqueous or organic solvents were unsuccessful. The composition of the pigment was therefore examined by histochemical methods and Table I summarizes the results obtained.

The occluding material gave strong positive reactions with PARS, phloroglucinol-HCl and Sudan Black B tests indicating the major components to be carbohydrate, lignin and lipid, the blue response obtained with Nile blue sulphate indicating that acidic binding sites are present. Acetylation of the polysaccharide hydroxyl groups greatly reduces the response to PARS reaction while pretreatment with dimedone has little effect, indicating the presence of carbohydrate (Table 1).

The mature occluding material does not react to tests for proteins, pectins, phenols, or terpenoid aldehydes. However, recent results (Rickard, 1979b, and under publication) shows that free phenols are present in the early stages of pigment formation. A positive response was obtained with rethenium red; this, however, was not affected by extraction with ammonium oxalate, indicating the absence of pectin, the observed reaction possibly being due to the presence of oxidized cellulosic material (Sterling, 1970). The blue-brown discoloration formed at wound surfaces was found to be histochemically similar to the xylem pigment except that lignin was not detected.

### Discussion

It was concluded by Noon and Booth (1977) that vascular discoloration is a physiological process as they were unable to isolate microorganisms from fresh discoloration is a physiological process as they were unable to isolate microorganisms from fresh discolored cassava tissue. The occlusions formed in such tissue are similar to those commonly occurring in response to vascular pathogens (VanderMolen, *et al.*, 1977) but microorganisms were not observed in freshly discolored cassava tissue during the present investigations, fully substantiating the hypothesis originally proposed by Drummond (1953) that the discoloration is a response to physiological stress rather than to microbial action. The occlusions observed in rose stems held under sterile conditions (Lineberger and Steponkus, 1976) were morphologically similar to those now described in cassava.

Cassava xylem in both pigmented and non-pigmented vessels is frequently occluded by tyloses, but there is no indication that tylose formation is essential to vascular discoloration. The rapid development of tyloses formation is essential to vascular discoloration. The rapid development of tyloses in infected xylem vessels has been proposed as a *Fusarium* and *Verticillium* wilt-resistance mechanism in banana (Beckman, Halmos and Mace, 1962) and cotton (Mace, 1978), but in cassava xylem where tylose formation was not associated with microbial infection, it appears to be a non-specific response to injury.

Observations by light-microscope and TEM both indicate that the pigmented material originates from adjacent cells and enters the xylem vessels via breaks in the pit membrane (Plates 1 and 2). This mechanism can be seen to differ from the process of gel



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formation which occurs in a variety of hosts in response to vascular pathogens (VanderMolen *et al.*, 1977). Their TEM observations showed that gels originating from perforation plates, end walls and pit membranes by a process of distension of primary wall and middle lamella constituents.

However, some host-pathogen interactions produce occlusions which are similar to those formed in cassava xylem in both appearance and apparent site of origin. Infected willow xylem becomes blocked by brown phenolic compounds, including leucoanthocyanins, which apparently diffuse into the lumen from adjacent cells. Although the mature occluding material in cassava xylem from water-stressed roots does not respond to tests for free phenolics, including leucoanthocyanins, are present in the early stages of pigment formation.

The occlusions formed in rose stems under sterile conditions were also found to originate from adjacent cells, and were thought to be due to the relocation of the cell constituents (Lineberger and Steponkus, 1976). Both rose stem and cassava root occlusions responded to tests for carbohydrates and lipid. While pectins and protein were detected in rose stems (Parups and Molnar, 1972) they were not in cassava. The presence of phenols and lignin in cassava occlusions is, however, similar to some microbially stimulated occlusions (Wong and Preece, 1978), though the latter were found to be composed mainly of pectinacious material (Beckman and Zargoogian, 1967; VanderMolen, 1977), which were not detected in pigmented cassava occlusions.

## Conclusions

The formation of vascular occlusions in cassava root xylem appears to be a physiological process which occurs as a response to wounding. The vessels become blocked due to the production of tyloses and of pigmented material which reacts positively to histochemical tests for lipids, lignins and carbohydrate. These two types of response can occur singularly or together.

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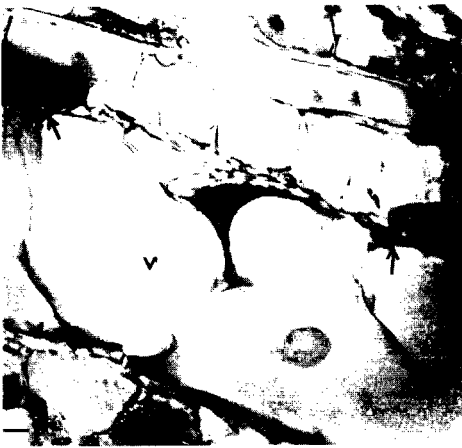
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Table 1. Histochemical Tests on Xylem Occlusions in Cassava Roots

Compound	Test	Result
<b>Polysaccharides</b>	a) Periodic acid-Schiff (PARS)	++
	b) Schiff	+
control a)	Acetylation-PARS	+
	Aldehyde block (Dimedone)-PARS	++
control b)	Aldehyde block - Schiff	-
	a) Alkaline hydroxylamine hydrochloride	-
<b>Pectins</b>	b) Ruthenium red	+
	control b)	Ammonium oxalate extraction- Ruthenium red
<b>Proteins</b>	2,4 dinitrofluorobenzene	-
<b>Lipids</b>	Sudan black	++
	Nile blue sulphate	++
	Acid haematein	++
<b>Terpenoid aldehydes</b>	Antimony trichloride	-
<b>Lignin</b>	Phloroglucinol-HCl	++
<b>Phenols</b>	Nitrous acid	-
	Ferric chloride	-

++ = positive response  
 + = partial response  
 - = no response

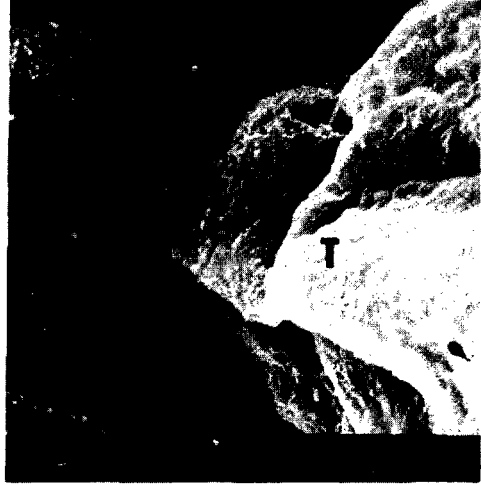
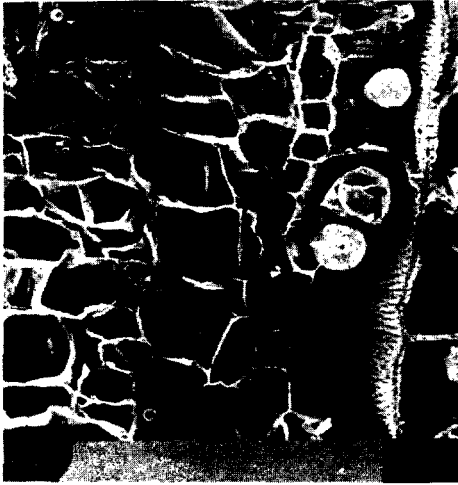


Unfixed, frozen sections of cassava root showing pigmented material entering the vessel lumen (v) from adjacent parenchyma cells via pit areas (arrows). Bar = 25  $\mu$ m.



Transmission electron micrograph of pigmented material (p) entering the vessel lumen (v) via a rupture in the pit membrane (pm) between areas of secondary thickening (s). Bar = 3  $\mu$ m.





Scanning electron micrographs of pigmented (3a and b) and unpigmented (4a and b) cassava xylem. Tylosis (T) are present in both cases but the pigmented vessel also contains a dense granular material adhering to the vessel wall obscuring the pit areas. This material is absent from the unpigmented vessels.

