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Accumulation of Hydroxycoumarins During Post-harvest Deterioration of Tuberous Roots of Cassava (*Manihot esculenta* Crantz)

HOLGER BUSCHMANN*‡, MARIA X. RODRIGUEZ‡§, JOE TOHME§ and JOHN R. BEECHING†‡

‡*Department of Biology & Biochemistry, University of Bath, Bath, BA2 7AY, UK and* §*Centro International de Agricultura Tropical, CIAT, Cali, A.A. 6713, Colombia*

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The use of the root crop Cassava (*Manihot esculenta* Crantz) is constrained by its rapid deterioration after harvest. Chemical and spectroscopic examination revealed the accumulation of four hydroxycoumarins (esculin, esculetin, scopolin and scopoletin), compounds derived from the phenylpropanoid pathway, during the time course of post-harvest deterioration. Fluorescence-microscopy revealed their localization in the apoplast of the parenchyma. Scopoletin and scopolin showed the most dramatic increases in concentration, peaking by day 2 after harvesting. A smaller secondary peak of scopoletin tended to be more pronounced in cultivars showing lower susceptibility to deterioration. Evidence for the metabolism of scopoletin to an insoluble coloured product by means of a peroxidase is presented. This product may be the cause of the discolouration of the vascular tissue during storage.

Key words: Cassava, hydroxycoumarins, *Manihot esculenta*, peroxidases, post-harvest physiological deterioration, wound response.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is an important crop plant, especially for developing countries in Africa, Asia and Latin-America (Ravi *et al*., 1996). Specific attributes, such as a high efficiency in carbohydrate production, and tolerance to drought and different soil qualities make cassava a very attractive crop, especially to smallholding farmers. Despite its economic importance, this crop suffers from two major disadvantages. All parts of the plant contain cyanogenic glycosides that can release toxic cyanide. Thorough processing or preparation of the root or other edible parts of the plant significantly reduces or eliminates this toxicity prior to consumption. However, the major constraint for the use of cassava is that farmers, processors and consumers suffer substantial losses during storage of the root tubers. This is due to rapid physiological deterioration processes that initiate within 24 to 48 h after harvest (Beeching *et al*., 1998), followed by a second stage (after 5 to 7 d) involving microbial decay. The biochemical processes and histological changes of the first stage (summarized by Ravi and Aked, 1996) are known as post-harvest physiological deterioration (PPD) or vascular streaking.

A discolouration of the vascular tissue to blue-black is the first visible sign of PPD, followed by a browning of the parenchymatic tissues. Microscopically, observations of the early processes reveal occlusions in the xylem vessels, as well as the occurrence of tyloses (Rickard and Gahan, 1983). A rapid accumulation of fluorescent compounds in the parenchyma has been described by various authors (Tanaka *et al*., 1983; Uritani *et al*., 1984*a*,*b*). These compounds have been identified by the authors as the hydroxycoumarins, scopolin, scopoletin and esculin. Wheatley (1982) describes a 150 to 200-fold increase of scopoletin during the first 24 to 48 h after wounding, and this finding led Wheatley and Schwabe (1985) to the assumption that scopoletin may be involved in PPD. Other compounds that have been identified from cassava roots and that may play a role in PPD are the leucoanthocyanins, cyanidin and delphinidin (Akinrele, 1964), the flavan-3-ols, (+)-catechin and (+)-gallocatechin (Uritani *et al*., 1984*a*), and 22 diterpenoid compounds (Sakai and Nakagawa, 1988). Besides the accumulation of secondary metabolites, the increasing activities of enzymes like phenylalanine ammonia lyase (PAL; Tanaka *et al*., 1983), the key entry enzyme of the phenylpropanoid pathway, have been described. Evidence from cycloheximide treatment of root discs (which partially inhibited symptoms), *in vivo* labelling of proteins that were newly synthesized during deterioration time courses, and cDNA cloning and Northern hybridization experiments, clearly demonstrates that PPD is an active process involving the increase and *de novo* synthesis of proteins, and changes in gene expression (Beeching *et al*., 1998; K Reilly and J R Beeching, unpubl. res.).

These findings confirm that PPD is an abiotic stress response, and that the histological and biochemical changes observed in cassava are typical of wound responses described in other plant systems (Bonsen and Kucera, 1990; Bennett and Wallsgrove, 1994; Matsuki, 1996; Dixon

* Present address: Inst. of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany.
† For correspondence. Fax +44 1225 826779, e-mail J.R.Beeching@bath.ac.uk

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and Paiva, 1995). Whereas these responses can culminate in wound healing and survival in other plants, this is not the case for cassava roots. Plant wounding produces, or induces, the production of signalling components that initiate the wound response: this includes the production of defensive compounds and enzymes, the preparation of the plant for the potential extension of wounding, and wound repair, which is followed by the inhibition of signals. These aspects of the wound response are present in the cassava root. However, the wound repair and the resultant down-modulation of the signals are inadequate, which leads to continuous cascades of wound responses that spread throughout the cassava root. The incomplete wound response is observed as PPD (Beeching *et al*., 1998). Cassava storage roots are not propagules, but serve primarily as repositories of carbohydrate for the plant. In contrast to the detached root tuber, wound repair does occur if the root remains attached to the plant (Mwenje *et al*., 1998).

Comparative evaluation of the visual symptoms of PPD in various cultivars of cassava shows that there are differences in their susceptibility to deterioration (Iglesias *et al*., 1996). These differences can provide breeders and biologists with the opportunity to use the genetic variability to improve the crop.

This study identifies all the hydroxycoumarins present in cassava roots, and focuses on their biological function and their potential involvement in the PPD process. Moreover, it provides information on the accumulation of these compounds and a comparison of various economically important cassava cultivars that show differences in their susceptibility towards PPD.

MATERIALS AND METHODS

Plant materials

Root tubers of seven different cassava cultivars (CM 7033-3, MBRA 337, MCOL 22, MDOM 5, MNGA 1, MNGA 2 and SM 985-9) were harvested in 1998 and 1999 at CIAT, Cali, Colombia. The cultivars MCOL 22 and SM 985-9 show high susceptibility to PPD, MNGA 1 and MNGA 2 medium, and CM 7033-3, MBRA 337 and MDOM 5 low, according to CIAT (M Bonierbale, pers. comm.). Additionally, roots from plants grown in the tropical glasshouses at the University of Bath, UK, were used.

Storage and extraction of root tubers

All roots were cut transversely into 2-cm thick slices and stored for 7 d under controlled conditions (dark, 29°C, 80–90 % relative humidity) as described by Sakai and Nakagawa (1988). One slice per day from each cultivar was homogenized in absolute ethanol (Rathburn, UK) by means of a blender (1 g f. wt in 10 ml EtOH). The extracts were filtered through paper (Whatman, No. 1), evaporated to an end volume of 3 ml and stored until use at −20°C.

Chromatographic analysis and spectroscopy

For high performance liquid chromatography (HPLC), a system (Gilson) combined with a diode array detector (Hewlett Packard) and an analytical reversed phase column (Techsphere ODS-BDS, 250 × 4·6 mm, 5 µm, HPLC Technology, UK) was used. The best separation of compounds was achieved by using 0·5 % aqueous H_3PO_4 in a gradient of 2–100 % acetonitrile (Rathburn, UK) over 55 min and under a constant flow of 1·3 ml min^{-1}. Compounds were detected simultaneously at 215, 280 and 350 nm. The compounds were identified by their retention times, by co-chromatography with identical references (caffeic acid, esculin, esculetin, ferrulic acid, p-coumaric acid and scopoletin, Sigma; scopolin kindly provided by Dr Goro Taguchi) and by UV- and mass-spectroscopy. Quantification was performed on HPLC by using these reference compounds as external standards. LC-MS experiments were performed on a Finnigan TSQ 700 under atmosphere pressure chemical ionization (APCI), as described elsewhere (Renukappa *et al*., 1999).

For thin layer chromatography (TLC), the aliquots (corresponding to 0·1 g f. wt) of ethanolic extracts were spotted onto HPTLC plates (silica gel 60 F254, 20 × 20, Merck) and developed in a liquid phase of chloroform : ethyl acetate : methanol (2 : 2 : 1). The separated compounds were detected under a UV lamp (Camag) at 254 and 366 nm.

Enzyme assays

The enzyme assays for peroxidases were based on van den Berg *et al*. (1983) and van Gestelen *et al*. (1998) except that 5 g of root tissue was homogenized together with 0·2 g polyvinyl poly-pyrrolidone in 10 ml of extraction buffer (potassium phosphate buffer, 50 mM, pH 7·0; 1 mM Na-EDTA) and 1 mM DTT. The reaction mixture contained 50 mM potassium phosphate buffer (pH 6·0), 10 mM 3,5-dichoro-2-hydroxy-benzenesulfonic acid, 1 mM 4-aminoantipyrine, 10 mM H_2O_2 and 100 µl enzyme extract in a total volume of 3 ml. The reaction was started by adding H_2O_2, and the change in absorbance at 510 nm was measured using a Beckman spectrophotometer.

Light and fluorescence microscopy

Hand-cut cross sections of root tubers at different stages of storage were transferred and mounted in glycerol/water (1 : 1; v/v). The samples were examined with a Aristoplan microscope (Leitz), using either bright-field illumination or fluorescence excitation at 340–380 nm (Leitz filter combination A) or excitation at 355–424 nm (Leitz filter combination D). Staining for neutral fats and fatty acids was carried out with Sudan III (Merck) according to Nielsen (1973), and for lignin with phloroglucinol according to Sass (1968). For the induction of secondary fluorescence of flavonoids, a 1 % methanolic diphenyl boric acid 2-aminoethylester solution was used for localization, according to Hutzler *et al*. (1998). After 15 min the polyphenols can be detected by their intense flourescence by using the Leitz filter combination A.

Statistical analysis

The data sets were processed using regression analysis by means of the software package Minitab (Minitab, 1998).

RESULTS

Visible and microscopic observations

Visible observations (daylight and at 366 nm) confirmed the onset and progress of vascular streaking or PPD in the root slices (Fig. 1A–C). The cassava cultivars analysed showed different susceptibilities to PPD. In root slices of CM 7033-3, MCOL 22, MNGA 1, MNGA 2 and SM 985-9, first signs of PPD were obvious after 24 h, while the cultivars MBRA 337 and MDOM 5 were less susceptible and showed signs of PPD after 48 h (data not shown). The high PPD susceptibility that we observed with MNGA 1, MNGA 2 and CM 7033-3 contrasted with those given at CIAT, where these cultivars were scored as low, medium and medium, respectively (M Bonierbale, pers. comm.). However, the variability in the PPD responses that we observed was very high in individual roots and not dependent on whether the plants were grown at CIAT or Bath. This variability in PPD response within cultivars has been observed by others (M Fregene, pers. comm.). Because of this the quantification of secondary metabolites was performed with extracts from four roots from different plants of the same cultivar.

Microscopic observations of cross- and longitudinal-sections of the root tubers confirmed that in an early stage of PPD tylose formation and brownish occlusions were visible in the xylem vessels (Fig. 1D), and that after some time these occlusions spread into the xylem and storage parenchyma. Approximately 6 to 12 h after harvest, the first signs of blue fluorescence were detected around the xylem vessels, by means of fluorescence microscopy (Fig. 1E), whereas almost no fluorescence was visible directly after harvest (image not shown). After 48 h the fluorescence was detected all over the vascular tissue and in the parenchyma (Fig. 1F). Examination of roots stored for over 6 d showed a decrease in fluorescence as well as an increase of visible dark black-brown colouration of all tissues.

Identification of hydroxycoumarins

For the identification of secondary metabolites in cassava root extracts, we used spectroscopic methods (UV, MS) directly combined with liquid chromatography (LC), as described elsewhere (Wolfender *et al.*, 1995; Sumner *et al.*, 1996; Vogler *et al.*, 1998; Renukappa *et al.*, 1999). This analysis resulted in the detection of the four hydroxycoumarins, esculetin, esculin, scopoletin and scopolin (Fig. 2) in all the cassava cultivars examined (spectroscopic data given in Table 1).

Besides HPLC, thin layer chromatography was used. This resulted in the detection of many UV-absorbing bands, of which only four showed blue auto-fluorescence at 254 and 366 nm. The retention factors of these four bands were identical to the hydroxycoumarin reference compounds esculin, esculetin, scopolin and scopoletin.

Quantification of esculetin, esculin, scopoletin and scopolin

The HPLC parameters used, as well as the UV-detection at 350 nm, resulted in good identifiable and separated peaks of all four hydroxycoumarins. As a result it was possible to quantify these compounds by means of identical reference compounds. The typical quantitative development of these hydroxycoumarins during the first 6 d of storage of cassava root slices (data given for MNGA 2, which we found to be highly susceptible) is illustrated in Fig. 3. Directly after harvest, only small amounts of scopolin, esculetin and scopoletin were detectable (Fig. 3A). After a storage time of 3 d, scopolin and scopoletin increased dramatically, whereas esculin and esculetin showed only a small increase (Fig. 3B). The amounts of esculin and esculetin remained at a low level even after 6 d of storage, but the amounts of scopolin and scopoletin started to decrease (Fig. 3C).

The results of the quantification of scopolin and scopoletin from four different cassava root tubers (highly susceptible cultivar MCOL 22) over a storage period of 7 d are summarized in Fig. 4. Even though these results show high individual quantitative differences between the roots, which reflect the visual and microscopic observations on PPD responses, it reveals certain trends. Scopoletin shows a rapid accumulation during the first 24 to 48 h, followed by a decline, with a second smaller increase at 4 to 6 d. In all cases, the glycoside (scopolin) accumulates with a lag of 1 or 2 d after the aglycone (scopoletin).

Generally, these descriptions reflect the accumulation of the hydroxycoumarins in all the cassava cultivars examined (Table 2). All fresh harvested roots contained these four compounds in detectable amounts. During at least one stage of the storage time the coumarins accumulated dramatically, reaching 221 nmol g^{-1} f. wt in some cases. There were obvious differences between the cultivars, but these differences do not clearly correlate with the cultivar's susceptibility towards PPD. The root tubers of all cultivars accumulated scopoletin, scopolin and esculetin during the first 24 to 48 h of storage, followed by a more or less rapid

TABLE 1. *Mass- and UV-spectroscopic data of the identified hydroxycoumarins of cassava root tubers*

Compound	Molecular formula	Mass $[M + H]^+$ (rel. intensity)	Fragments (rel. intensity)	UV_{max} (50 % CH_3CN)
Esculin	$C_{15}H_{16}O_9$	341 (55)	227 (100), 179 (18), 147 (10)	224, 244, 292, 330
Esculetin	$C_9H_6O_4$	179 (70)	163 (100), 131 (45)	226, 252, 288, 338
Scopolin	$C_{16}H_{18}O_9$	355 (10)	193 (100)	202, 226, 246, 254, 288, 336
Scopoletin	$C_{10}H_8O_4$	193 (100)	163 (5)	202, 226, 256, 294, 342

Ion-Source = APCI + Q1MS; LC-Gradient = 2–65 % CH_3CN in 55 min.

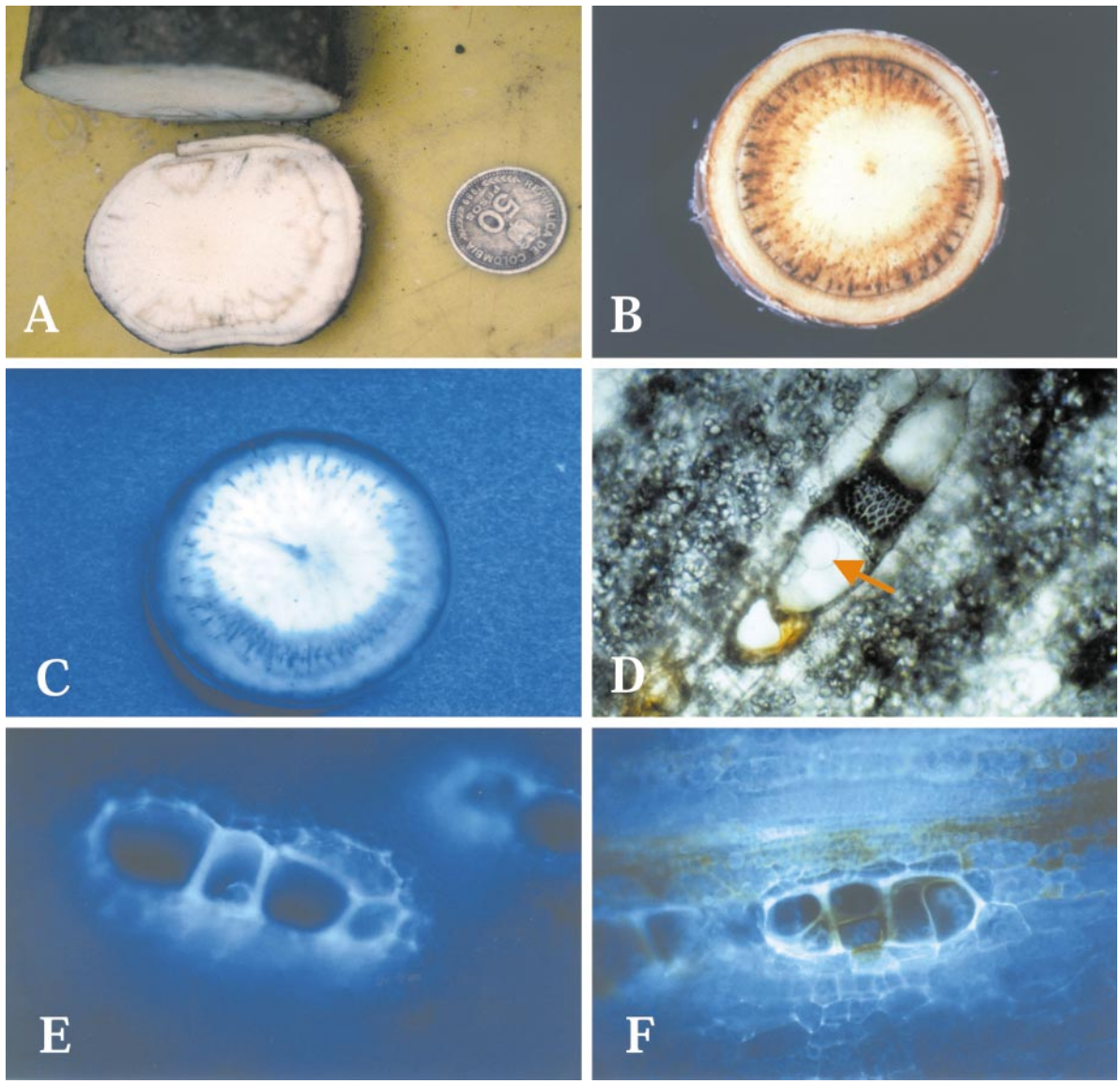


FIG. 1. Aspects of post-harvest physiological deterioration in tuberous roots of cassava. A. Cross-section of cassava (MCOL 22, highly susceptible) stored for 24 h. B, Cross-section of MCOL 22 after 4 d of storage. C, Same as B but under UV light (366 nm). D, Light microscopic image (×400) of a cross-section of a cassava root tuber (MCOL 22) after 2 d of storage [brown occlusions on the cell walls of the xylem vessels and tyloses (arrow)]. E, Fluorescence microscopic image (×400) of a cross-section of a cassava root tuber (MCOL 22) after 1 d of storage. F, Fluorescence microscopic image (×400) of a cross-section of a cassava root tuber (MCOL 22) after 4 d of storage.

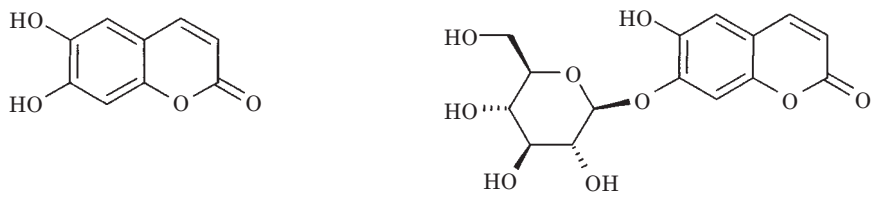
decline. In contrast, the concentration of esculin did not increase that dramatically. However, the most obvious difference between cultivars of high and low susceptibility is the second accumulation of scopoletin after 5 to 6 d. Both low susceptible cultivars, MBRA 337 and MDOM 5, accumulate high concentrations, whereas the other cultivars accumulate only small amounts or do not show any increase at all. After a storage time of 6 to 10 d the content of all hydroxycoumarins decreased until, after further storage (up to 14 d), they were nearly undetectable (data not shown).

During the first 6 d of storage, the accumulation of the aglycone (esculetin, scopoletin) is followed after a short lag by the accumulation of the glycoside (esculin, scopolin) in most of the investigated cultivars.

The activities of peroxidases over the storage period of 7 d are summarized in Fig. 5 for the cultivar MNGA 2, together with the corresponding scopoletin concentrations. Small changes of activity are visible during the first 24 to 48 h, whereas after 4 d there is a steady increase in activity. With this increase, there is a simultaneous decrease in the scopoletin concentration (Fig. 5), as well as increasing black-blue colouration of the vascular and parenchymatic tissue (data not shown).

DISCUSSION

Cytological and histological investigations of cassava root tissues confirmed the results described by other authors



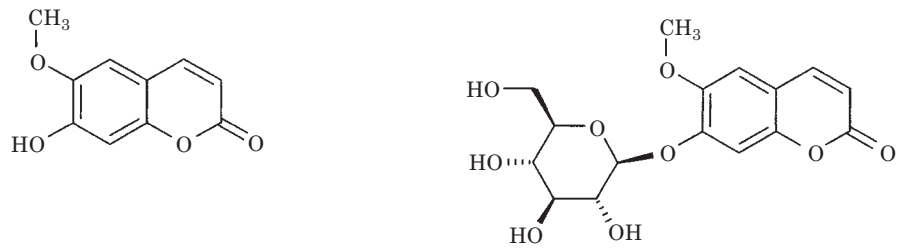


FIG. 2. Hydroxycoumarins identified from cassava root extracts.

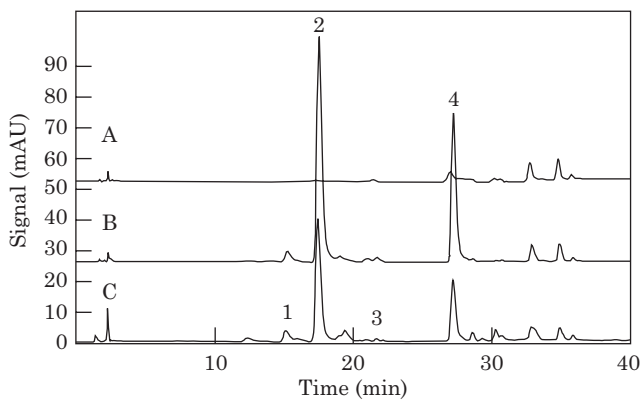


FIG. 3. HPLC chromatograms (detection wavelength 350 nm) of three cassava root extracts (cultivar MNGA 2) showing the four peaks identified as hydroxycoumarins (1 = esculin, 2 = scopolin, 3 = esculetin, 4 = scopoletin). A is based on a root extract prepared directly after harvest, B is based on an extract taken from a root slice stored for 3 d, C is based on an extract taken from a root slice stored for 6 d. In all three chromatograms the injected extract is derived from 0·1 g f. wt.

(Rickard and Gahan, 1983) regarding the formation of occlusions on the cell walls of xylem vessels, the occurrence of tyloses and, subsequently, the browning of the parenchymatic tissue. Investigation by fluorescence microscopy revealed the accumulation of blue fluorescent compounds, which were first visible in the cell walls of the xylem tissue but then spread all over the root tissues (except the periderm). This accumulation of blue fluorescent compounds is similar to reactions described for cassava stem tissue after infection with *Xanthomonas axonopodis* pv. *manihotis* (Kpemoua *et al.*, 1996).

These fluorescent compounds were identified as hydroxycoumarins, three of which (scopolin, esculin and

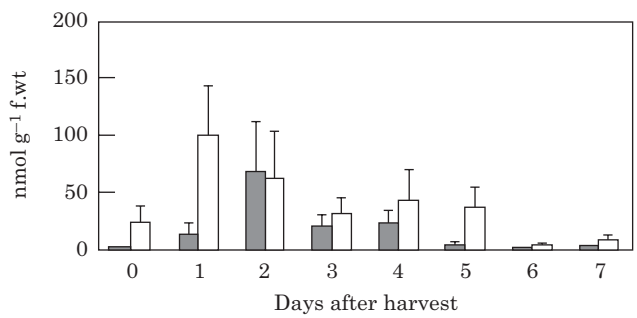


FIG. 4. Quantification of scopolin (■) and scopoletin (□) accumulation in cassava root (cultivar MCOL 22) slices that had been stored for up to 7 d. The columns represent the mean of four different root tubers taken from different plants. Bars = s.d.

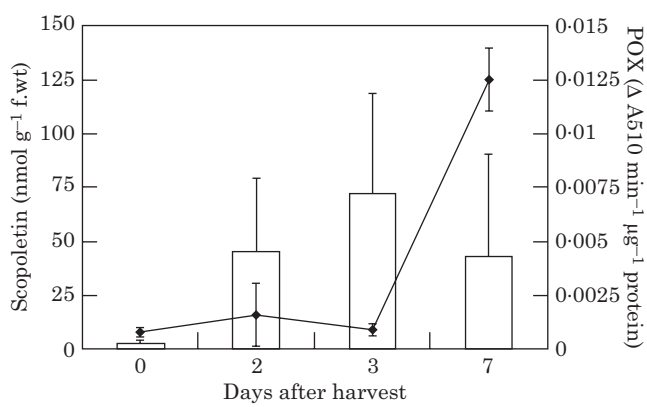


FIG. 5. Scopoletin concentration (□) and peroxidase (POX) activity (—◆—) of corresponding cassava root tubers (MNGA 2) over a storage time of 7 d. Columns and line represent the results obtained from three different roots (POX mean ± s.d.).

TABLE 2. *Quantification of hydroxycoumarins (nmol g^{-1} f. wt) in cassava root tubers over a storage time of 6 d*

	S. Cv.	High CM 7033-3	Low MBRA 337	High MCOL 22	Low MDOM 5	High MNGA 1	High MNGA 2	High SM 985-9
Compound	DAH							
Esculin	0	0·23 ± 0·20	0·44 ± 0·25	2·58 ± 1·70	1·37 ± 1·34	1·03 ± 0·95	2·04 ± 2·12	0·22 ± 0·16
	2	6·74 ± 8·07	7·58 ± 4·16	5·30 ± 3·65	30·49 ± 35·64	1·19 ± 0·83	2·88 ± 1·40	2·08 ± 3·27
	4	6·75 ± 3·38	4·97 ± 4·68	4·33 ± 3·74	16·25 ± 14·83	2·91 ± 1·25	3·44 ± 1·71	1·85 ± 2·57
	6	5·87 ± 3·52	15·42 ± 14·65	0·77 ± 0·27	45·86 ± 44·68	2·52 ± 1·83	3·08 ± 2·18	34·08 ± 43·79
Esculetin	0	4·75 ± 4·79	4·43 ± 6·04	3·43 ± 2·38	6·03 ± 3·42	3·90 ± 1·33	3·64 ± 2·38	15·37 ± 22·91
	2	16·94 ± 26·33	11·01 ± 13·63	6·39 ± 4·53	4·70 ± 0·73	2·86 ± 1·64	1·71 ± 1·19	13·43 ± 13·46
	4	7·45 ± 4·90	24·78 ± 19·53	3·46 ± 2·86	10·09 ± 5·84	6·61 ± 3·38	2·96 ± 4·16	32·87 ± 38·54
	6	11·32 ± 15·44	17·92 ± 9·20	1·87 ± 1·51	15·26 ± 8·79	3·84 ± 1·63	5·19 ± 1·35	12·95 ± 8·39
Scopolin	0	4·38 ± 5·49	1·89 ± 1·24	1·81 ± 1·10	12·49 ± 10·17	4·31 ± 2·07	13·96 ± 7·47	7·49 ± 6·53
	2	29·98 ± 15·65	10·65 ± 10·51	68·68 ± 44·96	6·74 ± 5·51	16·24 ± 11·72	66·86 ± 36·70	21·58 ± 19·16
	4	58·89 ± 41·80	24·97 ± 22·84	23·26 ± 11·02	87·38 ± 71·35	27·99 ± 33·57	79·99 ± 36·42	4·06 ± 2·41
	6	35·71 ± 12·16	101·05 ± 56·27	1·50 ± 1·00	23·54 ± 19·22	15·24 ± 14·92	48·13 ± 53·27	12·37 ± 12·06
Scopoletin	0	10·37 ± 9·88	20·23 ± 12·17	24·51 ± 14·30	45·09 ± 42·21	20·64 ± 13·74	28·38 ± 21·19	28·30 ± 28·75
	2	64·98 ± 85·15	21·53 ± 2·48	62·87 ± 41·84	90·29 ± 85·48	53·02 ± 11·19	43·24 ± 9·33	16·94 ± 17·26
	4	44·85 ± 37·92	25·58 ± 8·07	43·33 ± 27·65	49·98 ± 34·92	25·23 ± 8·96	15·67 ± 8·72	15·34 ± 2·27
	6	42·61 ± 41·37	101·16 ± 62·81	4·67 ± 1·51	123·94 ± 73·36	38·39 ± 26·70	34·12 ± 20·11	51·80 ± 39·03

Cv, cultivar; DAH, days after harvest; S, susceptibility to PPD; mean ± s.d.; $n = 4$.

scopoletin) had been previously described as occurring in cassava roots (Tanaka *et al*., 1983), whereas esculetin is described here for the first time. It is not surprising to find the aglycone of esculin in the plant tissues, as well as esculin itself. Rodriguez *et al*. (2000) examined possible functions of these compounds, and showed that scopoletin isolated from cassava had anti-microbial activities against a variety of different fungal and bacterial organisms, whereas esculin, scopolin and esculetin did not.

The results of fluorescence microscopy showed that the early increase of hydroxycoumarins (after 1 d) is due to their accumulation in the cell walls of the xylem vessels (Fig. 1E), whereas in later stages they accumulate particularly in the parenchymatic cells (Fig. 1F).

The cassava cultivars investigated were evaluated in terms of their PPD susceptibility by observation, histology and chemical analysis. This revealed a high variability between the tuberous roots of even the same cultivar, and this contributes to the high standard deviations for all measurements (Table 1). This great variability in PPD scores and the major influence of environmental parameters on the PPD response has also been observed at CIAT (M Fregene and H Ceballos, pers. comm.).

In the cassava cultivars studied, esculetin and scopoletin accumulated in one or two different stages. After 24 to 48 h these compounds increased and then gradually decreased, and a second less pronounced accumulation was sometimes observed after 4 to 6 d. The first increase can be interpreted as a direct response to wounding, whereas the second may be a defensive response to the invasion of micro-organisms. Observable differences between the cassava cultivars were based on timing and absolute amounts of initial coumarin accumulation, especially of scopoletin, and on the timing of the secondary accumulation. These results do not clearly correlate with differences in susceptibility of these cultivars towards PPD. However, there seems to be a relationship between susceptibility towards PPD and the absolute amount of scopoletin accumulation after 5 to 6 d, even if this can not be related to the PPD process itself. More cassava cultivars together with a larger number of roots, need to be investigated in order to determine whether this is a significant correlation. The correlation of hydroxycoumarin accumulation, fluorescence, 'vascular streaking' and PPD susceptibility could be a useful and objective tool to plant breeders for the evaluation of cassava cultivars for their PPD response; however, it first needs to be proved in more extensive field tests.

The concentration of all four hydroxycoumarins declined after a storage time of about 1 week, until those compounds were barely detectable. This can only be explained by further metabolic transformations. Recent research on the biosynthesis and metabolism of hydroxycoumarins in sunflower (Gutierrez *et al*., 1995; Edwards *et al*., 1997) showed that specific peroxidases can metabolize scopoletin into an insoluble blue-black precipitate *in vitro* and *in vivo*. In sunflowers, this precipitate pigments the vascular tissue in the stem. This is very similar to, and could explain, the blue-black pigmentation observed as 'vascular streaking', the initial visible symptoms of PPD in cassava roots, and the relationship between peroxidase activity and scopoletin concentrations illustrated in Fig. 5. The assumption of the occurrence of a scopoletin-degrading and specific peroxidase in cassava is supported by initial *in vitro* experiments (based on Edwards *et al*., 1997), showing a high substrate specificity of the reaction towards H_2O_2 and scopoletin, and leading as well to a blue-black precipitate. These data support Wheatley and Schwabe's (1985) assumption that there is a correlation between post-harvest physiological deterioration, the increase in content of scopoletin and

H_2O_2 in the root tissues, and the high activity of peroxidases.

The biosynthesis of hydroxycoumarins is still under debate. For esculetin and scopoletin, a separate synthesis from caffeic acid and ferrulic acid, respectively, was suggested by Strack (1997). In contrast to this, Cabello-Hurtado *et al.* (1998) suggest a synthesis starting from caffeic acid to esculetin which then is modified to scopoletin. Dewick (1988) suggested umbelliferone to be a general precursor for the biosynthesis of all other coumarins. It seems that there are different possible pathways and that plants may have developed convergent pathways leading to scopoletin. Statistical analysis of the results on quantification of esculetin and scopoletin in cassava roots give a correlation ($R^2 > 83\,\%$) between these hydroxycoumarins, suggesting that their biosynthesis is linked.

Biological activities of scopoletin have been described, but the toxic principle is still unclear. Coumarins show inhibitory effects on enzymes and interactions with DNA (Zobel, 1997; Ojala *et al.*, 1999). Scopoletin is five-times more potent as an inhibitor of prostaglandin synthase than aspirin (Farah and Samuelsson, 1992). Prostaglandin biosynthesis in mammals is very similar to the biosynthesis of the wound-induced signalling compound, jasmonic acid, in plants (Wasternack *et al.*, 1998), and aspirin inhibits hydroperoxide dehydrase which catalyses the synthesis of prostaglandin-like intermediates in the octadecanoid pathway to jasmonic acid (Peña-Cortés *et al.*, 1993). This raises intriguing questions as to the potential role of scopoletin in plant wound-responses in general and PPD in particular. If scopoletin is toxic, how does the plant cell cope with it? Strack (1997) suggests that coumarins are glycosylated during their synthesis into a non-toxic glycoside, and then stored in the vacuole. In response to stress, this glycoside is then transported into the apoplast where it is deglycosylated by a β-glycosidase into the active compound. This would explain the blue fluorescence due to scopolin and scopoletin that was observed in the cell walls of the xylem tissue and inside the parenchymatic cells of deteriorated cassava roots (Fig. 1E and F). But it would not explain why the increase of scopolin is detected with a lag of 24 h after the increase of scopoletin. Even if there is a strong compartmentalization of coumarins in the cells, the high concentrations of scopoletin may harm the plant cell itself. However, the activation of a specific peroxidase may detoxify this coumarin by metabolizing it into an insoluble and non-toxic product, as suggested for *Hevea brasiliensis* by Breton *et al.* (1997).

The results presented here confirm that there is a relationship between the accumulation of scopoletin and the visible symptoms of PPD (vascular streaking). However, an understanding of the biosynthesis and regulation of coumarins in cassava is required to fully understand their involvement in post-harvest physiological deterioration of cassava.

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