Localization of phenols and polyphenol oxidase in 'Jewel' sweet potatoes (*Ipomoea batatas* 'Jewel')[1]

W. E. SCHADEL AND W. M. WALTER, JR.

United States Department of Agriculture, Science and Education Administration, Agricultural Research, Southern Region, and North Carolina Agricultural Research Service, Department of Food Science, North Carolina State University, Raleigh, NC, U.S.A. 27650

Received February 23, 1981

SCHADEL, W. E., and W. M. WALTER, JR. 1981. Localization of phenols and polyphenol oxidase in 'Jewel' sweet potatoes (*Ipomoea batatas* 'Jewel'). Can. J. Bot. **59**: 1961–1967.

Histochemical tests for phenols and polyphenol oxidase were performed on fresh root tissue of *Ipomoea batatas* (L.) Lam. 'Jewel.' The phenolic compounds were localized in the phellem, phellogen, and phelloderm, in approximately 1 mm (ca. 10–15 cells) of the tissue directly beneath the periderm, in the latex of laticifers, in the phloem, in the cambium which separates the secondary phloem from the secondary xylem, in the anomalous secondary cambia of the central core, in the parenchyma cells adjacent to the xylem elements, and in the walls of the xylem elements. Polyphenol oxidase was localized primarily in the phellogen and phelloderm and most prominently in the latex of laticifers.

SCHADEL, W. E., et W. M. WALTER, JR. 1981. Localization of phenols and polyphenol oxidase in 'Jewel' sweet potatoes (*Ipomoea batatas* 'Jewel'). Can. J. Bot. **59**: 1961–1967.

Des tests histochimiques pour les phénols et la polyphénol oxydase ont été effectués sur des tissus racinaires frais de l'*Ipomoea batatas* (L.) Lam. 'Jewel.' Les composés phénoliques sont localisés dans le phellème, le phellogène et le phelloderme; dans environ 1 mm (environ 10–15 cellules) du tissu situé immédiatement sous le périderme; dans le latex des canaux laticifères; dans le phloème; dans le cambium qui sépare le phloème secondaire du xylème secondaire; dans les cambiums secondaires anormaux de la partie centrale; dans les cellules parenchymateuses adjacentes aux éléments de xylème et dans les parois des éléments de xylème. La polyphénol oxydase se rencontre surtout dans le phellogène et le phelloderme et, de manière très prononcée, dans le latex des canaux laticifères.

[Traduit par le journal]

Introduction

Phenols and polyphenol oxidase (*o*-diphenol:oxygen oxidoreductase EC 1.10.3.1) in sweet potatoes (*Ipomoea batatas* (L.) Lam.) have been investigated previously for their involvement in wound response (McClure 1960; Hyodo and Uritani 1967; Tanaka and Uritani 1977) and in discoloration (Scott et al. 1944; Arthur and McLemore 1956; Buescher et al. 1975; Porter et al. 1976; Walter and Purcell 1980). The distribution of phenols in 'Jewel' sweet potato roots has recently been chemically quantitated (W. M. Walter and W. E. Schadel, submitted for publication). Histochemical tests for sweet potato phenolics (Tanaka and Uritani 1977) have shown that the red-colored nitroso derivatives of the phenols are localized in the periderm, cambium, and vascular bundles but not in parenchymatous cells containing many starch granules. Histochemical tests for polyphenol oxidase activity (Scott et al. 1944; Scott and Kattan 1957) have demonstrated the presence of the enzyme in the area of the periderm, cambium, and parenchyma adjacent to the xylem in some cultivars and throughout the entire root in other cultivars. These histochemical investigations of phenols and polyphenol oxidase have been macroscopic evaluations (with the unaided eye). However, microscopic evaluation of histochemical tests can provide more accurate anatomical localization than macroscopic evaluation.

The present study was undertaken to define the anatomical distribution of the phenols and polyphenol oxidase in sweet potato roots ('Jewel') using microscopic as well as macroscopic examination of histochemical tests. A more accurate understanding of this distribution will enhance future investigations of the role of phenols and polyphenol oxidase in wound response and discoloration of sweet potatoes.

Materials and methods

Sweet potatoes (*Ipomoea batatas* (L.) Lam. 'Jewel') were harvested in mid-October and cured for 8 days at 29°C, 85% relative humidity and then stored at 13°C, 85% relative humidity until used. Ten roots for qualitative histochemical

[1]Paper No. 6756 of the journal series of the North Carolina Agricultural Research Service, Raleigh, NC. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or North Carolina State University, nor does it imply approval to the exclusion of other products that may be suitable.

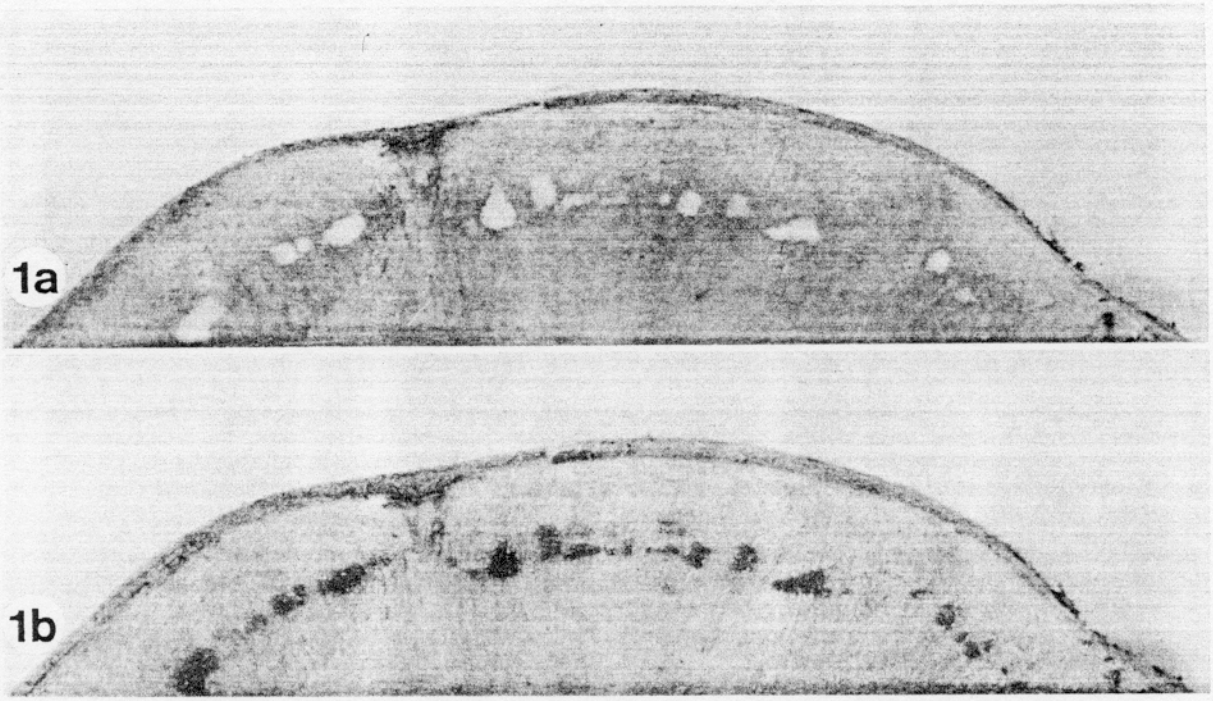


FIG. 1. (*a* and *b*) Cross section of 'Jewel' sweet potato root tissue showing the white appearance of the latex immediately after sectioning (*a*) and the distinct discoloration of the latex 1 h after sectioning (*b*).

tests were selected randomly from the stored roots, washed thoroughly with tap water, sectioned transversely and longitudinally at varying thicknesses with razor blades, and photographed following localization treatments for phenols and polyphenol oxidase activity as described below. Transverse and longitudinal sections of the same 10 roots were left untreated to serve as controls. These control sections were observed for a period of several hours to note any discoloration resulting from sectioning. The same 10 roots were analyzed quantitatively to verify the presence of large amounts of phenols localized by histochemical analysis in latex-containing tissue. Six of the 10 roots were randomly selected and also quantitatively analyzed to verify the large amount of polyphenol oxidase activity localized by histochemical analysis in latex-containing tissue.

Qualitative histochemical tests

Phenolic compounds were localized by a nitrosation histochemical method using nitrous acid, followed by application of aqueous sodium hydroxide (Reeve 1951). Chlorogenic and isochlorogenic acids, the principal phenolic compounds produced in sweet potato root tissue (Walter et al. 1979), reacted to form the same red-colored nitroso derivatives.

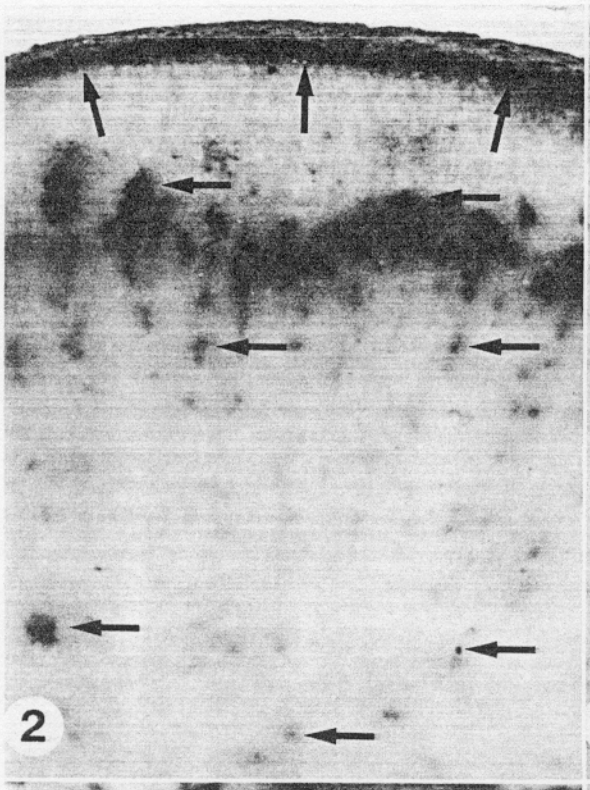
Organic acids which include the chlorogenic and isochlorogenic acids in the vacuoles of living root cells were localized by vital staining methods using neutral red and methylene blue (Bolay 1960).

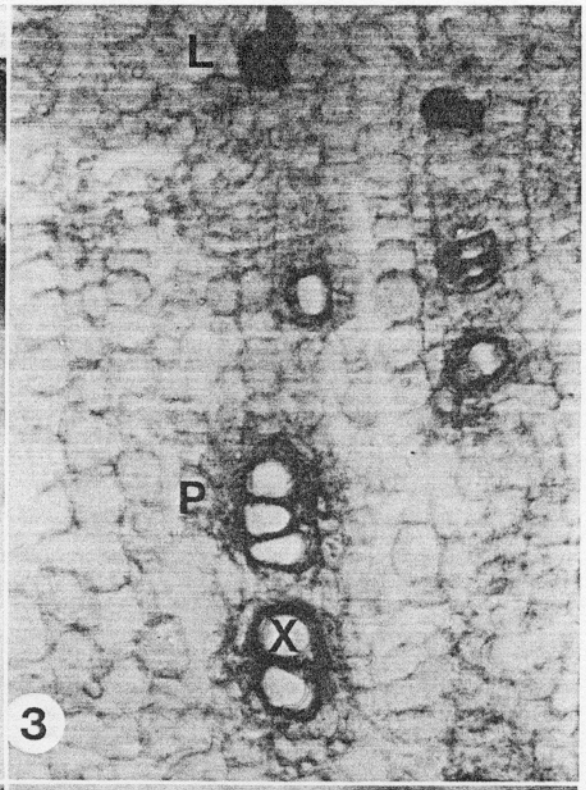
Polyphenol oxidase was localized using a 0.04 *M* aqueous solution of catechol as a substrate applied by lightly misting the root slices with a sprayer.

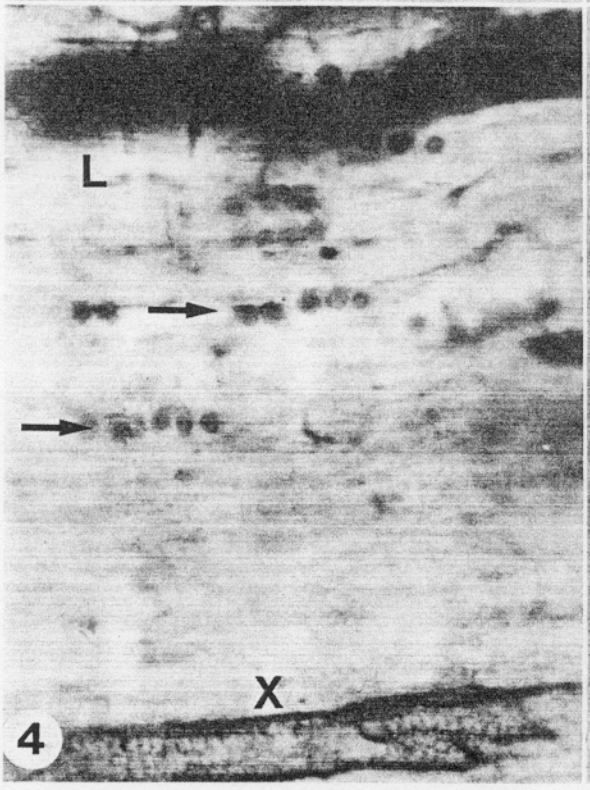
Quantitative analytical tests

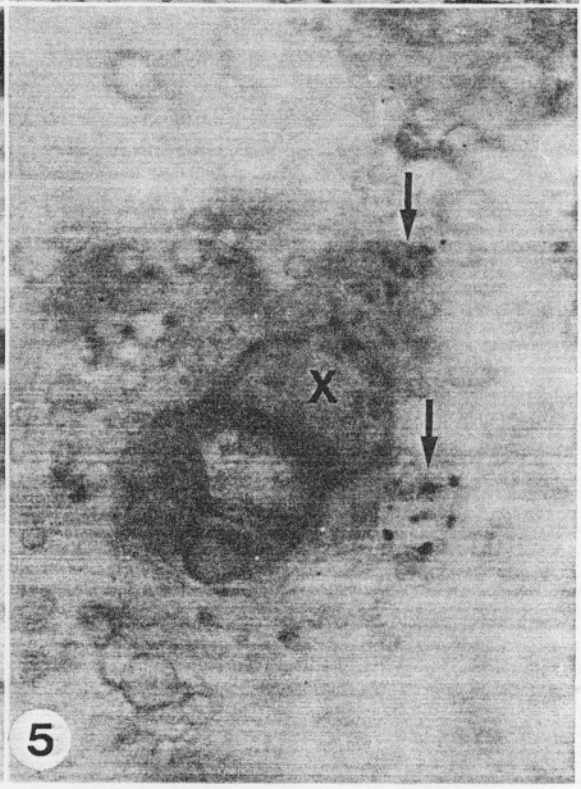
Prior to extraction and determination of phenolic compounds and polyphenol oxidase, root tissue was excised from midway between the proximal (stem) and distal (root) ends of the roots. This excised tissue was dissected into two groups: (1) secondary root tissue containing laticifers (tissue located approximately 3 mm to the interior of the periderm and immediately to the exterior of the cambium) and (2) secondary root tissue not containing latex (tissue located immediately to the interior of the cambium in which the xylem elements show a typical radial arrangement). Measurement of both poly-

FIGS. 2–5. Localization of phenolic compounds in root tissue of 'Jewel' sweet potato. Fig. 2. Dark nitroso derivatives of the phenolic compounds are present in this cross section (arrows). ×5. Fig. 3. Dark nitroso derivatives of the phenolic compounds are localized in the latex of cortical laticifers (L), in the parenchyma (P) adjacent to the xylem elements, and in the walls of the xylem elements (X) in this cross section. ×150. Fig. 4. Note the appearance of the dark nitroso derivatives in the latex and xylem elements in this longitudinal section. Numerous calcium oxalate crystals (arrows) are present in the layers of cells beneath the darkened latex. ×225. Fig. 5. Vital staining with neutral red shows the presence of organic acids in the vacuoles of parenchyma cells (arrows) adjacent to the xylem elements. ×560.


2


L
P
X
3


L
X
4


X
5

phenol oxidase activity and phenol levels was performed on tissue groups 1 and 2 as previously described (Walter and Purcell 1980).

Results

Qualitative histochemical tests

The transverse and longitudinal sections of sweet potato root tissue which served as controls for the histochemical tests demonstrated very distinct discoloration of the latex 1 h after sectioning (Figs. 1*a*, 1*b*). The nitrosation indicated that the red-colored nitroso derivatives of the phenolic compounds were localized in the phellem, phellogen, and phelloderm, in approximately 1 mm (ca. 10–15 cells) of the tissue directly beneath the periderm, in the latex of laticifers, in the phloem, in the cambium which separates the secondary phloem from the secondary xylem, in the parenchyma cells with few starch granules which are adjacent to the xylem elements, and in the walls of the xylem elements (Figs. 2, 3, 4). The phenols were not localized in starch-filled secondary phloem parenchyma cells exterior to the cambium or in starch-filled parenchyma cells of the central core.

The vital staining tests showed that the organic acids in the vacuoles (Fig. 5) of root cells were localized in the same areas as localized by the nitrosation of the phenols. The exceptions to this correlation were the phellem and the xylem elements which contain phenolic compounds in their walls but have no cellular contents at maturity that can absorb the vital stains.

Polyphenol oxidase was localized in the phellogen and phelloderm and most prominently in the latex of laticifers (Figs. 6, 7, 8, 9). Only a very small amount was observed by histochemical analysis in the parenchyma adjacent to the xylem elements.

Qualitative analytical tests

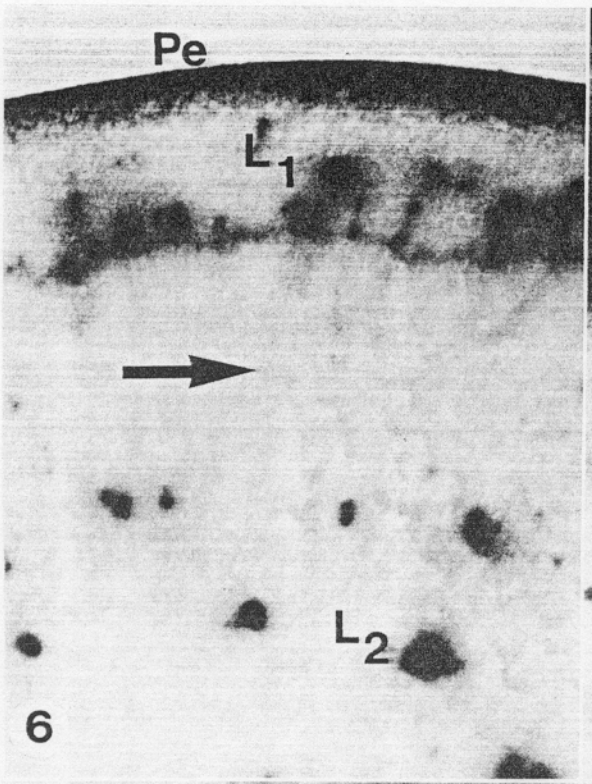
The secondary root tissue in which numerous laticifers are located (zone 1, Fig. 10) had a significantly greater amount of phenols and higher polyphenol oxidase activity than the nonlaticifer tissue (zone 2, Fig. 10; Table 1).

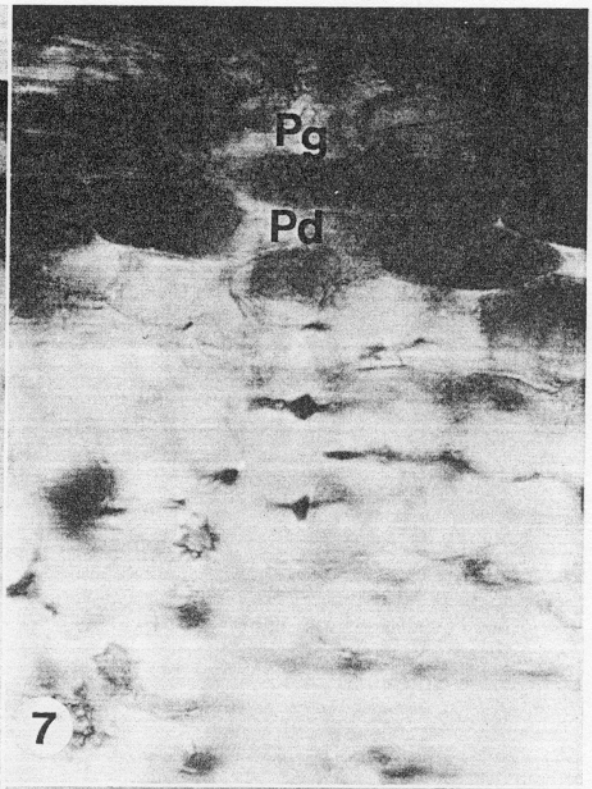
Discussion

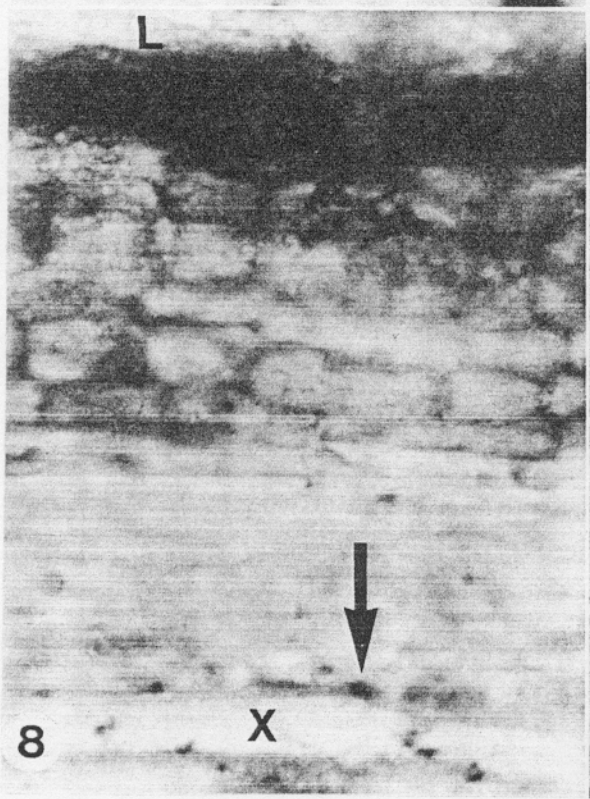
According to Walter and Purcell (1980), the majority of sweet potato phenolics are the chlorogenic acids which are esters formed between quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) and the *o*-dihydroxyphenol, caffeic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid). McClure (1960) postulates the involvement of chlorogenic acids in the suberization of sweet potato wounds, since chlorogenic acid accumulation is one of the first responses to occur at a wound and because of the high correlation between the amount of chlorogenic acid and amount of suberization. Furthermore, chlorogenic acids and other phenolics which are oxidized by polyphenol oxidase are known to result in discoloration of plant products (Scott et al. 1944; Arthur and McLemore 1956; Buescher et al. 1975). The *o*-dihydroxyphenols are oxidized by polyphenol oxidase to the corresponding *o*-quinones resulting in discoloration of plant tissue.

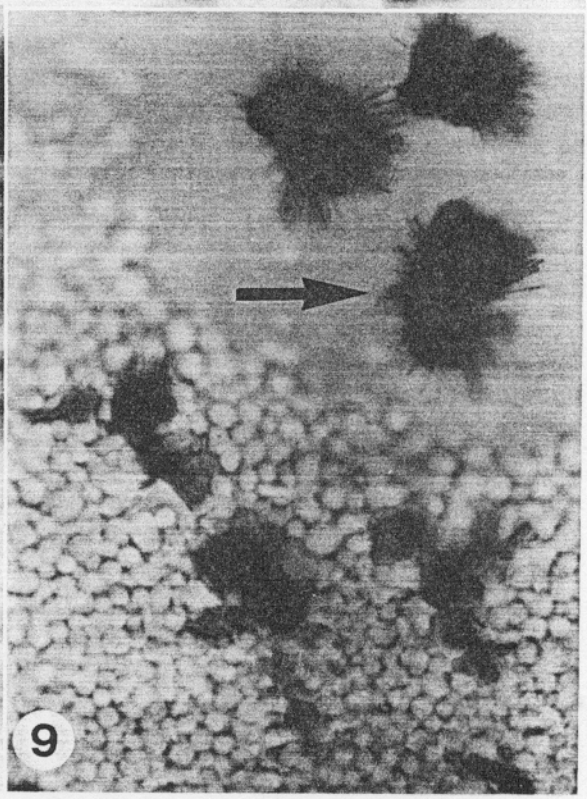
Polyphenol oxidase activity has previously been demonstrated in the latex of the rubber tree, *Hevea brasiliensis* (Kunth), Müll. Arg. (Coupé et al. 1972; Brzozowska-Hanower et al. 1978), and in the latex of the opium poppy, *Papaver somniferum* L. var. Halle (Roberts 1971, 1974). In *Ipomoea batatas* (L.) Lam. 'Jewel,' the polyphenol oxidase and phenolic substrates are both present but do not react in the latex of intact laticifers. If, however, the laticifers' cellular organization and compartmentalization are disrupted, the enzyme and substrate interact. This subsequently leads to the discoloration of the latex by reactive quinones which polymerize to form brown reaction products. Artschwager (1924) and Hayward (1938) observed that the vertical rows of laticiferous cells which form the latex vessels in sweet potatoes retain their transverse walls. They differ in this respect from plants having a latex system in which a continuous tube is formed by the resorption of the end walls. The prominent, articulated, nonanastomosing latex vessels may be recognized in young tissue by their large size and by their white, viscid contents. In older tissue, they are less prominent when viewed in cross section, since the cells of the tissue surrounding them are of equal size or larger. When examined in longitudinal sections, however, the latex vessels may be two or three times the length of adjacent parenchyma cells which are isodiametric.

FIGS. 6–9. Localization of polyphenol oxidase activity using catechol as a substrate in the root tissue of 'Jewel' sweet potato. Fig. 6. The dark enzyme reaction product is present in this cross section primarily in the area of the periderm (Pe) and in the latex of secondary phloem laticifers (L_1) and laticifers associated with the numerous anomalous secondary cambia (L_2). The reaction product is almost completely absent in the tissue (arrow) internal to the cambium in which the radially arranged xylem elements are located. ×5. Fig. 7. The dark enzymic reaction product demonstrating the presence of polyphenol oxidase is localized in this cross section in the phellogen (Pg) and phelloderm (Pd) of the periderm. ×300. Fig. 8. Polyphenol oxidase activity is evident in the dark latex of the laticifers (L) of this longitudinal section of root tissue. Only a very small amount of activity is histochemically localized (arrow) near the xylem elements (X). ×225. Fig. 9. The interaction between the enzyme and the applied catechol substrate is demonstrated in a drop of latex on a microscope slide to show the formation of the dark reaction product (arrow). ×1500.


Pe
L_1
L_2
6


Pg
Pd
7


L
X
8


9

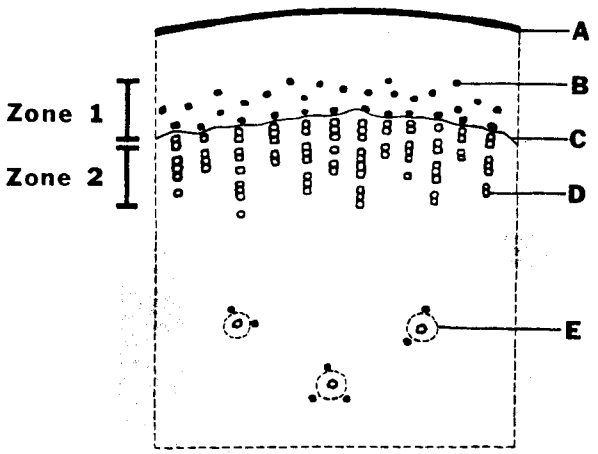


FIG. 10. Diagram of a partial transverse section of sweet potato root. Zone 1, secondary root tissue containing numerous laticifers; zone 2, secondary root tissue internal to the cambium in which the xylem elements have a radial arrangement. A, periderm; B, laticifer; C, cambium; D, xylem element; E, anomalous secondary cambium.

TABLE 1. Phenolic content and polyphenol oxidase (PPO) activity in tissue with latex and tissue without latex from 'Jewel' sweet potatoes

	Phenol content[a,c]	PPO activity[b,c]
Tissue with latex[d]	0.436 (±0.091)	5.22 (±1.26)
Tissue without latex[e]	0.249 (±0.056)	0.67 (±0.19)

[a]Milligrams chlorogenic acid per gram of tissue (fresh weight). Mean (and standard deviation) of 10 roots.

[b]ΔA_{450} per minute per gram of tissue (fresh weight). Mean (and standard deviation) of six roots.

[c]Means in vertical columns are significantly different at $P < 0.001$.

[d]Secondary root tissue containing numerous laticifers which is located external to the cambium.

[e]Secondary root tissue not containing laticifers which is located internal to the cambium.

The vital staining with neutral red and methylene blue demonstrates the presence of cellular vacuoles containing organic acids, such as phenolics, which retain basic dyestuffs (Bolay 1960; Bancher and Hölzl 1960; Politis 1964; Levitt 1974; Wernicke and Kohlenbach 1976). We found that the readily identifiable stained vacuoles were in the same cells that were shown to contain phenols by nitrosation. Although vital staining alone does not confirm the presence of phenolic substances, it is significant that the distribution of phenols as localized by nitrosation was similar to the distribution of organic acid localized by the vital staining.

The quantitative analytical results for latex-containing secondary root tissue and secondary root tissue not containing latex agree with the histochemical tests which show that the phenolic substrates and polyphenol oxidase are highly concentrated in the latex of laticifers. Reeve et al. (1969) emphasize that histochemical results may be very different from the results obtained with analytical methods and that the intensity of histochemical color reactions may be affected by the size and structure of cell types. This pertains to results that structurally can be compared only on a volumetric basis as amounts of tissue involved in a quantitative analysis. Without sufficient recognition of this aspect of histochemistry, comparisons with analytical results can have little meaning.

Further investigations of the laticifers and the phenolic and polyphenol oxidase content of the latex are warranted to discern the importance of the role of latex and its contents to wound response and discoloration in sweet potatoes.

ARTHUR, J. C., JR., and T. A. MCLEMORE. 1956. Properties of polyphenolases causing discoloration of sweet potatoes during processing. J. Agric. Food Chem. **4**: 553–555.

ARSTSCHWAGER, E. 1924. On the anatomy of the sweet potato root, with notes on the internal breakdown. J. Agric. Res. (Washington, D.C.), **27**: 157–166.

BANCHER, E., and J. HÖLZL. 1960. Chlorogensäure in Zellsäft der Knolle von *Solanum tuberosum*. Mikroskopie, **15**: 210–218.

BOLAY, E. 1960. Die Vitalfärbung voller Zellsäfte und ihre cytochemische Interpretation. Sitzungsber. Oesterr. Akad. Wiss. Math. Naturwiss. Kl. Abt. 1, **169**: 269–317.

BRZOZOWSKA-HANOWER, J., P. HANOWER, and C. LIORET. 1978. Etude du mécanisme de la coagulation de latex d'*Hevea brasiliensis* (Kunth) Mull. Arg. II. Systèmes enzymatiques impliqués dans le processus I. Phénol oxydases. Physiol. Veg. **16**: 231–254.

BUESCHER, R. W., W. A. SISTRUNK, and P. L. BRADY. 1975. Effects of ethylene on metabolic and quality attributes in sweet potato roots. J. Food Sci. **40**: 1018–1020.

COUPÉ, M., S. PUJARNISCLE, and J. D'AUZAC. 1972. Compartimentation de diverses oxydoréductases (peroxydase, *o*-diphénol oxydase et malate déshydrogénase) dans le latex d'*Hevea brasiliensis* (Kunth) Mull. Arg. Physiol. Veg. **10**: 459–480.

HAYWARD, H. E. 1938. Convolvulaceae. *Ipomoea batatus*. *In* The structure of economic plants. The MacMillan Company, New York. pp. 485–513.

HYODO, H., and I. URITANI. 1967. Properties of polyphenol oxidases produced in sweet potato tissue after wounding. Arch. Biochem. Biophys. **122**: 299–309.

LEVITT, J. 1974. Introduction to plant physiology. Mosby Company, St. Louis, MO. pp. 14–15, 49–50, 89–90.

MCCLURE, T. T. 1960. Chlorogenic acid accumulation and wound healing in sweet potato roots. Am. J. Bot. **47**: 277–280.

POLITIS, J. 1964. Untersuchung über die cytologische Bildung der chlorogensäuren bei einigen Scrophulariaceen und Convolvulaceen. Flora (Jena), **155**: 3–9.

PORTER, W. C., D. M. PHARR, L. J. KUSHMAN, and D. T. POPE. 1976. Discoloration of chilled sweet potato (*Ipomoea*

batatas (L.) Lam.) roots: factors related to cultivar differences. J. Food. Sci. **41**: 938–941.

REEVE, R. M. 1951. Histochemical tests for polyphenols in plant tissues. Stain Technol. **26**: 91–96.

REEVE, R. M., E. HAUTALA, and M. L. WEAVER. 1969. Anatomy and compositional variation within potatoes. II. Phenolics, enzymes, and other minor components. Am. Potato J. **46**: 374–386.

ROBERTS, M. F. 1971. Polyphenolases in the 1000 g fraction of *Papaver somniferum* latex. Phytochemistry, **10**: 3021–3027.

——— 1974. Oxidation of tyrosine by *Papaver somniferum* latex. Phytochemistry, **13**: 119–123.

SCOTT, L. E., C. O. APPLEMAN, and M. WILSON. 1944. The discoloration of sweet potatoes during preparation for processing and the oxidase in the root. Md. Agric. Exp. Stn. Bull. A33. pp. 11–26.

SCOTT, L. E., and A. A. KATTAN. 1957. Varietal differences in the catechol oxidase content of the sweet potato root. Proc. Am. Soc. Hort. Sci. **69**: 436–442.

TANAKA, Y., and I. URITANI. 1977. Polarity of production of polyphenols and development of various enzyme activities in cut-injured sweet potato root tissue. Plant Physiol. **60**: 563–566.

WALTER, W. M., JR., and A. E. PURCELL. 1980. Effect of substrate levels and polyphenol oxidase activity on darkening in sweet potato cultivars. J. Agric. Food Chem. **28**: 941–944.

WALTER, W. M., JR., A. E. PURCELL, and G. K. MCCOLLUM. 1979. Use of high-pressure liquid chromatography for analysis of sweet potato phenolics. J. Agric. Food Chem. **27**: 938–941.

WERNICKE, W., and H. W. KOHLENBACH. 1976. Akkumulation von Chlorogensäure in isolierten Mesophyllzellen von *Ipomoea batatas* Poir. Z. Pflanzenphysiol. **77**: 464–470.