Literature Review on Wound Healing in Root and Tuber Crops (with a special focus on sweet potato)

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Contents

I The general process of wound healing

The general processes of wound healing have been described in various reviews (Davies, 1987; Kahl, 1982;1983). Specific details of wound healing in root and tuber crops have been given for potatoes e.g. (Roensen and Karlsen, 1978; Thompson *et al*., 1995), in sweet potatoes (Artschwager and Starrett, 1931; Morris and Mann, 1955; Oirschot van, 2000) and other root crops e.g. in yam (Passam *et al*., 1976a and b; Ikediobi *et al*., 1989).

Wound healing is markedly influenced by moisture availability, temperature and oxygen tension. Optimal conditions for potato wound healing have been investigated by a number of researchers (Morris *et al*., 1989; Wigginton, 1974). Suberisation and wound periderm formation in sweet potatoes is most rapid at temperatures between 27^{o} and 34.5^{o}C and at a relative humidity of 90% (Artschwager and Starrett, 1931; Nielsen and Johnson, 1974). Apple twigs with 83% moisture content (m.c.) failed to wound heal at 12^{o}C. Lignification and periderm formation accelerated as the temperature was increased from 16 to 24^{o}C. Healing slowed down if the m.c. of the twigs was below 80 % or if the trees were heavily fruiting (Chen *et al*., 1982).

Potato tissues become responsive to gibberellins after wounding leading to enhanced RNA synthesis (Wielgat and Kahl, 1979). Wounded shoots of fruit trees produced wound hormones and attracted a high supply of sugars which transformed into proteins, cellulose and other compounds (Feucht *et al*., 1985). Several hormones were shown to stimulate wound healing in apple trees e.g. IAA, IBA, GA3 and NAA (Woloszyn *et al*., 1980).

II Biochemistry of lignification

Lignification is a normal process of secondary cell wall development found in the native periderm of many plant organs as well as in specialised tissues such as xylem vessels. Lignification is also one of the more universal responses of plant tissues to wounding. Lignin formation provides a mechanical barrier which retards or inhibits the penetration of injured tissue by pathogens. Lignification is stimulated by temperatures of 25-30^{o}C and high humidities (96-98%) (Ismail and Brown, 1975).

The basic outlines of the lignin monomer biosynthetic pathway have been know for about 30 years but there are still ambiguities about how lignin monomers, or monolignols are made, where they are stored, and how they are polymerized into lignins. Recent reviews of the synthesis of monolignols include those by Boudet *et al.* (1995); Douglass (1996) and Whetten and Sederoff (1995). The first important enzyme in phenylpropanoid biosynthesis is phenylalanine ammonium lyase (PAL). A review about the induction and regulation of PAL are provided by Jones (1984).

A brief description of monolignol biosynthesis is given here, summarised from Whetten *et al.* (1998). The monolignol biosynthetic pathway converts phenylalanine to one of three monolignols i.e. *p*-coumaryl, sinapyl and coniferyl alcohols. PAL deaminates the phenylalanine to *trans*-cinnamic acid. Genetic engineering of plants with reduced PAL activity have been used to show that the enzyme is a key step in the regulation of phenylpropanoid biosynthesis and is the rate-limiting stem for carbon flux into lignin at levels 3-4-fold below wild-type (Bate *et al*., 1994). *trans*-cinnamic acid is the substrate for a P-450 hydroxylase, cinnamate 4-hydroxylase (C4H) and is converted to *para*-coumaric acid. The next stage is not clear. Traditionally it was thought to involved hydroxylation by *p*-coumarate 3-hydroxylase (C3H) to give caffeic acid. An alternative pathway is the conversion of *p*-coumaric acid in to *p*-coumaroyl-CoA by 4-coumarate:CoA ligase followed by hydroxylation to produce caffeoyl-CoA. The next step in the traditional route is for caffeic acid to be methylated to ferulic acid by caffeic acid O-methyltransferase (COMT). More recently it has

been suggested that a caffeoyl-CoA O-methyltransferase (CCoAOMT) enzyme plays a role in monolignol biosynthesis (Ye *et al*, 1994). Ferulic acid and *p*-coumarate can be activated with coenzyme A to CoA thioesters which are believed to be the intermediates in the synthesis of coniferyl and coumaryl alcohols respectively. The synthesis of sinapyl alcohols is not so clear. The traditional route involves the conversion of ferulic acid to 5-hydroxy-ferulic acid and this is converted to sinapic acid before coenzyme A activation.The CoA thioesters are thought to be the substrates for cinnamoyl-CoA reductase (CCR) which reduces them to their corresponding aldehydes. Transgenic experiments have shown that reduction in CCR activity, reduces lignin content. The cinnamaldehydes in turn are substrates for cinnamyl alcohol dehydrogenase (CAD) which reduces them to alcohols, yielding the three monolignols. It was believed that CAD could be used as a molecular marker specific for lignin synthesis (Walter *et al*., 1988) and when the main CAD enzyme associated with lignification is down regulated, there is often an accumulation of its cinnamaldehyde substrates. However, in some plants, even a severe reduction in CAD has only a small effect on total lignin content. It is suggested that other enzymes can play this role if primary CAD activity is reduced (Whetten *et al*., 1998). In potato tubers, irradiation impairs wound healing and reduces lignification. This has been correlated with a decrease in the induced activity of C4H and CAD (Pendharkar and Nair, 1987; Ramamurthy *et al*., 2000).

Boron appears to be vital to lignin synthesis and a potential role for BO_3 in regulating the hydroxylase and oxidase activities of the phenolases involved in the biosynthesis of caffeic and hydroxyferulic acids has been suggested (Lewis, 1980).

The monolignols are polymerised outside the cell, however, the process by which mono-lignols are secreted from the cell for lignin synthesis are unknown. Possibly they are exported as glycosides which are hydrolysed in the cell wall by glucosidases. The mechanism of lignin synthesis also remains unclear. Peroxidases are involved in linking the mono-lignols although the origin of the required H_2O_2 is unknown. Wounding in chickpea seedlings causes a rapid increase in a cell wall copper amine oxidase (CuAO) mRNA and enzyme activity. Aminoguanidine, a specific CuAO inhibitor, decreased the deposition of lignin-suberin barrier along the lesion. CuAO may be a limiting factor in H_2O_2 production in the cell wall of chickpea seedlings (Rea *et al*., 1998). Reducing power (NADPH) for lignin synthesis is probably from the pentose phosphate pathway (Pryke and ap Rees, 1977).

The composition of lignins varies widely between plants. The lignin binds covalently to the cellulose in the cell walls. It is assumed that other cell wall components e.g. cell wall proteins or polysaccharide-phenolic esters (e.g. ferulates) act as nucleation points for lignin deposition. The presence of alkyl ferulates has been noted in wounded potato tubers (Bernards and Lewis, 1992).

The changing activity of enzymes (peroxidase, aromatic alcohol dehydrogenase, phenylalanine transaminase, O-methyl transferase, shikimate dehydrogenase, ferulyl CoA reductase, PAL, cinnamic acid-4-hydroxylase and hydroxycinnamate CoA ligase) involved in lignin biosynthesis in swede root disks was investigated by Rhodes *et al*., 1976).

Immunogold locatisations in wounded hypocotyl tissue of French bean (*Phaseolus vulgaris* L.) showed that PAL and cinnamate-4-hydroxylase (C4H) accumulated to high levels throughout cell types in wound sites, especially in the epidermal cells. At the subcellular level, PAL was localized in the cytosol in the wounded cells; however, because of the loss of membrane through mechanical damage, association with membrane structures, particularly endoplasmic reticulum, in unwounded cells could not be ruled out. C4H was associated with membranes when these were preserved. In wounded tissue, the peroxidase was found at the growing edges of tylose-like structures in the vascular xylem (Smith *et al*., 1994).

During wound healing of potato tubers, the levels of chlorogenic acid (including neo and crypto-isomers), caffeic acid, p-coumaric acid and ferulic acid increase many-fold and this is accompanied by a parallel rise in PAL activity (McClure, 1960). The chlorogenic acid isomers account for about 88% of the phenolics formed in potatoes as determined by HPLC (Ramamurthy *et al*., 1992) and the major polyphenols in sweet potato roots ((Kojima and Uritani, 1972). The biosynthetic pathway and specific role of chlorogenic acid and its isomers in wound healing is not clear (Rhodes and Wooltorton, 1978).

III The importance of wound healing as a marker of storeability of root and tuber crops

Evidence of the role of wound healing in the storeability of sweet potatoes is mixed. Walter *et al*, 1988) suggest that the relationship is indirect, whereas van Oirschot (2000) suggested that wound healing ability is a major factor for the shelf-life of sweet potato cultivars under tropical storage conditions.

The temperature of the soil from which sweet potatoes were harvest influenced the degree of wound lignification up to 3 days of curing. After 16 weeks of storage, roots harvested at soil temperatures of 10 degrees to 12 degrees and 22 to 25 degrees had lost more weight and developed more rots than did roots harvested at 15 degrees to 17 degrees, despite the fact that wound healing progress was similar. Thus, factors other than wound healing strongly influence storage stability (Walter *et al*., 1989).

Varietal ranking of wound healing potential in UK maincrop potato cultivars was moderately consistent over 4 experiments in 2 year (McGee *et al*., 1985).

IV Detailed review of wound healing processes in root and tuber crops

a) Stage 1: Cells are damaged causing immediate responses

i. Decompartmentation of damaged cell components leading to mixing of substrates and enzymes normally held apart in the cell, e.g. polyphenol oxidases and their substrates.

ii. Fatty acids are released from damaged membranes. These undergo peroxidation to form volatiles such as ethane, ethylene, aldehydes etc. some of which may act as wound signals (Galliard, 1978). Lipoxygenase activity increases in wounded potato tubers (Lulai, 1988).

iii. Cell wall fragments are released e.g. polygalacturonic acid (PGA) which may act as wound signals and elicitors of various responses. Elicitors have been shown to cause a rapid rise in the production of H_2O_2 (Apostol *et al*., 1989) which may play a role in subsequence defense responses e.g. the synthesis of phytoalexins and other defense products. PGA has shown to be a potent inducer of proteinase inhibitors (Bowles, 1990) and in sweet potato, stimulates synthesis of beta-amylase (Ohto *et al*., 1992). Elicitor oligosaccharides have been shown to cause the depolarisation of cell membranes (see below in iv.) in tomato leaf cells (Thain *et al*., 1990).

iv. Depolarization of intact cell membranes occurs in the cell layers below the wound. This leads to a net efflux of K^+ and Cl^- and influx of H^+ and Ca^{2+}. These ionic changes may initiate action potentials which act as signals from the wound site. Within 30 min, the membranes repolarize but sometimes to levels less than normal resting potential for 24h or more e.g. in potato tissues (Tomiyama *et al*., 1987). A possible flux of ABA out of

cells into cell walls may occur in response to decreased cytosolic pH which changes ABA into a non-ionic (polar) form.

v. Activation of protein phosphoylation by kinase proteins (Felix *et al*., 1991;Mikolajczyk *et al*., 2000; Seo *et al*., 1995). This has been shown to follow an increase in cytosolic calcium ion concentration (Takahashi *et al*., 1997).

vi. Stimulation of callose synthetase, cellulose synthetase and ACC oxidase, the latter leading to ethylene synthesis. In potato tubers, the first observed response to wounding was the production, within 15 min, of callose at the sites of plasmodesmata and sieve-plate pores (Thompson *et al*, 1994).

vii. Wound respiration is stimulated, the free fatty acids are thought to be the major substrates (Laties, 1978; Theologis and Laties, 1981). However, in sweet potato roots and other cyanide-resistant plant tissues, fresh tissue slices did not show measurable levels of lipid degradation (Theologis and Laties, 1980).

Cell suspension cultures are a useful model system for investigating the initial stress responses of plant tissues. These cultured plant cells have been shown to respond immediately to stressors such as osmotic stress, microbial elicitors, heavy metals, ozone or mechanical stress with ion fluxes (including rapid extracellular alkalinisation), production of oxygen reactive species, the activation of mitogen-activated protein (MAP) -kinase pathways and ACC synthase. In tomato cells, lowering the medium osmolality by as little as 10 mosmol (an osmotic potential of about 0.2 bar) caused immediate and significant induction of medium alkalinisation (Felix *et al*., 2000) and references therein). An osmosensing signal transduction pathway has been described for yeast cells (Maeda *et al*., 1994).

b) Stage 2: Outer cell layers start to dessicate and sugars are synthesized.

Desiccation of the outer cell layers is the first observable morphological event in the wound healing of sweet potato roots (Artschwager and Starrett, 1931; Morris and Mann, 1955, van Oirschot, 2000). When healing is rapid, this layer is between 4 to 6 cells deep, however, if healing is slow, for example due to low humidities, the number of desiccated cell layers increases (Strider and McCombs, 1958). Up to 25 layers have been recorded (van Oirschot, 2000). A wound periderm is seldom formed under such circumstances (Strider and McCombs, 1958).

The response of many plant tissues to both mild and extreme water stress is to rapidly increase sucrose synthesis and inhibit starch synthesis and stimulate starch degradation e.g. in leaves (Quick *et al*., 1989; Zrenner and Stitt, 1991). In the discs of potato tubers, increasing water deficit leads to a switch from sucrose degradation to sucrose synthesis (Geigenberger *et al*., 1997). This appears to be via stimulation of the activity of sucrose phosphate synthase (SPS), controlled by reversible phosphorylation (Toroser and Huber, 1997). Furthermore, a fall in 3-phosphoglycerate levels leads to an inhibition of ADP glucose pyrophosphorylase (AGPase) which restricts the rate of starch synthesis (Geigenberger *et al*., 1997).

At least one role of the sucrose is to increase the osmotic potential of the tissues so as to reduce their water loss i.e. act as an osmolyte (Hare *et al*., 1998; Ingram and Bartels, 1996). An increased turnover of hexoses to hexose-phosphates may act as an important internal signal which regulates the expression of a wide variety of genes involved in photosynthesis, respiration, starch and sucrose synthesis and degradation and nitrogen metabolism (Koch, 1996). During water stress, the essentially irreversible hexokinase reaction is also likely to be an important determinant of whether hexoses are essentially respired or accummulated, possibly for osmotic purposes (Hare *et al*., 1998). The role of invertases is examined below in section c).

It well established that because of the high membrane permeability of the protonated, uncharged species ABA.H and low permeability of the anionic species ABA^-, ABA accumulates in the most alkaline compartment of cells. Upon dehydration, ABA levels increase greatly in the apoplastic fluid as a consequence of large increases in apoplastic pH (Hartung *et al*., 1988). It has been suggested that ABA may regulate the production of ethylene in water stressed tissues by reducing the amount of ACC. On the other hand auxin and cytokinin application appeared to stimulate ACC synthesis under the same conditions (McKeon *et al*, 1982).

There is evidence that sweet potato cultivars with a high dry matter content (dmc) have a reduced capacity for wound healing at low to moderate humidities (58-70% r.h.). It has been postulated that this may be due to wounds on roots with a lower moisture content (high dmc) desiccating too fast such that wound healing metabolic activity is impaired (van Oirschot, 2000).

c) Stage 3: Induction of synthesis of a wide range of enzymes:

Transcription and translation activities increase to produce many different enzymes including proteinase inhibitors, acidic peroxidases, chitinases, polyphenol oxidases, enzymes for lignin (Lamb, 1982) and suberin synthesis. In sweet potatoes, PAL is synthesized *de novo* with a lag of 3h and a maximum level of activity after 12h. Although activity decreases thereafter, synthesis continues, making it likely that inhibition or degradation of the enzyme is responsible for this decrease in activity (Tanaka and Uritani, 1977). In many plant tissues, including sweet potatoes, ethylene appears to play a role in the induction of enzyme activity (see section e) below).

In many tissues, proteinase inhibitors are induced e.g. Inhibitors I and II of wild tomato fruits and potato tubers (Ryan, 1988). Two proteins, sporamin and beta-amylase are induced in sweet potato tissues by wounding or application of sucrose, PGA or chitosan (Nakamura *et al*., 1991). Sporamin, located in cell vacuoles, appears to be the primary storage protein in the roots, however, there is circumstantial evidence that it may play a defence role as a proteinase inhibitor. The physiological role of beta-amylase, however, is quite unclear. It appears to be generally located in the vacuole of vegetative tissues (Lin *et al*., 1988) and does not seem to play an essential role in the normal metabolism of starch. Ohto *et al* 1992) speculate, however, that beta-amylase might degrade starch in the vacuole under certain conditions. The same authors show that ABA (but not methyl jasmonate) also stimulates the expression of the sporamin and beta-amylase genes, so ABA may participate in the transfer of PGA-induced signals in sweet potato. ABA has also been shown to be involved in the wound-induced expression of Inhibitor I and II genes of potato and tomato (Pena-Cortes *et al*., 1989).

Vacuolar (acid) invertases increase in water stressed and wounded tissues, for example, water stressed maize leaves (Pelleschi *et al*., 1997). In fresh sweet potato slices acid invertase is formed *de novo* with a lag of a couple of hours, reaches a peak after about 18 hours and then declines. (Matsushita and Uritani, 1974). Transgenic potato plants expressing elevated levels of invertase in different cell compartments, showed symptoms resembling those of water stress (Bussis *et al*., 1997). It was speculated that in vacuolar transformants, the conversion of sucrose to hexoses might act to increase vacuolar osmotic pressure, with sucrose, in turn increasing in the cytosol for the same purpose. Acid invertase also may supply monosaccharides for respiration (see section d below).

In potatoes, dramatically raised levels of the enzyme tyramine hydroxycinnamoyl transferase (THT) occurs 3-4 hours after wounding. THT catalyses the synthesis of hydroxycinnamoyltyramines which appear to be rapidly incorporated into the cell walls of suberized periderm (Negrel *et al*., 1993). This process appears to occur well before the lipid

components of suberin are synthesised and may be associated with the synthesis of the aromatic part of suberin (see below in section f).

d) Stage 4: Increased respiration and starch degradation

In sweet potatoes, wound respiration dramatically increases after a lag of a couple of hours, doubling in rate within 20 hours. Enzymes of the glycolitic and pentose phosphate pathways have been found to increase as have oxidative processes in the mitochondria. The major initial substrate appears to be glucose-6-phosphate, followed by sucrose and then finally starch (Kato and Uritani, 1976; Uritani, 19??). During the curing period for sweet potatoes, the sucrose levels rise sharply and there was a general tendency for monosaccharides to increase during curing (Picha, 1986). It is perhaps relevant to note that Weimer and Harter (1931) observed that in sweet potatoes, a wound periderm was formed more readily near to the vascular ring compared to the centre (assuming that the transport system still functions within the detached root).

Generally, in root and tuber crops, starch grains disappear from cell layers adjacent to the wound site (Espelie *et al*., 1986;). This probably serves as a source of energy for the wound healing process (Barckhausen, 1978). In both potato and sweet potato, starch is decomposed to glucose with a lag of about 12 hours. The activity of both alpha and beta amylases increases but not to so marked an extent (Kato and Uritani, 1976).

e) Stage 5: Lignin synthesis

(See section on the biochemistry of lignification)

Cells immediately under the damaged layer begin to lay down lignin like compounds in the corners and outer layers of their cell walls. This is believed to protect against root- and tuber-invading bacteria and fungi which lack the enzymes needed to degrade lignified cell walls (Maher and Kelman, 1984). A significant negative correlation was found between the degree of wound lignification in sweet potatoes and root weight loss at day 4 after wounding (van Oirschot, 2000). However, the same author found no such relationship between the wound transpiration rate and degree of lignification at day 3 after wounding even though transpiration had decreased markedly. It is not clear which cell wall component/s is or are required to inhibit wound water loss in sweet potatoes, however van Oirschot (2000) noted that the desiccated cell layers probably retard water loss to some extent.

Ethylene appears to stimulate aspects of wound healing in roots and tubers, for example in potatoes (Ilker *et al*., 1977). In swede root, ethylene stimulates the pathway of phenolic-acid biosynthesis leading to lignification. Cytological studies showed that the secondary xylem parenchyma lignified in response to ethylene. In the case of the disks, the response was restricted to a layer 2-3 cells thick on the surface of the disks (Rhodes and Wooltorton, 1973; Rhodes *et al*., 1976). An increase in PAL activity and lignin synthesis was stimulated in cucumber roots by ferulic and p-coumaric acids but only when ethylene was synthesised (Politycka, 1999).

In sweet potato root slices, exogenous ethylene (10 ppm) is known to stimulate PAL, cinnamyl-alcohol dehydrogenase CAH, HC coA ligase and lignin synthesis (Imaseki *et al*, 1968). Furthermore in whole sweet potato roots, wounding induces ethylene within 24 h (Randle and Woodson, 1986; Sakai, *et al*. 1970; Saltveit and Locy, 1982). Ethylene production preceded wound lignification and wound periderm formation by 24 and 48 h, respectively (St Amand and Randle, 1989). Blocking ethylene action with 2,5-norbornadiene increased ethylene production, blocked wound periderm formation for up to 12 d, and strongly suppressed and delayed lignification. Blocking ethylene synthesis with AOA or $CoCl_2$ decreased ethylene production to 10% of the control value. Lignification and wound

periderm formation were also suppressed and initiation delayed (St Amand and Randle, 1989). Multiple regression analysis indicated statistically significant and moderate correlations between selected daily ethylene production for 18 cultivars and their cell lignification (r2 = 0.74) and wound periderm formation (r2 = 0.73). These observations suggest that measurement of ethylene produced by wounded roots may be useful in helping to screen large numbers of sweet potato breeding lines and their progeny for the ability to produce lignified and wound periderm cell layers (St Amand and Randle, 1991).

In sweet potato roots, peroxidase activity is stimulated by both wounding and ethylene and at least some of this activity appears to be due to *de novo* synthesis (Shannon *et al*., 1971). It appears that wounding and ethylene treatments have different effects on the expression of isoperoxidases (Bireka *et al*., 1976).

Where plant wound responses include both lignification and suberisation, it appears that the former process occurs first, followed by the latter (Rittinger *et al*., 1987). There is no evidence that meristematic activity is needed in root tissues for lignification to occur (Obrucheva, 1974).

f) Stage 6: Suberin synthesis

Suberin is an important component of native periderm and suberization is a common response to wounding of plant organs (Kolattukudy 1983). The composition of suberin in the native periderm of sweet potato and other root crops was investigated by Kolattukudy *et al*, 1975). Suberization results in the development of a diffusion barrier and thus seals off the wound from further water loss (Kolattukudy and Dean, 1974). Some studies suggest suberin has anti-fungal properties (Kolattukudy, 1984).

The deposition of suberin in the wound periderm of potatoes has been the subject of numerous studies (Cottle and Kolattukudy, 1982; Lapierre *et al*., 1996). Various stains have been identified that can allow detection of suberin in potato peridermis (Vaughn and Lulai, 1991). Current understanding of the biochemistry of suberin synthesis is still limited and has not moved on much since the descriptions by Kolattukudy (1981;1984) and for potatoes specifically, by Cottle and Kolattukudy (1982a). Recent evidence suggests that glycerol is a monomer for suberin synthesis (Moire *et al*., 1999)

The process of suberisation in potato wound periderm was followed by nuclear magnetic resonance (nmr) techniques. This showed that suberin is deposited at the outermost intact cell wall surface during the first 7 d of wound healing (Stark *et al*., 1994). These outer, non-dessciated cell layers begin to lay down pro-suberin lamellae and finally suberin on the inner layers of the cell walls i.e. just outside the cell membrane. As the suberisation process proceeds, the cellular content degenerate (Kolattukudy, 1983). SER accumulation has been linked to lipid and suberin synthesis in potato tubers. Peroxidases have been implicated in the deposition of wound suberin in potatoes (Espelie *et al*., 1986; Bernards *et al*, 1999). The appearance of an anionic peroxidase which is localised in the inner side of the suberising walls shows the same time course of activity as the suberisation process (Espelie *et al*, 1986).

There is evidence that it is the waxes associated with the suberin polymer, rather than the polymer itself which constitute the major diffusion barrier in native and wound periderm (Espelie *et al*., 1980; Soliday *et al*., 1979; Vogt *et al*., 1983). There is strong evidence that suberin contains both aliphatic and lignin-like aromatic domains however, how the domains are associated and attached the cell wall, is not clear (Lulai and Morgan, 1992; Schmidt and Schonherr, 1982; Kolattukudy, 1981; 1983; 1984). Riley and Kolattukudy (1975) found that the lignin-like domain of suberin in sweet potatoes contains ferulates that they suggested might covalently attach the suberin to the cell walls. In potatoes, the accumulation of alkyl ferulates is temporally and spatially correlated with suberin formation (Bernards and Lewis,

1992). There is also recent evidence that the suberin phenolic and aliphatic domains may play different roles in providing resistance against pathogens in potato tubers (Lulai and Corsini, 1998).

Cell suberisation was stimulated by the application of auxin to wounded white yam (Mozie, 1981-1982). The synthesis of suberin during wound healing has been investigated in jade leaves, tomato fruit and bean pods (Dean and Kolattukudy, 1976). Iron deficiency has been shown to inhibit suberin synthesis in beans apparently due to a decrease in peroxidase activity (Sijmons *et al*., 1979).

Suberization in potatoes was inhibited by mM levels of IAA, unaffected by traumatic acid and stimulated by ABA at 10^{-4} mM (Soliday *et al*., 1978). The decrease of vapour conductance in potato wounds is stimulated by ABA (Lulai and Orr, 1995). ABA appears to induce a suberin-associated isozyme of peroxidase and stimulates the deposition of both the polymeric aliphatic and aromatic components of suberin (Cottle and Kolattukudy, 1982b). Furthermore, omega-hydroxypalmitic acid hydroxycinnamoyl transferase (HHT), which is a good marker of suberin biosynthesis, was induced by ABA as was to a lesser extent tyramine hydroxycinnamoyl transferase (THT) (Negrel *et al*., 1995).

g) Stage 7: Meristematic activity

Meristematic activity is induced in a single layer of cells known as the wound or cork cambium. This is observed as periclinal divisions in cells immediately below the wound reaction cells. The cambium gives rise to cork (phellem) cells which also become lignified and then suberized forming a wound periderm. This is described in detail for potatoes by Thompson *et al*. (1995). In sweet potatoes the wound periderm appears, on a biochemical basis, to be identical to normal periderm and varies in thickness from 4 to 10 cell layers (Morris and Mann, 1955; Walter and Schadel, 1983).

In sweet potato roots, formation of a lignified layer appears to be a vital prerequisite for the formation of a wound periderm (Walter and Schadel, 1982; van Oirschot, 2000). Not all cultivars of sweet potato produce lignified cell layers and a new periderm under sub-optimal curing conditions (van Oirschot, 2000).

h) General Points

In sweet potatoes, the macroscopic progress of wound healing can be followed using a saturated solution of phloroglucinol (PG) in 18% HCl (Walter and Schadel, 1982). Within a 7 day curing period at 30^{o}C, 84-88% r.h., 4-5 PG-positive cell-layers can be detected under the several outer layers of desiccated cells. These layers only stain very weakly with Sudan IV for lipophilic compounds. The opposite result of staining was found for the new periderm layers forming by meristematic activity. These cell layers stained strongly with Sudan IV and only weakly with PG reagent and this is very similar to the native periderm of the roots. Chemical analysis of both skin and wound tissues of sweet potato revealed large amounts of guaiacyl residues, moderate amounts of p-hydroxy-phenyl residues and small amounts of syringyl residues. Wound tissues (non-periderm) released about 7 times less 1,18-octadecene diol than did periderm tissues. This compound has been used to estimate the amount of suberin present (Kolattukudy and Dean, 1974) and suggests that the sweet potato lignified layers is much less suberized than normal periderm (Walter and Schadel, 1983).

The induction of various enzymes (PAL, C4H, acid invertase and peroxidase) and the appeaance of polyphenols was shown to a marked polarity in cut-injured sweet potato root tissue - cv. Kokei 14 (Tanaka and Uritani, 1977). Activity was pronounced in the proximal side of tissue pieces compared to the distal side, although polyphenols were produced in the narrow layer of cells beneath the distal surface (but not at all in its interior tissues). It was

suggested that this might be related to acropetal movement of IAA in the roots of various plants. Treatment of the distal side of the tissues with IAA caused polyphenol production but not when gibberellic acid, ABA, kinetin or ethylene were applied.

Bruise-type wounds heal slowly and irregularly in sweet potato (Strider and McComb, 1958) and in bruised potatoes, where cells are separated from other tissues by fissures, healing was even slower and in some cases the separated cells died (Thompson *et al*., 1995). It has been postulated that the slowness in healing could be due to a lack of oxygen (Weimer and Harter, 1921) or decreased transpiration (Strider and McComb, 1958).

Studies using excised cores, discs and slices of potato indicate the presence of suberin-associated phenolic and aliphatic components typically appearing about 2-3 days (48-72h) after wounding and healing being completed after 6 days (Borg-Oliver and Monties, 1993; Cottle and Kolattukudy, 1982a; Espelie *et al*., 1986; Kolattukudy, 1984). However, a study by Thompson *et al*. (1995) revealed that wound healing is initiated much earlier (4h) and completed much earlier (within 1 day) in cortical tissues of freshly harvested tubers.

V Summary

The most likely events that occur in sweet potato after wounding are as follows:

Cell fragments acting as elicitors cause immediate flux of calcium into the cell, which initiates a protein kinase cascade. A number of key enzymes are activated including ACC synthase leading to the production of wound ethylene.

The osmotic stress from the open wound causes protons to flux out of the cell causing an acidification of the cytoplasm. ABA moves out of the cell into the apoplast where it attaches to receptors on the cell membranes and initiates a further signalling cascade. This includes the activation by phosphorylation of sucrose phosphate synthase leading to increased cytosolic sucrose synthesis and the production of beta-amylase in the vacuole. Presumably any damaged vascular tissues are blocked with callose.

Within a few hours, transcription begins of enzymes involved in the phenylpropanoid pathway. Ethylene appears to be vital to this process. Lignin and possibly aliphatic compounds start to be synthesised within 24 h and are lain down with in the cell walls. These components slow down water loss from the wound.

Transcription also begins of enzymes involved in carbohydrate metabolism including acid invertase which may increase the levels of osmotically active hexoses in the cell vacuoles. The activity of enzymes involved in the degradation of starch increases within 12 h producing energy required for the synthetic reactions required in wound periderm formation which begins about 48-36 h after wounding.

(nb. The timing of events is dependent on both the environmental conditions and the genotype of sweet potato).

VI Somequestions that remain to be answered about wound healing in sweet potato

1. What component/s of the healing wound first acts to reduce transpiration from the wound surface and how quickly?
2. Is callose synthesized in damaged vascular tissues?
3. Is there an aliphatic (suberin and wax) component lain down with the lignin in the first reaction layers?

4. If so, what is the timing of this biosynthesis compared to other cellular events after wounding?
5. Are some of the desiccated cell layers also lignified i.e. do some layers lignify before they dry out? (this is suggested by data in Oirshot, 2000 where the thickness of both the desiccated cell layers and the lignified cell layers increases with time - fig 6.2, pp. 129).
6. What is the role of chlorogenic acid and its isomers in phenylpropanoid biochemistry?
7. Is the maintenance of an adequate osmotic pressure in the outer cell layers, vital to the wound response?
8. If so, how does this relate to the carbohydrate status of the tissues?
9. What roles (if any) do ABA and IAA play in the wound response of sweet potato?
10. Does acropetal development of a wound response impact upon wound healing in sweet potatoes (see Tanaka and Uritani, 1977)
11. Which of all these responses might vary from cultivar to cultivar in response to increased osmotic stress?!

VII Hypotheses

These first two hypotheses would concentrate on the very earliest events in wound healing.

1. Sweet potatoes with intrinsically high hexose levels (or other osmolytes) in the vacuole and/or sucrose levels in the cytoplasm (due to higher tissue osmotic potential) are less sensitive to water loss than those with low intrinsic sugar levels. This gives cells longer to initiate a synthetic response to the water stress.
2. Upon wounding, hexoses from the vacuole are converted to sucrose in the cytosol of wound tissues thus raising their osmotic potential. This reaction is slower in some sweet potato cultivars due to intrinsically low levels of a) ABA (for signalling osmotic stress) or b) phosphate (for protein and/or hexose phosphoylations or c) UTP (for glucose phosphate activation in sucrose synthesis) or SPS or ???.

VIII Suggested experimental work

1. Confirm relationship between sugar levels and wound healing
2. Confirm whether or not there is variation in wound healing between cultivars at high humidity.
3. Choose a couple of cultivars that consistently show good or bad wound healing at medium humidity for further physiological and biochemical studies.
4. Confirm whether or not, different cultivars lose water at different rates, pre-curing.
5. Measure the changing osmotic potential of sweet potato root wound tissue in relation to its wound healing capacity
6. Investigate the phosphate status of sweet potato tissues in different cultivars.
7. Assess the impact of ABA and IAA on sweet potato wound healing
8. Using lipophylic dyes (e.g. Sudan IV and fluorescent dyes) investigate the synthesis of aliphatic compounds in the wound tissues in relation to the timing of decreased transpiration.
9. Measure of ethylene production by different cultivars under different humidities.
10. Assay for induction of one or more key enzymes in the lignification process e.g. phenylalanine ammonia lyase (PAL), peroxidase; 4-coumaryl CoA ligase; caffeate O-methyltransferase (COMT), cinnamoyl-CoA reductase (CCR) and cinnamyl-alcohol dehydrogenase (CAD).
11. Determine the mineral content of different roots, looking for differences in minerals that might be critical activators for key enzymes in the wound healing process (Bo, Fe, Cu, Zn etc.)

(Even at 25oC with a 94% humidity, the water potential of the air is -84.95 bar which is not really severe but is lower than ordinarily found in soils)

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