CHAPTER 6

Curing and the physiology of wound healing

Q.E.A. van Oirschot, D. Rees, J. Aked, A. Kihurani, C. Lucas, D. Maina, T. Mcharo and J. Bohac

6.1 Introduction

6.1.1 The nature of wound healing

Damage is inevitable during handling and marketing of sweetpotato and is exacerbated by practices such as overpacking sacks as shown in the picture. Most plant tissues have mechanisms for healing wounds. This is exploited to improve storability of root crops after harvest by 'curing', where they are placed in an environment to promote healing of wounds incurred during harvesting and handling. Sweetpotato is similar in this respect to other root and tuber crops such as potato, cassava and yam (Lulai and Orr, 1995; Rickard, 1985; Passam *et al.,* 1976).

Descriptions of wound healing in sweetpotato date from the 1920s when Weimer and Harter (1921) described how moisture and temperature affect wound periderm formation and the efficiency of the wound cork in preventing infection. Artschwager and Starrett (1931) distinguished three stages of healing:

i) desiccation of surface cell layers
ii) thickening of cell walls (suberization or lignification) in underlying cell layers
iii) formation of a new 'wound' periderm underneath the lignified cells.

Each of these processes is described in more detail below.

Desiccation of surface cell layers

The first response after wounding is desiccation of the cell layers where the cells on the surface dry out and die. Under sub-optimal curing conditions (lower

humidities), this layer of desiccated cells may be thicker, which is unfavourable for the shelf-life of the roots as it favours the growth of pathogens (Nielsen and Johnson, 1974). The effect of cultivar on the thickness of the desiccated layer in sweetpotatoes has not been reported.

Lignification

Lignification is probably the most crucial step in the wound healing process. Cell walls below the desiccated cell layers become thickened. There is some uncertainty about the exact chemical nature of this thickening, i.e. whether the thickening is primarily due to the addition of lignin or suberin (Walter and Schadel, 1982, 1983). Both molecules are large polymers: lignin consists of phenolic sub-units, it is hydrophobic thereby reducing water movement and also has specific antifungal properties; suberin contains more aliphatic (lipid) components and so also reduces water movement. Artschwager and Starrett (1931) reported that the thickened cell layers absorb crystal violet which indicates suberization. Later, McClure (1960) found that these cells have a much stronger affinity for a saturated solution of phloroglucinol in 18% HCl, which indicates a lignin-like structure. With mass spectroscopy, Walter and Schadel (1983) confirmed that the polymeric compounds in these cells had the chemical properties of lignin. Thus, the cell wall thickening probably consists of both lignin and suberin. Once this layer is formed, a new wound periderm will form underneath, even if the roots are removed from curing conditions (Walter and Schadel, 1982; Morris and Mann, 1955), although it develops more quickly under curing conditions.

Wound periderm

The wound periderm consists of cell layers stacked in a similar way to the native periderm, and conferring the same protective barrier. The thickness of the wound periderm may vary according to cultivar. Morris and Mann (1955) found thicknesses varying from 4 to 10 layers, while Walter and Schadel (1983) and St Amand and Randle (1991) reported thicknesses between 5 and 6.7 layers. Walter and Schadel (1982) considered a wound periderm needed to be approximately 4.2 cell layers thick to be effective against water loss and pathogen invasion.

6.1.2 Conditions that promote wound healing

Wounds in sweetpotatoes cure most efficiently when the roots are exposed to temperatures of 28–30 ºC and a relative humidity (RH) greater than 85% (Kushman and Wright, 1969). Although curing is practised commercially in temperate areas, it is often assumed that it takes place naturally in the tropics (Collins and Walter, 1985; Woolfe, 1992) and is not actively practised. Jenkins (1982) reported that artificial curing under tropical conditions in Bangladesh did not reduce weight losses. However, the high levels of weight loss and very short shelf-life often seen in the tropics put into doubt whether wound healing takes place.

6.1.3 Objectives

In the previous chapter, it was shown that in storage trials carried out under simulated marketing conditions in Tanzania, rates of root weight loss and rotting varied considerably among cultivars. The work reported in this chapter was conducted to determine whether this was due to the characteristics of root wound healing. Some variability has been found among sweetpotato cultivars in the rate of wound healing (Strider and McCombs, 1958; St Amand and Randle, 1991). However, prior to this study, little was known about the wound healing characteristics of African germplasm, how this relates to shelf-life and also how the process is affected by sub-optimal humidities. A better understanding of the wound healing process under sub-optimal conditions may contribute to efforts to extend shelf-life by improved handling and cultivar selection.

A rapid method to assess wound healing efficiency is described that can be used by sweetpotato breeding programmes in developing countries where sophisticated equipment is not available. This method was validated to establish the relationship between wound healing characteristics of sweetpotato cultivars and their keeping qualities, including water loss, susceptibility to micro-organisms and shelf-life.

Further details of these studies are given in van Oirschot (2000) and van Oirschot *et al.* (2001).

6.2 Methods

Artificial wounds were inflicted by peeling a portion of the root surface with a potato peeler. An area of tissue approximately 2 x 5 cm and 1.7 mm deep was removed. The wounds were then left to heal under conditions chosen to simulate the marketing environment. Several studies were then conducted to look at wound healing by microscopy. Staining lignin by phloroglucinol is simple and gives a red stain that can be seen by the naked eye. This was, therefore, developed into a means of assessing wound healing efficiency by a lignification index. This was tested as a measure of functional wound healing by comparing it with water loss through wounds and susceptibility to rots. Details of the root supply, healing environments and methods of microscopy are given below.

Root Supply and Wound Healing Trials

Eight storage trials were conducted. Table 6.1 presents relevant field and experimental information for each of the trials, while Table 6.2 presents the cultivars included. The storage conditions used were as follows.

Method A (Trials 1 and 2, based at NRI, UK)
Twelve plastic dustbins (B&Q) were placed in a controlled-temperature room at 26 ºC. Within each of these, approximately 20 sweetpotato roots (weighing about 5 kg in total) were placed on a platform. The platform was constructed from a plastic plant support and plastic covered chicken wire, and was supported at a height of about 30 cm. In order to maintain high humidity, a layer of water (approximately 70 mm) was placed in the bottom of each bin, and air was bubbled through this at a rate of approximately 3 l/min/bin. A single pump (Charles Austin Pumps Ltd, UK) was used to provide an air flow which was divided using a manifold to supply all 12 bins. The humidity was measured at hourly intervals in 6 of the 12 bins using humidity probes (Vaisala, Helsinki, Finland), recorded by data-loggers (Grant Instruments Ltd, Barrington, Cambridge) and was found to remain between 76 and 100%.

Method B (Trial 3, based at NRI, UK)
One randomly selected root/cultivar/trial was placed in each of eight cardboard boxes (22 roots/box), and kept for 10 weeks in a controlled-temperature room at NRI, maintained at 25 ºC and 60% RH. Temperature and relative humidity (25 ± 0.5 ºC and 55% ± 6% RH) were recorded using Tinytalk data-loggers (Gemini, Chichester, UK). .

Method C (Trials 4, 5, 6 and 7 based at NARL, Nairobi, Kenya)
Roots were stored in crates. Each crate contained up to 30 roots with an equal number of roots for each cultivar. During the first 2 days, the boxes were lined with plastic sheets or dustbin liners to simulate the high humidity in closed sacks to which the roots would be exposed when transported to the market. In six boxes, the relative humidity was measured every 30 min, using RH probes and recorded using data-loggers as described above. The temperature fluctuated between 18 ºC and 27 ºC and relative humidity fluctuated between 45% and 95%.

Method D (Trial 8 based at NRI, UK)
The roots were maintained at three different levels of humidity, in three chambers located within a controlled-temperature room maintained at 25 ºC. In one chamber, a high relative humidity was maintained by means of an air flow of 3.5 l/h through a layer of water in the base of the chamber. Humidification of the air was improved by using fish-tank stones for air dispersal; 97% relative humidity was achieved.

For the two other chambers, an intermediate humidity was maintained using two supplies of air, one of low humidity (sourced from outside the controlled-temperature room) and one of high humidity (obtained by bubbling through water). The supply of these two sources of air was controlled using an adjustable humidity sensor placed within the chamber. The humidities attained were in the range of 56.6–62.3% and 64.5–70.5%, with an average of 58% and 65%, respectively.

Root Supply and Wound Healing Trials

Both fresh and embedded tissue sections were studied. Fresh sections were hand cut with a razor blade (Wilkinson Sword) at a thickness of 2–7 cells. The sections were stained with phloroglucinol (1% in 95% ethanol) for 2 min, transferred to concentrated HCl for 30 s, then rinsed in water for 30 s. Four sections per wound were assessed.

For the preparation of embedded sections, tissue blocks of 7 x 7 x 7 mm, including both wound surface and native periderm, were cut and fixed in a formalin acetic acid solution (ethanol 70%, formalin 5%, acetic acid 5%). The tissue blocks were than dehydrated in toluene (99%) and embedded in paraffin wax (Paraplast Plus, Sigma). Sections of 15 mm thickness were cut using a microtome. Before staining, the embedded sections were dehydrated in a series of toluene (2 x 100%) and ethanol (2 x 100%, 1 x 90% (last)). Sections were stained for lignin with phloroglucinol and HCl as described above. The morphology of the lignified layer was assessed at 100x magnification using a microscope (Leitz, UK) equipped with a graticule. Micrographs were taken using a Minolta X-700 camera mounted on the microscope.

Method for Measuring the 'Lignification Index'

Four thin cross-sections with a depth of 10 mm and approximately 0.5 mm thick were cut from the wounds using a razor blade. The sections were stained with phloroglucinol as described above. Each wound was given a score between 0 and 1 based on the continuity of lignification across the wound (see Table 6.3 for examples). The average lignification score for the four sections of each wound was called the 'lignification index' (LI).

Table 6.1 Overview of location, field design, planting and harvesting dates, experimental set-up and conditions for each of the trials

	Field location	Number of cultivars	Field design	Date of planting	Date of harvesting	Storage during curing	Curing experiment location	Temperature	Relative humidity
Trial 1	CIP	5	-	15-7-96	22-1-97	Method A	NRI	26.1 ± 0.5 ºC	82.2 ± 4%
Trial 2	CIP	5	-	15-7-96	17-3-97	Method A	NRI	26.1± 0.1 ºC	73.2±-7.3%
Trial 3[†]	CIP	22[‡]	RCBD	Oct 2000	Mar 2001	Method B	NRI	25 ± 0.5 ºC	60 ± 6%
Trial 4	CIP	10	CRD*, 3 rep, 90/120 plants per cultivar	25-5-98	27-10-98	Method C	NARL	21.1 ± 1.7 ºC	71%
Trial 5	CIP	10	CRD*, 3 rep, 90/120 plants per cultivar	June 98	11-11-98	Method C	NARL	20.7 ± 1.9 ºC	75.9%
Trial 6	CIP	8	-	July 98	1-12-98	Method C	NARL	21 ºC	67.3%
Trial 7	CIP	10	-	July 98	7-1-99	Method C	NARL	26 ºC	85–90%
Trial 8	CIP LZARDI (Lake Site)	10 3	-	Nov 98 Nov 98	March 99 March 99	Method D	NRI	26 ºC	Low 58% Intermediate 65% High 97%

* CRD = Complete randomized design.

[†] Trial 3 was replanted in January 1997.

[‡] Trial 3 has two sets of cultivars as the roots were part of different experiments.

Location of growth of sweetpotatoes: CIP = International Potato Center, Nairobi, Kenya; LZARDI = Lake Zone Agriculture Research and Development Institute, main station and lake site station.

Experiment location: NRI = Natural Resources Institute, UK; NARL = National Agricultural Research Laboratories.

Table 6.2 Overview of the cultivars used in each of the trials

Trial	Cultivars	Cultivars	Cultivars	Cultivars	Cultivars
Trial 1	Kemb 10	KSP 20	SPK 004	Yan Shu 1	Zapallo
Trial 2	Kemb 10	KSP 20	SPK 004	Yan Shu 1	Zapallo
Trial 3	Jewel Budagala* Yanshu Beauregard	Sinia KSP 20* Sinia B Kemb 10*	Zapallo* SPN/0 Hernandez Polista	Kagole Iboja Bilagala Mwananmonde SPK 004	L-86-33
Trial 4	BP1-SP-2 Caplina	Julian Kemb 10	KSP 20 Salyboro	SPK 004 Yarada	Yan Shu 1 Zapallo
Trial 5	BP1-SP-2 Caplina	Julian Kemb 10	KSP 20 Salyboro	SPK 004 Yarada	Yan Shu 1 Zapallo
Trial 6	BP1-SP-2 Caplina	Julian Kemb 10	KSP 20 Salyboro	SPK 004 Yarada	Yan Shu 1 Zapallo
Trial 7	BP1-SP-2 Caplina	Julian Kemb 10	KSP 20 Salyboro	SPK 004	Yan Shu 1 Zapallo
Trial 8	BP1-SP-2 Caplina	Julian Kemb 10	KSP 20 Salyboro SPK 004	Yarada Yan Shu 1 Zapallo	Polista SPN/0 SP/93/2

* Roots of these cultivars grown in two separate field trials were considered separately.

Table 6.3 Scores for lignification of sweetpotato wound sections representing continuity of lignified layer

		Completeness of the lignin layer		
	Lignification score	Presence of lignin	Completeness of lignification	Distribution of lignin in wound = lignin
Complete lignification	1	1	1	
Patchy lignification	0.5	1	0	
No lignification at all	0	0	0	

The physiological purpose of wound healing is to prevent water loss and inhibit microbial invasion. The LI was tested for its validity as a measure of functional wound healing by comparing it with rates of water loss and the susceptibility of the wound to rotting. Water loss was measured using a porometer (as described in Chapter 5, section 5.2.5). Susceptibility to rotting was determined by artificially inoculating wounds with the rot, *Rhizopus oryzae* after specific periods of healing.

Method Used to Assess Susceptibility to Microbial Invasion

Roots with wounds were kept under sub-optimal conditions for 3, 6 and 10 days after which they were assessed for susceptibility to *Rhizopus oryzae*. Mycelial discs (9 mm) were cut from the border of a 2-day-old potato dextrin agar (PDA) culture of *R. oryzae* and placed on the wound with the mycelial side facing down. Roots were incubated for 2 days in transparent polyethylene bags (40 x 50 cm), which were perforated with 16 holes for ventilation. The relative humidity and temperature in the bags were recorded using electronic data-loggers (Onset Computer Corporation 1998) and were found to be 94.2–97.5% and 21.7–24.0 ºC, respectively.

To assess the extent of tissue degradation by the inoculated pathogens, the roots/tubers were then cut longitudinally through the point of inoculation (Duarte and Clark, 1993) and measurements of the lesions taken. The wounds were further assessed for lignification as described above.

The results presented in this chapter include an assessment of wound healing efficiency using the LI for a wide range of sweetpotato germplasm. Details of the root supply and the screening trials are given below.

Cultivars Included in the Screening Programme for Wound Healing Efficiency

Set A: 16 cultivars were grown in Nairobi by CIP as part of a worldwide trial on germplasm by environment (GxE) interactions: Blesbok, Brondal, Mugande, Mafutha, Cemsa 74-228, Kemb 37, Jayalo, Naveto, Zapallo, Santo Amaro, Yan Shu 1, NC 1560, Xu Shu 18, Tainung No.64, Mogamba and Kemb 10. Five additional check cultivars (Yan Shu 1, Kemb 10, KSP 20, Zapallo and SPK 004) were planted in a separate field trial. Trials were planted in January 2000 and were harvested in May and July 2000.

Set B: 18 cultivars were grown by Janice Bohac of the US Vegetable Laboratory (USDA-ARS): Beau Regard, PI 538354, PI 595856, PI 595873, Picadito, Regal, SC 1149-19, Sumor, Tanzanian, Tinian, W287 Ruddy, W-308, W-317, W-325, W-341, W-345, W364 97k-11and White Regal. The first season trials were planted in May and harvested October 1999, then cured and stored for 2 months before being assessed in January 2000. The second season trials were planted May 2000 and harvested in November 2000.

Set C: cultivars were grown in Nairobi, Kenya by CIP. These included eight Tanzanian cultivars (Bilagala, Budagala, Iboja, Kagole, Mwanamonde, Polista, Sinia B and SPN/0), five check cultivars (Kemb 10, KSP 20, SPK 004, Yanshu 1 and Zapallo) and four cultivars from North and South America (Beauregard, Jewel, Hernandez and L-86-33). The first season trials were planted in May and harvested in September 2000; the second season trials were planted in November 2000 and harvested in February 2001.

The post-harvest experiments were conducted at the Natural Resources Institute (NRI), Chatham, UK. Assessment of wound healing efficiency at moderate humidity was conducted with the roots placed in three controlled environment chambers maintained at 65% RH and 26 ºC. For assessment at high humidity, the roots were placed in an enclosed bin with a layer of water in the base; relative humidity was greater than 95% throughout the assessment. The humidity and temperature of the storage environment were recorded using Tinytalk miniature data-loggers (Gemini, Chichester, UK). A minimum of 12 roots per cultivar were assessed at moderate humidity and a minimum of four roots per cultivar at high humidity.

6.3 Results and discussion

6.3.1 Physiology of wound healing at sub-optimal humidities

Relative humidity affects the pattern of wound healing and reduces its efficiency in sweetpotato roots, but the effects vary by cultivar. Figure 6.1 shows the cross-section of roots of eight sweetpotato cultivars after wounding and subsequent storage for 3 days and 6 days at 97, 65 and 58% RH. Roots that were kept at lower humidity after wounding show sunken wound surfaces, presumably due to desiccation. The response of roots to lower humidities appears to be cultivar dependent, with tissue shrinkage more pronounced for the cultivars SPK 004, Kemb 10, KSP 20 and Caplina. A thick desiccated crust formed in these cultivars, which was difficult to cut. Less shrinkage and much thinner desiccated crusts were observed for the cultivars Zapallo, Salyboro, Yan Shu 1 and Julian. These cultivars appear to heal more efficiently at the lower humidities.

Figure 6.2 shows micrographs of sections through wounds healed at 71% RH for three contrasting cultivars – Zapallo, Kemb10 and KSP 20. Lignification started at the periphery of the wound under the periderm, and subsequently developed towards the centre of the wound (Figure 6.3c) All cultivars show surface layers of desiccated cells which are flattened and appear white due to the concentration of starch granules as the cells lose water. This has been described previously for sweetpotato by Artschwager and Starrett (1931) and for yam (*Dioscorea* spp.) by Passam *et al.* (1976). Consistent with the observations above, the micrographs show that Zapallo has a much thinner desiccated layer than Kemb10 or KSP 20.

Lignified layers started to develop below the desiccated layer from 2 days after wounding for most cultivars, although development started after 1 day in roots of some cultivars, notably Yan Shu 1 and KSP 20. Figure 6.3a–c shows micrographs of wounds from three contrasting cultivars. Zapallo developed lignified layers close to the surface (Figure 6.3a). For some cultivars, a continuous lignin layer never developed, the layer remaining patchy/discontinuous (Figure 6.3b), or even absent (Figure 6.3c) at the centre of the wound.

Thickness of desiccated and lignified layers

More detailed studies were carried out on five of the eight cultivars. The number of lignified cell layers observed by microscopy increased for 5 days after wounding and healing at 82% RH (Table 6.4). The mean number of lignified cell layers for the five cultivars was significantly different and varied between 0.47 and 3.65 layers after 4 days and 1.75 and 3.36 layers after 5 days. This is a thinner layer than reported for curing at high humidity by Walter and Schadel

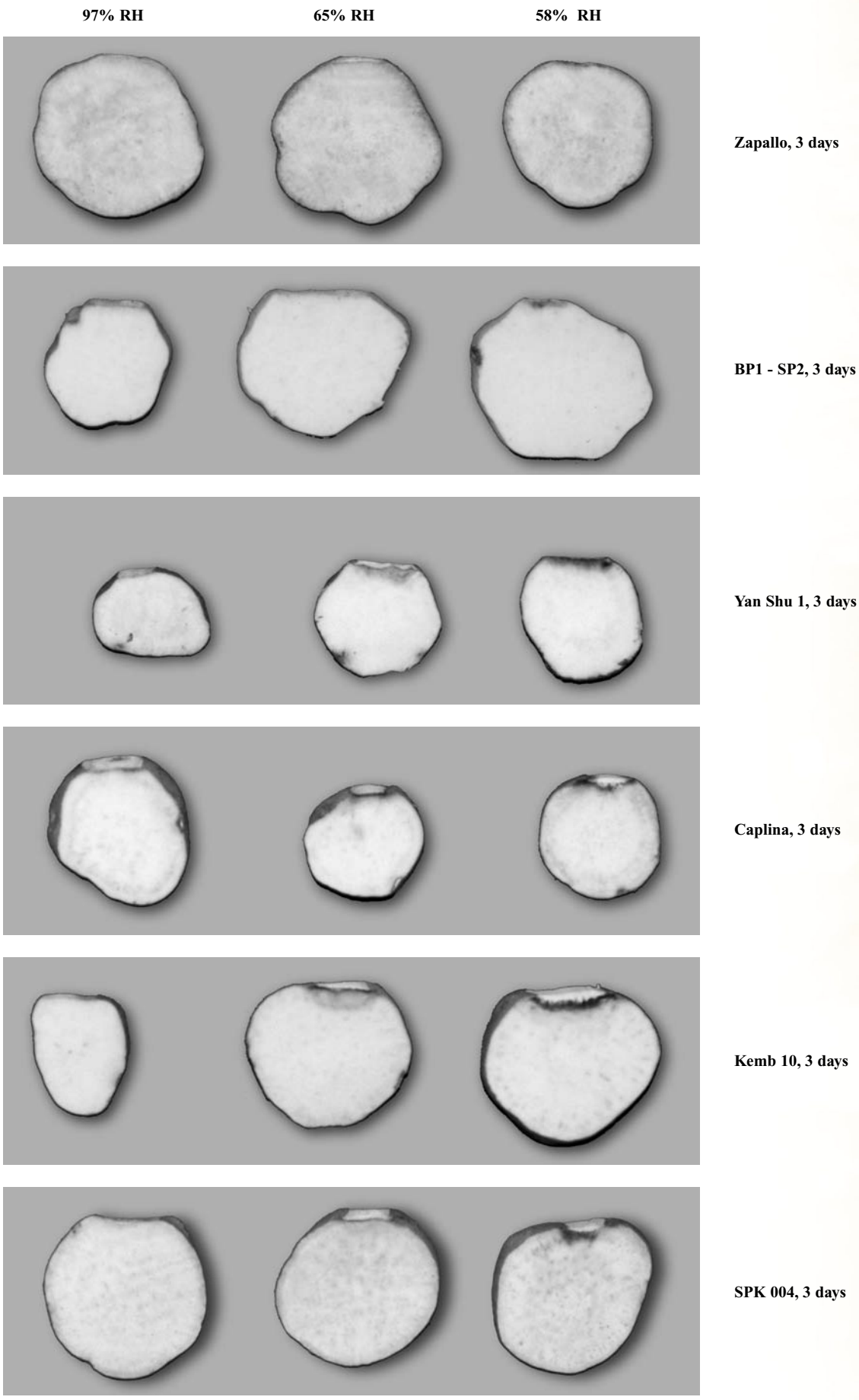
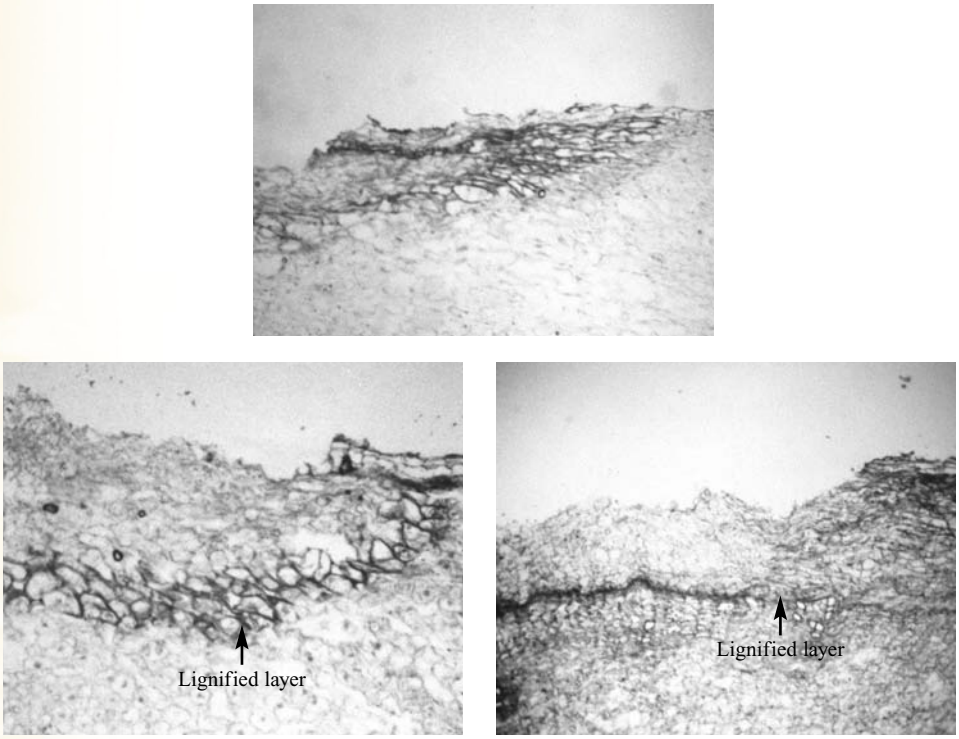
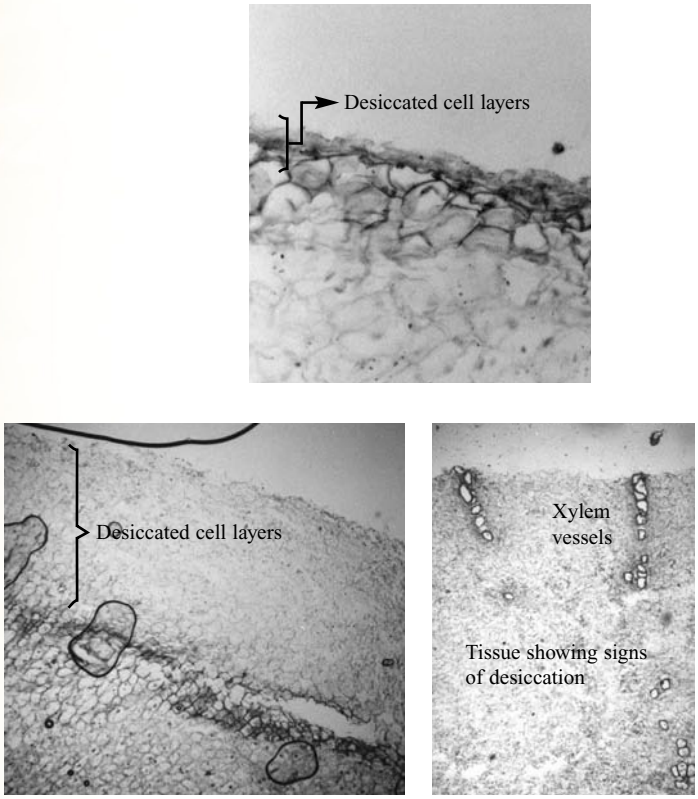


Figure 6.1 Slices of sweetpotato with wounds; variability in depth of desiccation depending on cultivar and relative humidity



The bar represents around 200 mm. Sections: 15 mm thick, stained with Phloroglucinol/HCl, which stains the lignin red. The sections were taken from (a) Zapallo, (b) Kemb 10 and (c) KSP 20.

Figure 6.2 Lignification starts at the wound boundary. The onset of the lignin layer in wounds of sweetpotato kept at 71% RH at 6 days after wounding



Sections were stained with phloroglucinol (1% in ethanol 95%) and concentrated HCl. Magnification: x 40 or x 100. The bar represents 100 mm. (a) Zapallo: thin desiccated cell layer (x 100), (b) KSP 20: 20 to 25 desiccated cell layers above patchy lignification (x40), (c) SPK 004: no lignified cell layers (x 40).

Figure 6.3 Variability in depth of desiccation. Typical sections through sweetpotato wounds at 6 days after wounding when the roots were kept at 71.1% RH and 20.9 ± 1.6 ˚C

Table 6.4 The number of lignified cell layers following artificial wounding and healing in roots of five cultivars of sweetpotato

Days after wounding	2	3	4	5
Yan Shu 1	1.41	1.29	2.04	2.28
Kemb 10	0.96	2.19	2.39	3.36
KSP 20	0.56	1.56	3.65	3.09
Zapallo	1.21	1.96	2.15	2.88
SPK 004	0.47	0.97	0.47	1.75
Cultivar effect (*P* value)	ns	ns	< 0.001	0.076
LSD	0.85	1.00	1.24	1.73

Roots were obtained from Trial 1 after 1 and 4 weeks of storage. Measurements were taken for five wounds per cultivar for each storage time. Healing conditions: 26 °C and 82% RH. No significant effects of storage time were found.

LSD = least significant difference.

(1983), who found on average 4.3 layers of suberized cells after 5–7 days.

Given that it is not possible to differentiate cells in the desiccated layer, a better indication of the thickness of both desiccated and lignified layers could be obtained by using a microscope fitted with a graticule to measure the actual dimensions. Table 6.5 shows data obtained for roots that had been stored for 1 and 6 weeks after harvest. For the lignified layer, highly significant differences among cultivars were observed for 2, 4, 6 and 10 days of healing. Results were less clear for the desiccated cell layers, but cultivar differences were significant after 4 days and highly significant after 10 days. An effect of storage time was only apparent after 10 days of healing, at which time SPK 004 and Kemb 10 had much thinner lignified layers, and thicker desiccated layers, while the other three cultivars showed no change. The cultivars SPK 004 and Kemb 10 had the thinnest lignified layers throughout healing with some roots completely failing to lignify. Yan Shu 1 and Zapallo had the thickest lignified layers. The results confirmed the findings of Walter and Schadel (1983) that after 4 days lignification is complete, but disagreed with the results of St Amand and Randle (1991), who described an almost linear increase in the number of lignified cell layers from 0 to 7 layers between 3 and 12 days after wounding (at 29 ºC and 85% RH).

6.3.2 Continuity and depth of the lignified layer as an indication of wound healing efficiency

Average thickness of the lignified layer gives no indication of the completeness of the layer. As mentioned above, for some wounds, lignification occurred in a patchy pattern, with 5–6 lignified layers in some places, but no lignification in others, while some wounds completely failed to produce lignin. Failure to lignify was observed in 30 out of 46 roots of SPK 004 and 26 out of 42 roots for Kemb 10.

The thickness of the desiccated cell layer, and hence the depth of the lignified layer (Table 6.5), appears to be related to the efficiency of the healing process. Absence of a lignified layer usually coincided with a very thick desiccated layer and development of a hard wound surface. In these cases, the desiccated layer stained bright red with safranin-fast green, indicating the presence of phenolics, consistent with disruption of lignin synthesis. On the other hand, in those cultivars with efficient lignin synthesis, the desiccated layer tended to be thin and the lignified cell layers close to the surface (e.g. Zapallo, Figure 6.2). Strider and McCombs (1958) observed such a pattern when comparing roots cured at different humidities. Thus, they reported a thick desiccated layer (17 cell layers) where the roots were kept at 21 ºC and 60% RH, compared to a depth of 4–6 layers where roots were healed at 95% RH. These authors did not compare different cultivars.

6.3.3 Lignification index as a measure of wound healing efficiency

A range of methods to measure progress of wound healing have been reported in the literature. Cell layers may be counted using microscopy (as above and Strider and McCombs, 1958). Walter and Schadel (1982) developed a rapid method in which artificially inflicted wounds are lifted off the tissue after healing and stained with phloroglucinol. The colour intensity was used to indicate the level of lignification. It was found that 1.4 layers of lignified cells stained pink, 2.6 layers stained red, and above 4 layers stained reddish-purple. Lulai and Orr (1995) measured the wound healing efficiency in potato by determining the transpiration rate through the wound surface using a porometer. These methods generally require sophisticated equipment, or are too time consuming to be used to screen germplasm. In addition, from our observations, we considered that the continuity of lignification is more important in the healing of wounds than the thickness of the lignified layer, and that there appears to be little relationship between thickness of the lignified layer and continuity. Thus Yan Shu 1 tends to have good continuity, but thin lignified layers. We, therefore, developed a rapid method to assess continuity of the lignified layer to determine the wound healing efficiency of cultivars at lower humidities. After staining lignin with phloroglucinol, the continuity of the lignified layer can be easily assessed and scored by the naked eye in tissue sections cut by hand from wounds left to heal for 5 days. The average score for each wound (0–1) was termed the lignification index (LI) (see section 6.2 for further details). This method is quick, as the staining is rapid

Table 6.5 The thickness of the desiccated and lignified cell layers (µm) during healing after artificial wounding of roots of five cultivars of sweetpotato

Days of healing		2 days		4 days		6 days		10 days	
	Storage time from harvest	Desiccated cells	Lignified cells	Desiccated cells	Lignified cells	Desiccated cells	Lignified cells	Desiccated cells	Lignified cells
Yan Shu 1	1 week	23.2	22.8	24.4	22.7	18.2	25.3	22.6	27.4
	6 weeks							20.5	29.2
Kemb 10	1 week	19.4	5.8	26.5	20	14.2	6.4	21	22
	6 weeks							75	2
KSP 20	1 week	19.4	13.7	38.6	28.4	22.7	38.4	44	34.2
	6 weeks							16	32.5
Zapallo	1 week	15.5	20.1	21.4	32.6	11.9	29.1	16.8	27.2
	6 weeks							16.2	19.8
SPK 004	1 week	19.8	3.9	46	5.3	24.8	12.4	58	19.6
	6 weeks							122.8	3.5
Storage effect *P* value		ns	ns	ns	ns	ns	ns	0.013	0.019
Cultivar effect *P* value		ns	< 0.001	0.045	0.011	ns	<0.001	< 0.001	0.001
Cultivar storage effect *P* value				0.004				<0.001	
LSD cultivar		16.95	7.76		16.36		12.76		
LSD cultivar storage								32.04	16.84

Measurements were taken using a microscope equipped with a graticule for four sections per root, after 1 and 6 weeks of storage. Five and four roots obtained from Trial 2 were assessed per cultivar after 1 and 6 weeks, respectively. In some cases it was not possible to determine the thickness of the desiccated cell layers, and this was treated as missing data. Healing conditions: 26 °C and 73% RH.
LSD = least significant difference.

(3 min), requires minimal equipment and can thus be used in developing countries of the tropics where laboratory facilities with microscopes are not available.

Table 6.6 shows the LI for the five cultivars, measured in seven trials. Consistently high LIs were observed for the cultivars Zapallo and Yan Shu 1, while SPK 004 was consistently poor. The cultivars Kemb 10 and KSP 20 were more variable, and appeared to show some dependence on relative humidity.

The relationship between LI and humidity is examined in more detail in Figure 6.4 which shows the LI for 13 cultivars measured at three relative humidities (58%, 65% and 97%). At high humidity, the LI is close to 1 for all cultivars but there is a wide range among cultivars in the ability to lignify at lower humidities.

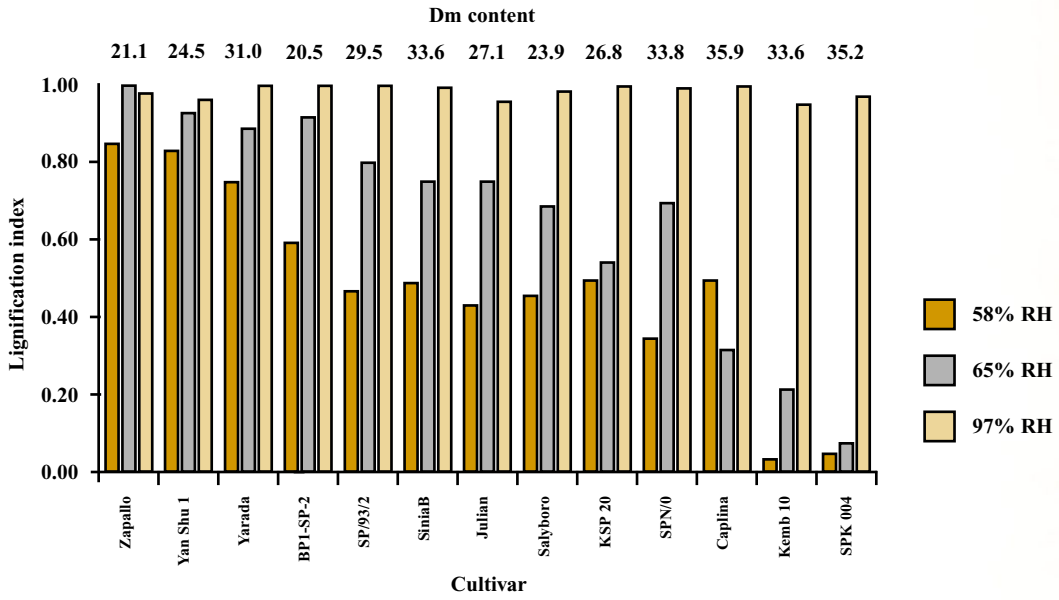
Testing the validity of the lignification index as an indicator of healing and storability

Wound healing is considered important both to reduce water loss through a wound, and also to prevent the entry of pathogens. We tested the validity of the LI as a measure of wound healing in terms of both these aspects.

(i) The relationship between the LI and water loss through a wound

Water loss through wounds was measured directly in terms of transpiration rate using a modified leaf porometer. Figure 6.5 shows the transpiration rate over time through wounds healed at 76% RH for the five key cultivars. The transpiration rate decreases during the wound healing process. This is partly due to desiccation of the top cell layers under the wound, and partly through lignification and formation of the wound periderm. Although cultivars showed a similar pattern, significant differences were observed among them at all time points. Consistent with the LI, the transpiration rates through wounds in Zapallo and Yan Shu 1 were always lower than for Kemb 10, KSP 20 and SPK 004.

In Figure 6.5, the transpiration rates are also shown for two potato cultivars. It is worth noting that the water loss profiles of potato were different from those of sweetpotato. The transpiration rate through wounds in potato decreased more rapidly after wounding, confirming the findings of Lulai *et al.* (1996). Thus the barrier under a potato wound forms more rapidly, or has a more effective sealing capacity than in



Roots were obtained from Trial 8. At least four roots were used for each measurement. Cultivar root dry matter content measured using three roots per cultivar is given at the top of the graph.

Figure 6.4 The LI of thirteen sweetpotato cultivars measured after healing at three relative humidities (58%, 65% and 97%)

Table 6.6 The lignification index of five sweetpotato cultivars as determined in five trials

	Trial 1	Trial 1 (4 weeks)	Trial 2	Trial 2 (6 weeks)	Trial 4 (6 weeks)	Trial 5	Trial 7
Temperature (ºC)	**26**	**26**	**26**	**26**	**21**	**20**	**23**
RH (%)	**82**	**82**	**73**	**73**	**71**	**76**	**65**
Zapallo	1	0.82	1	0.95	0.89	1	0.79
Yan Shu 1	0.8	0.9	1	1	0.96	0.98	0.95
KSP 20	0.85	0.91	0.8	0.9	0.3	0.58	0.33
Kemb 10	1	0.6	0.45	0.16	0.39	0.79	0.25
SPK 004	0.29	0.35	0.38	0.3	0.15	0.31	0.15

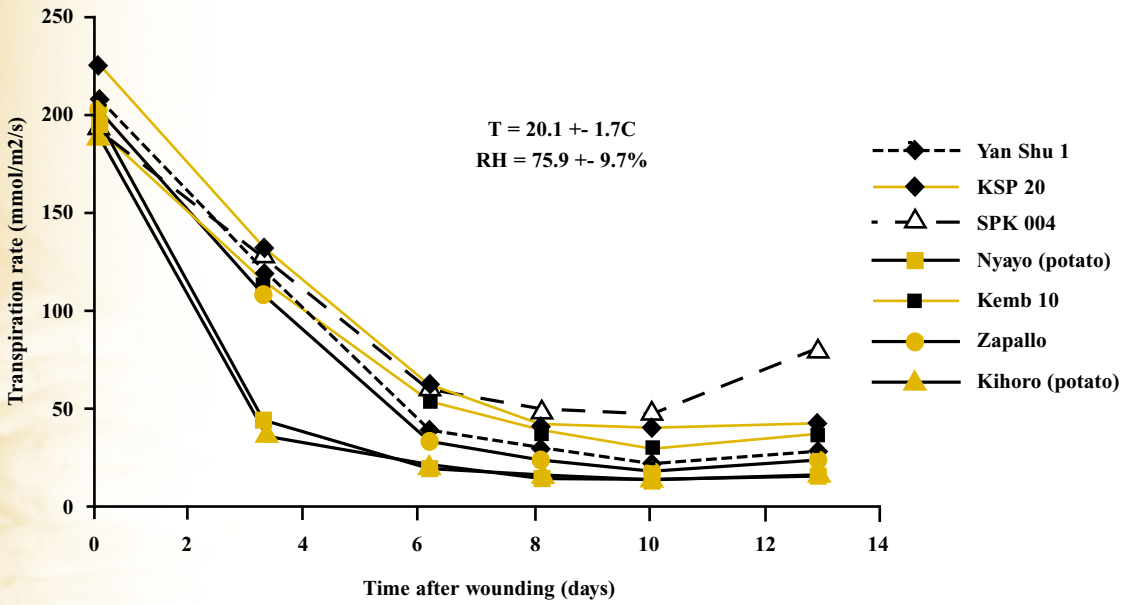
For Trials 1 and 2, measurements were repeated after 4 and 6 weeks of storage, respectively. At least four roots were assessed per cultivar in each trial.

sweetpotato. The barrier formed in potato does not stain with phloroglucinol/HCl, but stains with Sudan III and is assumed, therefore, to consist mainly of suberin (Lulai and Morgan, 1992). The barrier in sweetpotato on the other hand stains bright red with phloroglucinol/HCl and is believed to be a ligno-suberin-like substance with more lignin character (McClure, 1960).

The association between the presence/continuity of lignin and transpiration rate was assessed using statistical tests. These indicated that completeness of the lignified layer (high LI) was related to lower transpiration rates (Table 6.7). Thus, the distribution of the levels of transpiration rate were divided into three categories (i.e. low, intermediate and high) and lignification was divided into two categories, according to the completeness of lignification. Pearson chi square tests indicated that lignification was significantly associated with lower transpiration rates at 6, 8, 10 and 13 days after wounding. No association with transpiration rate was only indicated on day 3 when presumably the wound healing process was not completed.

(ii) The relationship between the lignification index and pathogen invasion of the wound

The effectiveness of wound healing in protecting the wound against pathogen invasion was tested by placing mycelia of *Rhizopus oryzae* directly on to wounds at various stages during the healing process. Figure 6.6 shows the dimensions of lesions allowed to develop over 2 days for twelve cultivars. SPK 004 is notable in the development of lesions on wounds even after 6 days of healing. A contingency table relating the incidence of rots to either the presence of lignin, or to the completeness of the lignified layer (Table 6.8), indicates that the latter is much more important in preventing rotting.



Roots were obtained from Trial 5. Each value is the mean of 5–10 measurements taken with a porometer at the wound site. Healing conditions: 20 °C, 76% RH.

Figure 6.5 Transpiration rate through artificially inflicted wounds for five sweetpotato and two potato cultivars

Table 6.7 Association between lignification and the rate of water loss through wounds after 3, 6, 8, 10 and 13 days of healing

Time after wounding	Transpiration (T) (mmol/s/m²)				Counts of roots: No lignin or patchy lignin	Counts of roots: Continuous lignin layer	Chi square value	*P* value
3 days	Low	76.2	> T		5	9	3.26	= 0.196
	Intermediate	76.2	< T <	89.2	7	4		
	High		T >	89.2	10	5		
6 days	Low	43.2	> T		0	12	11.80	= 0.003
	Intermediate	43.2	< T <	57.8	8	4		
	High		T >	57.8	5	8		
8 days	Low	37.25	> T		3	9	17.20	< 0.001
	Intermediate	37.25	< T <	63.75	1	10		
	High		T >	63.75	10	1		
10 days	Low	29.6	> T		1	9	8.36	= 0.015
	Intermediate	29.6	< T <	69.4	3	8		
	High		T >	69.4	7	3		
13 days	Low	14	> T		2	9	8.71	= 0.013
	Intermediate	14	< T <	19.4	0	12		
	High		T >	19.4	6	6		

Data was collected from Trial 4. Healing conditions: 21 °C, 71% RH.

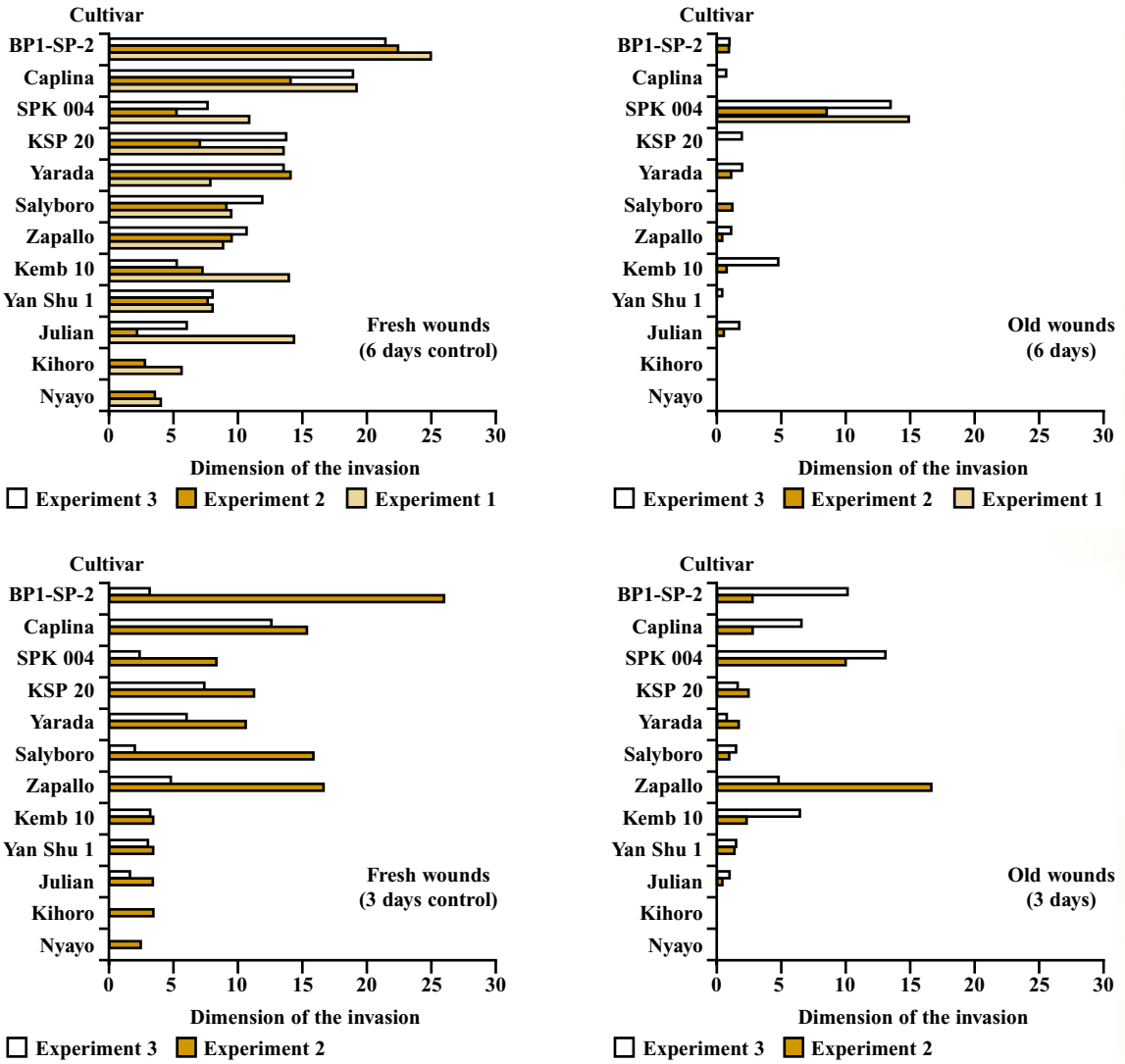
Lignification index and storability (weight loss)

The results above establish that continuity of the lignin layer and, therefore, the LI provide a valid indication of functional wound healing for sweetpotatoes at sub-optimal humidities. In Chapter 5, we postulated that water loss is the main cause of deterioration for roots during marketing in the tropics (Rees *et al.*, in press) and suggested that during marketing most water loss occurs through new or incompletely healed wounds (van Oirschot, 2000). This suggests that the LI for a cultivar could provide an important indication of the potential shelf-life during marketing. Consistent with this, we find that high weight losses occur during storage for cultivars with poor lignification efficiency

Table 6.8 Contingency table using the incidence of roots rotting and/or lignification

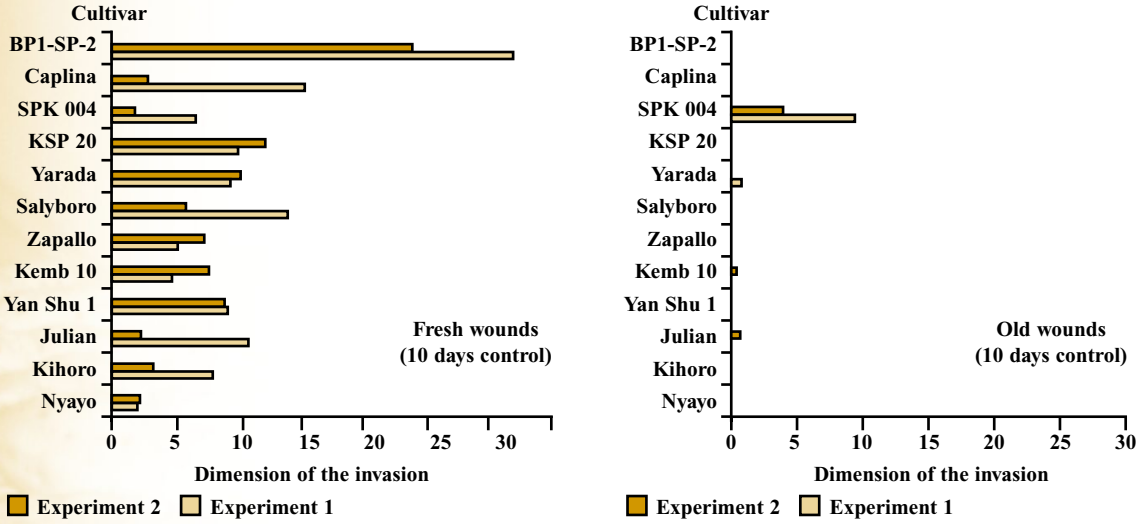
Time after wounding	Rotting	(A) Presence of lignin		(B) Completeness of lignified layer	
		No lignin	Patchy lignin Complete lignification	No lignin Patchy lignin	Complete lignification
Day 3	No rotting	11	26	14	23
	Rotting	18	31	28	21
		Pearson chi square = 0.46 $P = 0.496$ Fisher's exact test: $P = 0.6455$		Pearson chi square = 6.71 $P = 0.010$ Fisher's exact test: $P = 0.01544$	
Day 6	No rotting	5	28	5	28
	Rotting	15	19	26	8
		Pearson chi square = 3.14 $P = 0.076$ Fisher's exact test: $P = 0.0861$		Pearson chi square = 25.33 $P < 0.001$ Fisher's exact test: $P < 0.001$	

In (A) patchy lignified roots were grouped with complete lignified roots, and in (B) patchy lignification was grouped with 'no lignin'.



Freshly cut wounds were used as controls. Mycelial plugs were placed on the wound and the roots were incubated for 2 days in plastic bags to maintain humidity (at 21–25 ºC, 95% RH) ($LSD_{3\ days}$ = 6.96; $LSD_{6\ days}$= 5.74; $LSD_{10\ days}$ = 7.02).

Figure 6.6 The dimensions of lesions of *R. oryzae* placed on 3, 6 or 10-day-old wounds



Freshly cut wounds were used as controls. Mycelial plugs were placed on the wound and the roots were incubated for 2 days in plastic bags to maintain humidity (at 21–25 ºC, 95% RH) ($LSD_{3\ days}$ = 6.96; $LSD_{6\ days}$= 5.74; $LSD_{10\ days}$ = 7.02).

Figure 6.6 ***cont.***

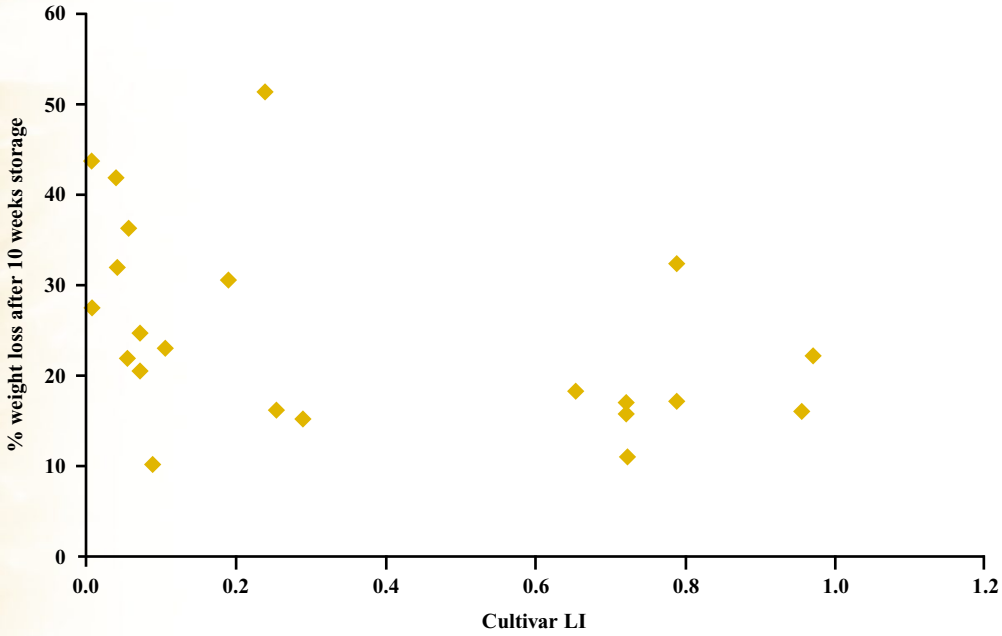


Figure 6.7 The relationship between cultivar LI and percentage root weight loss after 10 weeks of storage. Each point corresponds to a cultivar

(Figure 6.7, correlation coefficient -0.472, $P = 0.027$). This is in contrast with the findings of Walter *et al.* (1989) that the rate of lignification did not relate to storability.

6.3.4 Screening of sweetpotato germplasm using the lignification index

Once we were confident that the LI was a valid measure of wound healing efficiency, we initiated a screening programme of sweetpotato germplasm from all areas of the world to find cultivars that were particularly efficient at healing at lower humidities. These cultivars would be valuable in breeding programmes.

Three sets of cultivars were assessed (see section 6.2. for details). For all sets, a large range in LI was found. Moreover, a comparison of the results obtained for the two harvests of Set A, and for the two seasons for Sets B and C, indicated that cultivar behaviour was fairly consistent (Figure 6.8a–c). Correlation coefficients (r) of 0.80 ($P< 0.001$), 0.47 ($P<0.001$) and 0.82 ($P<0.05$)

were calculated for sets A, B and C, respectively. The lower correlation seen for Set B may be because the roots were stored prior to screening in the first season.

Table 6.9 presents the mean LI for each cultivar measured both at moderate and high humidity. As previously suggested (van Oirschot, 2000) almost all cultivars have high LI at high humidity, although there are a few exceptions, for example, Beauregard, Hernandez and L86-33.

Why does wound healing efficiency vary among cultivars?

A key question is what is the physiological basis for differences in wound healing efficiency among sweetpotato cultivars. If we could understand this, then it might help in the selection of better cultivars, and may even provide the basis for genetic modification in future decades. Research is ongoing in the investigation of this issue. We found a relationship between dry matter (DM) content and wound healing efficiency in several trials. High DM cultivars tended to be less efficient at wound healing at moderate humidities. This was in agreement with Rees *et al.* (1998) who reported that high dry matter is often related to short shelf-life.

The finding that cultivars with higher DM content have less efficient wound healing would be unwelcome. High DM is associated with sensory characteristics (*Flouriness*) important for consumer acceptability in East Africa (Kapinga *et al*., 1997) and is a key attribute for processing. The characteristic is considered so important worldwide that CIP has a specific initiative to breed for higher DM cultivars. It thus becomes important to determine whether it is possible to breed for cultivars with high DM content and good wound healing characteristics.

Figure 6.9 classifies the cultivars screened according to their origin. It is apparent that the cultivars cluster by origin, for both DM and LI. For example, cultivars originating from East Africa had higher DM content and lower LI than cultivars from the USA or Central/South America. Although for the whole data set, there is a significant negative correlation between DM and LI, correlation analysis carried out separately for cultivars of each origin did not reveal any significant relationships between DM and LI.

To investigate this matter further, a set of five experiments was conducted on a selection of cultivars in which DM content and LI at moderate humidity was measured for each individual root. In this way it was possible to use multivariate linear regression analysis to model LI in terms of cultivar and DM content. The linear regression models obtained from the five experiments, with their significance levels, are presented in Table 6.10. In all cases, cultivar was the

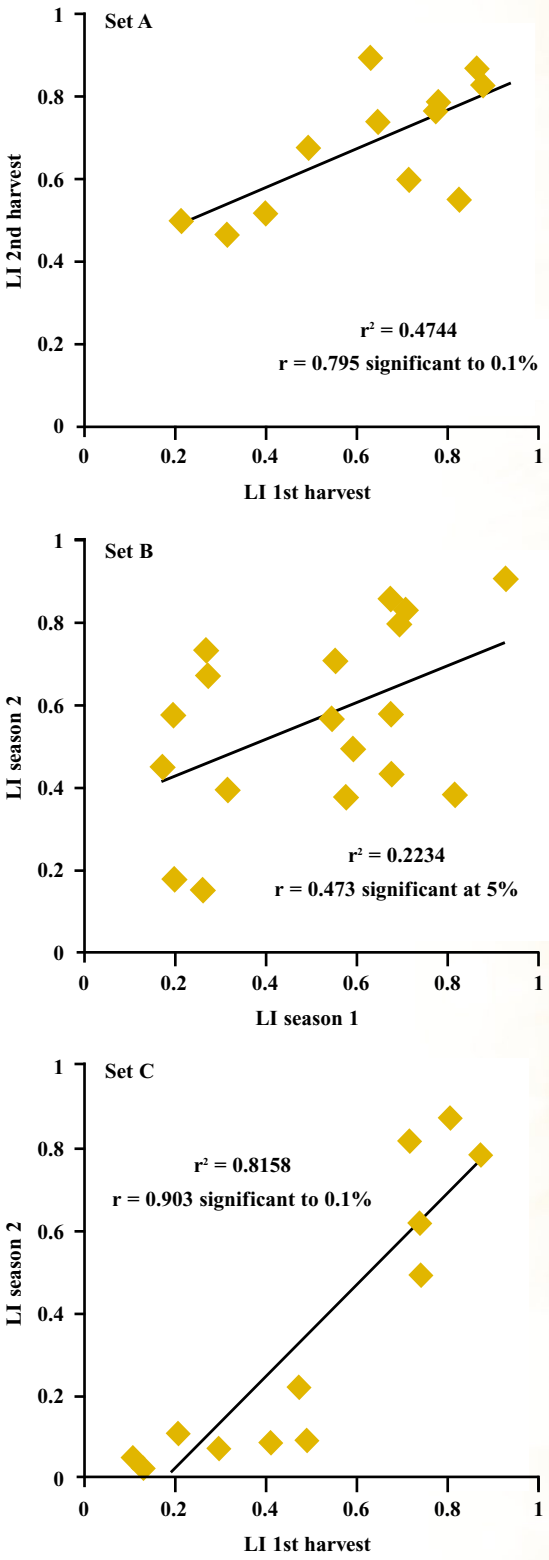


Figure 6.8 Comparison of the LI measured at moderate humidity by cultivar: (a) Set A, first and second harvest; (b) Set B, first and second season; (c) Set C, first and second season

most important factor. However, in each case, root DM content made an extra contribution, albeit a small one, to the strength of the model. The general conclusion from this data is that DM content does affect wound

Table 6.9 Mean LIs measured at high and moderate relative humidity, for all cultivars screened

Cultivar	LI high RH	LI moderate RH
W-308	0.99	0.93
Blesbok	0.83	0.87
Yan Shu 1	0.98	0.86
Zapallo	0.91	0.80
Jewel	0.78	0.78
Cemsa 74-228	0.90	0.77
Xu Shu 18	0.83	0.77
PI 595856	0.96	0.75
W-287	0.78	0.74
Sumor	0.93	0.71
Tainung No 64	0.78	0.71
Beauregard	0.64	0.68
Brondal	0.90	0.68
Jayalo	-	0.68
Naveto	0.90	0.68
W-325	0.91	0.67
Tinian	0.95	0.63
Sinia	0.95	0.60
W-317	0.94	0.57
L-86-33	0.64	0.57
PI 595873	0.97	0.56
Santo Amaro	0.90	0.55
W-341	1.00	0.55
W-345	0.94	0.48
Mafutha	0.93	0.45
Mugande	0.98	0.39
97K-11	1.00	0.38
Mogamba	0.90	0.37
Regal	0.98	0.35
SC 1149-19	0.95	0.33
Kemb 37	0.85	0.31
SPN/0	0.84	0.29
Bilagala	0.73	0.29
White Regal	0.96	0.27
Picadito	0.94	0.26
KSP 20	0.96	0.23
Kemb 10	0.94	0.22
Tanzania	0.98	0.20
NC 1560	0.95	0.20
PI 538354	0.96	0.18
Hernandez	0.61	0.13
Budagala	0.94	0.13
Polista	1.00	0.10
Kagole	0.96	0.08
SPK 004	0.94	0.05
Iboja	1.00	0.05
Mwanamonde	1.00	0.05

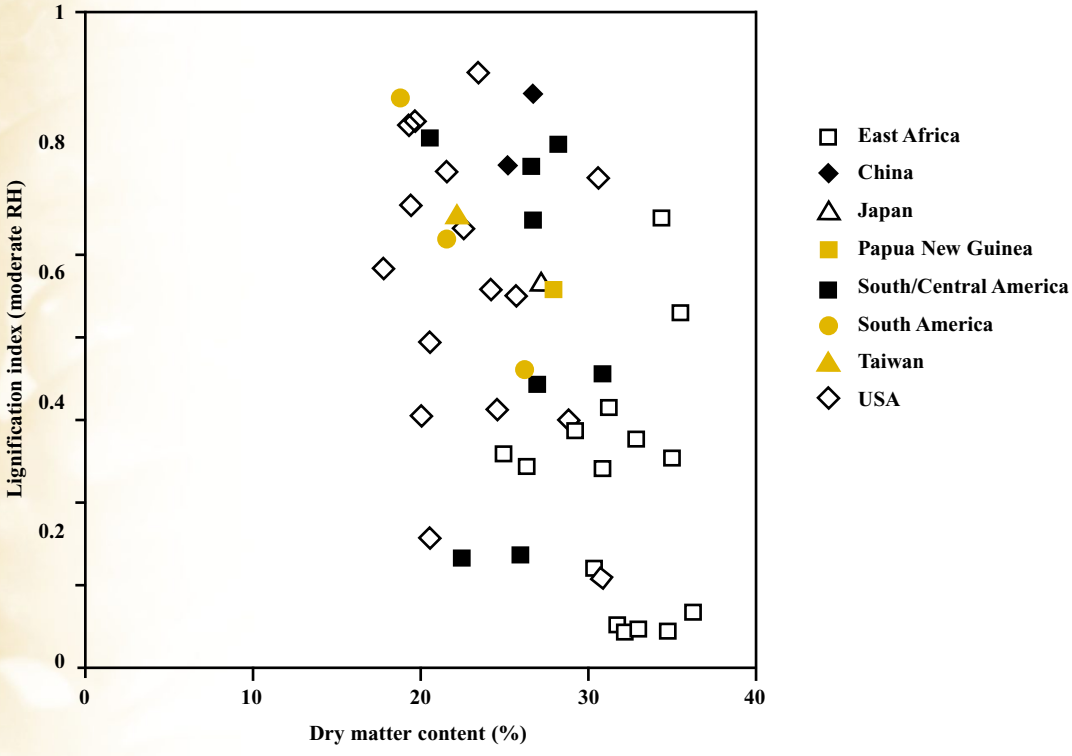


Figure 6.9 Relationship between LI (at moderate relative humidity) and dry matter content for all cultivars tested, classified by location

Table 6.10 Multivariate linear regression models for LI at moderate humidity

Experiment	Model (cultivar)	Model (DM and cultivar)
1	LI = 0.303 + constant* cultivar *P*<0.001 34.3% variance accounted for	LI = 1.182–0.030 DMCi + constant* cultivar *P*<0.001 36% variance accounted for
2	LI = 0.625 + constant* cultivar *P*<0.001 23.1% variance accounted for	LI = 1.276–0.036 DMCi + constant* cultivar *P*<0.001 28.2% variance accounted for
3	LI = 0.272 + constant* cultivar *P*<0.001 40.0% variance accounted for	LI = 0.902–0.021 DMCi + constant* cultivar *P*<0.001 41.7 % variance accounted for
4	LI = 0.224 + constant* cultivar *P*<0.001 43.1% variance accounted for	LI = 1.075–0.027 DMCi + constant* cultivar *P*<0.001 45.4% variance accounted for
5	LI = 0.37 + constant* cultivar *P*<0.045 7.2% variance accounted for	LI = 1.59–0.058 DMCi + constant* cultivar *P*<0.001 18.3% variance accounted for

DMCi = Initial DM content. For experiments 1–4, this was estimated using final DM content and weight loss during the experiment. For experiment 5, the root was cut in halves at the start of the experiment, and one half used to measure DMC.

Experiment 1: 18 roots each of 10 cultivars; Experiment 2: 13–18 roots each of 13 cultivars; Experiment 3: 182 roots each of 12 cultivars; Experiment 4: 17–18 roots each of 11 cultivars; Experiment 5: 12 roots each of 9 cultivars.

healing ability at moderate humidity, but there are other cultivar factors that are much more important.

This matter is still under investigation (further information can be obtained by contacting Q. van Oirschot or D. Rees).

6.4 Conclusions and implications

Under sub-optimal humidities (65% ± 10) the wound healing process in sweetpotato follows a similar pattern to wound healing under curing conditions. However, the thickness of the desiccated cell layer, and hence the depth of the lignified layer, is affected by both cultivar and humidity. Some cultivars consistently failed to produce a lignified layer while in others the layer is often not continuous. The continuity of the lignified layer is more important for effectiveness of wound healing than the actual thickness.

Wound healing efficiency as measured by lignification was found to be a major factor in the shelf-life of sweet potato cultivars. Lignification of wounds correlates with reduced rate of weight loss and fungal infection.

A method for assessing efficiency of wound healing termed the lignification index, based on assessing the continuity of lignified layers has been developed. This quick and simple method estimates the probability that wound healing occurs, and does not require a microscope. This could be a suitable method by which breeding programmes could assess their germplasm.

References

ARTSCHWAGER, E. and STARRETT, R.C. (1931) Suberization and wound-periderm formation in sweetpotato and gladiolus as affected by temperature and relative humidity. *Journal of Agricultural Research*, **43** (3): 353–364.

COLLINS, W.W. and WALTER, W.M. (1985) Fresh roots for human consumption. pp. 153–173. In: *Sweet Potato Products: A Natural Resource for the Tropics*. Bouwkamp, J.C. (ed.). Boca Raton: CRC Press Inc.

DUARTE, V. and CLARK, C.A. (1993) Interaction of *Erwinia chrysanthemi* and *Fusarium solani* on sweet potato. *Plant Disease*, **77**: 733–735.

JENKINS, P.D. (1982) Losses in sweet potatoes (*Ipomoea batatas*) stored under traditional conditions in Bangladesh. *Tropical Science*, **24** (1): 17–28.

KAPINGA, R.E., JEREMIAH, S.C., RWIZA, E.J. and REES, D. (1997) Preferences and Selection Criteria of Sweetpotato in Urban Areas of the Lake Zone of Tanzania. Chatham, UK: Natural Resources Institute. (unpublished)

KUSHMAN, L.J. and WRIGHT, F.S. (1969) Sweet potato storage. *USDA Agriculture Handbook*, No. 358.

LULAI, E.C., GLYNN, M.T. and ORR, P.H. (1996) Cellular changes and physiological responses to tuber pressure bruising. *American Potato Journal*, **73**: 197–209.

LULAI, E.C. and MORGAN, W.C. (1992) Histochemical probing of potato periderm with neutral red: a sensitive cytofluorochrome for hydrophobic domain of suberin. *Biotechnic and Histochemistry*, **67**: 185–195.

LULAI, E.C. and ORR, P.H. (1995) Porometer measurements indicate wound severity and tuber maturity affect the early stages of wound-healing. *American Potato Journal*, **72**: 225–241.

MCCLURE, T.T. (1960) Chlorogenic acid accumulation and wound healing in sweet potato roots. *American Journal of Botany*, **47**: 277–280.

MORRIS, L.L. and MANN, L.K. (1955) Wound healing, keeping quality, and compositional changes during curing and storage of sweet potatoes. *Hilgardia*, **24** (7): 143–183.

NIELSEN, L.W. and JOHNSON, J.T. (1974) Postharvest temperature effects on wound healing and surface rot in sweetpotato. *Phytopathology*, **64**: 967–970.

PASSAM, H.C., READ, S.J. and RICKARD, J.E. (1976) Wound repair in yam tubers: physiological processes during repair. *New Phytologist*, **77**: 325–331.

REES, D., KAPINGA, R., RWIZA, E., MOHAMMED, R., VAN OIRSCHOT, Q., CAREY, E. and WESTBY, A. (1998) The potential for extending shelf-life of sweet potato in East Africa through cultivar selection. *Tropical Agriculture (Trinidad)*, **75**: 208–211.

REES, D., VAN OIRSCHOT, Q.E.A., AMOUR, R., RWIZA, E., KAPINGA, R. and CAREY, T. (in press) Cultivar variation in keeping quality of sweetpotatoes. *Postharvest Biology and Technology*.

RICKARD, J.E. (1985) Physiological deterioration in cassava roots. *Journal of Science of Food and Agriculture,* **36**: 167–176.

ST AMAND, P.C. and RANDLE, W.M. (1991) Ethylene production as a possible indicator of wound healing in roots of several sweet potato cultivars. *Euphytica*, **53**: 97–102.

STRIDER, D.L. and MCCOMBS, C.L. (1958) Rate of wound phellem formation in the sweet potato. *American Society for Horticultural Science*, **72**: 435–442.

VAN OIRSCHOT, Q.E.A. (2000) *Storability of Sweet Potatoes (Ipomoea batatas (L.)) under Tropical Conditions: Physiological and Sensory Aspects*. PhD thesis, Cranfield University.

VAN OIRSCHOT, Q.E., REES, D., LUCAS, C., MAINA, D., MCHARO, T. and BOHAC, J. (2001) Sweetpotato: germplasm evaluation for wound healing efficiency. *Acta Horticulturae*, **584**: 31–40.

WALTER, W.M., HAMMETT, L.K. and GIESBRECHT, F.G. (1989). Wound healing in sweet potato and stability during subsequent storage. *Journal of the American Society for Horticultural Science*, **114**: 94-100.

WALTER, W.M. and SCHADEL, W.E. (1982) A rapid method for evaluating curing progress in sweet potatoes. *Journal of the American Society for Horticultural Science*, **107**: 1129–1133.

WALTER, W.M. and SCHADEL, W.E. (1983) Structure and composition of normal skin (periderm) and wound tissue from cured sweet potatoes. *Journal of the American Society for Horticultural Science*, **108**: 909–914.

WEIMER, J.L. and HARTER, L.L. (1921) Wound cork formation in the sweet potato. *Journal of Agricultural Research*, **21** (9): 637–647.

WOOLFE, J.A. (1992) *Sweet Potato. An Untapped Food Resource*. Cambridge: Cambridge University Press.