CHAPTER 7

Improving long-term storage under tropical conditions: role of cultivar selection

D. Rees, Q.E.A. van Oirschot, L. B. Mbilinyi, M. Muhanna and K. I. Tomlins

7.1 Introduction

7.1.1 The potential for long-term storage under tropical conditions

Storage temperature has a large effect on the keeping qualities of sweetpotato. The ideal storage temperature is 13–15 ºC; lower temperatures can cause physiological damage, whereas higher temperatures promote fast metabolic rates and, therefore, increase rates of deterioration. Thus, where temperature-controlled storage is feasible, roots can be stored for extended periods of time. For example, in the USA, sweetpotatoes are often stored for up to a year (Picha, 1986). In some cooler areas of the tropics, long-term fresh storage is also routinely practised. For example, in the highlands of south-west Tanzania, sweetpotatoes are traditionally stored in pits under banana trees for several months (Kapinga *et al.*, 1995).

Despite increased metabolic rates, it has been demonstrated that even in warmer regions, fresh storage for several months is technically feasible. This has been demonstrated in Uganda, where sweetpotatoes were stored for up to 4 months in pits and clamps (Hall and Devereau, 2000). At this time, there is still an element of risk in such storage; some of the trials failed with most of the roots rotting. Mbeza *et al.* (1997) reported that although sweetpotatoes can

be stored in pits for up to 5 months, the market value obtained thereafter is low and unattractive. The main reasons are losses in weight due to shrinkage and attack by moulds. To what extent success or failure is due to

the design of the store, or due to variations in climate during storage, has not yet been established.

7.1.2 Root characteristics desirable for long-term storage

In Chapter 5, we distinguished between root 'shelf-life' and 'storability', and discussed the fact that characteristics that make a cultivar suitable for long-term storage may not be the same as those that give a cultivar a long shelf-life and, therefore, make it suitable for marketing.

Resistance to rotting

We have established that for long shelf-life during marketing and handling, a cultivar should have a low susceptibility to water loss and efficient wound healing (Chapters 5 and 6). However, long-term storage structures are usually designed to maintain a high humidity. In the high temperatures experienced in the tropics, curing will occur naturally, and efficiency of wound healing is less of an issue. During marketing and handling, the main controlling factor is the extent of unhealed surface wounds through which pathogens causing rots can enter, whereas under storage environments, the intrinsic resistance of tissues to pathogen growth is likely to be more important.

Root respiration

Where ventilation is low, root respiration will lead to a decrease in levels of oxygen and an increase in carbon dioxide. If the change in atmospheric composition is too great, then roots may be unable to metabolize normally, and may switch to anaerobic respiration (see below), which in turn leads to root damage. Thus, cultivars with low respiration rates and resistance to switching to anaerobic respiration would be advantageous.

Anaerobic respiration in sweetpotato roots

For all plant tissue, when oxygen levels fall below a certain level or carbon dioxide rises above a certain level, the tissues switch to anaerobic respiration. The subsequent build-up of ethanol and acetaldehyde leads to the build-up of off-flavours and eventually leads to irreversible tissue damage and death. Compared to other root crops, sweetpotato is particularly susceptible to anaerobiosis, as the switch in metabolism occurs at a higher oxygen and lower carbon dioxide level. This becomes a particular problem given the elevated rates of respiration during storage at tropical temperatures. The susceptibility to low oxygen levels in sweetpotato is probably also an important reason why the sweetpotato crop is particularly susceptible to waterlogging.

Little work has been carried out to determine the range of characteristics in storage roots (such as rates of respiration and susceptibility to anaerobiosis) that would affect long-term storage under tropical conditions. However, studies on US cultivars have indicated not only a large range of respiratory rates, but significant variation in the level of oxygen at which roots switch to anaerobic respiration, and their ability to re-metabolize the products of anaerobic respiration on return to normal atmospheric composition (Kays, 1985). The fact that cultivars differ in susceptibility to waterlogging is also noteworthy (Paul Thompson, University of Mississippi, personal communication).

7.1.3 Objectives

While work is still ongoing in the improvement of store design, the main emphasis in this chapter is to examine the extent to which cultivar selection might affect the success of long-term storage. The main characteristics identified as being desirable for long-term storage are intrinsic resistance to rots, low respiration rates, and a reduced tendency to switch to anaerobic respiration. Trials conducted to examine cultivar effects on all of these parameters are presented, with some discussion as to the relationship with storability. (For information on store design contact K. Tomlins, Q. van Oirschot, T. Ndengello or E. Rwiza.)

7.2 Methods

7.2.1 Laboratory trials to test cultivar reaction to storage environment

A study was conducted at Sokoine University of Agriculture to examine the effect of storage environment on the keeping qualities of key Tanzanian sweetpotato cultivars. Five cultivars, namely Budagala, Iboja, Mwanamonde, Sinia and SPN/O, were harvested and subjected to four different storage environments. These differed in degree of ventilation, i.e. an open sack rolled down, a closed sack, a double layered closed sack and a lined closed sack (waterproof polythene bag closed in closed sack).

Roots were assessed weekly throughout the experiment for a range of quality attributes such as weight, external appearance (rotting) and internal appearance, by cutting the roots into longitudinal and cross-sections. The methods used are similar to those described in Chapter 5.

7.2.2 Trials conducted to assess sweetpotato cultivars for susceptibility to rots (*Rhizopus oryzae*)

In order to assess the intrinsic resistance of roots to rot growth (as distinct from rot entry through wounds, see section 7.1), trials were conducted in which roots were artificially inoculated with pathogens causing rots. Inoculation was carried out by removing a disc of root tissue and inserting a disc of mycelia obtained from the edge of a culture grown on agar. Roots were then stored under controlled conditions and the growth of the pathogen assessed in terms of lesion size and weight.

Trials Conducted to Assess Susceptibility to Rots

Initial trials were conducted in 1998 and 1999 on Tanzanian cultivars grown on-station at Sokoine University of Agriculture, Tanzania. Later experiments were conducted at Silsoe College, UK using roots which had been grown by the International Potato Center (CIP) in Nairobi, and air-freighted to the UK. A summary of the cultivars used is given in Tables 7.1 and 7.2.

Table 7.1 Cultivars grown in Tanzania used in trials to assess susceptibility to rots

Experiments 1, 2 and 3 conducted in 1998	Experiments 4, 5 and 6 conducted in September 1999	Experiments 7 and 8 conducted in November/December 1999
Mwanamonde	Mwanamonde	SPN/0
SPN/0	SPN/0	Iboja
Iboja	Iboja	Sinia
Budagala	Sinia	Budagala
Sinia	Ukerewe	
Hali ya mtumwa		
Chenzeru		
Sindano		
Elias		
Ukerewe		

7.2.3 Assaying for antifungal compounds by testing growth of pathogens on agar produced from sweetpotato extract

In order to determine the nature of tissue resistance to pathogens, experiments were conducted in which tissue extracts were taken from infected roots at various locations, and used to make agar, on which the growth of *Rhizopus oryzae* was tested.

Table 7.2 Cultivars grown by CIP used in trials conducted in the UK to assess susceptibility to rots

Experiments 9, 10 and 11 conducted in May/August 2000
KSP 20
SPK004
Kemb 10
Yan Shu 1
Zapallo

Experiments to Assay for Antifungal Compounds

Sweetpotato roots were inoculated using the method described in section 7.2.2 and kept in plastic bags for 3 days. After 3 days, roots were removed from the plastic bags. Using a sharp knife, the infected sweetpotato root tissue (2 mm thick) was cut at points 0.5 cm (border tissue), 5 cm (middle tissue) and 10 cm (healthy tissue) from the edge of the lesion. The sweetpotato tissues were then freeze-dried, and ground into a fine powder. Thus, for each cultivar there were three powder types: border tissue powder, middle tissue powder and healthy tissue powder. Different sweetpotato meal agar (SMAs) were then prepared as follows.

(i) A mixture of powdered sweetpotato (7.5 g) and water (250 ml) was boiled over a water bath for 1 h.

(ii) The contents were filtered through a muslin cloth.

(iii) Sterile distilled water was then added to make up the volume to 250 ml.

(iv) Oxoid agar (5 g) was added and the mixture boiled until the agar dissolved.

(v) Sterilization was carried out at 15 psi for 15 min.

(vi) After cooling the agar was poured into petri dishes.

During the assessment of the rate and extent of growth of *R. oryzae* on different SMAs, a 3 mm disc of mycelia was cut at the periphery of a 2-day-old culture of *R. oryzae* on PDA using a 3 mm diameter sterile corkborer. The disc was placed at the centre of the SMA media in the petri dish (about 4.5 cm from the periphery). The petri dishes containing SMAs and the pathogen were then put in sterile ventilated bags. The pathogen was allowed to grow for 1 day on SMA. The rate of growth of *R. oryzae* was estimated by measuring the diameter of the circle of pathogen mycelia after every 6 h, taking the initial point of placement of the agar disc as the reference. After 1 day, the form of growth on different SMAs was estimated using the following scale.

Class	Description
1	No growth
2	Weak growth (very sparse and slender mycelia)
3	Good growth (relatively thick and compact mycelia)
4	Very good growth (very thick and compact mycelia)

7.2.4 Laboratory trials to assess respiration rates

A set of experiments was carried out at the Natural Resources Institute (NRI) to examine the respiratory characteristics of three cultivars, as it was hypothesized that these characteristics would be important for the storability of the cultivars. In particular we were interested in the rates of respiration, and the susceptibility of the roots to switch to anaerobic respiration as the storage atmosphere became modified (i.e. decreased oxygen levels, increased carbon dioxide levels). The scientific literature suggests that there is variation among sweetpotato cultivars in the levels of oxygen at which the switch to anaerobic respiration occurs.

Respiration Rate Trials

Roots of three sweetpotato cultivars were transported from the Lake Zone Agricultural Research and Development Institute (LZARDI), Ukiriguru to NRI in the luggage hold of a commercial airline. The roots were well insulated during the flight. A tinytalk data-logger recorded the temperature continuously. The minimum and maximum temperatures to which the roots were exposed were *x* and *y*, respectively. Once at NRI, the roots were stored under ambient temperatures at high humidity (>80%).

Two experiments were conducted to measure the rates of respiration. For each, six good roots (low levels of damage, free from rots or insect damage) were selected for each cultivar. Each root was weighed and placed in a 3 litre jar, in a controlled-temperature room set to 25 ºC. The 18 jars containing roots were left open in the controlled-temperature room for 1 h to allow temperature equilibration, and were then closed with an air-tight seal. Air samples were removed at intervals from each jar through a rubber septum using an air-tight syringe. Levels of carbon dioxide and oxygen were measured by gas chromatography using a molecular sieve (to separate carbon dioxide from oxygen/nitrogen) and poropak column (to separate oxygen and nitrogen), arranged so that they could be run alternately in series and with the poropak bypassed. Levels of the gases were measured using a thermal conductivity detector. Oxygen and carbon dioxide concentrations were approximated by assuming that nitrogen comprised 78% air, and that the thermal conductivity of the three gases is identical. (This leads to inaccuracies, but does not affect the validity of comparisons.)

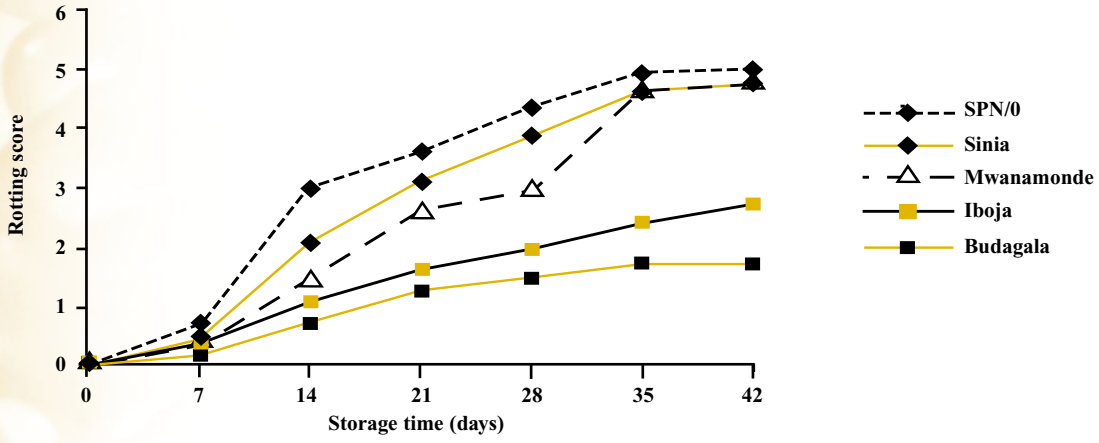
The first experiment was terminated after 30 h while the second continued to 180 h.

7.3 Results and discussion

7.3.1 Cultivar differences in keeping qualities under long-term storage conditions

Significant cultivar differences in susceptibility to rotting were found. This is illustrated in Figure 7.1, which shows the rate of rotting for roots stored in the highest humidity treatment (lined closed sacks).

The ranking of cultivar storability was not the same as that when roots were stored under simulated marketing conditions (low humidity). At the highest humidities, the cultivar Budagala appeared to be the least susceptible to rotting, but at lower humidities it did not keep well.



Source: Mbilinyi *et al*. (2000).

Figure 7.1 The rate of rotting for five key Tanzanian cultivars stored under high humidity conditions

7.3.2 Susceptibility of sweetpotato cultivars to rotting

A detailed study of the susceptibility of sweetpotato cultivars to rot was carried out in the Morogoro region of Tanzania using artificial inoculation methods. In that region, the main storage rot was identified as *Rhizopus oryzae*. Screening of cultivars over successive seasons indicated that cultivar differences in susceptibility did exist. For many cultivars, susceptibility varied with growing conditions, but some cultivars were more stable. Budagala, mentioned above, and Sinia were consistently resistant, while SPN/0 was consistently susceptible (Table 7.3).

7.3.3 Tissue defence mechanisms against rotting

There are a variety of ways in which plant tissues protect themselves against attack by pathogens causing rot. Healing of surface wounds is an important defence mechanism, but other mechanisms come into force once infection has occurred.

It has been observed in a number of tuber crops that infection can lead to hypersensitive cell death. In this case, following infection, a barrier is created by cell death that effectively halts pathogen growth. This has been studied in potato (e.g. Doke and Chai, 1985), but currently there is no information on a hypersensitive reaction in sweetpotato roots as a result of pathogen infection.

Other defence mechanisms involve antifungal chemicals. Some of these are present in tissues prior to infection, but most appear to be induced afterwards.

Kojima and Uritani (1974, 1978) studied some pre-existing compounds of high molecular weight, including a spore agglutinating factor composed primarily of polygalacturonic acid, which agglutinates germinating spores of certain fungi and inhibits growth. Latex in some plants acts as a natural defence system against certain insects. The roots of some sweetpotato cultivars produce substantial quantities of latex when cut. The potential of latex as a defence mechanism against the sweetpotato weevil, *Cylas formicarius*, has been demonstrated, and it is likely that it is also involved in resistance to some post-harvest pathogens.

Phytoalexins are low molecular weight antimicrobial compounds which are synthesized by, and accumulate in, plant cells after microbial infection (Paxton, 1981). In sweetpotato, the phytoalexin, ipomeamarone, a furanoterpenoid, has been identified. Accumulation of ipomeamarone has been found to occur in sweetpotato roots infected by a range of pathogens. Imaseki and Uritani (1964) reported that ipomeamarone accumulated in roots after infection by the black rot fungus (*Ceratocystis fimbriata*).

Arinze and Smith (1980) observed that ipomeamarone concentrations were greater in restricted lesions than expanding lesions caused by fungal post-harvest pathogens, which is consistent with its defensive role.

As well as acting as precursors in the formation of physical barriers such as lignin and suberin, phenolics also contribute to resistance through chemical inhibition of pathogen growth and cell wall degrading enzymes. Arinze and Smith (1982) found elevated levels of phenolics, polyphenol oxidase and peroxidase, both within the lesions of five fungal rots and the surrounding healthy tissue. A greater accumulation of these compounds was found in fungal rots with limited lesions. Inhibition of the fungal polygalacturonase by extracts of infected sweetpotato roots was also demonstrated.

7.3.4 The physiological basis for differences in sweetpotato cultivars in susceptibility to rotting

In order to determine the basis of the cultivar differences in resistance to rots under high humidity (sections 7.3.1 and 7.3.2), experiments were conducted

Table 7.3 Ranking of cultivars in terms of decreasing susceptibility to *Rhizopus oryzae* as measured by rot weight following artificial inoculation

Experiments 1, 2 and 3 conducted in 1998	Experiments 4, 5 and 6 conducted in September 1999	Experiments 7 and 8 conducted in November/December 1999
Ukerewe	SPN/0	Iboja
SPN/0	Iboja	SPN/0
Elias	Mwanamonde	Sinia
Chenzeru	Ukerewe	Budagala
Sindano	Sinia	
Iboja		
H/mtumwa		
Budagala		
Sinia		
Mwanamonde		

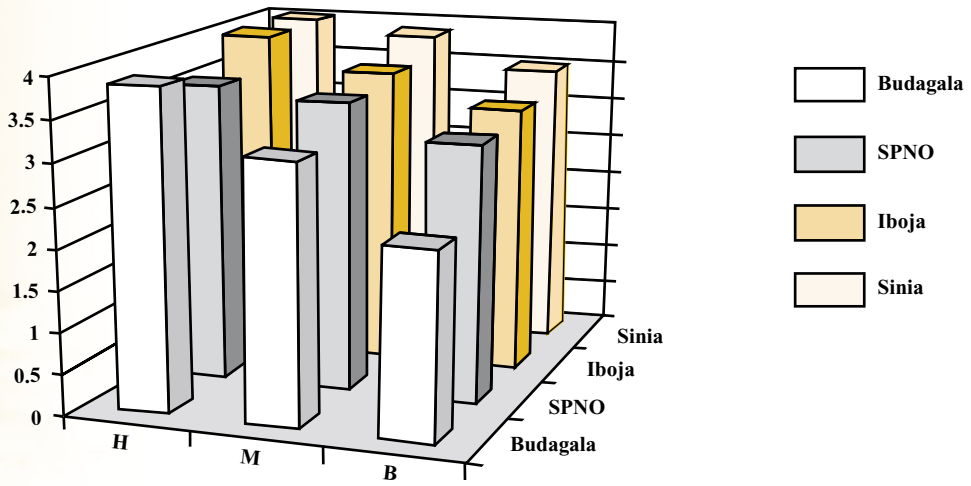


Figure 7.2 Relative growth rates of *R. oryzae* on SMA agar made with tissue extracts taken 10 cm (H), 5 cm (M) and 1 cm (B) from the disease lesion on roots of four cultivars. LSD = 0.8. Interaction significant at 5% level of probability. Growth was scored as described on p.89.

to distinguish whether resistance was due to tissue mechanical characteristics, barrier formation or chemical characteristics.

Figure 7.2 shows the results of an experiment in which extracts were made of tissue isolated from infected sweetpotato roots, and used to make agar on which the rate of pathogen growth was assessed. It was found that agar made from tissue extracted from the border of lesions inhibited growth of *Rhizopus oryzae*, and that the effect was cultivar specific. The greatest effects were seen for Budagala, a cultivar noted for its resistance to *R. oryzae*. This suggests that the resistance is at least partly due to the induction of an antifungal chemical. This chemical (or chemicals) must be heat resistant, as it survived autoclaving during the production of the agar. This agrees with previous studies in which antifungal compounds have not been destroyed by heating at 100 ºC for 5 min (Kojima and Uritani, 1976) and are stable under autoclaving (Jenkins, 1981).

7.3.5 Assessment of cultivar respiration rates

The results of two experiments to compare the respiration rates for three cultivars: SPN/0, Sinia and Polista are summarized in Table 7.4. The rate of respiration was calculated for each root from the rate of increase of carbon dioxide over the first 4–9 h (Respiration 1) and over the first 24–30 h (Respiration 2) in an enclosed jar. Due to wide variability among roots, cultivar effects were not significant for either individual experiment, but a combined analysis indicates that Polista had a significantly higher rate of respiration than SPN/0 and Sinia, with no significant difference between the latter two cultivars.

When measuring respiration rates in a closed system, as here, the atmosphere becomes modified over time, and can affect the behaviour of the roots. For the first measurement (Respiration 1), it is unlikely that the modification of the atmosphere would have significantly affected the rates of respiration. However, for the second measurement, carbon dioxide levels

Table 7.4 Rates of respiration for roots of three sweetpotato cultivars

Cultivar	Experiment 1		Experiment 2		Combined analysis	
	Resp 1	Resp 2	Resp 1	Resp 2	Resp 1	Resp 2
SPN/0	35.6	34.7	56.6	51.2	46.1	43.0
Sinia	44.2	41.0	45.8	44.5	45.0	42.8
Polista	80.6	70.3	75.3	69.0	77.9	69.7
Cultivar effect (*P* value)	0.098	0.111	n.s.	n.s.	0.032	0.043
LSD	43.8	36.1	38.5	35.5	27.3	23.7

Respiration rates are given in ml CO_2/kg/h. Respiration 1 is calculated from CO_2 levels measured after 4–8 h, assuming the initial CO_2 level was 0. Respiration 2 is calculated from CO_2 levels measured after 24–30 h, assuming the initial CO_2 level was 0. For Experiments 1 and 2, each value is the mean of data measured for six roots.

could be as high as 16%, and oxygen levels as low as 7%, so that some decrease in rate of oxygen consumption might be expected. Respiration 2 was in fact lower that Respiration 1 for all three cultivars in both experiments, but the effect was small.

7.3.6 Assessment of cultivar susceptibility to switch to anaerobic respiration

In Experiment 2, the roots were kept in sealed jars for 6 days, and the modification of the atmosphere monitored for this period. This allowed calculation of the respiratory quotient (carbon dioxide produced/oxygen consumed) for each root over a range of oxygen and carbon dioxide levels. This data can give information about the atmospheric composition at which the roots switch from aerobic to anaerobic respiration, because at the switch point, the respiratory quotient will rise.

Figure 7.3a–b shows the respiratory quotient for each root at each time interval, plotted against the average oxygen level and average carbon dioxide level for that time period. This indicates that sweetpotatoes switch metabolism at 2–3% oxygen and 23–24% carbon dioxide. There is, however, no indication from this data of any differences between the cultivars.

7.4 Conclusions and implications

From studies of East African germplasm, we have demonstrated that sweetpotato cultivars differ in their susceptibility to pathogens responsible for rotting when maintained at high humidity, and that this is at least partly due to the induction of antifungal chemicals. We have also demonstrated that differences in respiration rate exist, although we have no evidence of a difference in tendency to switch to anaerobic respiration.

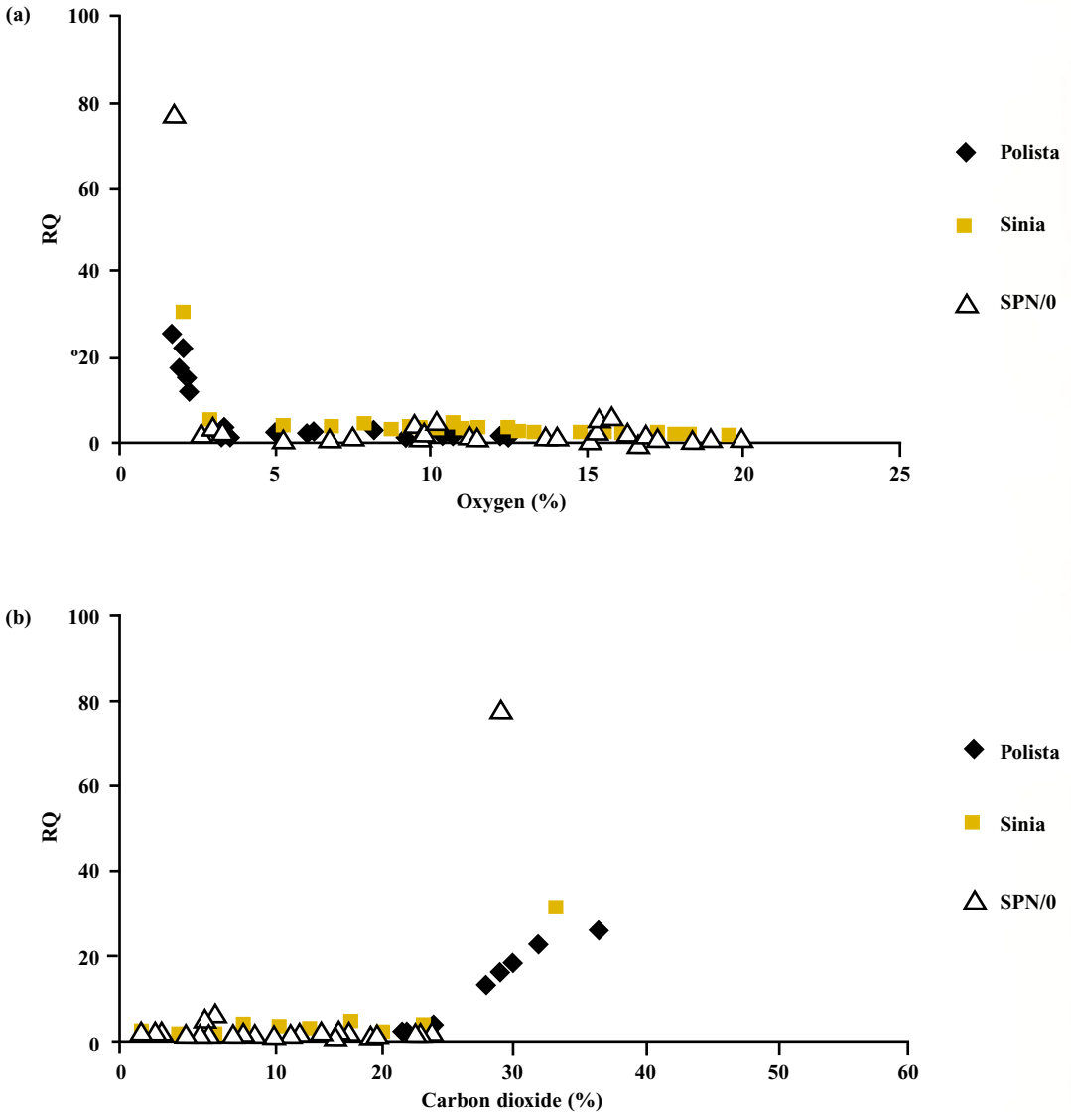


Figure 7.3 **Respiratory quotient plotted against (a) oxygen concentration and (b) carbon dioxide concentration for each individual root over each time period of measurement in Experiment 2**

At the time of writing, a set of storage trials is underway in Tanzania to test different storage designs, and to determine the relative importance of storage design and cultivar selection. These storage trials are using three cultivars: Sinia, SPN/0 and Polista. So far we have no information on the susceptibility of Polista to rot pathogens, and the information we have for SPN/0 and Sinia is confusing. In section 7.3.2, SPN/0 appeared to be consistently more susceptible to rotting than Sinia, but this was contrary to the findings of section 7.3.1. With respect to respiratory characteristics, we have found that Polista has a higher rate of respiration than the other two cultivars.

From this data, if cultivar effects are important for successful storage, we might predict that Polista would be a poor storer due to its higher respiration rates. Indeed, consistent with this, initial results suggest that Polista shows higher rates of shrivelling. However, until these trials are complete, we cannot make firm conclusions as to the value of selecting cultivars for long-term storage on the basis of respiratory characteristics or resistance to rotting.

References

ARINZE, A.E. and SMITH, I.M. (1980) Antifungal furanoterpenoids of sweet potato in relation to pathogenic and non-pathogenic fungi. *Physiological Plant Pathology*, **17**: 145–155.

ARINZE, A.E. and SMITH, I.M. (1982) Distribution of polygalacturonase, total phenolics substances, polyphenol oxidase and peroxidase in root zones in sweet potato. *Plant Pathology*, **31**: 119–122.

DOKE, N. and CHAI, H.B. (1985) Activation of superoxide generation and enhancement of resistance against compatible races of *Phytophthora infestans* in potato plants treated with digitonin. *Physiological Plant Pathology*, **27**: 323–334.

HALL, A.J. and DEVEREAU, A.D. (2000) Low-cost storage of fresh sweet potatoes in Uganda: lessons from participatory and on-station approaches to technology choice and adapative testing. *Outlook on Agriculture*, **29**: 275–282.

IMASEKI, H. and URITANI, I. (1964) Ipomeamarone accumulation and lipid metabolism in sweet potato infected by the black rot fungus. Accumulation mechanism of ipomeamarone in the infected region with special regard to contribution of the non-infected tissue. *Plant and Cell Physiology*, **5**: 133–142.

JENKINS, P.D. (1981) Differences in susceptibility of sweet potato cultivars to infection by storage fungi in Bangladesh. *Phytopathologische Zeitschrift*, **102**: 247–256.

KAPINGA, R.E., EWELL, P.T., JEREMIAH, S.C. and KILEO, R. (1995*) Sweetpotato in Tanzanian Farming and Food Systems: Implications for Research.* Nairobi: International Potato Center (CIP) and Ministry of Agriculture, Tanzania.

KAYS, S.J. (1985) The physiology of yield in sweet potato. In: *Sweet Potato Products: A Natural Resource for the Tropics.* Bouwkamp J.C. (ed.). Boca Raton: CRC Press Inc.

KOJIMA, M. and URITANI, I. (1974) Possible involvement of spore agglutinating factors of the host plants in manifestation of host specificity by various strains of black rot fungus, *Ceratocystis fimbriata*. *Plant Cell Physiology*, **15**: 733–737.

KOJIMA, M. and URITANI, I. (1976) Possible involvement of furanoterpenoid phytoalexins in establishing host parasite specificity between sweet potato and various strains of *Ceratocystis fimbriata. Physiological Plant Pathology*, **8:** 97–111.

KOJIMA, M. and URITANI, I. (1978) Studies on factor(s) in sweet potato which specifically inhibits germ tube growth of imcompatible isolates of *Ceratocystis fimbriata. Plant and Cell Physiology* **19:** 71–81.

MBEZA, H.F. (1997) Storage of Sweet Potato in Malawi – Second Year Report. Bunda College of Agriculture, PO Box 219, Lilongwe, Malawi. (unpublished)

MBILINYI, L.B., MULUNGU, L.S., REUBEN, S.O.W.M., SENKONDO, F.J., MABAGALA, R.B., REES, D. and KAPINGA, R. (2000) The effect of storage environment on the shelf-life of sweetpotato. pp. 513–519. In: *Fifth Triennial Congress of the African Potato Association, Kampala, Uganda, 29 May–2 June 2000. APA Conference Proceedings,* Volume 5 (A).

MUHANNA, M. (2001) *Physiological and Biochemical Factors Determining Resistance of Sweetpotatoes to Post-harvest Pathogens in Tanzania*. M.Phil thesis, Cranfield University.

PAXTON, J.D. (1981) Phytoalexins – a working redefinition. *Phytopathologische Zeitschrift*, **101**: 106–109.

PICHA, D.H. (1986) Weight loss in sweet potatoes during curing and storage: contribution of transpiration and respiration. *Journal of the American Society for Horticultural Science*, **11**: 889–892.