Storage Root Production in Sweetpotato

(*Ipomoea batatas* (L.) Lam.)

Stephen L. Lewthwaite

2004

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Storage root production in sweetpotato (*Ipomoea batatas* (L.) Lam.)

A thesis presented in partial fulfilment of the requirements for

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New Zealand

Stephen L. Lewthwaite

2004

To my wife

Michelle

1966 – 1993

&

our daughter

Holly

Abstract

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a root crop well established throughout the world's tropical and sub-tropical regions. Despite sweepotato's importance, it has been under-researched relative to many other major crops. The main objective of this thesis is to contribute to a fuller understanding of the genetic and physiological factors underlying the production of sweetpotato storage roots for human consumption.

The sweetpotato genome is diverse and subject to high levels of natural somatic mutation. Applying the AFLP (amplified fragment length polymorphism) technique allowed a direct comparison of inter and intra-cultivar DNA (deoxyribonucleic acid) base sequence variation. Analysis of the variation indicated that although sweetpotatoes are clonally propagated, clones show a lack of genetic fidelity to their source. Further, the level of genetic variation within the cultivar 'Owairaka Red' indicated the continuing emergence of distinct new strains.

Plant field establishment represents the interaction of both propagation and growth phases of storage root production. A range of establishment techniques were investigated in a field trial under commercial conditions. Sprouts cut from seed roots and held for six days rather than immediate planting improved establishment as measured by growth, at little expense. Plug raised plants also improved establishment, while potentially reducing the degree of intra-cultivar genetic variation.

Plant carbohydrate partitioning in three cultivars, 'Beauregard', 'Beniazuma' and 'Owairaka Red' was examined by field trial over the period of storage root growth. While cultivars differed in the proportions of dry matter partitioned to leaf, stem and root organs, the cultivar specific ratios of leaf to stem dry weight were relatively stable over time. Total storage root dry weight increased with time for all cultivars, but the distribution of storage root grades by size was cultivar specific. Within the storage roots % dry weight increased over time in all cultivars, but total sugar concentration only increased for 'Beauregard' and 'Beniazuma'.

Finally, a storage root disorder called 'brown centre' curtails the temperate growing season so was investigated using low temperature storage and a field trial with various nutrition regimes. The disorder was found to be associated with susceptible germplasm, high soil nitrogen and harvest time.

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List of Abbreviations

a.i.	active ingredient
AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
AVRDC	Asian Vegetable Research and Development Centre
B	boron
B.C.	Before Christ
BC	brown centre
bp	base pair
BT	brown tissue
C	cavity
c.	*circa* or approximately
^{o}C	degrees Centigrade
C&FR	New Zealand Institute for Crop & Food Research Ltd.
CIP	International Potato Centre
cm	centimetre
CTAB	hexadecyltrimethylammonium bromide
cv.	cultivar
^{11}C	Carbon isotope 11
^{14}C	Carbon isotope 14
DAT	days after transplanting
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside 5'-triphosphate
%DW	percent dry weight
EDTA	ethylenediaminetetra-acetic acid disodium salt
FAA	formol-acetic-alcohol
g.	g-force
g	gram
ha	hectare
HC	Hardcore
HI	harvest index

HPLC	high performance liquid chromatography
K	Potassium
Kb	kilobase
kg	kilogram
KLC	potassium chloride
L.	Linnaeus
LA	Individual leaf area
LAD	Leaf area duration
LAI	leaf area index
Lam.	Lamarck
LAR	leaf area ratio
Lat.	Latitude
LDM	leaf dry matter
LDW	Individual leaf dry weight
Log_e	natural logarithm
LSD	least significant difference
LSU	Louisiana State University
m	metre
μg	microgram
μL	microlitre
μM	micromole
me	milliequivalent
mL	millilitre
mm	millimetre
mM	micromole
N	Nitrogen
NAR	net assimilation rate
NASA	National Aeronautics and Space Administration
ng	nanograms
NIWA	National Institute of Water and Atmospheric Research Ltd.
P	Phosphorous
P	probability
PAUP	phylogenetic analysis using parsimony
PCR	polymerase chain reaction

pH	potential of hydrogen or acidity/alkalinity
pmol	picomole
R^2	coefficient of determination
RDM	root dry matter
REML	Residual maximum likelihood
RGR	relative growth rate
rpm	revolutions per minute
s	second
s.e.	standard error of the mean
SDM	stem dry matter
SLA	specific leaf area
t	metric ton
TAE	Tris acetate EDTA
TDM	total plant dry matter
TS	total sugar
UPGMA	unweighted pair group method with arithmetic averaging
USA	United States of America
v/v	volume per volume
VAM	Vesicular-Arbuscular Mycorrhizae
w/v	weight per volume

Chapter 1

General introduction

Sweetpotato is an ancient crop developed within the prehistoric civilizations of Central and South America (O'Brien 1972). The contemporary version of the crop is grown throughout the tropical and subtropical regions of the globe, across cultures and continents (Huaccho & Hijmans 2000). In the future sweetpotato may well be grown in space, as suggested by preliminary ground-based experiments (Mortley et al. 1998) conducted on behalf of the US National Aeronautics and Space Administration (NASA) and China's sweetpotato experiments undertaken in space using recoverable satellites (Guangqin et al. 2002).

Despite sweetpotato's history as an important food stuff it has long been an under-researched crop (Bouwkamp 1985a; Hill et al. 1992; Jones & Bouwkamp 1992; Clark 1998). This is partially due to its perception as a food for animals or the poor, even within developing countries (Sheng et al. 1992; Jackson 1996), and its historic use as an emergency food during famine in developed countries (Duell 1985). However perceptions are rapidly changing, driven primarily by the development of novel cultivars suitable for processing or with added health benefits (Yamakawa 1998). Coincident with rising commercial interest in the sweetpotato and its diversity, is the realization of systematic limitations in our research based knowledge of the crop and its management (Jones & Bouwkamp 1992; Hill et al. 1992; La Bonte et al. 1998).

While much of the world's sweetpotato production is still fed directly to animals or used for industrial processing (Sheng et al. 1992; Woolfe 1992), this thesis examines sweetpotato cultivars cropped for human consumption. Various parts of the sweetpotato plant have been used as a food source, including leaves (Lin et al. 1985; YanHwa et al. 2000), vine tips (Villareal et al. 1985; Guo et al. 2001) and storage roots (Collins & Walter 1985; Sheng et al. 1992). However the sweetpotato crop is primarily grown for its swollen storage roots and this is the organ most commonly traded and consumed around the world.

Scientific attention is increasingly being focused on developing cultivars with storage roots for specific niche markets. This includes optimising production of the carbohydrates starch and sugar for flavour (Lewthwaite et al. 1997) and for use in the manufacture of noodles, drinking alcohol and sugar syrups (Suganuma & Kitahara 1998). Sweetpotato root tissue is also increasingly traded in processed pre-prepared form (crisps, fries, frozen peeled roots) or in a form (puree, flour, chunks) readily incorporated into composite products (Lewthwaite 1998; Tamaru 1998). As typical sweetpotato cultivars may be considered excessively sweet for use as a staple in some cultures, clones with alternative flavours have been produced (Kays et al. 1998). Of particular importance is the development of novel cultivars with roots contributing to the human diet, both as a basic food stuff but with added physiological functions such as antioxidant or specific nutritional properties (Cevallos-Casals & Cisneros-Zevallos 2002; Yamakawa & Yoshimoto 2002). In the future, the value of sweetpotato as a basic food stuff may be considered secondary to its ability to synthesize useful phytochemicals, such as natural food colourants (Odake 1998).

Escalating diversity in sweetpotato use, concomitant with increasing cultivar specialty, has highlighted research priorities which are recognised by the international research community and which motivate the research in this thesis. This study examines storage root production in the sweetpotato crop, based on genotypic effects, field establishment, storage root development and seasonal limitations.

Genotypic effects: A basic necessity for efficient crop production is the ability to select and maintain distinct cultivars. However even carefully conserved sweetpotato cultivars appear to decline in yield and quality over time (Edmond & Ammerman 1971; Villordon & La Bonte 1995). While this effect may be due to the accumulation of somatic mutations (La Bonte et al. 2000), assessing the contribution of mutation to cultivar decline is confounded by potentially increasing virus titre through vegetative propagation (Villordon & La Bonte 1996; Fletcher et al. 2000). In this investigation the level of intra-cultivar somatic mutation using molecular techniques, is assessed.

Field establishment: Once a cultivar is selected for production it must be mass produced then efficiently established in the field, while minimising the inclusion of

mutant types. As propagation and transplanting are significantly expensive phases in sweetpotato production, they are areas recognised for further research (Jones 1992; Fukazawa 1998). Novel field establishment techniques suggested by scientific literature and growers' experience are evaluated in this thesis.

Storage root development: Kays (1992) states that current knowledge of sweetpotato root chemistry is limited, with notable advances in only three main areas; toxic stress metabolites which are primarily produced as a defence response to specific fungal infections (Wilson & Burka 1984), the storage root enzyme β-amylase, which is involved in the hydrolysis of starch to maltose during cooking (Morrison et al. 1993; Lewthwaite et al. 1997) and the molecular genetics of the principal storage root protein sporamin (Nakamura 1992). There is little information available on the carbohydrate composition of raw roots, particularly during plant development (La Bonte et al. 2000), which is a fundamental requirement for the selection and growing of market specific cultivars. The partitioning of carbohydrates during plant growth for three cultivars is investigated.

Seasonal limitations: The sweetpotato is a perennial crop which is generally grown on an annual basis (Bouwkamp 1984). Storage roots do not ripen or mature, as can be broadly illustrated by the ability of roots to produce sprouts throughout their growth, the lack of a natural quiescent or dormant phase, and the inability of consumers to discern differences in eating quality based on harvest date. As storage roots do not reach a final stage of development the growing season is potentially open-ended, with harvest times based on storage root size distribution. In Australia the sweetpotato growing season typically varies from 16 to 25 weeks (Loader et al. 2000). However the longer storage roots are retained in the ground, the more potential exposure to quality diminishing damage through diseases, pests and environmental conditions. In the final section of this thesis season-limiting factors related to environmental conditions within a temperate climate are examined.

The objective of this study is to examine the factors underlying crop management at four critical stages of sweetpotato storage root production: genotype maintenance, field establishment, storage root development and season length.

Chapter 2

Literature review

2.1 Introduction

The sweetpotato (*Ipomoea batatas* (L.) Lam.) is a herbaceous dicotyledonous perennial plant grown primarily as a root crop. The crop is well established around the world, primarily through historical dispersal within subsistence agriculture but now by acceptance into modern horticultural systems.

The sweetpotato is thought to have originated in Central America or northwestern South America, with early cultivation beginning about 3000 B.C. (O'Brien 1972). By 2500 to 2000 B.C. the sweetpotato was widely grown in Peru and Mexico (O'Brien 1972). The most common names for this plant in Latin America are 'batata', 'camote', 'boniato', 'batata doce', 'apichu', and 'kumara' (Huáman 1997). The New Zealand Maori also knew the sweetpotato as 'kumara' (Yen 1974), suggesting an ancient connection between Polynesia and the Americas which has yet to be explained (Clark 2001; Rossel et al. 2001).

In Europe, Linnaeus first (1753) described this species using the Latin binomial system, as *Convolvulus batatas.* However in 1791, Lamarck re-classified the species within the genus *Ipomoea* on the basis of stigma shape and the surface of pollen grains, changing the name to its current form, *Ipomoea batatas* (L.) Lam. (Huáman 1997). To distinguish the sweetpotato from the tuberous potato (*Solanum tuberosum* L.), the internationally accepted convention for the common English name is now the one word spelling 'sweetpotato' (Janick 1978), rather than the older version, 'sweet potato'.

Although sweetpotato shoot tips and leaves may be eaten, the swollen root is the main organ used for human consumption. The swollen root is generally called a 'storage root' (Hill et al. 1992) and by classical botanical definition is an enlarged true root

(Kays et al. 1992), although there has been some discussion on adopting the word 'tuber' and 'tuberization' (Wilson & Wickham 1992a, 1992b).

Ranked by current world production, sweetpotato is the tenth major crop, after sugar cane, maize, wheat, rice, potatoes, sugar beet, cassava, soybeans and barley (Huaccho & Hijmans 2000). Of the ten leading crops consumed globally, sweetpotato ranks in the top five in terms of labour efficiency, as well as caloric and biomass production per unit area (Bouwkamp 1985a). World sweetpotato production (1998) was estimated at 129,164,392 t from 8,867,267 ha (Huaccho & Hijmans 2000), and is generally considered fairly stable at around 100 million metric tons per year. The main sweetpotato production regions by area, include; Asia (78.4%), Africa (17.1%), North America (1.8%), South America (1.3%) and Oceania (1.2%). Approximately two thirds of the world production is utilized in China (Villareal 1982), while over 95% of the crop is produced in developing countries (Yencho et al. 2002). Sweetpotato is an important traditional crop in New Zealand, however the area cropped locally is only about 0.01 % of the world's total (Lewthwaite 2002b).

Based on a review of current knowledge, the objective of this study is to examine the factors underlying crop management at four critical stages of sweetpotato storage root production: genotype maintenance, field establishment, storage root development and season length. Previous research pertaining to this objective is summarised in this and ensuing chapters.

2.2 Genetics

In systematic plant taxonomy, the sweetpotato is assigned to the family Convolvulaceae Juss., which comprises 55 genera. *Ipomoea* L., the largest genus within the Convolvulaceae (Austin & Huáman 1996), contains *c.* 500 species (McDonald & Mabry 1992). The sweetpotato is further classified in subgenus *Eriospermum*, section *Eriospermum* and series *Batatas* (Austin & Huáman 1996).

Sweetpotato is phylogenetically related to thirteen wild species in section *Batatas* (McDonald & Austin 1990; Huáman 1992). The cultivated species, *I. batatas*, is an

outcrossing hexaploid (6x=2n=90) and is well known for its large edible storage roots. Some of the wild species in section *Batatas*, namely *I. littoralis* Blume, *I. tabascana* McDonald & Austin and *I. tiliacea* (Willdenow) Choisy are tetraploid (4x=2n=60), while the other species are diploid (2x=2n=30), apart from *I. trifida* (H.B.K) G. Don which includes plants that are 2x, 3x, 4x and 6x (Huáman 1992, 1997). Species in section *Batatas* are divided into two groups, based on their cross-compatibility with sweetpotato. Group I includes species which are cross-compatible with sweetpotato, while Group II includes those which are cross-incompatible (Zhang et al. 2002). There is evidence that the fibrous roots in some of the wild species are capable of thickening to various degrees (Nishiyama 1982), and that thickening is influenced by ploidy level. Species *I. cordato-triloba* Dennstedt, *I. tiliacea* and *I. ramosissma* (Poiret) Choisy all exhibit root thickening (Diaz et al. 1992). Thickened roots have also been found in diploid *I. trifida* (Komaki & Katayama 1999), which has been used in hybridisation studies with *I. batatas* (Oreja et al 1992). *I. trifida* is considered a possible ancestor of the modern sweetpotato (Jarret et al. 1992).

The cultigen *I. batatas* is not known in the wild state (Huáman 1997) and escapes do not appear to survive effectively (Kays 1985). It is still uncertain how sweetpotato's hexaploid genome was derived, by autopolyploidy or allopolyploidy. Allopolyploidy occurs when the genomes of different species are combined into hybrids and then duplicated by unreduced gametes at fertilization, autopolyploidy occurs by duplication of the genome within a species. Based on morphological characteristics, it has been hypothesised that *I. triloba* and *I. trifida* contributed to an allopolyploid sweetpotato genome (Austin 1988). However cytogenetic and molecular evaluation suggests the genome may be autopolyploid (Shiotani 1988; Ukoskit & Thompson 1997). Regardless of the sweetpotato genome's derivation, the species is highly variable, with most of the important horticultural characteristics inherited quantitatively (Jones et al. 1986; Martin & Jones 1986).

Gene flow within the species *I. batatas* itself is restricted by both widespread self-incompatibility and cross-incompatibility (Onwueme 1978). For applied breeding purposes, sweetpotato are divided into cross-incompatible groups (Martin & Cabanillas 1968; Vimala 1989; Murata & Matsuda 2003). The incompatibility classification

currently used in Japan recognises 18 groups (Reynoso et al. 1997). Incompatibility in *I. batatas* is generally considered sporophytic (Kowyama et al. 2000), but other limiting factors also occur (Murata & Matsuda 2003). Tissue culture and molecular techniques have been employed to allow the introgression of genes from different sweetpotato compatibility groups (Wang et al. 1997), section *Batatas* Group II species (Zhang et al. 2002) and wider sources (Woodward et al. 1999). It should be noted that sweetpotato are subject to a high level of natural somatic mutation, so that although diversity produced through hybridisation is relatively fixed and conserved by subsequent clonal propagation, genetic fidelity is only approximated even under conditions of strict germplasm management (Edmond & Ammerman 1971; Huett 1982).

Due to the crop's historic origins, natural hybridization, systematic breeding and mutation, sweetpotato clones vary widely in phenotypic and developmental characteristics such as growth habit, canopy structure and leaf shape, root arrangement, storage root shape and pigmentation, time to maturity, as well as disease and pest resistance (Broadhurst et al. 1997; Huáman 1997). A very large number of sweetpotato cultivars exist, broadly divided into three groups on the basis of cooked root texture; (a) firm, dry, and mealy, (b) soft, moist, and gelatinous, (c) coarse roots for animal feed (Onwueme 1978).

2.3 Root anatomy

The sweetpotato plant is generally propagated as a cutting rather than a seedling (Weaver & Bruner 1927; Coleman 1972), so all initial roots are adventitious in derivation and originate within the underground stem tissue (Esau 1977). The sweetpotato's ability to root easily may be due to the presence of pericycle and endodermal tissue layers in the stem (Onwueme 1978; Caveness et al. 1983). Lateral roots arise directly from the adventitious roots, dramatically increasing the root volume with primary, secondary and occasionally tertiary laterals (Weaver & Bruner 1927).

The first adventitious roots develop from pre-formed root primordia which are commonly visible on the stem at the time of cutting (Hahn & Hozyo 1983). These primordia form in pairs either side of the stem just below the point of petiole attachment

(Togari 1950). As the leaves are arranged in a spiral alternate pattern, the pairs of adventitious roots derived from nodes are efficiently arrayed in three dimensional space without mutual interference. Sweetpotato have a 2/5 phyllotaxis, where five leaves are arranged spirally in two revolutions of the stem for any two leaves to be in the same plane (Onwueme 1978; Huáman 1992). The buried cut stem end forms callus tissue, from which further adventitious roots may form. Adventitious roots derived from nodal and callus tissue all bear lateral roots. Swollen edible storage roots develop from the initial adventitious roots, primarily those formed at nodes rather than the callus.

During early development adventitious root diameter is related to the size of the apical meristem (Artschwager 1924; Togari 1950). At this stage, adventitious roots can be divided by their diameter into two classes, 'thin' and 'thick'. While it has been reported that 'thick' roots tend to form at nodes and 'thin' roots on inter-nodal regions (Togari 1950), it is commonly observed that no roots at all form within internodal regions. In cross section, the stele structures of 'thin' and 'thick' young roots differ in anatomy, with thin roots having a tetrarch arrangement of their vasculature and thick roots having pentarch, hexarch or higher polyarch (McCormick 1916; Wilson & Lowe 1973a). The cross section of a tetrarch root stele, for example, shows a vascular cylinder arranged with four xylem poles (or points) alternating with phloem tissue. It should be noted that roots may change from a pentarch or hexarch structure to tetrarch along their length (Wilson 1982). The first formed adventitious roots tend to be of the 'thick' category and have the potential to become storage roots. At an early stage potential storage roots can also often be recognised through destructive examination by the presence of a central pith possibly with a central metaxylem cell (Artschwager 1924; Togari 1950; Wilson & Lowe 1973a). Metaxylem is part of the primary vascular system, differentiating after the protoxylem but before secondary xylem forms (Esau 1977).

A developmental series can then be predicted based on root diameter. 'Thick' adventitious roots may first develop into 'fibrous roots' (<5mm diameter), then 'pencil roots' (<15mm diameter) and finally 'storage roots (>15mm diameter). The proportion of roots in each of these categories is determined by the interaction of the cultivar and environment through time, so the development of potential storage roots can be temporarily or permanently frustrated or blocked at any one of these developmental

stages (Togari 1950; Kays 1985). The 'thin' roots generally become fibrous roots, although some may become pencil roots.

Fibrous roots do not have a carbohydrate storage function, but anchor the plant and provide extensive access to soil moisture and mineral reserves. Permanent fibrous roots, rather than those going through a transitory stage, are incapable of further lateral growth as lignification of the vascular stele permanently impairs the ability of the cambium to provide secondary thickening (Wilson & Lowe 1973a). Pencil roots have a limited carbohydrate storage function, as the stele is only partially lignified allowing some secondary lateral thickening (Wilson 1970). The development of pigmentation in sweetpotato roots is indicative of the onset of secondary growth (Wilson & Lowe 1973a), which may lead either to the differentiation of lignified cells which frustrate increases in girth or to the proliferation of the parenchyma tissue observed in storage root initiation. Storage roots primarily develop from 'thick' adventitious roots with pentarch or higher polyarch steles which remain unlignified, allowing relatively unhindered cell division. Storage roots are the principal carbohydrate storage organs in sweetpotato.

The width of the storage root is due to the contribution of cells from two distinct types of cambia. In cross section the normal vascular cambium within a developing storage root eventually forms a circle, with xylem forming on the inner face gradually displacing the cambium outward, while phloem forms to the outside (Esau 1977). The secondary xylem also contains a large proportion of parenchyma storage cells. However, anomalous cambia are common in sweetpotato storage roots and occur within various tissue types. The anomalous cambia may form around protoxylem groups, the central metaxylem cell, within secondary xylem derived from the vascular cambium, within xylem derived from previous anomalous cambia, around protophloem groups, or even independently of vascular groups (Artschwager 1924; Wilson & Lowe 1973a). The anomalous cambia mainly develop in the parenchyma cells around individual secondary xylem vessels or vessel groups, producing a few tracheary elements (xylem) towards the vessels, a few sieve tubes (phloem) and laticifers (latex ducts) away from the vessels and considerable storage parenchyma in both directions. So phloem elements form within tissue that originally differentiated as xylem tissue (Esau 1977).

The relative importance of the different cambia is cultivar specific, storage roots thickened primarily by the activity of the normal vascular cambium tend to be uniformly narrow while the involvement of the anomalous cambia leads to more globular storage roots (Wilson 1982).

Storage root growth begins by the deposition of carbohydrates at the distal (farthest) end of the developing storage root and then proceeds upward to the proximal end (Kays et al. 1982). A relatively unthickened root stalk attaches the proximal end of the storage root to the plant's stem, while at the distal end of the storage root a thin fibrous root may continue down into the soil. Both the root stalk and end root delimit the storage root, and may become lignified as the storage section develops (Wilson 1982). Root diameter increases under secondary growth, so the original storage root cortex is sloughed off along with the epidermis and endodermis (Artschwager 1924; Esau 1977).

A cork cambium (phellogen) forms in the outer part of the pericycle as the root develops, producing a cork (phellem) layer on the outside of the storage root and a layer of phelloderm (parenchymatous tissue) to the inside, just beneath the root's surface (Artschwager 1924). These three layers together constitute the periderm or skin, which does not contribute much bulk to the storage root but is important for protection against pathogen entry and water loss particularly after harvest.

There are a number of environmental conditions that temporarily or permanently inhibit the specific formation of storage roots, although the root itself may continue to grow with the complete or partial absence of a storage function. Exposure of the root system to light inhibits storage root formation, until the root is returned to dark conditions (Tsuno & Fujise 1965; Hozyo & Kato 1976). Oxygen deficiency in the root zone through waterlogged or high bulk density soils will also inhibit storage root formation, although the effect is reversible if the oxygen concentration is raised (Togari 1950; Watanabe et al. 1968; Chua & Kays 1981). Dry compact soil may produce relatively high numbers of pencil roots due to partial lignification of the stele (Togari 1950). High fertiliser rates, particularly nitrogen supply, may adversely affect the development of storage root numbers through increased shoot competition for photosythates (Samuels

1967; Watanabe & Nakayama 1970; Acock & Garner 1984). In general, if the roots are limited in carbohydrate supply, experience reduced cambial activity, develop increased lignification of the vascular stele or are exposed to light, the capacity for storage root formation diminishes.

It should be noted that the sweetpotato is capable of developing carbohydrate storage sites in organs other than roots, provided the environmental conditions listed above are taken into account. In sprouted storage roots, buried stem bases have been observed to swell into storage organs (Sirju-charran & Wickham 1988).

The life cycle of the storage root is completed by the production of sprouts for the following season's planting. There is strong proximal dominance in sweetpotato storage roots, with most of the sprouts forming at the root stalk end following harvest but developing elsewhere as the roots age (Kays & Stutte 1979). Sprouts originate in the vascular cambium region (Schlimme 1966) opposite the xylem rays (Wilson & Lowe 1973a).

2.4 Propagation

As a perennial plant the sweetpotato may be cropped continuously within suitably warm climates. Under tropical conditions new fields may be planted with vine cuttings taken from concurrent established crops. Vine cuttings are usually about 25 cm long and include the apical growing tip. There has been some research on the optimum vine cutting length and location, suggesting that the use of relatively large apical cuttings with intact foliage favours early root establishment and growth (Villamayor & Perez 1988; Schultheis & Cantliffe 1994). The use of vine cuttings in continuous production systems increases the risk of disease and pest epidemics by providing a constant supply of host material.

Under temperate climates sweetpotato is grown as an annual crop (Yen 1974). Harvested roots are stored over winter and healthy well shaped 'seed' roots are selected the following spring (Coleman 1972). Seed roots are laid flat in propagation beds, minimising inter-root contact, and covered with soil. The raised beds are constructed

from free draining soil which has not been previously used for sweetpotato production, to reduce the pathogen load. Beds are covered by clear plastic cloches to increase the temperature and to protect the emerging sprouts from inclement weather (Coleman 1972). Drip tape or overhead irrigation systems are often set into the beds to enable the sprouting roots to be watered and the humidity raised. Sprouts appear within about two weeks, depending on the cultivar, and are of sufficient height for cutting about two to four weeks later (Wilson & Averre 1989). Prior to field transplanting the bed cloches are opened to allow the sprouts to harden up, through exposure to conditions approximating those of the field. Sprouts are cut at about 25 to 30cm length, above soil level to avoid transferring pathogens from the seed root or propagation soil. Several subsequent crops of sprouts may be taken from the same propagation beds (Onwueme 1978).

At transplanting, about half to two thirds of the sprout are placed under the soil, depending on the cultivar (Onwueme 1978). Typically three nodes with fully opened leaves are inserted under the soil, with another three at the apical end of the cut sprout exposed. The number of nodes placed under the soil can influence yield (Hall 1987b). Cultivars' differ in both their rate of sprout production and sprout internodal distance, so the choice of propagule size may be limited or the insertion of more nodes required to anchor the plant securely. Sprouts pulled from the 'seed' root, rather than cut above soil level have been used in the past (Coleman 1962b), but although pulled plants establish well with their pre-formed roots, the risk of spreading disease outweighs the potential benefits.

Normally roots are stored at about 16°C and 85 to 90% relative humidity (Wilson & Averre 1989). Sprouting is promoted by conditions equivalent to those used in post harvest storage root curing, with temperatures of about 30°C and 90-97% relative humidity (Kushman 1975; Hall 1987a). In cultivars that have naturally low rates of sprout production, a pre-sprouting treatment using raised temperature and humidity may be applied to roots in storage, prior to placement in propagation beds (Hall 1986a, 1987a). As mentioned in the root anatomy section, sweetpotato storage roots have pronounced proximal dominance which can be clearly observed during sprouting (Kays & Stutte 1979). Although trials have been conducted on sprout production from cut

root pieces to reduce proximal dominance, the technique has not entered commercial practice (Hall 1988).

Historically, New Zealand Maori propagated the pre-European sweetpotato cultivars by directly field planting small cut pieces of sprouting storage root (Best 1925), in a manner similar to that used in potato (*Solanum tuberosum* L.) where intact or cut tubers are directly planted. Recently studies on this planting method for sweetpotato have been conducted in the USA and Japan, in order to reduce plant production inputs (Bouwkamp & Scott 1972; Kays & Stutte 1979; Fukazawa 1998). These studies suggest choice of cultivar is of critical importance for direct planting, both for pathogen susceptibility but particularly as the original planted root pieces may further swell as storage sites in competition with new storage root formation (Bouwkamp 1982).

Transplant field establishment is a crucial stage in sweetpotato production, to both ensure an even plant stand and to maximise storage root quality and yield. Erratic initial plant spacing or subsequent plant death increases variation in storage root size at harvest (Wilson & Averre 1989). In New Zealand, ridges are generally 0.75m wide and with inter plant spacing of 0.3m. This gives a planting density of just less than 45,000 plants ha^{-1}, within the optimum range for total yield of 40,000 to 50,000 plants ha^{-1} suggested by extrapolation from a number of research studies (Bouwkamp 1985b). However, manipulation of inter-plant spacing allows growers to plan for a relatively early or late harvest (Schultheis et al. 1999).

A number of studies have examined options for improving sweetpotato transplant establishment, including sprout storage, planting orientation and depth. Growers planting schedules are frequently held up, but refrigerated storage of transplants at 13.3°C for seven days gave a transplant survival rate equivalent to those direct planted (Hammett 1985). Storage beyond a week appeared detrimental to transplant survival (Hall 1985). Of particular interest is research suggesting that storing sprouts for several days, rather than field planting directly after cutting, potentially improves yield (Nwinyi 1991; Nakatani 1993). The effect of transplant length, planting orientation and depth appears to be cultivar specific (Hall 1986b, 1987b), and requires more investigation. However sweetpotato transplants are fairly robust, as when the polarity of cultivar 'Red

Jewel' transplants were completely inverted they still yielded the same as conventional plantings with the acropetal end (shoot apex) above ground (Hall 1994).

2.5 Plant growth

Sweetpotato plant growth can be divided into three phases following the field transplanting of cuttings: establishment of a fibrous root system with little leaf growth, extensive vine growth and storage root initiation, and finally storage root bulking with little further vine growth while the leaf area remains constant or declines. Maintenance of leaf area is dynamic, resulting from the balance between new leaf production and the rate of leaf abscission (Somda & Kays 1990). The first phase may last for approximately 10 weeks, the second for 7 weeks and the third for the remainder of the season (Scott 1950). However under tropical conditions the first two phases may be more rapid, followed by a long bulking period (Wilson & Lowe 1973b). In sub-tropical Australia, two growth phases could be distinguished from the change in harvest index (storage root to total plant weight ratio) with time (Huett & O'Neill 1976). The first phase was dominated by canopy growth while the later phase was dominated by storage root growth. Cultivars can be classified into short duration (12-17 weeks), medium duration (17-21 weeks), and long duration (>21 weeks) categories, on the basis of the time taken for storage roots to initiate and bulk (Yanfu et al. 1989).

Sweetpotato plants continue to produce new leaves until harvest (Hahn & Hozyo 1983). Most sweetpotato leaves are cordate (heart shaped), but leaf shape is highly variable amongst cultivars with many having a deeply divided lamina (Yen 1974). In sweetpotato the efficiency of light interception is reduced by the mutual shading of leaves within the canopy (Tsuno & Fujise 1963). When sweetpotato vines were grown on a trellis, exposing more of the leaves to full sunlight, the storage root yield was increased (Chapman & Cowling 1965). Leaf shedding is a common feature of sweetpotato plants later in the growing season, due to both leaf age and low light interception (Somda et al. 1991). The photosynthetic rate of 60 day old leaves was reported as only 14% of that measured in 20 day old leaves (Bhagsari 1988). Petiole length tends to increase in leaves developing later in the growing season (Somda & Kays 1990), shading previously formed leaves. However during early growth, the ratio

of leaf to land area (leaf area index) correlates with canopy photosynthesis (Bhagsari 1990). Cultivars with deeply lobed leaves had 16 to 27% higher canopy photosynthesis than the common cordate leaves (Bhagsari 1986). Leaf area is dependent on the rate of leaf development at apical meristems, the rate of leaf growth and leaf longevity (Hahn & Hozyo 1983). The relationship between the plant canopy as both a photosynthate source and sink early in the season, with storage roots as the principal sinks later in the season, is discussed further in chapter five.

The plant's foliage is structurally supported and connected to the root system by vines or stems. The growth habit of sweetpotato cultivars may be categorised as erect, semi-erect, spreading and very spreading (Huáman 1997). A low spreading vine system is the predominant growth habit. The stems in some cultivars have a twining habit and vines may twine about each other, rising above the general canopy. In cross section, the stem is circular or slightly angular. The stem is covered by an epidermis, which varies from glabrous (hairless) to very pubescent. Glabrous shoot tips are preferred for human consumption when the crop is harvested for its leaves and shoots. Beneath the epidermis, lies the cortex which contains latex ducts, an endodermis and a pericycle. The vascular bundles are bi-collateral, with phloem at both inner and outer edges. The two phloem zones are separated by xylem with cambium on the inner and outer faces (Onwueme 1978). Carbon fixed within the sweetpotato leaf is translocated out of the leaf as sucrose. The rate at which photosynthate is translocated along the main stem has been estimated using radioactive tracers. With ^{14}C-photosynthate in the cultivar 'Okinawa 100' it was shown that there was bidirectional transport, at a translocation speed of 48 cm $hour^{-1}$ toward the shoot apex and 128 cm $hour^{-1}$ towards the storage root (Kato & Hozyo 1974). In the cultivar 'Jewel' the translocation speed of $^{11}CO_2$-photosynthate varied with the time of day and position along the stem, with an average speed of 150 cm $hour^{-1}$ (Kays et al. 1982). When the leaves of a whole plant were exposed to $^{11}CO_2$ it was found that almost all of the photosynthate was translocated towards the roots (Kays et al. 1987). While acropetal translocation (towards the shoot apex) in the main stem was negligible, lateral stem branches showed some acropetal translocation.

2.5.1 Growth analysis

The term 'growth' may be loosely defined as an irreversible change in plant size (Hunt 1978). Plant growth analysis provides a method with which to analyse the absolute and relative changes in the size of plants and their organs over time. It provides a structured approach, allowing a comparison of the growth strategies between different species or cultivars, while broadly indicating the underlying biological processes (Woolhouse 1980). An understanding of the relationships between plant organs over time may facilitate the development of more efficient sweetpotato cultivars (Kays 1985) or allow the development of predictive growth models, as used in a range of crops (Reid & English 2000).

In practice, a number of plant measurements are made at approximately uniform intervals over time. The measurements may include the entire plant, plant organs or even compounds within organs, usually on a dry weight basis for data stability. Leaf area is often recorded, as it represents the major photosynthetic 'catchment' (Hunt 1978). While leaves are the major source of photosynthates, they are also photosynthate sinks as are the organs which comprise the remainder of the plant. In general, a plant organ is broadly defined as a source or sink depending on whether it is a net exporter or importer of photosynthates. However organs may change roles throughout the life of the plant, depending on specific growth or storage requirements.

Once the plant data is collected, curves are fitted to allow interpolation between harvests and delineation of the major trends over time. While the fitted curves approximate plant growth over time, the difference between any two points on the curve represents the absolute growth rate for that specific time interval.

Blackman (1919) suggested an efficiency index called relative growth rate (RGR), which measures the rate of increase as a proportion of plant size. Generally growth data requires transformation to stabilise the variance over time before curve fitting and natural logarithms are commonly used. If natural logarithms of the raw data are plotted against time and a curve fitted, according to calculus the relative growth rate is the slope of the curve at any specific time.

A further measure of plant efficiency termed the net assimilation rate (NAR) was also suggested (Gregory 1918), in which the net gain in weight is calculated per unit of leaf area. NAR approximates the difference between photosynthetic gain via the leaves and respiratory loss from the total plant mass. Briggs et al. (1920) suggested a term to give a leafiness index, leaf area ratio (LAR) quantifies the ratio of leaf area to total plant dry weight. In general, the relationship between these three indices is approximated by RGR=NAR ×LAR.

In sweetpotato, RGR increases over the early part of the season as the crop canopy is established, then declines during storage root bulking in a cultivar specific way (Huett & O'Neill 1976; Roberts-Nkrumah et al. 1986). NAR also declines in a cultivar specific way over the later growth period, as mutual shading of leaves within the canopy increases (Huett & O'Neill 1976; Tsuno & Fujise 1965). A maximum NAR of 9 to 10 g m^{-2} day^{-1} has been reported in Japan (Tsuno & Fujise 1965). These indices allow direct comparison of plant growth efficiencies in dissimilar sweetpotato cultivars, bearing in mind the importance of the rate of assimilate movement to the storage root itself (Austin & Aung 1973).

2.6 Water requirements

Water supply has a significant effect on sweetpotato storage root production. Flooding or drought at various stages of plant development may severely reduce root yield and quality. Uniform plant establishment is critical to high yields at a later date. During vine growth, canopy development may be affected in that an inadequate water supply inhibits shoot growth, while excess water promotes the shoot through altering the root to shoot ratio (Ehara & Sekioka 1962; Gollifer 1980). Flooded or dry soil conditions may cause specific roots to lignify, irreversibly limiting their potential to become storage roots (Togari 1950; Ravi & Indira 1996).

2.6.1 Drought

Sweetpotatoes are often produced on unirrigated land, but while sweetpotato is considered a drought tolerant crop (Jones 1961; Constantin et al. 1974), the response to drought stress varies with cultivar (Villareal et al. 1979). Selection for cultivar based drought resistance is of current international interest (Ekanayake 1997). Yields may be significantly reduced if drought occurs within the first six weeks from planting (Edmond & Ammerman 1971). However fairly good yields have been reported under low moisture and minimum input conditions such as 13 t ha^{-1} in a season of 129 mm of rainfall (Villareal et al. 1979), when 530 to 660 mm of precipitation over a growing season (four to five months) is considered optimal (Edmond & Ammerman 1971; Chukwu 1995). Moisture requirements vary with soil type, as loamy soils require maintenance at about 25% of field capacity and sandy soils require 50% or more up to about 75% (Bonsi et al. 1992). Using water loss as a measure of sweetpotato requirements gave evapotranspiration rates of 2.6 mm day^{-1} over the first 45 days, 3.9 mm day^{-1} over the second 45 days and 2.5 mm day^{-1} for the last 30 days (Jones 1961). The sweetpotato is a deep rooted plant (Weaver & Bruner 1927) so can tap into low water tables. However supplementary irrigation may significantly improve root yield and quality when the rainfall distribution is erratic or inadequate.

2.6.2 Flood

During the early stages of plant growth, flooding can repress potential storage root formation and enlargement (Chua & Kays 1981). It is suggested that extended wet periods of 3 to 10 weeks following planting reduces the number of storage roots formed (Chua & Kays 1981). Flooding may regulate storage root production by modifying the oxygen concentration in the root zone. However the plant's capacity for storage root formation is not permanently impaired by the roots passing through limited anoxic conditions (Chua & Kays 1981; King 1985). Cultivars differ in susceptibility to flood injury, as seen with drought conditions (Martin 1983; Martin & Cramer 1985). The development of storage roots is inhibited by the increasing height of the water table or

flood level (Li & Kao 1985), but sweetpotatoes can produce acceptable roots in soils with a water table at or below 50 cm (Ghuman & Lal 1983).

The time of flooding determines the degree of crop damage, with mid season floods causing a greater reduction in yield than late season flooding (Roberts & Russo 1991). Flooding during the growing season and just before harvest may cause loss of storage roots due to rotting in the soil or during subsequent storage (Ton & Hernandez 1978). Ethanol concentration increased in storage roots from flooded soil (Corey & Collins 1982; Corey et al. 1982). If sweetpotato vines are removed for harvest, just prior to flooding, yield is not unduly effected but the storage loss of apparently sound roots is increased (Corey & Collins 1982).

2.7 Mineral nutrition

Adequate levels of potassium, nitrogen and phosphorus are required to ensure an acceptable yield of good quality storage roots. These are the three predominant nutrients in sweetpotato root tissue, so soils may require their replenishment following repeated harvests (O'Sullivan et al. 1997). Generally nutrients are delivered to the plant as a base fertilizer, soil incorporated prior to planting (Coleman 1972; Wilson & Averre 1989). However some side dressing and foliar applications are used, to allow more control over application time (Jett et al. 1996) or to address specific nutrient deficiencies. Growers may use starter solutions of dissolved nutrients to water in transplants at establishment, although there is no research based evidence to demonstrate their effectiveness in sweetpotato (McKee 1981: Thompson & Hurley 1987).

Sweetpotato can tolerate a wide range in soil pH, but a range of 5.8 to 6.2 is recommended (Wilson & Averre 1989) with regard to both storage root yield and quality (Jones et al. 1977).

2.7.1 Potassium

Like other root crops, sweetpotatoes have a high potassium requirement (O'Sullivan et al. 1997). Potassium is the most common mineral in sweetpotato roots and the addition of potassium fertilizer to deficient soils generally provides a yield increase. Potassium deficiency tends to have a greater effect on storage root yield than on the plant canopy, unlike nitrogen or phosphorous deficiency (Bourke 1985). The addition of potassium can offset excessive nitrogen effects such as disproportionate canopy growth and reduced yields (Bouwkamp 1985b). Root yield response to additions of potassium are frequently linear, with approximately 1.2 t ha^{-1} per 28 kg ha^{-1} of applied potassium (Jones et al. 1979). While Scott & Bouwkamp (1974) suggest applying 116 kg ha^{-1} of potassium, in previously uncropped soils 32 kg ha^{-1} of potassium was sufficient for a good crop (Godfrey-Sam-Aggrey 1976). However Hammett et al. (1984) obtained higher yields with 140 kg ha^{-1} than 70 or 280 kg ha^{-1} of applied potassium. As a general measure of total potassium requirement, it is estimated that a crop yielding 12 t ha^{-1} would remove 90 kg ha^{-1} of potassium, of which 33% would be in the foliage (O'Sullivan et al. 1997). As for root quality, fibre content increased with soil potassium levels, while percent dry matter, protein content and flesh firmness declined (Fujise & Tsuno 1967; Constantin et al. 1977).

2.7.2 Nitrogen

The effects of varying soil nitrogen levels have been thoroughly investigated in sweetpotato, as nitrogen strongly influences dry matter production and distribution. Excess nitrogen may stimulate foliage growth at the expense of storage root development, although cultivars vary widely in their responses to nitrogen (O'Sullivan et al. 1997). Under excess nitrogen conditions the leaf area index may become too high and cause leaf shedding through mutual shading (Tsuno & Fujise 1965; Haynes et al. 1967). Increasing nitrogen levels from 0 to 101 kg ha^{-1} produced roots with less fibre and carotene but firmer processed flesh (Constantin et al. 1984). Improved nitrogen supply may also produce higher protein concentrations in the storage roots (Constantin et al. 1974; Purcell et al. 1982). Storage roots develop a more elongated shape with

increasing nitrogen concentration (Knavel 1971). As the rate of nitrogen was increased from 101 to 202 kg ha^{-1}, root weight loss during storage increased and dry matter content decreased (Hammett & Miller 1982). Sweetpotato can form associations with nitrogen fixing soil bacteria such as *Azospirillum brasilense* (Hill & Bacon 1984; Yoneyama et al. 1998). It is estimated that a crop yielding 12 t ha^{-1} would remove 52 kg ha^{-1} of nitrogen, of which 50% would be in the foliage (O'Sullivan et al. 1997).

Root yield and quality are modified by the interaction of nitrogen and potassium levels. High nitrogen and potassium rates modify the length to diameter ratio and dry matter content of storage roots (Tsuno & Fujise 1968). Soil nitrogen to potassium ratios of 1:1.4 are recommended for sweetpotato roots with good keeping quality (Hammett & Miller 1982).

2.7.3 Phosphorus

Sweetpotatoes do not require large quantities of phosphorus to produce good yields and are relatively tolerant to low soil phosphorus levels (de Geus 1967). Applications of phosphorus have little effect on sweetpotato root yield, grade, or quality (Constantin et al. 1974; Nicholaides et al. 1985). However, under particularly low soil phosphorus conditions such as in volcanic ash soils, additions of phosphorus produce a yield increase (Floyd et al. 1988; Halavatau et al. 1996). Sweetpotato forms an association with vesicular-arbuscular mycorrhizae (VAM). The VAM invade sweetpotato fibrous roots gaining a sugar supply and at the same time increasing the plant's ability to take up phosphorus on low phosphorus soils (Bouwkamp 1985b; Floyd et al. 1988). While VAM are effective on low phosphorus soils, there may be a neutral or negative effect on crops well supplied with phosphorus (Negeve & Roncadori 1985). A 12 t ha^{-1} crop would remove approximately 9 kg ha^{-1} of phosphate, of which 33% would be in the foliage (O'Sullivan et al. 1997).

2.7.4 Storage root disorders

There is a number of recognised storage root disorders directly associated with mineral deficiencies. Low nitrogen levels may produce abnormal skin colours, as do low iron levels (Clark & Moyer 1988). Potassium deficiency produces pale flesh in high carotene cultivars (O'Sullivan et al. 1997). Low calcium may cause the production of soft, small and misshapen storage roots (Clark & Moyer 1988). Copper deficiency causes the development of storage roots which are normal externally, but contain brown streaks in the flesh (Pillai et al. 1986). Boron deficiency may cause high carotene storage roots to be paler than normal, produce irregular root shapes, and brown necrotic areas in the flesh (Nusbaum 1946). Sulphur deficiency may cause storage roots to become more rounded (Clark & Moyer 1988). While zinc deficiency does not affect storage root shape, it can produce brown flesh discolouration (Pillai et al. 1986).

Further detailed and specific literature reviews relevant to the subject matter of individual portions of the thesis are included within the appropriate chapters.

Chapter 3

Genotypic variability

3.1 Introduction

A plant's phenotype or appearance is the product of an interaction between its inherited genotype and exposure to an environment (Raven et al. 1976). As the high level of phenotypic diversity in sweetpotato *Ipomoea batatas* (L.) Lam. directly affects commercial storage root production, this investigation begins by developing a genetic frame of reference. The heterogenous nature of the modern hexaploid sweetpotato is an expression of its origins, its sexual reproduction system and vegetative propagation.

Thousands of sweetpotato cultivars are grown around the globe (in tropical, sub-tropical and warm temperate regions), varying widely in growth habit, morphology and biochemistry (Huáman 1991). Historically, local conditions and ethnic preferences have driven selection criteria for new sweetpotato cultivars, whether derived through natural hybridisation or systematic breeding. Preference in North American has been for storage roots with an intense orange colour, moist mouth-feel and very sweet taste (Martin & Jones 1986). In Asia and the Pacific red skinned roots have been selected, with white to yellow flesh, dry to moderately dry texture, and sweet to moderately sweet taste (Lin et al. 1985).

Currently, cultivar preferences in storage roots have become more global, with international germplasm exchange and systematic breeding for a wide range of attributes (Lewthwaite 1991a). Classical breeding programs are modifying integral aspects of storage root biochemistry. The physiological function of sweetpotato roots in the human diet is being enhanced (Yamakawa & Yoshimoto 2002), as exhibited by high carotenoid (Kays & Kays 1998) and anthocyanin levels (Philpott et al. 2003). Sugar components (Lewthwaite et al. 1997) and enzyme systems (Morrison et al. 1993) are being changed to produce varyingly sweet and non-sweet cultivars. The starch content and pasting properties are being modified to improve processing potential (Katayama et al. 1999). Levels of root resistance to pests (Thompson et al. 1999) and diseases

(Wright et al. 2003) are also being increased. More recently tissue culture and molecular techniques have been employed to introduce genes into the sweetpotato genome that were previously unavailable (Hinchee 1998; Otani et al. 2003).

The extreme variability seen between sweetpotato cultivars is a result of the species' derivation and polyploid sexual reproduction system. However, vegetative or clonal propagation for horticultural production paradoxically incurs a significant third source of genetic variation, somatic mutation (Hernandez et al. 1964). Generally, clones are defined as having strict genetic fidelity to their source (Abercrombie et al. 1990), but intra-clonal variation occurs in many species at both a primary sequence level and in expression (Lushai & Loxdale 2002). Sweetpotato exhibits such high levels of natural somatic mutation that annual selection is required to maintain each cultivar's storage root yield, shape and colour (Edmond & Ammerman 1971; Huett 1982).

All organs and possibly physiological processes in the sweetpotato plant appear subject to these somatic mutations, but of the phenotypic changes, root colour mutations are most easily observed. Storage root flesh colour mutation rates can vary from 1 to 18%, depending on the cultivar (Hernandez et al. 1964). For comparison, the estimated mutation rate in potato (*Solanum tuberosum* L.) ranges from 0.0005 to 0.001% (Heiken 1958). Aside from qualitative skin and flesh colour mutations, measured quantitative storage root mutations may affect yield, flavour, dry matter content, and storage life (Edmond & Ammerman 1971). A mutation which delays storage root formation has also been observed (Nakatani et al. 2002).

Storage root mutations can take the form of a 'bud sport' where all tissues in the new sprout are modified, or various chimeras where only one tissue layer or a fraction of it changes. Adventitious sprouts produced from tissues containing somatic mutations tend to maintain the mutation in subsequent plants. The vegetative propagation system of the sweetpotato contributes to genetic variability, as propagation from pre-existing meristematic regions such as leaf nodes produces more genetically uniform plants than those derived from normal adventitious origins (Villordon & La Bonte 1996).

Early New Zealand sweetpotato cultivars rarely flowered, so clones were maintained or improved by single plant storage root selection. Selection was intermittently applied,

based on root yield, shape and appearance (Coleman 1972). However, as most phenotypic mutants are undesirable, clonal selection is primarily used to reduce cultivar decline (Edmond & Ammerman 1971). It should be noted that intra-clonal yield and quality differences putatively due to mutations may be confounded (Martin & Jones 1986) with those produced by varying virus titre (Fletcher et al. 2000; Carroll 2003; Gutierrez et al. 2003). High virus titre is also promoted by commercial vegetative propagation systems, unless pathogen tested material derived through tissue culture is incorporated into the system. Currently global research is focussed on identifying mutation events, viral effects and their interaction (Clark & Valverde 2000; La Bonte et al. 2000).

Mutations resulting in spectacular colour changes are readily observed in harvested sweetpotato storage roots, however more subtle mutations are often overlooked. An examination of genetic fidelity at the molecular level would allow the degree of genetic deviation to be estimated across the whole genome. This study examined the degree of DNA (deoxyribonucleic acid) base sequence variation in sweetpotatoes bred or selected primarily for storage root production, using the Amplified Fragment Length Polymorphism (AFLP) 'fingerprinting' technique (Vos et al. 1995; Zhang et al. 2000; Fajardo et al. 2002). The germplasm evaluated varies in source, breeding system and level of clonal selection. Specifically, this experiment tested the hypothesis that intra-clonal genetic diversity may be of a magnitude equivalent to that observed between recognised commercial cultivars.

3.2 Materials and methods

3.2.1 Germplasm

Specimen storage roots exhibiting natural field mutations were collected from both commercial cultivars and advanced sweetpotato clones over three seasons, then maintained as separate clonal selections. This material was collected to observe the nature of visible field mutations in storage roots, across cultivars.

For AFLP analysis, DNA was extracted from the leaves of selected clones. The leaves were produced on sprouted storage roots, selected for their apparent genetic integrity as demonstrated by lack of observable phenotypic mutation. The samples of healthy young leaves were collected and dried rapidly on self indicating silica gel (October 2001). Leaves from 29 *I. batatas* selections and an *I. plummerae* A. Gray (McDonald 1995) seedling were included in the study. The sampled germplasm was selected in a nested manner, to form a gradient of diminishing genetic variability, from inter-specific differences, sexually hybridised international cultivars, nominally recognised and new clonal selections, then finally adventitious sprouts from the same storage root.

The species *I. plummerae* (McDonald 1995), included in this study for reference as an out-group, forms swollen albeit small storage roots, which are globose or ellipsoid in shape. The infrageneric classification of species *I. batatas* and *I. plummerae* diverge at the subgenera level (Austin & Huáman 1996). *I. batatas* is classified in subgenus *Eriospermum*, section *Eriospermum* and series *Batatas* (Austin & Huáman 1996), while *I. plummerae* is placed in the subgenus *Quamoclit*, section *Leptocallis* (McDonald 1995).

The *I. batatas* selections included cultivar 'Beniazuma' (Bz) representing germplasm from Japan (Shiga et al. 1985) and 'Beauregard' (Rolston et al. 1987) representing USA germplasm and breeding priorities. 'Beauregard' was included from two sources, from material maintained in tissue culture (Btc) since its 1991 New Zealand importation and from material field grown since 1992 (Bfd). The New Zealand commercial cultivar 'Toka Toka Gold' (TTG) was also included (Lewthwaite 1998), as it produces unusually high numbers of slightly thickened pencil roots.

The remaining cultivars were all derived from the historic New Zealand cultivar 'Waina', through storage root clonal selection (Lewthwaite 1998). 'Waina' (W) and the named derivatives, cvs. 'Tauranga Red' (TR), 'Gisborne Red' (GR), 'Owairaka Pink' (OP) and 'Owairaka Red' (ORC0) were selected by early New Zealand growers then maintained in Japan from 1969 (Tarumoto et al. 1992). 'Owairaka Red' remains the predominant New Zealand cultivar and local growers have intermittently practiced clonal selection on the cultivar since 1969. Ten commercial 'Owairaka Red' growers' strains were sampled (ORC1 to ORC10). A four year annual field cycle of clonal

selection pressure for improved storage root yield and shape was placed on a random collection of commercial 'Owairaka Red' roots, five of the resulting clones were sampled (C1 to C5). Finally, five sprouts were selected at random from one 'Owairaka Red' storage root (S1 to S5).

3.2.2 DNA extraction and AFLP analysis

Approximately 50 mg of dried leaf material was frozen in liquid nitrogen, ground to powder and the DNA extracted by a modified CTAB (hexadecyltrimethylammonium bromide) procedure (Doyle & Doyle 1990). CTAB isolation buffer (2% [w/v] CTAB, 1% [w/v] polyvinyl-pyrrolidone [PVP], 1.4M sodium chloride [NaCl], 100mM Tris-HCl at pH 8.0) and 20 mM ethylenediaminetetra-acetic acid disodium salt [EDTA]) was mixed with the ground leaf material and incubated at 65°C for approximately 30 minutes. Chloroform was added to this isolation mixture, the sample tube inverted several times and then centrifuged at 2,700 x *g* for approximately 5 seconds. The organic and aqueous phases of the mixture were clearly separated. The top aqueous phase containing DNA was removed and gently combined with isopropanol. Following incubation on ice for 10 minutes, the DNA precipitated out of solution. The DNA precipitate was carefully removed and gently but repeatedly (three to four times) washed in 80% ethanol, to remove any contaminants. The washed DNA was centrifuged at 20,800 x *g* for approximately 5 seconds to form a pellet. Ethanol was decanted off and the sample tube left inverted overnight on a paper towel. The dried pellet of DNA was dissolved in Milli-Q H_2O and stored at 4°C.

The yield of extracted DNA was quantified by electrophoreses on an 0.8% w/v SeaKem LE agarose gel (FMC BioProducts) in 1× Tris acetate EDTA (TAE) buffer (40mM Tris acetate, 1mM EDTA at pH 8.0) with a High Mass DNA Ladder™ – Invitrogen as a mass and size standard. The samples were visualised under UV light by staining with a 0.5 $\mu g\ mL^{-1}$ ethidium bromide solution.

Restriction endonuclease digestion of the DNA was carried out with the enzyme combination *Mse*I and *Eco*RI in a reaction mixture containing: 1 × restriction buffer (50mM potassium acetate [KOAc][Sigma], 10mM magnesium acetate [MgOAc]

[Sigma] and 10mM Tris-HCL at pH 7.5), 1U *Eco*RI (Roche), 4U *Mse*I (New England Biolabs) and 50 to 75 ng of genomic DNA in a total volume of 25 µL. The reactions were incubated for 2 hours at 37°C. The digested DNA was electrophoresed on a 0.8% w/v agarose gel along with a 1 Kb Plus DNA Ladder™ - Invitrogen as a size standard, to ensure digestion was complete. Digestions were incubated at 70°C to denature the restriction endonucleases and stored at –20°C.

Double stranded adapters were prepared by annealing two single stranded oligonucleotides (Table 3.1) at 95°C for 4 minutes then cooling slowly to room temperature before storage at –20°C. The adapters were ligated to the restriction fragments in 20 µL reaction mixture containing 1× ligation buffer (66mM Tris-HCl, 5mM $MgCl_2$, 1mM dithioerythitol, 1mM ATP at pH 7.5 [Roche]), 50pmol *Mse*I adapter, 5pmol *Eco*RI adapter, 1 Weiss U T4 DNA ligase (Roche) and approximately 15 ng of digested genomic DNA. The ligation reactions were incubated overnight at 4°C and then stored at –80°C.

Pre-selective amplifications were performed using primers with one selective base *Eco*RI-A and *Mse*I-C (Table 3.1) using the Polymerase Chain Reaction (PCR). Each PCR reaction consisted of 1× Q solution (Qiagen), 1× PCR buffer (10mM Tris-HCl, 1.5mM $MgCl_2$, 50mM KCl at pH 8.3, [Roche]), 250 µmol of each dNTP (Roche), 10pmol *Eco*RI+A pre-selective primer, 10pmol *Mse*I+C pre-selective primer, 1 U *Taq* polymerase (Roche) and 1 µL of ligation product in a total volume of 20 µL. PCR was carried out in a thermal cycler: 20 cycles of 94°C for 30s, 56°C for 60s, 72°C for 60s; then held and stored at 4°C. Ramping speed was set at 1°C s^{-1}. The adapters and pre-selective primers theoretically allowed systematic selection of a subset of the restriction fragments (about $\frac{1}{16}$) for PCR amplification.

For selective amplification, combinations of *Eco*RI selective primers with three selective bases and *Mse*I selective primers with four selective bases (Table 3.1) were chosen to optimise band clarity and the level of polymorphism (Clarke 2001). The two selective primer combinations used were *Eco*RI-ATA with *Mse*I-CAAC, and *Eco*RI-AAA with *Mse*I-CAAG. Each PCR reaction consisted of 1× PCR buffer, 50mM $MgCl_2$, 250µmol of each dNTP, 10pmol *Eco*RI+ANN selective primer, 10pmol

*Mse*I+CNNN selective primer, 1U *Taq* DNA polymerase and 1 μL of pre-selective amplification product, in a total volume of 20 μL. PCR was performed in a thermal cycler: held at 94°C for 2 minutes; 6 cycles of 94°C for 30s, 65°C for 30s, 72°C for 60s; 6 cycles of 94°C for 30s, 60°C for 30s, 72°C for 60s; 24 cycles of 94°C for 30s, 56°C for 30s, 72°C for 60s; held at 72°C for 5 minutes; held at 4°C. Ramping speed was set at 1°C s^{-1}. The PCR products were then stored at –20°C. In the selective PCR, theoretically only about $\frac{1}{256}$ of total genome fragments are amplified.

The selective amplification PCR products were prepared for polyacrylamide electrophoresis by adding 10 μL of formamide loading dye to the entire 20 μL PCR reaction. The samples were then irreversibly denatured at 95°C for 4 minutes before rapid cooling to 4°C. A 6 μL aliquot of each selective amplification PCR product solution was loaded on to the denaturing 5% w/v polyacrylamide gel. A 100bp DNA Ladder™ – Invitrogen was used as a size standard on the AFLP gels. AFLP gels were run at 45W until the xylene cyanol dye front was approximately 5 cm from the gel base.

The AFLP profiles were visualized by silver staining according to Promega Corporation (1998) protocol. Two AFLP gels were run, the first with selective primers *Eco*RI-ATA and *Mse*I-CAAC, the second with *Eco*RI-AAA and *Mse*I-CAAG. Choice of selective primer combinations was based on previous work in *I. batatas* (Clarke 2001), which produced high yields of unambiguous polymorphic bands. Banding pattern reproducibility was monitored by replicating the entire procedure for all samples, apart from the 'Owairaka Red' unnamed sub-cultivar level samples. This degree of replication provided a procedural test, while retaining all genotypes on a common gel for each primer combination.

Table 3.1 Oligonucleotide sequences[1] used in *Ipomoea* AFLP analysis.

Class	Contraction	Oligonucleotide sequence (5' - 3')
Adapters	*Eco*RI Linker:	
	Linker E1	CTCGTAGACTGCGTACC
	Linker E2	AATTGGTACGCAGTCTAC
	*Mse*I Linker:	
	Linker M1	TACTCAGGACTCAT
	Linker M2	GACGATGAGTCCTGAG
Pre-selective amplification	*Eco*RI Primer:	
	*Eco*RI-A	GACTGCGTACCAATTC A
	*Mse*I Primer	
	*Mse*I-C	GATGAGTCCTGAGTAA C
Selective amplification	*Eco*RI Primers:	
	*Eco*RI-ATA	GACTGCGTACCAATTC ATA
	*Eco*RI-AAA	GACTGCGTACCAATTC AAA
	*Mse*I Primers[2]:	
	*Mse*I-CAAC	--- GAGTCCTGAGTAA CAAC
	*Mse*I-CAAG	--- GAGTCCTGAGTAA CAAG

[1]Supplied by Invitrogen Life Technologies, Auckland, New Zealand.
[2]The *Mse*I primers were shortened at the 5' end by three nucleotides, to compensate for the fourth base at the 3' end.

3.2.3 Statistical analysis

Genotypes were scored for the presence or absence of each polymorphic band and transcribed into binary format (1 and 0 respectively). Monomorphic bands were not scored, as they did not contribute to genome discrimination. Similarity matrices were produced from the binary data within the hierarchical clustering procedure of the GENSTAT ® statistical computer package. To establish data robustness, pair-wise similarity coefficients were calculated using both Euclidean (Sneath & Sokal 1973) and Jaccard (1908) distances (Table 3.2). The Euclidean coefficient includes the pair-wise dual absence of a band as a measure of similarity, while Jaccard's coefficient is more conservative as it rejects dual absence of bands by denying their contribution to the coefficient.

Table 3.2 Pair-wise similarity coefficients for binary data in sample x_i and x_j. The measure of similarity is produced by multiplying each contribution by the corresponding weight, summing all these values, and then dividing by the sum of the weights (GENSTAT®: Hierarchical clustering procedure).

Similarity coefficient	Contribution	Weight
Euclidean $1-(x_i-x_j)^2$	if $x_i=x_j$, then 1	1
	if $x_i \neq x_j$, then 0	1
Jaccard	if $x_i=x_j=1$, then 1	1
	if $x_i=x_j=0$, then 0	0
	if $x_i \neq x_j$, then 0	1

Dendrograms of approximately homogeneous groups were constructed from the matrices of Euclidean and Jaccard's similarity coefficients, using hierarchical cluster analysis. The agglomerative algorithms; single linkage (nearest neighbour), UPGMA (unweighted pair group method with arithmetic averaging) and complete linkage (furthest neighbour) were used to evaluate the robustness of group fusions across

joining methods. A bootstrap re-sampling statistical software procedure, phylogenetic analysis using parsimony (PAUP* 4.0) was used to obtain confidence levels for UPGMA clusters (Swofford 1998), as represented by the percentage of bootstrap re-samples containing each cluster in the 50% majority-rule consensus of 1000 re-samples.

Non-hierarchical cluster analysis was used to test the optimum grouping of the clones within arbitrarily chosen numbers of clusters, based on the original binary data and using statistical software GENSTAT®. The clustering was tested by arbitrarily dividing the samples into groups, ranging in number from two to ten. Two alternate initial classifications were imposed on the data set; (a) 'equal sized groups by input order', which initially produces groups of nearly equal size and (b) 'automatic by distance', in which the clones that are furthest apart in multi-dimensional space as defined by the data variates are used as nuclei for group formation. Between-group interchanges were set as transfers and swaps between groups, where one direct swap is equivalent to two transfers. The classifications were optimised under two alternative criteria; (a) 'between-group sum of squares' and (b) 'maximal predictive classification' (Gower 1974). The 'between-group sum of squares' criterion minimises the total within-class sum of squares, while the 'maximal predictive classification' is optimised when the within-class homogeneity criterion (W) is maximised and the between-class heterogeneity subsidiary criterion (B) is minimised.

Principal co-ordinate analysis (Gower 1966) was carried out based on the Euclidean similarity matrix. Examination of the first three principal co-ordinates allowed visualization of the distribution of the genotypes in a three dimensional scatter plot.

3.3 Results

3.3.1 Field mutations

The wide range of sweetpotato cultivars grown in New Zealand and their typical coloration is presented in Plate 3.1. Storage root skin and flesh colour mutations were clearly observable in all commercial cultivars (Plate 3.2). While they occurred with varying frequency, chimeric colour mutations affected both storage root skin and flesh, either independently or in combination. Shoot bud mutations were also observed, where all the storage roots borne on subsequent transplants showed the same mutation, such as white skin mutants with a total absence of anthocyanins, compared to the original high anthocyanic red skin in 'Owairaka Red'.

Phenotypic plasticity is common for all aspects of sweetpotato growth, including storage root coloration. However most root colour mutations were clearly discernable from the more subtle changes seen through plasticity due to local environmental effects. Mutations were maintained across growing environments and under subsequent clonal propagation, as opposed to transitory phenotypic changes induced by the immediate environment.

Biochemical pathways represented by observed storage root colour changes particularly included anthocyanin and carotene production. Typically, observable mutations resulted in either the loss or gain of biochemical pathways leading to orange or purple/red pigmentation of the skin or flesh. Within the flesh, chimeric mutations may affect only specific tissues (Plate 3.3), such as the loss of anthocyanin production within xylem tissue. Mutation also occurred in other plant organs such as leaves and stems, where natural field mutation was observed in the development of chlorophyll varigation (Plate 3.3).

Storage roots were produced by *I. plummerae* seedlings (Plate 3.4), however phenotypic evidence of mutations was not observable in the fifteen seedlings grown.

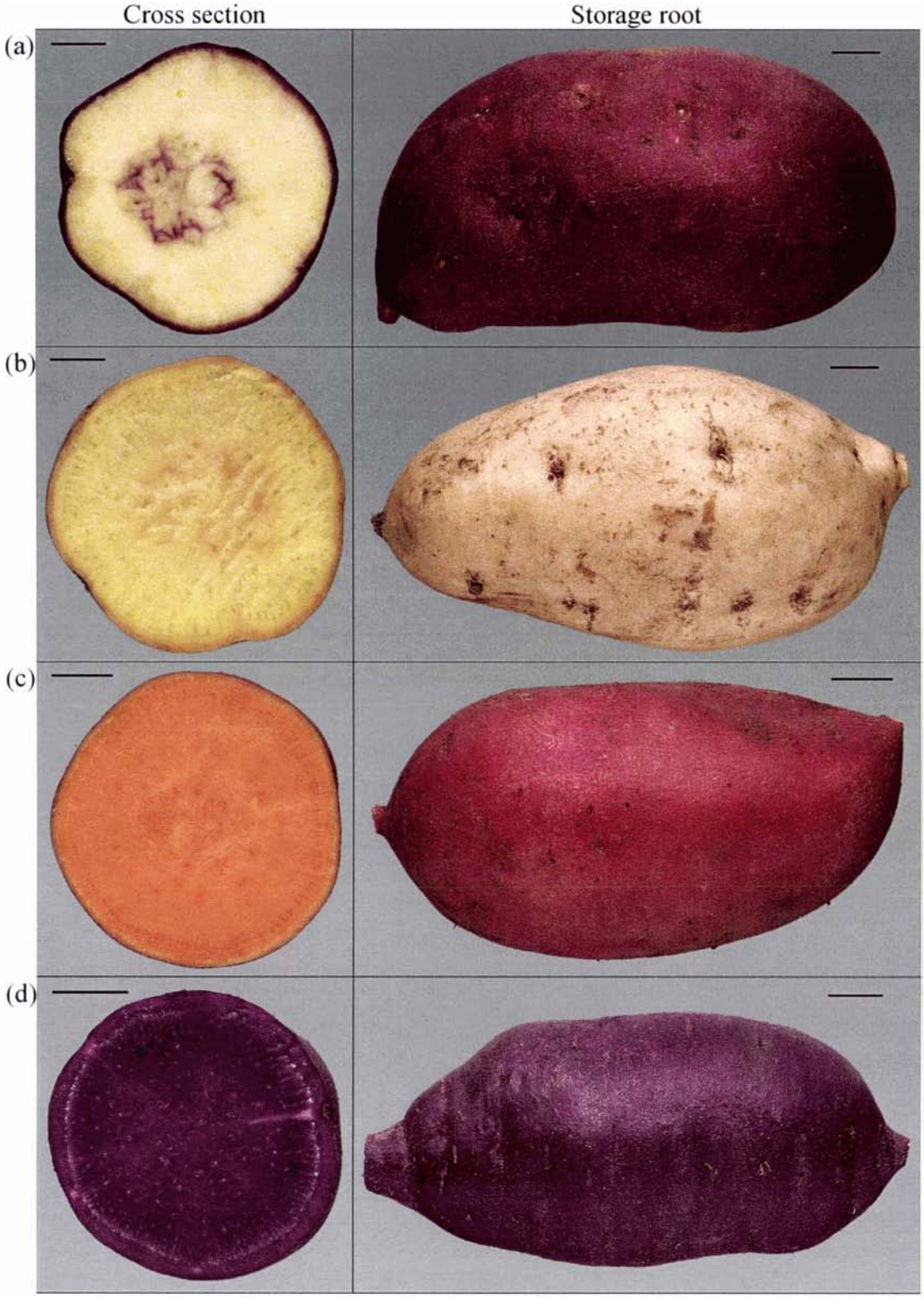


Plate 3.1 Storage roots of sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars currently grown in New Zealand, (a) 'Owairaka Red', (b) 'Toka Toka Gold', (c) 'Beauregard', and a new selection, (d) '99N1/222' (scale bar, 10 mm length).

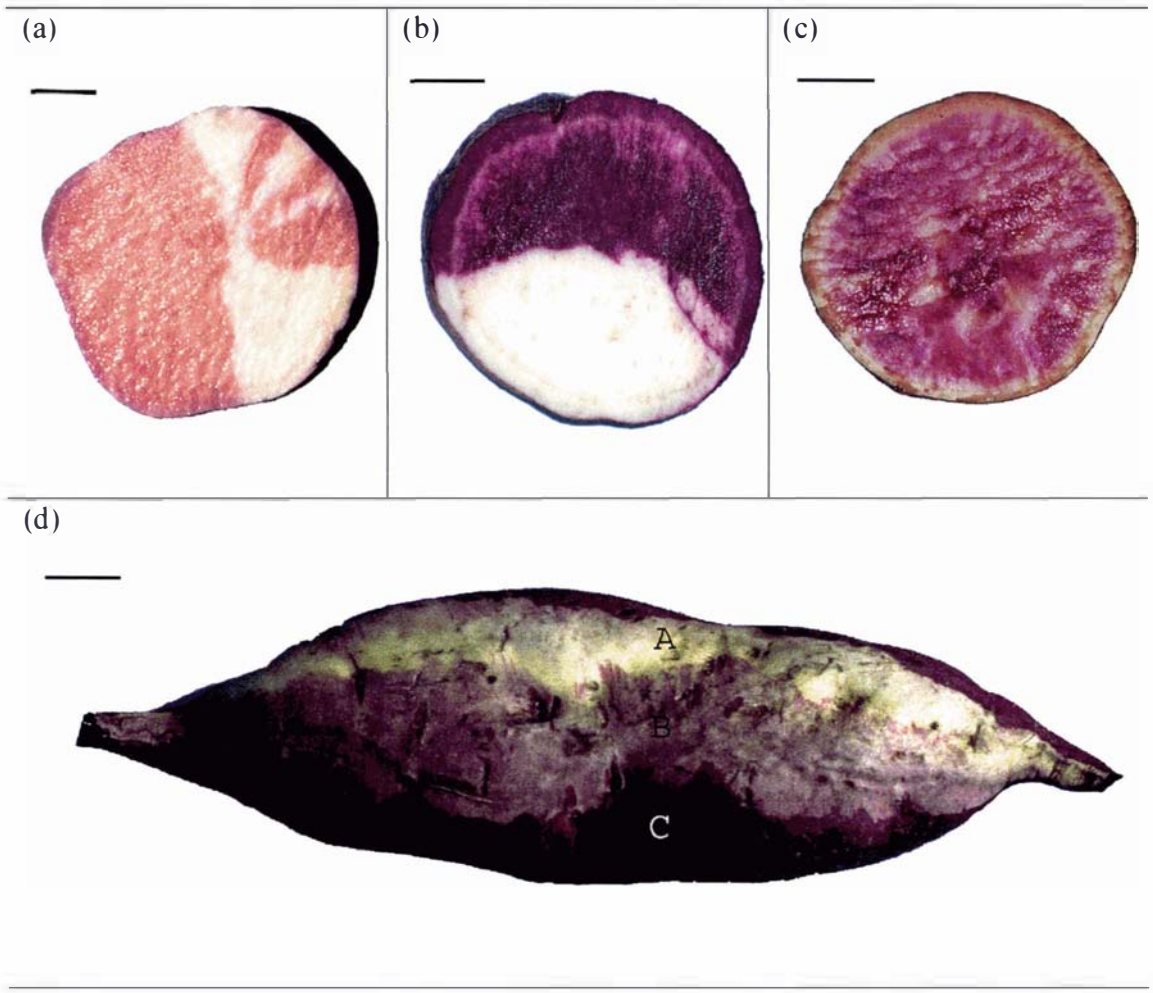
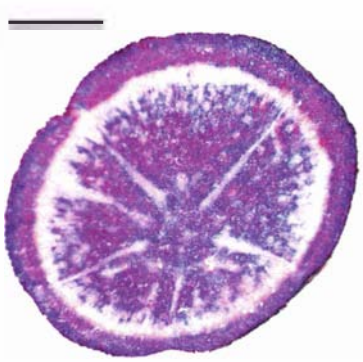


Plate 3.2 (a) Transverse section of an orange fleshed sweetpotato (*Ipomoea batatas* (L.) Lam., cv. 'Beauregard') storage root, showing field induced sectorial chimeric mutation into low carotene white fleshed sectors. (b) Transverse section of a high anthocyanic sweetpotato storage root of clone '99N1/222', showing a natural sectorial chimeric mutation into a low anthocyanic white sector. (c) Transverse section of a normally yellow fleshed sweetpotato storage root of 'Toka Toka Gold', showing a bud mutation for high anthocyanin production. (d) White (A) and pink (B) mericlinal chimeric colour mutations in a normally red (C) skinned sweetpotato 'Owairaka Red' storage root (scale bars, 10 mm length).

(a)



(b)

Plate 3.3 (a) Transverse section of a high anthocyanic sweetpotato (*Ipomoea batatas* (L.) Lam., clone '99N1/222') storage root, showing the development of low anthocyanic xylem tissue induced by field mutation. (b) Naturally occurring leaf chlorophyll mutation within a sweetpotato plant, 'Beauregard' (scale bars, 10 mm length).

Plate 3.4 Enlarged storage roots of *Ipomoea plummerae* A. Gray, (scale bar, 10 mm length).

3.3.2 DNA

High quality genomic DNA was readily extracted from leaf tissue. Approximately 50 mg of dried leaf tissue was required per sample to yield sufficient DNA. The sample DNA concentrations were adjusted to average *c.* 50 ng μL^{-1} by comparison of the undigested high molecular weight band with a high DNA mass ladder on an electrophoresed agarose gel (Plate 3.5). While RNA was present it was both negligible and subsequently diluted in the PCR reactions, so was not removed by RNase. Following adjustment for concentration, the extracted DNA was successfully digested by restriction endonucleases *Eco*RI and *Mse*I, as indicated by the absence of a high molecular weight band and the presence of a smear of low molecular weight DNA between *c.* 100 and 1500 bp on an electrophoresed agarose gel (Plate 3.5).

Comparison of the relative yields of clear polymorphic bands by various primer combinations in previous sweetpotato research (Clarke 2001) allowed the prior selection of high yielding primer combinations. A total of 91 unambiguous polymorphic bands were generated across the 30 germplasm samples by the two selected primer combinations. The primer pair combination *Mse*I + CAAC and *Eco*RI + ATA produced 49 unambiguous polymorphic bands (Plate 3.6) while the primer pair combination *Mse*I + CAAG and *Eco*RI + AAA generated 42 (Plate 3.7).

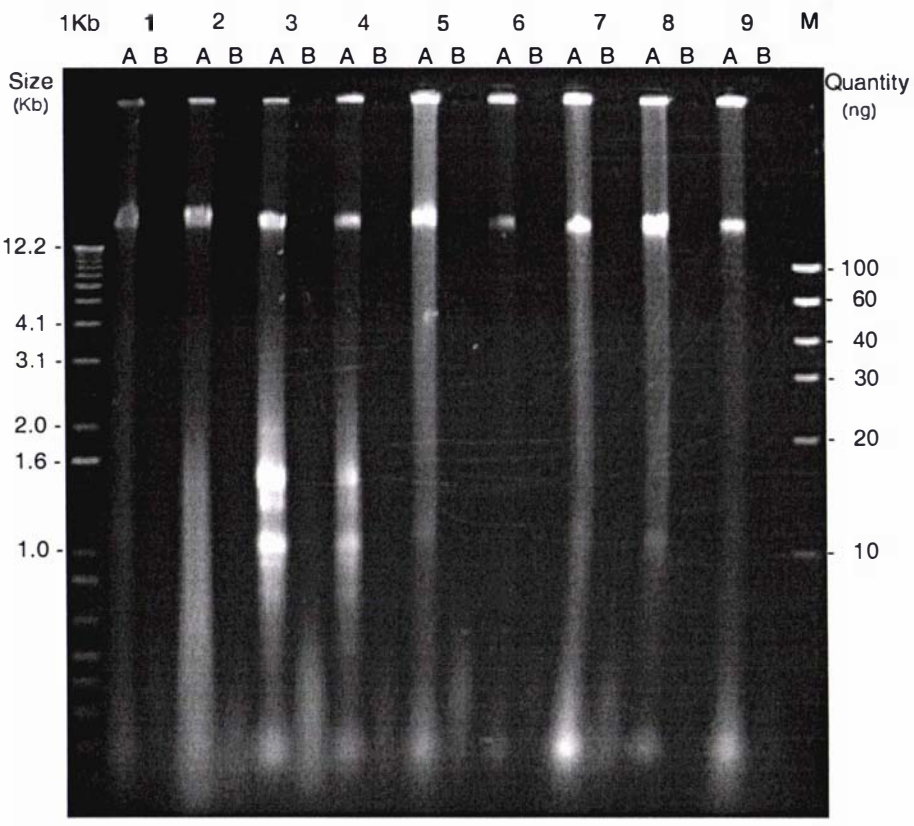


Plate 3.5 DNA extraction products from the leaves of *Ipomoea* spp., electrophoresed on agarose gel. Paired lanes contain alternating (A) undigested and (B) digested (restriction endonucleases *Eco*RI and *Mse*I) genomic DNA from one *I. plummerae* A. Gray seedling and eight sweetpotato (*I. batatas* (L.) Lam.) cultivars. Abbreviations; 1 'Gisborne Red', 2 'Owairaka Pink', 3 'Tauranga Red', 4 'Waina', 5 'Toka Toka Gold', 6 'Beauregard', 7 'Beniazuma', 8 *I. plummerae*, 9 'Owairaka Red'. A 1Kb ladder (1 Kb Plus DNA Ladder™ - Invitrogen) and a High Mass DNA Ladder™ - Invitrogen (M) allow estimation of undigested DNA molecular size (> 12Kb) and weight (> 100ng).

Plate 3.6 Primer pair combination *Mse*I + CAAC and *Eco*RI + ATA. Polyacrylamide gel of selective amplification PCR products following application of the AFLP technique to genomic DNA extracted from leaves of *Ipomoea* spp, using the above primer pair combination. Lanes are duplicated and labelled; Ladder (100bp DNA Ladder™ - Invitrogen), I. plum (*I. plummerae* A. Gray seedling) and *I. batatas* (L.) Lam. cultivars; Bz 'Beniazuma', Btc 'Beauregard'-tissue cultured, Bfd 'Beauregard'-field grown, TTG 'Toka Toka Gold', GR 'Gisborne Red', OP 'Owairaka Pink', W 'Waina', TR 'Tauranga Red' and ORC0 'Owairaka Red' ex. Japan.

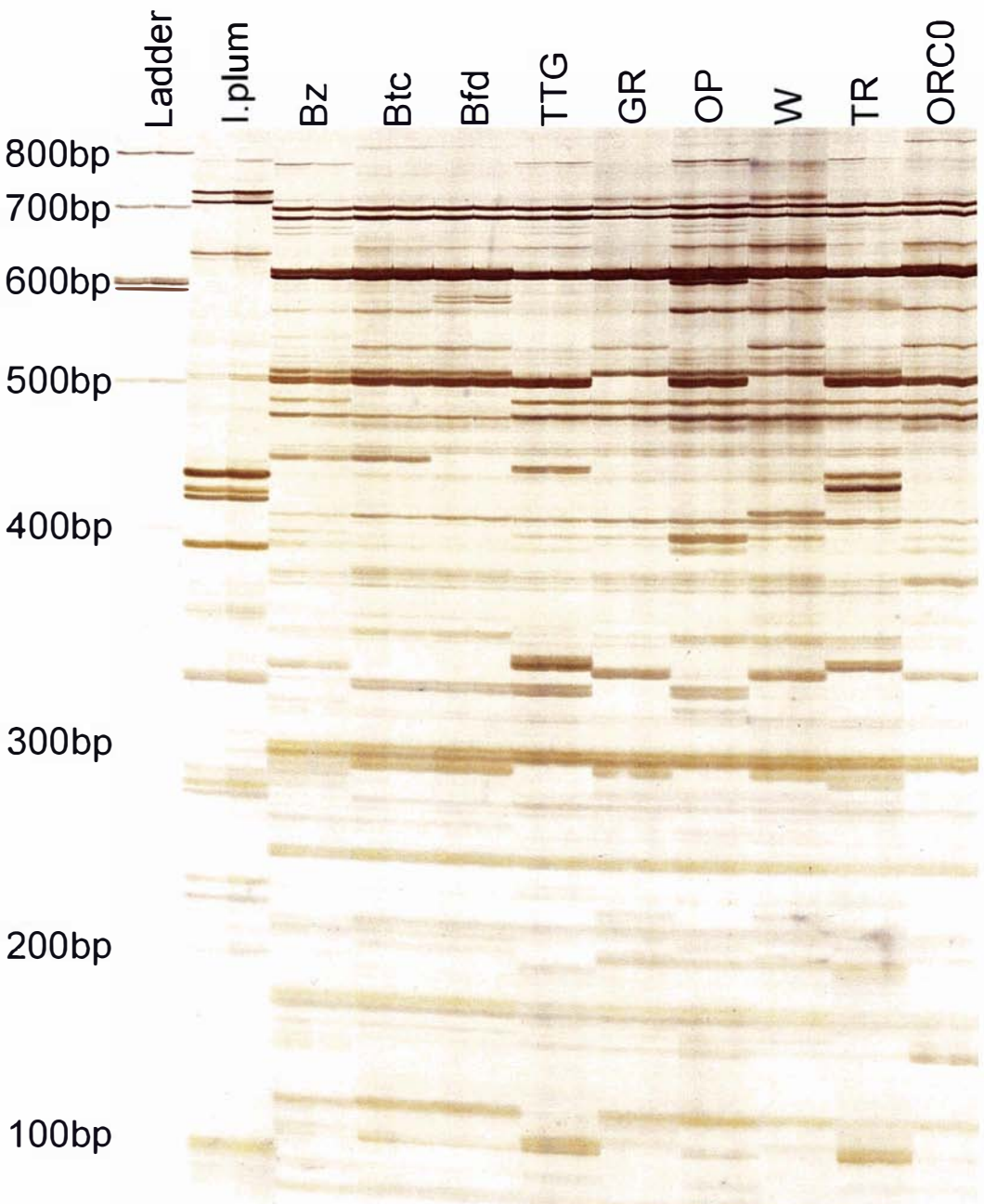
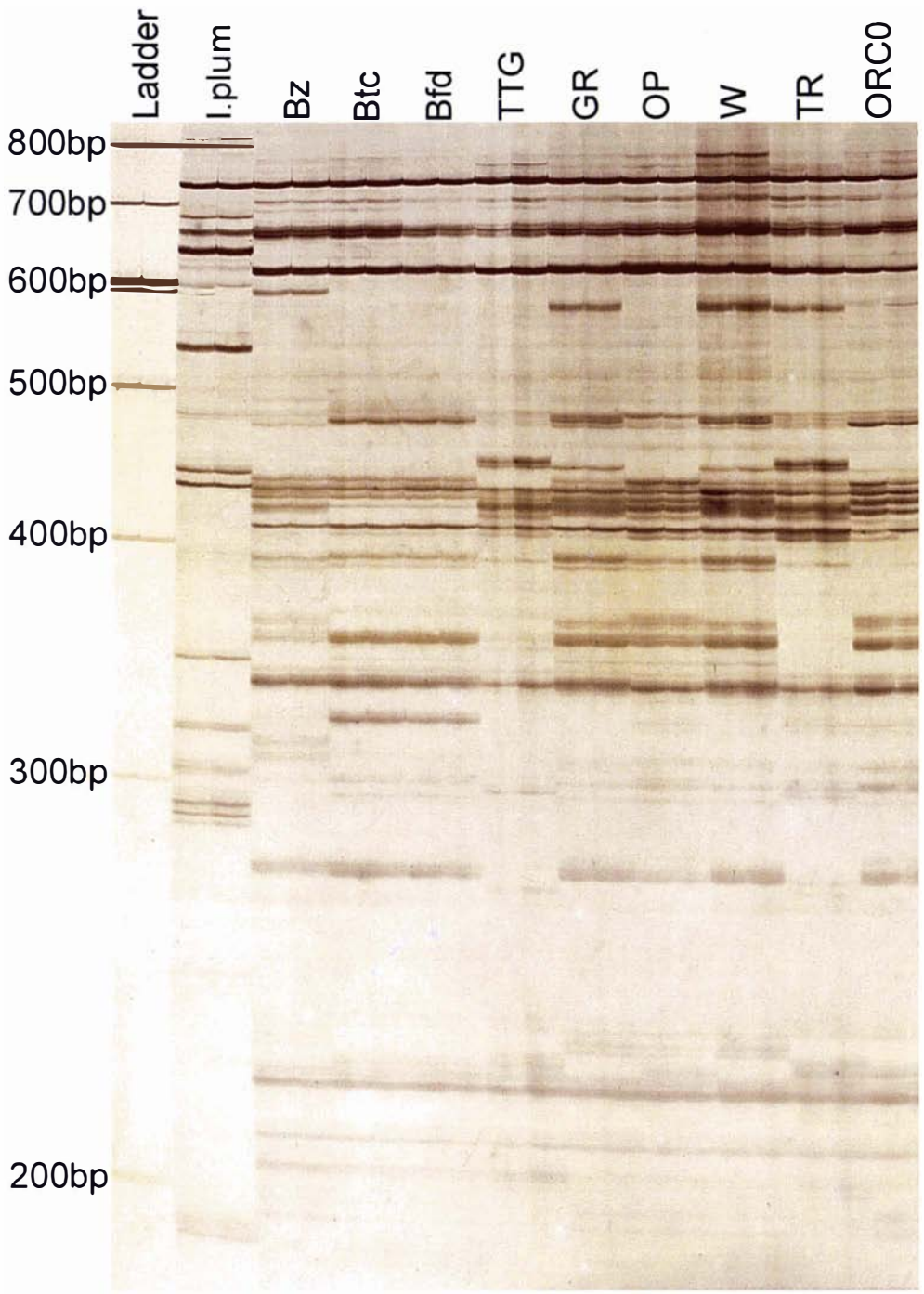


Plate 3.7 Primer pair combination *Mse*I + CAAG and *Eco*RI + AAA. Polyacrylamide gel of selective amplification PCR products following application of the AFLP technique to genomic DNA extracted from leaves of *Ipomoea* spp, using the above primer pair combination. Lanes are duplicated and labelled; Ladder (100bp DNA Ladder™ - Invitrogen), I. plum (*I. plummerae* A. Gray seedling) and *I. batatas* (L.) Lam. cultivars; Bz 'Beniazuma', Btc 'Beauregard'-tissue cultured, Bfd 'Beauregard'-field grown, TTG 'Toka Toka Gold', GR 'Gisborne Red', OP 'Owairaka Pink', W 'Waina', TR 'Tauranga Red' and ORC0 'Owairaka Red' ex. Japan.



3.3.3 Cluster analysis

For hierarchical cluster analysis, pair-wise similarity coefficients were calculated using both Euclidean and Jaccard distances. As both coefficients produced similar dendrogram topologies, only results based on the Euclidean coefficient are presented here. The agglomerative algorithms; single linkage, UPGMA and complete linkage were used to evaluate the robustness of group fusions across joining methods. Single linkage was included as the most facile group joining method, while complete linkage was included as the most discriminating method. General dendrogram topology was robust to changes in algorithm, as only minor changes were distinguishable across these three methods. Having compared the effects of different similarity coefficients and group joining methods, it was concluded that a UPGMA dendrogram derived from Euclidean similarity coefficients produced a statistically robust description of the data (Figure 3.1).

While hierarchical cluster analysis indicated relatively stable and conserved patterns of genotype groupings across similarity levels, non-hierarchical cluster analysis was employed to robustly determine the number of distinct clusters (Figure 3.1). The initial classification method 'equal sized groups by input order' produced divergent results under the two alternative optimisation criteria 'between-group sum of squares' and 'maximal predictive classification', so the dispersion of genotypes into groups by this approach was considered sub-optimal. However the initial classification method 'automatic by distance' produced identical results under the two alternative optimisation criteria 'between-group sum of squares' and 'maximal predictive classification', so genotype grouping appeared to be optimised (Table 3.3). Based on the convergence of optimisation tests for 'between-group sum of squares' and 'maximal predictive classification', eight groups appeared optimal (Figure 3.1).

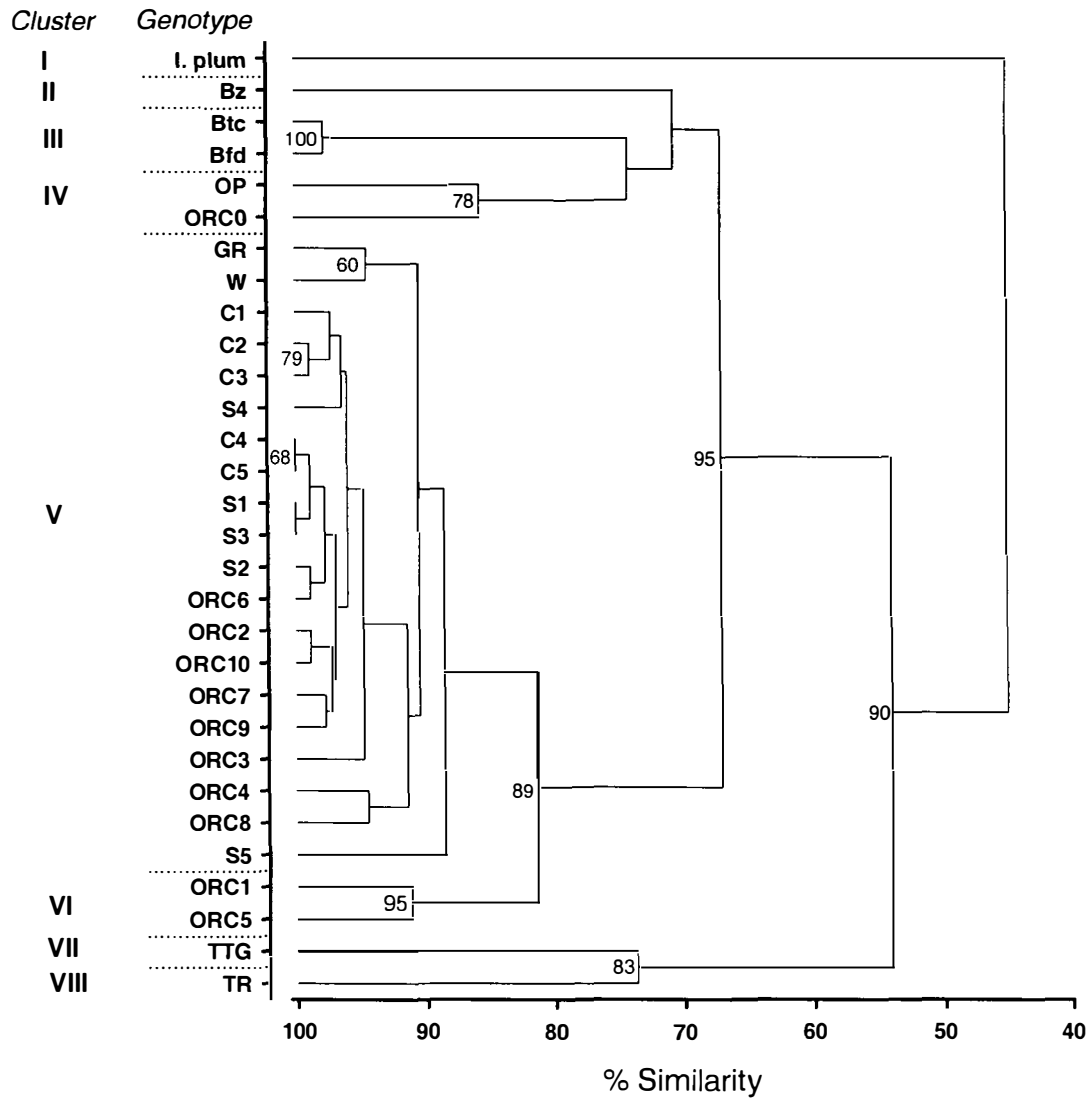


Figure 3.1 UPGMA cluster analysis dendrogram based on Euclidean similarity coefficients of AFLP polymorphic variation amongst 30 *Ipomoea* genotypes. Bootstrap values are indicated at nodes present under the 50% majority-rule consensus of 1000 re-samplings. I.plum (*I. plummerae* A. Gray) and *I. batatas* (L.) Lam. genotypes; Bz 'Beniazuma', Btc 'Beauregard'-tissue cultured, Bfd 'Beauregard'-field grown, TTG 'Toka Toka Gold', GR 'Gisbome Red', OP 'Owairaka Pink', W 'Waina', TR 'Tauranga Red' and ORC0 'Owairaka Red' ex. Japan, ORC1 to ORC10 ('Owairaka Red' commercial growers' strains), C1 to C5 ('Owairaka Red' clones following clonal selection), S1 to S5 (sprouts from one 'Owairaka Red' storage root). The indicated clusters (I to VIII) were established by non-hierarchical cluster analysis.

Table 3.3 Group optimisation for the non-hierarchical classification of 30 *Ipomoea* genotypes, based on polymorphic DNA bands derived by AFLP. Genotypes were initially classified using the 'automatic by distance' option of the GENSTAT®: Non-hierarchical classification procedure.

Classification method	Optimisation criterion	Number of groups		
		7	8	9
Between-group sum of squares	Within class homogeneity	79	59	53
Maximal predictive classification	W (within class homogeneity)	2616	2647	2660
	B (between class heterogeneity)	1644	1631	1678

Cluster I comprised *I. plummerae.* The remaining seven clusters contain the *I. batatas* genotypes as follows; Cluster II 'Beniazuma', Cluster III 'Beauregard' (both tissue cultured and field grown), Cluster IV 'Owairaka Pink' and ORC0 ('Owairaka Red' ex. Japan), Cluster V 'Waina', 'Gisborne Red,' ORC2, ORC3, ORC4, ORC6, ORC7, ORC8, ORC9, ORC10 (eight of the 'Owairaka Red' commercial growers' strains), C1 to 5 ('Owairaka Red' following clonal selection), and S1 to 5 (sprouts produced on the same 'Owairaka Red' storage root), Cluster VI ORC1 and ORC5 (two 'Owairaka Red' commercial growers' strains), Cluster VII 'Toka Toka Gold' and Cluster VII 'Tauranga Red'.

In general bootstrap values supported separation into the groups indicated by non-hierarchical analysis, with acceptable values using the 50% majority-rule. However, the two nodes immediately underlying the internal separation of Clusters II, III and IV were not robustly supported at bootstrap levels greater than 50% (Figure 3.1).

3.3.4 Similarity Matrix

The similarity matrix (Table 3.4) based on Euclidean distance, offers another method of assessing the resemblance between different DNA profiles. The species *I. plummerae* (Cluster I) produced relatively low similarity coefficients with the *I. batatas* genotypes of 35 to 52%. Cultivar 'Beniazuma' in Cluster II had similarity coefficients with the other *I. batatas* genotypes ranging from 53 to 75%. Tissue cultured 'Beauregard' in Cluster III had a 98% similarity with the field grown version. In general 'Beauregard' similarity with the *I. batatas* genotypes ranged from 53 to 77%. The 'Owairaka Pink' and ORC0 of Cluster IV showed a 58 to 77% similarity with the other *I. batatas* genotypes.

The relative similarities within the largest and most closely related group, Cluster V, range from similarity coefficients of 88 to 100%. Within subsets of the group, sprouts (S1 to S5) from the same 'Owairaka Red' storage root produced similarity coefficients ranging from 92 to 100%, while amongst 'Owairaka Red' clonal selections (C1 to C5) coefficients ranged from 95 to 99%. The commercial growers' strains within Cluster V ranged from 89 to 99%, while the lowest similarity incurred by including 'Gisborne Red' and 'Waina' was 88%.

The growers' selections in Cluster VI, ORC1 and ORC5, produced similarity coefficients of 53 to 88% against the other *I. batatas* genotypes. The remaining two clusters, Cluster VII 'Toka Toka Gold' and Cluster VIII 'Tauranga Red' produced similarity coefficients with the other *I. batatas* genotypes ranging from 43 to 74% and 52 to 74% respectively.

Table 3.4 Similarity matrix (%) for 30 *Ipomoea* samples by Euclidean distance, based on polymorphic AFLP bands. Clusters supported by non-hierarchical cluster analysis are indicated by dotted lines.

	I.plum.	TTG	TR	Bz	Btc	Bfd	OP	0 ORC	1 ORC	5 ORC	GR	W	4 ORC	8 ORC	3 ORC	9 ORC	7 ORC	10 ORC	6 ORC	2 ORC	C1	C2	C3	C4	C5	S5	S4	S2	S1	S3
I. plum.	100																													
TTG	52	100																												
TR	45	74	100																											
Bz	35	53	53	100																										
Btc	48	53	55	69	100																									
Bfd	48	53	55	67	98	100																								
OP	41	58	56	75	77	77	100																							
ORC0	44	66	62	71	71	71	86	100																						
ORC1	46	53	59	63	65	65	70	69	100																					
ORC5	42	53	59	71	67	65	70	69	91	100																				
GR	44	44	51	67	67	67	66	67	78	78	100																			
W	41	43	52	68	70	70	69	68	81	81	95	100																		
ORC4	38	47	54	68	68	68	67	66	84	88	88	91	100																	
ORC8	40	46	55	69	69	67	68	67	82	87	89	92	95	100																
ORC3	41	49	56	66	68	66	65	66	77	84	90	89	89	92	100															
ORC9	45	52	56	68	68	68	67	68	81	86	92	91	91	92	96	100														
ORC7	45	52	54	66	70	70	69	68	81	84	92	91	91	92	96	98	100													
ORC10	43	49	56	68	70	70	69	68	81	86	92	93	93	95	96	98	98	100												
ORC6	43	49	54	68	70	68	67	68	79	86	92	91	91	95	98	98	98	98	100											
ORC2	42	48	55	67	69	69	68	67	80	85	91	95	95	96	95	97	97	99	97	100										
C1	44	48	53	65	67	67	66	67	80	82	96	92	92	91	95	97	97	97	97	96	100									
C2	45	52	52	66	68	68	67	68	77	81	92	89	91	90	93	96	96	96	96	95	97	100								
C3	46	51	53	67	69	69	68	69	78	82	93	90	92	91	95	97	97	97	97	96	98	99	100							
C4	42	51	55	69	69	69	70	71	80	85	91	92	92	91	95	97	97	97	97	96	96	95	96	100						
C5	42	51	55	69	69	69	70	71	80	85	91	92	92	91	95	97	97	97	97	96	96	95	96	100	100					
S5	43	49	52	64	66	68	67	68	79	84	88	87	91	86	89	91	91	91	91	90	92	91	92	95	95	100				
S4	45	47	52	66	70	70	67	68	79	84	92	91	93	90	93	96	96	96	96	95	97	96	97	97	97	96	100			
S2	42	48	53	69	71	69	68	69	80	87	91	92	92	93	97	97	97	97	99	96	96	95	96	98	98	92	97	100		
S1	43	49	54	68	70	70	69	70	81	86	92	93	93	92	96	98	98	98	98	97	97	96	97	99	99	93	98	99	100	
S3	43	49	54	68	70	70	69	70	81	86	92	93	93	92	96	98	98	98	98	97	97	96	97	99	99	93	98	99	100	100

3.3.5 Principal co-ordinate analysis

A three dimensional representation of the *Ipomoea* genotypes was explored by principal co-ordinate analysis based on Euclidean similarity coefficients and visualised within a scatter plot (Figure 3.2). The three primary principal co-ordinate axes explained a total of 60.2% of the variation. The main axis (PC-1) explained 35.7% of the variation, the second axis (PC-2) 13.7% and the third axis (PC-3) 10.8%. Addition of axes in further dimensions produced a reducing return relative to ease of visualization.

In general, the genotypes were well dispersed by the three axes. *I. plummerae* was located in an extreme position on all three axes. In contrast, most of the 'Owairaka Red' genotypes clustered (OR) strongly together in an almost diametrically opposite position to *I. plummerae*, in a group that encompassed the current genetic version of their putative historic source, 'Waina'. The dense OR cluster also included the cultivar 'Gisbome Red', which is recorded as derived from 'Owairaka Red' although historically given a separate identity within commercial production.

For *I. batatas* genotypes, the PC-1 axis was of primary importance in the distribution of 'Owairaka Red' clones, indicating the relative genetic separation of a number of 'Owairaka Red' clones from the main OR group. The cultivar 'Owairaka Pink' is a historic mutant of 'Owairaka Red', having earned a separate name during commercial production. However, the clone ORC0 (ex Japan) and the commercial growers' strains ORC5 and ORC1 are still currently recognised as 'Owairaka Red', despite their clear genetic differentiation by AFLP-based principal co-ordinate analysis.

The cultivar 'Tauranga Red' is putatively an historic mutant of 'Waina', however this analysis positioned it well away from the main OR group on the basis of axis PC-1 alone, placing it at the extreme end of 'Owairaka Red' derived clonal selections. The inclusion of axis PC-2 in positioning 'Tauranga Red' substantially highlighted its dissimilarity from other 'Owairaka Red' derived clones and particularly its putative originator 'Waina' (within OR group). The third axis, PC-3, gave little differentiation between clones related to 'Owairaka Red'.

The position of cultivar 'Toka Toka Gold' was primarily determined by axes PC-1 and PC-2, relative to the majority of the *Ipomoea* genotypes. There is no historic record of the relationship between 'Toka Toka Gold' and other germplasm, however under this analysis 'Toka Toka Gold' remained distinct but positioned adjacent to 'Tauranga Red'. While it is well established that the commercial cultivar 'Toka Toka Gold' has the propensity to produce extremely high numbers of adventitious pencil roots, this rare growth habit was also observed in 'Tauranga Red' over the course of this study (Plate 3.8).

The Japanese cultivar 'Beniazuma', while distinct, remained positioned within the general 'Owairaka Red' derived clones on the basis of axes PC-1 and 2. Aside from complementing the distinctiveness of *I. plummerae* as demonstrated by the first two axes, axis PC-3 primarily illustrated the separation of cultivar 'Beauregard' from the majority of the clones. Cultivar 'Beauregard's genetic distance from 'Toka Toka Gold' and 'Tauranga Red' was further highlighted by axis PC-2. The tissue cultured and field grown sources of 'Beauregard' showed little differentiation at this level of resolution. Principal co-ordinate analysis supported the aggregation and dispersion pattern shown in the cluster analysis.

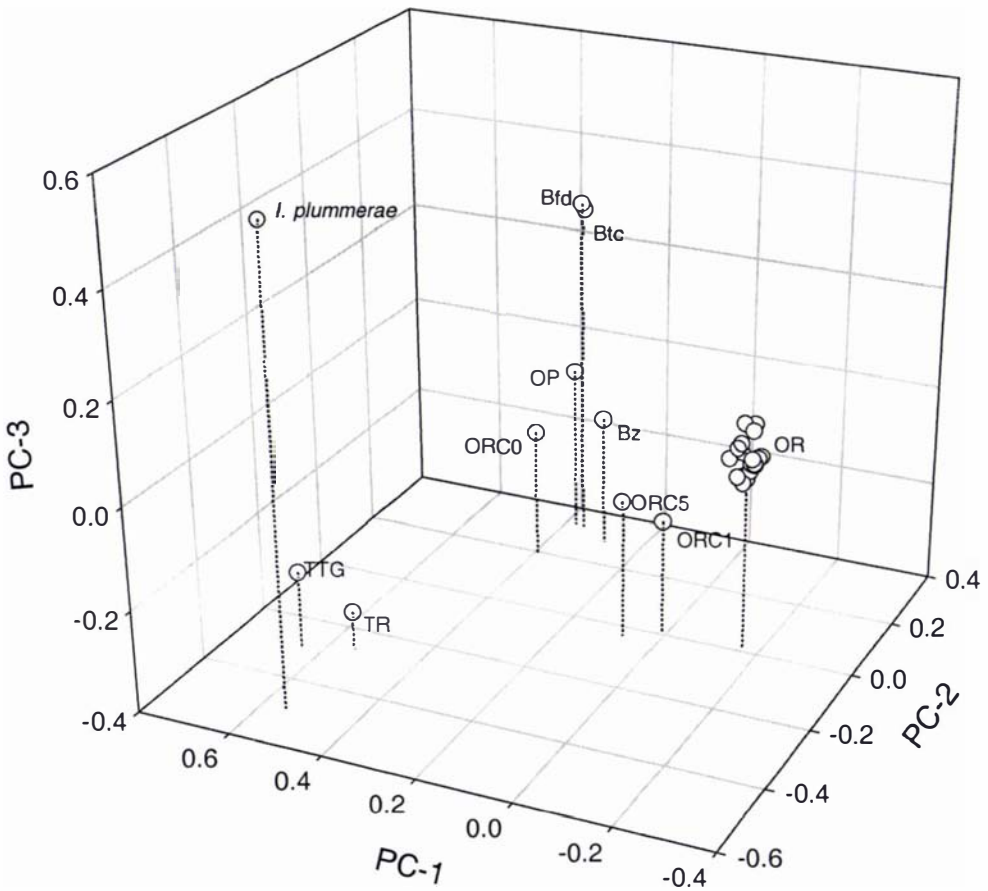


Figure 3.2 Scatter plot showing the three dimensional distribution of *Ipomoea plummerae* A. Gray and *I. batatas* (L.) Lam. genotypes through principal co-ordinate analysis of a Euclidean similarity matrix based on AFLP derived polymorphic bands. *I. batatas* genotypes; Bz 'Beniazuma', Btc 'Beauregard'-tissue cultured, Bfd 'Beauregard'-field grown, TTG 'Toka Toka Gold', OP 'Owairaka Pink', TR 'Tauranga Red' and ORC0 'Owairaka Red' ex. Japan, ORC1 and ORC5 ('Owairaka Red' commercial growers' strains). The cluster denoted OR contains all the remaining 'Owairaka Red' genotypes included in the study.

(a)   (b)

Plate 3.8 Adventitious pencil roots on unburied vine nodes of field grown sweetpotato (*Ipomoea batatas* (L.) Lam. cultivars (a) 'Toka Toka Gold' and (b) 'Tauranga Red' (scale bars, 10 mm length).

3.4 Discussion

When attempting to determine relationships within plant germplasm, historical records can be of limited reliability, especially when recorded retrospectively. However, such records provide a starting point for comparative analysis using molecular genetic techniques. The historic development of sweetpotato germplasm within New Zealand provides a unique genetic base for assessing genetic drift within commercial production systems.

3.4.1 Historical perspective

The cultivar 'Waina' was a post-European introduction to New Zealand, which supplied the genetic base required to develop the present sweetpotato industry. The most reliable details of its introduction are recorded by Berridge (1913). In the 1850s a whaling ship put into Opotiki for repairs to its boats. The ship's stores contained sweetpotato roots

obtained in the islands of Rarotonga. Some of these roots were shared amongst local Maori, and the cultivar was dispersed both north and into the Bay of Plenty. This sweetpotato was called 'Waina' by the Maori as it was propagated by plants or parts of the vine ('waina' being the Maori approximation for the word 'vine', as there is no 'v' in the Maori language). By 1913 this cultivar was grown more widely than any other in New Zealand.

By 1925 'Waina' became almost the only cultivar used for a general crop. It had very heavy yields, a robust habit and had superseded the older cultivars with their smaller storage roots, although some were still grown in various districts as a special delicacy (Best 1925). Following the introduction of the original clone 'Waina', a number of skin colour mutations were recognised. Commercial mutant strains of 'Waina' developed around New Zealand, among these were the cultivars 'Gisborne Red', 'Tauranga Red', 'New Zealand Red' and their pink-skinned mutants. Dr. H. Mouatt and Mr. J. Hunter of the Department of Scientific and Industrial Research (DSIR) noted a promising mutation (*c.* 1950) of 'Tauranga Red' and called it 'Owairaka Red' after the Auckland district in which it was selected (Coleman 1969).

The two main cultivars grown in 1948 were the 'Waina' derivatives, 'Tauranga Red' and 'New Zealand Pink'. About 24 hectares of sweetpotato were grown in the Northland and Auckland regions alone, with more grown elsewhere in New Zealand (Gillard 1948). In 1951 'Owairaka Red' was passed to the Department of Agriculture for commercial evaluation. At that time commercial sweetpotatoes were mainly pink-skinned. Mr. S. Gillard collected a number of commercial strains from around New Zealand and evaluated them during the 1952/53 season at Owairaka, Auckland (Bennett 1953). Thirteen cultivars were selected from this replicated trial and were evaluated again during the 1953/54 season. Amongst these 13 cultivars were five clones derived from 'Waina' and six clones supplied in 1950 by the University of Louisiana, USA (Department of Agriculture 1954).

Five clones were retained from the 1953/54 trial and tested again in a replicated trial in 1954/55. The five clones consisted of the 'Waina' mutants, 'Tauranga Red', 'Owairaka Red', 'Owairaka Pink', 'New Zealand Pink' and 'Gisborne Red'. Although yield differences were small, the cultivars 'Tauranga Red' and 'Owairaka Red' were

considered outstanding in appearance in comparison with the other five in trial. 'Owairaka Red' was a better commercial proposition because of its uniformity and general appearance (Department of Agriculture 1955; Forbes 1956a).

By 1954 these clones were released in limited quantities to growers and nurserymen (Forbes 1956b). In 1957 sales declined, demonstrating that the growers' immediate demand for the five selected lines was satisfied (Forbes 1957). However maintenance by deliberate clonal selection to allow provision of sweetpotato nucleus stock for further commercial distribution continued (Vegfed 1958; Wilson 1961) and growers were encouraged to make their own selections (Coleman 1962a). Between 1959 and 1969 there was a rapid expansion of sweetpotato production based on 'Owairaka Red', especially in the Ruawai Flats of Northland, where the area increased from 20 to 200 hectares (Coleman 1969). It is estimated that by 1969 commercial sweetpotato production was comprised entirely of the 'Owairaka Red' cultivar. The cultivar had high sprout production, vigorous trailing growth and produced good yields of red to purple-skinned roots with cream flesh, which occasionally exhibited purple streaks. Growers' application of clonal selection techniques began to decline.

Commercial evaluation of sweetpotato clones appears to have continued under Mr. Coleman (Department of Agriculture) until his death in January 1972 (Vegfed 1972). A sweetpotato clone with cream skin and cream flesh streaked with orange was selected from a trial established by Mr. Coleman but harvested by Mr. I. McKinley (grower) and Mr. W. Stacy (Ministry of Agriculture and Fisheries) in 1972. This selection was subsequently named 'Toka Toka Gold' after a distinctive local volcano (Mr. I. McKinley, pers. comm.) and has become a significant secondary commercial cultivar.

In 1991 the cultivar 'Beauregard' (Rolston et al. 1987) was imported from the USA (Lewthwaite 1991b). This cultivar was evaluated in New Zealand and released to growers (1993) by the New Zealand Institute for Crop & Food Research Ltd. The cultivar 'Beauregard' is now well established within New Zealand, at over 4% of total production (Lewthwaite 1998).

The most easily recognised mutation events in sweetpotato storage roots are those which are colour coded. The range of clearly expressed qualitative mutations found

within commercial sweetpotato cultivars suggest an even greater level of unrecognised quantitative variation (Edmond & Ammerman 1971; La Bonte et al. 2000). The historic development of new cultivars through observation and clonal selection emphasises the importance of mutation within the sweetpotato crop, but does not accurately portray the underlying level of genetic heterogeneity.

3.4.2 AFLP analysis

While analytical molecular approaches may indicate the amount of sweetpotato genome heterogeneity, they do not necessarily represent observable variation at the level of phenotypic expression. Other intermediate analytical approaches for measuring sweetpotato variation have employed enzyme (Kennedy & Thompson 1991) and protein analysis (Stegemann et al. 1992). The AFLP technique has been used on sweetpotato by other researchers (Zhang et al. 2000; Clarke 2001; Rossel et al. 2001; Saha & Mandal 2001) and provided an efficient reproducible method of ascertaining genetic heterogeneity.

Estimation of the relative position of the original historic 'Waina' genotype is doubtful under such high levels of genetic drift across long time scales. However, the location of 'Waina' within a cluster containing the majority of growers' strains and including its derivative 'Gisbome Red' provides a clustering consensus for the commonality of the current version of 'Waina' (Figures 3.1 and 3.2). The cultivar 'Gisbome Red' as it is currently represented, appears so genetically similar to 'Waina' (similarity coefficient 95%) that it is surprising that it was distinguished commercially. By contrast, the similarity coefficients amongst sprouts grown on the same individual 'Owairaka Red' storage root range from 92 to 100% (Table 3.4). The similarity coefficients amongst the 'Owairaka Red' clonal selections (C1 to C5) at 95 to 100%, were similar to those of the sprouts. Generally the remaining genotypes in cluster V (Figure 3.1) were homogeneous, although growers' strains ORC4 and ORC8 may have demonstrated more heterogeneity if higher levels of resolution were applied in the AFLP procedure.

'Owairaka Red' is only propagated vegetatively and while flowers occasionally form there is no record of any seed set. However, the level of somatic mutation found in

vegetatively propagated sweetpotato has had a profound effect on clonal genotypic fidelity. As shown through AFLP-based cluster analysis (Figure 3.1) and supported by principal co-ordinate analysis (Figure 3.2) a number of 'Owairaka Red' strains have become so removed from the common 'Owairaka Red' genotype that they may be considered separate cultivars. Although distinct clonal strains were recognised in the past by field evaluation and renaming, new 'Owairaka Red' strains with subtle differences in phenotype are continuing to develop without formal recognition. The original hypothesis, that intra-clonal genetic diversity may be of a magnitude equivalent to that observed between recognised commercial cultivars, is supported by this experiment.

The occurrence of cultivar 'Owairaka Pink' with the 'Owairaka Red' strain ORC0 in cluster IV (Figure 3.1) lends support to their common historical derivation, however they are significantly separated from the consensus 'Owairaka Red' genotype represented by cluster V. While the growers' strains in cluster VI, namely ORC1 and ORC5, appear more similar to general 'Owairaka Red' germplasm than those in cluster IV, they are still distinctly different at this level of resolution.

The distinct separation of 'Tauranga Red' into cluster VII is of interest (Figure 3.1) as is its close proximity to 'Toka Toka Gold' (Figure 3.2). From a historical perspective, the cultivar 'Tauranga Red' is reputably recorded (Coleman 1969) as an intermediate mutation between 'Waina' and 'Owairaka Red'. Yet cluster analysis, supported by principal co-ordinate analysis, indicates 'Tauranga Red' is more genetically removed from any 'Owairaka Red' or 'Waina' germplasm than even the introduced hybrid cultivars 'Beauregard' and 'Beniazuma'. The degree of dissimilarity from 'Waina' and 'Owairaka Red' would suggest that either historical records of their relationship are incorrect or the source of 'Tauranga Red' germplasm has been compromised. The original source of cultivar 'Toka Toka Gold' germplasm is unknown (Lewthwaite 1998), however although distinct from 'Tauranga Red' it appears to cluster relatively closely (Figure 3.2). The suggestion of their genetic similarity is further reinforced by both 'Tauranga Red' and 'Toka Toka Gold' having the tendency to produce extremely high numbers of adventitious pencil roots, a relatively rare growth habit (Plate 3.8).

The *I. plummerae* seedling and *I. batatas* cultivars 'Beauregard' and 'Beniazuma' provided a useful frame of reference for the level of intra-clonal variation. At this level of resolution, the tissue cultured 'Beauregard' differed from the field grown version by only two polymorphic bands out of the 91 scored (similarity coefficient of 98%), following ten years of maintenance under different systems. While colour mutations are commonly seen, 'Beauregard' appears to have a relatively low mutation rate compared to other cultivars (La Bonte et al. 2000). Leaf samples of the New Zealand field grown 'Beauregard' and a local white fleshed version developed from a sectorial mutant (Plate 3.2a) were submitted to Louisiana State University as part of an international collaborative project. Of the two samples, only the white fleshed mutant was distinguishable from the United States 'Beauregard' clones by banding profile (La Bonte et al. 2000). While at the limits of accurate resolution, of the five 'Owairaka Red' sprouts (S1 to S5) produced here on the same individual storage root, two showed identical banding patterns while the other three sprout samples differed from them in one, two and six bands respectively (similarity coefficients of 100 to 92%).

Comparison of Chilean cultivars (Sagredo et al. 1998) against those of global origin by RAPDs (randomly amplified polymorphic DNA) showed that Chilean accessions possessed very little relative diversity. One suggested hypothesis is that Chilean cultivars are derived from a common origin, a single introduction, which has been vegetatively propagated and accumulated mutations for several generations. The Chilean result appears somewhat equivalent to that seen in New Zealand with 'Waina' derived material, however the recorded history of New Zealand sweetpotato allows a more definitive interpretation.

It has been shown that potato (*Solanum tuberosum* L.) plants are more stable if they are derived from preformed meristematic regions as opposed to regeneration via callus (Potter & Jones 1991). Field grown sweetpotato plants from *in vitro* sources are significantly more uniform than adventitiously derived plants (Templeton-Somers & Collins 1986). So the sweetpotato propagation system affects the level of mutation. A molecular comparison of nodal and adventitiously derived sweetpotato plants demonstrated that preformed meristematic regions at the nodes produced more genetically uniform plants (Villordon & La Bonte 1996). If plant propagation from

nodal material was incorporated into commercial production systems the level of intra-clonal genetic variability would decrease.

3.4.3 Chapter summary

The AFLP technique with two prior selected primer combinations showed adequate discriminating capacity between accessions. A nested selection of *Ipomoea* spp, *I. batatas*: cultivars, growers' strains, intra-clonal selections and sprouts, gave a gradient of increasing genetic uniformity (fewer polymorphic bands) at each sample level. The historic application of clonal selection techniques and the phenotypic variation observed in current crops suggests relatively high levels of somatic mutation. The degree of genetic heterogeneity as quantified by AFLP further demonstrated the underlying instability of sweetpotato cultivars. There is a paucity of commercially viable techniques to minimise or eliminate genetic drift, the only method currently suggested is plant propagation based on preformed meristematic regions rather than adventitiously derived plants. However, the formation of adventitiously derived storage roots in the field still provides a route for accruing mutations.

Chapter 4

Plant field establishment under modified techniques

4.1 Introduction

There has been limited research in New Zealand into field establishment of the sweetpotato crop. International research has generally been directed at increasing sprout production in the propagation beds rather than examining early field establishment. The early Maori propagation technique involved planting sprouted root pieces directly into the field (Best 1925). However, the introduction of cultivars such as 'Waina' allowed the most significant development in field establishment, propagation by vine cuttings or sprouts pulled from storage roots (Berridge 1913). At present about 80% of the commercial crop consists of the cultivar 'Owairaka Red', a mutant selected from 'Waina' and released commercially in 1954 (Lewthwaite 1998). Improved hygiene practices were adopted following outbreaks of various fungal diseases such as black rot (*Ceratosystis fimbriata*) (Coleman 1962b). The current recommended practice is to transplant sprouts produced on storage roots, but cut above the soil level. In New Zealand's temperate climate sweetpotato is cultivated as an annual crop, with most of the crop planted during November. Dargaville (Lat. 35° 55' S), New Zealand's main sweetpotato production area, has a mean air temperature of 15.4°C in November (50-year mean). Cool winds may occur during transplanting (Anon. 1999).

The experiment described in this chapter examined the effect of various transplanting systems on plant survival, transplant growth checks, and storage root initiation (Lewthwaite 2002a). Previous work (Lewthwaite & Triggs 1999) demonstrated that transplant quality may affect final yield. In the present trial both the transplant itself and the environment into which it is placed, were modified in order to maximise growth. The results of earlier trials which suggested that plug transplants may produce a yield increase in 'Owairaka Red' without loss of root quality (Lewthwaite & Triggs 1999), were further investigated, as were international reports that when sweetpotato sprouts are cut and held for several days prior to transplanting root yields can increase

(Hammett 1981; Nakatani 1993). It has been observed that smaller propagules may cope better than those of standard size when transplanted into cool, windy conditions and that sheltering the young plant within a hollow on the top of the mould may encourage early growth, so these management options were also examined. Finally, the application of anti-transpirants to reduce water loss or the use of fertilizer starter solutions to supply readily absorbed nutrients were investigated (McKee 1981). While international research into direct planting of sprouted root pieces has continued (Hosokawa et al. 1998), this technique was not included here as its effective use is cultivar specific (Fukazawa 1998).

The previous chapter established that there is a high level of intra-clonal genetic diversity in sweetpotato, requiring deliberate management to minimise. Variation in crop performance may also be due to the nature of the transplant and its vulnerability at establishment. This experiment tests the hypothesis that relatively minor modifications to commercial sweetpotato transplanting systems may lead to significant changes in the efficiency of plant establishment.

4.2 Materials and Methods

4.2.1 Treatments

A total of 16 plant establishment treatments were applied in this study (Table 4.1). As there has been limited published research on the topic, treatments were selected within a grower discussion group based on practical experience and on available literature. Sprouts were produced by bedding storage roots of the cultivar 'Owairaka Red' in trays of commercial potting mix in an unheated glasshouse. All the treatments including plants in plugs were prepared at the Pukekohe Research Centre, so that the entire trial could be transplanted into the field within a single day. All plant material was watered daily, including cut sprouts. After cutting, sprouts were placed in a shed open to external air conditions. The mean ambient air temperature during treatment preparation (November 1997) was 14.1°C.

Table 4.1: Sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', plant establishment treatments.

Treatment	Description
Control	Sprouts of commercial size (30 cm long, with 6 nodes), transplanted with 4 nodes inserted into the soil the day following cutting.
Held-1	As for the control, but held for 3 days under moist[1] conditions at ambient temperature prior to transplanting.
Held-2	As for the control, but held for 6 days under moist conditions at ambient temperature prior to transplanting.
Held-3	As for the control, but held for 9 days under moist conditions at ambient temperature prior to transplanting.
Sand-1	As for the control, but held for 3 days with 4 nodes inserted into river sand prior to transplanting.
Sand-2	As for the control, but held for 6 days with 4 nodes inserted into river sand prior to transplanting.
Sand-3	As for the control, but held for 9 days with 4 nodes inserted into river sand prior to transplanting.
Anti-1	As for the control, but with leaves dipped in an anti-transpirant solution (Vaporgard® at 1:50 parts water) just prior to transplanting.
Anti-2	As for the control, but with leaves dipped in an anti-transpirant solution (commercial fish oil (NPK 5-1-1) at 1% v/v) just prior to transplanting.
Start-1	As for the control, but watered in with 200 ml per sprout of monopotassium phosphate (NPK 0-52-34) in a 1% w/v solution.
Start-2	As for the control, but watered in with 200 ml per sprout of monoammonium phosphate (NPK 12-61-0) in a 1% w/v solution.
Mould	As for the control, but transplanted into a protective groove formed along the top of the soil ridge, to reduce exposure.
Size-1	Small sprouts (4 nodes) with 1 node inserted into the soil the day following cutting.
Size-2	Small sprouts (4 nodes) with 2 nodes inserted into the soil the day following cutting.
Size-3	Small sprouts (4 nodes) with 3 nodes inserted into the soil the day following cutting.
Plug	Small sprouts (3 nodes) with 1 node inserted into 45 ml plugs, 23 days before transplanting.

[1]Moist conditions were maintained by applying overhead watering to freely-draining cut sprouts, morning and evening.

4.2.2 Trial management

The experiment was conducted in a commercial field at Dargaville, New Zealand, in Kaipara clay soil. Superphosphate (NPK 0-10-0) was broadcast (1 t/ha) six months

prior to transplanting, then muriate of potash (NPK 0-0-50) at 0.5 t/ha and urea (NPK 46-0-0) at 0.1 t/ha five months later. The soil was sampled at planting with the following analysis (techniques in Appendix 1): phosphorus 74 μg/ml (Olsen P), potassium 1.83 me/100 g, calcium 18.9 me/100 g, magnesium 3.08 me/100 g, sodium 0.20 me/100 g, exchangeable cations 31.3 me/100 g, available nitrogen 86 kg/ha, pH 5.9, and a volume/weight ratio of 0.95 for dried ground soil. The trial was transplanted into the field on 28 November 1997 and then watered in without any additives, by a tractor-drawn tanker. Weed control was obtained by hand weeding and application of Gramoxone® at 0.5 l/ha (paraquat dichloride, 25 % a.i.), 30 days after transplanting (Lewthwaite & Triggs 2000).

The experiment was planted in a modified alpha row and column design (Williams & John 1989), essentially a latinized design with a two dimensional blocking structure. The trial was constructed using design software ALPHA+ (CSIRO, Australia) at four columns in width and twelve rows in length. There were 16 treatments with three replicates, giving a total of 48 plots. Each plot was further divided along its length into two sub-plots, one sub-plot for each of the two harvest dates. Each of the 96 sub-plots were fully buffered by guard plants on all sides. The within-row plant spacing was 30 cm, with 75 cm wide rows. Excluding buffer plants, the harvested area of each sub-plot was 2.4 m long by 1.5 m wide, and contained 16 plants (arranged in two rows of eight). All sub-plots were hand harvested.

4.2.3 Harvests

The two respective sets of trial sub-plots were harvested at different dates, the first on 20 January (53 days after transplanting) and the second on 1 April 1998 (124 days after transplanting). At the first harvest plant survival was recorded, and then plant tops were removed and divided into leaves and stems. Leaves were separated at the point where the petiole and lamina met. For each plot, all of the leaves were removed and weighed before a leaf sub-sample (60 leaves) was taken at random to estimate total leaf number, then the stem, leaf and sub-samples were oven dried at 80°C for 5 days and finally weighed. Individual fresh leaf area and oven dry weight of 50 randomly selected leaves with open lamina were recorded for each plot. Leaf area was obtained using a Delta-T

leaf area meter (Delta-T Devices Ltd, Cambridge, England). All root material was hand harvested, apart from the non pigmented (white) fine feeder roots. The harvested root material was divided into underground stems (from the original transplant), pencil roots (up to 15 mm in diameter) and storage roots (above 15 mm in diameter). Storage root stalk length was measured at full extension, from the point of stalk attachment on the underground stem to the shoulders of the storage root. Stalks of any storage roots broken during harvest or with ambiguous storage root shoulders, were not measured. Of all storage roots, 84% had their stalk length measured. The number of roots in each size class was then recorded and oven dried at 80°C for 5 days.

As one treatment (Plug) had unusually long storage root stalks, samples from Plug and Control treatments were fixed in formol-acetic-alcohol (F.A.A.) to allow the degree of tissue lignification to be tested at a later date. The F.A.A solution consisted of formalin (13 ml), glacial acetic acid (5 ml) and 50% ethanol (200 ml). Sections for lignin staining were immersed overnight in 50% ethanol prior to sectioning at *c.* 90 μm, using a vibratome. Lignin was stained using acidified phloroglucin (Sass 1951).

At the second harvest all of the storage roots were dug by hand. The roots were graded on the basis of diameter (Sterrett et al. 1987): Cull (< 2.5 cm), Canner (2.5-5 cm), No. 1 (5-9 cm) and Jumbo (> 9 cm). Root sub-samples for dry matter calculation were taken from each plot and oven dried at 80°C for 5 days. Data were analysed using the statistical software GenStat®: Residual maximum likelihood (REML) procedure (Patterson & Thompson 1971; Lewthwaite & Triggs 1998), to account for any two dimensional spatial effects. The root stalk length data of the first harvest was analysed using the GenStat®: ANOVA procedure (with and without storage root number as a covariate).

4.3 Results

4.3.1 Harvest 1

At the first harvest (Plate 4.1), 53 days after transplanting, there were no significant differences in plant survival rate among treatments ($P = 0.46$). While some treatments (Table 4.2) significantly modified plant weight, plants treated with anti-transpirants or fertilizer starter solutions did not differ from those in the commercial control ($P > 0.05$). Specific leaf area (SLA=Leaf Area/Leaf dry weight) did not differ significantly amongst the treatments, with a mean value of 230 $cm^2\ g^{-1}$ ($P=0.84$).

Irrespective of the number of nodes inserted into the soil, the 4 node plants had smaller total weights than the commercial (6 node) control ($P < 0.01$). Leaf number in these small plants was not significantly reduced ($P > 0.05$) but leaf weight, pencil root weight and pencil root number were reduced ($P < 0.05$).

The Plug and Held-2 treatments had significantly higher total plant weights than the commercial control ($P < 0.01$). While plants in the Plug treatment had a similar leaf weight to the control, they had a significantly higher leaf number ($P < 0.05$). The treatments Plug, Held-2 and Sand-2 produced plants with a higher total root weight than those in the control ($P < 0.05$). These three treatments had pencil root weights comparable to the control, but the Plug treatment had more pencil roots ($P < 0.05$). The treatments Sand-2 and Sand-3 had significantly higher storage root weights ($P < 0.05$) than the control, while treatments Plug and Held-2 showed increases in both storage root weight and number ($P < 0.05$).

Table 4.2: **The main components of sweetpotato cv. 'Owairaka Red' plants 53 days after transplanting, expressed as mean dry weights (g) and number per plant.**

Propagation treatment	Total plant weight	Leaf		Stem	Total root		Pencil root		Storage root	
		Weight	Number	weight	Weight	Number	Weight	Number	Weight	Number
Plug	22	7	67	6	9	9	3.2	7	6	1.7
Held-1	17	6	52	5	6	7	2.5	6	4	1.1
Held-2	22	7	47	6	7	7	2.8	6	5	1.2
Held-3	16	5	40	6	5	7	2.8	6	2	0.5
Sand-1	16	6	54	5	5	7	2.8	6	2	0.6
Sand-2	20	8	60	5	6	8	2.5	7	4	0.9
Sand-3	16	6	55	4	6	6	1.8	5	4	1.0
Mould	19	7	62	5	6	8	2.9	7	3	1.0
Control	16	6	46	5	4	6	2.7	6	2	0.7
Anti-1	16	6	55	4	6	7	2.4	6	3	0.8
Anti-2	16	6	56	5	4	7	3.0	7	1	0.4
Start-1	16	6	41	5	4	6	2.9	6	1	0.3
Start-2	17	6	61	5	5	6	2.1	5	3	1.0
Size-1	9	4	36	3	3	4	2.0	4	1	0.2
Size-2	11	4	38	3	3	4	1.9	4	1	0.3
Size-3	10	4	36	3	3	4	2.0	4	1	0.3
LSD (0.05)	3.5	1.5	16.2	1.6	2.0	1.3	0.66	1.3	2.1	0.52
LSD (0.01)	4.7	2.1	21.9	2.1	2.6	1.7	0.89	1.7	2.8	0.70
P-VALUE	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The number of storage roots produced under the various treatments at 53 days after transplanting differed significantly ($P < 0.01$), so a covariate analysis of stalk length on storage root number was conducted. However, any apparent covariate effect was due solely to the high storage root numbers and long stalk lengths within the Plug treatment alone. As there was no significant relationship between storage root number and stalk length across the treatments, a standard ANOVA was subsequently used. The treatments Start-1 and Size-2 produced storage root stalk lengths just significantly longer than the Control ($P < 0.05$). However the Plug treatment (Plate 4.2) produced much longer storage root stalks ($P < 0.001$), on average over two times the Control length (Fig. 4.1). By selectively staining transverse sections of storage root stalk tissue for lignin it was demonstrated there was greater lignification in the stalk (Plate 4.3) relative to storage tissue (Plate 4.4).

Plate 4.1: Sweetpotato (*Ipomoea batatas* (L.) Lam., cv. 'Owairaka Red') plant establishment trial, 53 days after transplanting in a commercial field at Dargaville, New Zealand.

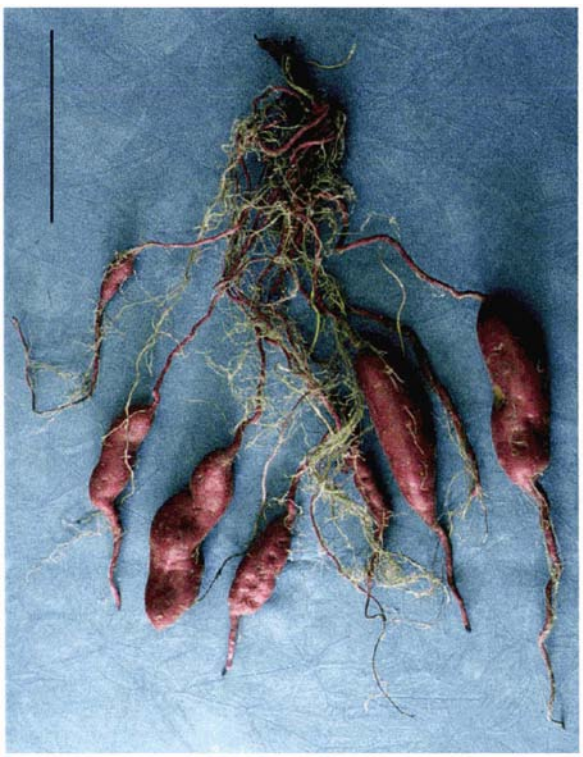


Plate 4.2: Storage roots of a plug-transplanted sweetpotato (*Ipomoea batatas* (L.) Lam., cv. 'Owairaka Red') plant, following 53 days of field growth. The plug has been teased apart to display storage root stalk length (scale bar, 10 cm length).

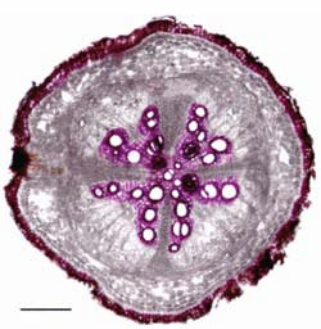


Plate 4.3: Transverse section (*c*. 90 μm thick) of a lignified sweetpotato (*Ipomoea batatas* (L.) Lam., cv. 'Owairaka Red') storage root stalk. Lignin is stained red with acidified phloroglucin (scale bar, 0.5mm length).

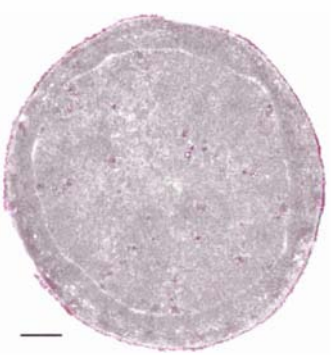


Plate 4.4: Transverse section (*c*. 90 μm thick) of a developing sweetpotato (*Ipomoea batatas* (L.) Lam., cv. 'Owairaka Red') storage root. Lignin is stained red with acidified phloroglucin (scale bar, 1 mm length).

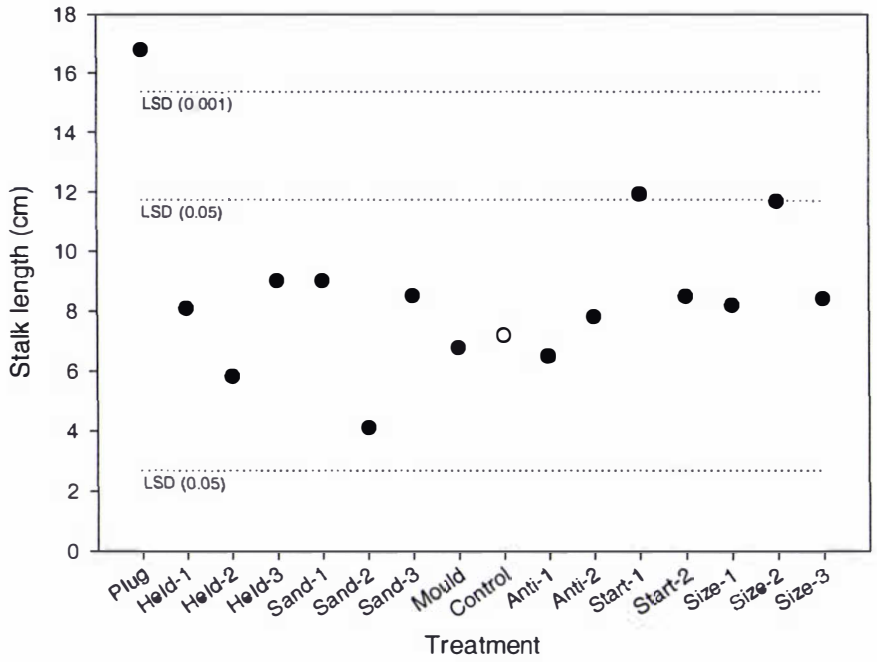


Figure 4.1: The average length of sweetpotato (*Ipomoea batatas* (L.) Lam., cv. 'Owairaka Red') storage root stalks under various transplant treatments, following 53 days of field growth. The open circle represents the control treatment. Least significant differences (LSD) relative to the Control treatment are shown at 5 and 0.1% levels.

4.3.2 Harvest 2

The main effect seen at the second harvest (Table 4.3), 124 days after transplanting, was the significant reduction in total root number for all of the 4-node plants (Size-1,-2,-3) compared with the commercial control ($P < 0.05$). The reduction in root number was observed in both the canner ($P < 0.05$) and No. 1 ($P < 0.05$) grades for the Size-1 and Size-2 treatments, but only in the canner grade for the Size-3 treatment. The treatment with one node inserted into the soil (Size-1) also produced plants with a significantly lower total root weight ($P < 0.05$). None of the parameters measured for the two treatments (Plug and Held-2) differed from the control at the 5% significance level.

Table 4.3: Mean graded root yields and root numbers produced by the sweetpotato cv. 'Owairaka Red' 124 days after transplanting, expressed as mean dry weights (g) and number per plant.

Propagation treatment	Total root		Combined grades Jumbo+No.1		Combined grades Jumbo+No.1+Canner	
	Weight	Number	Weight	Number	Weight	Number
Plug	206	5	142	1.8	203	4.3
Held-1	200	5	127	1.6	197	4.4
Held-2	200	4	143	1.8	198	3.8
Held-3	142	3	108	1.2	141	2.5
Sand-1	222	5	142	1.6	219	4.3
Sand-2	193	5	130	1.6	190	4.1
Sand-3	173	4	132	1.6	172	3.2
Mould	205	4	138	1.6	204	3.6
Control	180	4	124	1.5	178	3.8
Anti-1	176	4	119	1.4	173	3.3
Anti-2	208	5	109	1.3	206	4.2
Start-1	186	4	117	1.2	184	3.4
Start-2	186	4	132	1.5	184	3.3
Size-1	121	2	93	1.0	119	1.8
Size-2	137	3	104	1.1	136	2.3
Size-3	158	3	125	1.3	157	2.4
LSD (0.05)	46	1.2	43	0.40	46	0.90
LSD (0.01)	62	1.6	57	0.55	61	1.22
P-VALUE	0.003	<0.001	0.462	0.005	0.003	<0.001

4.4 Discussion

4.4.1 Harvest 1

The weather conditions were mild at transplanting and during the early establishment period (Fig. 4.2) so there was little plant loss. Plants treated with anti-transpirants or fertilizer starter solutions did not differ in plant weight from the commercial control. This confirms the results of Jett and Talbot (1998), who under Louisiana conditions evaluated a number of anti-transpirants including Vaporgard®, none of which improved plant survival or marketable yield.

All the 4 node treatments produced significantly lower total plant weights, with lower leaf weights, pencil root weights and pencil root numbers. Varying the number of buried nodes made no significant difference to the plant growth rate relative to the Control. These smaller plants never caught up with the growth of the Control plants. Pencil roots are precursors for storage roots and those with active meristems have the potential to swell into storage roots. In these three treatments (Size-1, -2, -3) the growth rate was compromised by a reduced leaf area, and potential storage root production was further limited by the development of fewer pencil roots.

The Plug and Held-2 treatments were of particular interest in this experiment as they both produced total plant weights significantly greater than the commercial control. The Plug treatment appeared to have the potential for a rapid increase in leaf area, as although it had a similar leaf weight to the control, leaf number was significantly higher. Having established that SLA did not differ significantly amongst treatments, the greater leaf number within the Plug treatment was due to an increase in young unexpanded leaves. The greater total root weight in the Plug, Held-2 and Sand-2 treatments, relative to the Control, demonstrated an efficient plant establishment phase. While all three treatments had pencil root weights comparable to the control, only the Plug treatment had more pencil roots. The greater numbers of pencil roots in the Plug treatment suggests a capacity for increased storage root production. The significantly higher storage root weights in the Sand-2 and Sand-3 treatments showed an improvement in plant establishment, but the Plug and Held-2 treatments produced increases in both storage root weight and number, demonstrating a greater storage potential.

In sweetpotato plants, roots form at the callus base of the transplanted sprout and at the buried leaf nodes (Sirju-charran & Wickham 1988). The Plug treatment was originally a 3 node sprout with only one node inserted into the soil, while the commercial control had 6 nodes with 4 inserted. Despite its smaller size and fewer points for root formation, the Plug treatment out yielded the commercial transplant for root weight and number. This is in direct contrast to all the 4 node treatments, which regardless of the number of nodes inserted into the soil produced reduced root yields under mild establishment conditions. The plugs had an extra 23 days of growth prior to

transplanting, allowing them to establish a comprehensive root system which was transplanted intact. Root growth in the Plug treatment more than compensated for the reduction in plant size and decreased sites for root initiation as it also diminished the growth check at transplanting. The Held-2 treatment was identical to the commercial control apart from being stored for 6 days. The 6-day storage allowed the formation of only small root initials, yet this treatment produced plants significantly larger than the commercial control 53 days after transplanting. The rapid establishment of the Plug, Held-2 and Sand-2 transplants demonstrated that even a small period of root initiation delivered significant gains by reducing the growth check at transplanting.

Among the treatments with stored sprouts there appeared to be an optimum holding period. The 3-day (Held-1) storage period may not have undergone enough root initiation to give a significant growth advantage, but the treatment stored for 6 days (Held-2) produced a significant gain in total plant weight ($P < 0.01$). By contrast, the 9-day (Held-3) treatment did not give a growth gain despite increased root development. Hammett (1981) suggested that relatively extensive bare root growth as seen in the 9-day treatment, is more easily damaged during the transplanting operation, negating any gains. Nakatani (1993) found that lignification of the root meristem also increases with the holding period, at a rate that varies with cultivar. Sprout storage in sand for 6 days (Sand-2) significantly increased total root weight and number ($P < 0.05$), but not total plant weight. Storage in sand for 3 (Sand-1) or 9 (Sand-3) days did not result in significant increases in total plant weight or total root weight. The significant growth increases seen in both of the 6-day storage treatments (Held-2 and Sand-2), but not for the 3 or 9-day storage periods, demonstrates the concept of an optimal holding period. Storing plants in air (Held-2) for relatively short periods produced disproportionate gains over the commercial control, gains that were retained well into the growing season. Nakatani (1993) suggested that root developmental stages are accelerated by holding treatments.

The significant growth advantages accumulated by the Plug and Held-2 treatments over the first half of the growing season demonstrated a decreased growth check at transplanting and the production of more robust plants during the early establishment phase.

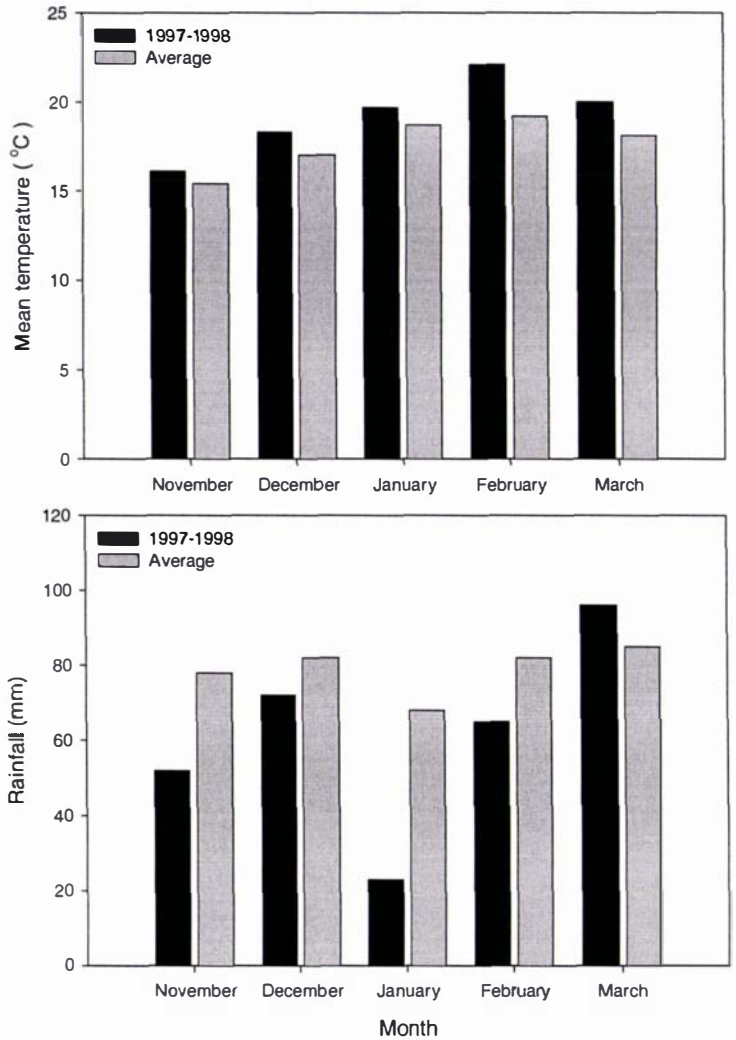


Figure 4.2: Mean monthly temperature (°C) and monthly rainfall (mm) at Dargaville over the 1997/98 growing season, contrasted with long term averages (50-55 years). Data courtesy of the National Institute of Water and Atmospheric Research Ltd.

Islam et al. (2002) found that propagating the cultivar 'Beniazuma' with plugs produced coiled storage roots with reduced fresh market value. In this trial, the Plug treatment did not produce abnormal storage roots (Plate 4.2), due to lignification (Wilson & Lowe 1973a) of the roots coiled within the plug (Plate 4.3). Only the unlignified extensions from these roots (Plate 4.4), developing outside the plug volume, had the capacity for a storage function. This process effectively moved the storage function down the root's length, leaving an extended root stalk (Fig. 4.1). Storage root formation occurred at the same soil depth for all the treatments, as the stalks in the Plug treatment were coiled. Root lignification in this trial was a natural consequence of the dry establishment

conditions (Fig. 4.2), especially high in the soil profile. However, root lignification contrived by passing plug transplants through a dry regime before being established in the field, could eliminate plug-induced storage root coiling in the future. Further work is required to establish the duration and degree of drought stress required to significantly lignify roots within a plug.

Sweetpotato roots are often categorised as feeder, pencil or storage roots, depending primarily on root thickness but also on anatomy (Kays 1985). In this trial, the Plug treatment in particular showed that these categories are quite arbitrary, as a root may exhibit all three elements simultaneously. These categories define roots by their most developed state, in a developmental series from feeder, through pencil, to storage root, but under-represent the complexity within an individual primary root.

4.4.2 Harvests 1 and 2

Commercial growers of cultivar 'Owairaka Red' typically plan a growing season of 120 days. However, after 124 days the small (4 node) plants still did not develop as many roots as the commercial control. The Size-1 treatment (with one node inserted into the soil) produced plants with a significantly lower total root weight, as expected from its limited number of primary rooting sites.

The two treatments (Plug and Held-2) showed considerable growth gains relative to the commercial control at the first harvest, but had lost those gains by the second harvest. This is in contrast to previous results, as in the 1994/95 season the Plug treatment displayed a yield increase under well watered conditions (Lewthwaite & Triggs 1999). Other researchers have also found variable results. Research in both the USA and Japan demonstrated commercial yield increases from holding plants in some years, but not others (Hammett 1982; Nakatani 1993), implying a seasonal effect. The seasonal differences may possibly be due to stress effects, such as drought. Loss of early transplant advantage over a growing season is not atypical of annual crops (Wurr et al. 1986).

In the Dargaville area, due to a limited water supply, the sweetpotato crop is not irrigated beyond watering in transplants at establishment with tractor-drawn water carts. The 1997/98 growing season was warmer than usual (Fig. 4.2) and the crop received less rain than average, particularly over the period of storage root development (January). Storage roots swell and develop from pencil roots through the activity of secondary and tertiary meristems (Wilson 1982). Under certain environmental conditions, such as drought, the meristems may become irreversibly lignified so that storage root development is limited (Togari 1950). On the basis of these and previous results it is suggested that in this experiment the dry season may have limited the potential commercial yield of the Plug and Held-2 treatments, despite their growth gains during early plant establishment.

This experiment demonstrated that sweetpotato transplant systems have a significant impact on plant establishment and storage root initiation. Relatively small changes to the system, such as holding the plants for 6 days after cutting or using sprouts with 6 nodes rather than 4, provided effects that endured well after the transplanting date. The plug technique was particularly remarkable, producing the most rapid early plant growth of all the systems tested, while relocating the storage root initiation sites. These results support the initial hypothesis that relatively minor modifications to commercial sweetpotato transplanting systems may lead to significant changes in the efficiency of plant establishment.

The two holding systems examined here, in the media of air and sand, mutually support the concept of an optimal holding period for sprouts, as does international research (Hammett 1982; Nakatani 1993). However this experiment can be considered preliminary, as further work is required to evaluate and confirm both optimum holding conditions and precise holding durations. Sweetpotato cultivars are classified by the proximity of storage root formation to the plant crown (Huáman 1991), so a comparison of the mechanism modifying stalk length between cultivars would also be an interesting study.

4.4.3 Chapter summary

There has been only limited international research into sweetpotato plant establishment, despite its particular importance within seasonally defined production systems. Sweetpotato is generally propagated and established in the field by transplanting unrooted sprouts. This study examined a range of modified plant establishment techniques, to reduce transplant shock and improve plant survival. At the first harvest (53 DAT), sprouts held in air or sand for six days, or rooted in plug trays, yielded a significantly higher total root weight and storage root weight than the commercial control. Experimental use of sweetpotato plug transplants in Japan has raised concerns about the production of misshapen roots. A procedure for avoiding the formation of storage roots within the plug volume is suggested here. If roots developing within the plug are lignified, they are no longer capable of the lateral growth required for storage root formation. By the second harvest (124 DAT), the yield and number of storage roots was significantly reduced in treatments grown from small four node transplants but was comparable with the control for other treatments. This study showed that by using appropriate propagation material and techniques, significant gains may be made in early plant establishment.

Chapter 5

Carbohydrate partitioning during storage root growth

5.1 Introduction

In this chapter, cultivar specific patterns of dry matter partitioning between major plant components are compared over the period of storage root development. Differences in storage root levels of fructose, glucose, sucrose and %DW (% dry weight) are also compared, for the three commercial sweetpotato cultivars 'Owairaka Red', 'Beniazuma' and 'Beauregard'.

The sweetpotato was first introduced to New Zealand by Polynesian voyagers, the Maori, who settled the country in the thirteenth century (Lewthwaite 1998). Since then there has been a succession of sweetpotato cultivars, from the early introductions until those of the present day. As stated in chapter 3, the predominant cultivar 'Owairaka Red' was selected as a natural mutant from 'Waina', a landrace cultivar introduced from the Pacific Islands of Rarotonga by whalers in the 1850s (Berridge 1913). The sweetpotato has been important throughout New Zealand's history and remains a traditional crop, within which 'Owairaka Red' has become an established traditional cultivar.

Contrasting with the early origins of 'Owairaka Red', modern sweetpotato breeding uses selected parent clones, which are deliberately crossed to produce the desired groupings of attributes. Science-based directional breeding, followed by screening for useful traits, produced the two commercial cultivars, 'Beauregard' (USA) and 'Beniazuma' (Japan). 'Beniazuma' was produced by crossing selected male and female parents in 1977. The clone was screened for useful characteristics until its final release in 1985 (Shiga et al. 1985). 'Beauregard' was produced in a polycross nursery (1981), where the female parent was known, but not the pollen source (Rolston et al. 1987). 'Beauregard' was screened for agronomically useful traits until its release in 1987. The cultivars 'Owairaka Red', 'Beniazuma' and 'Beauregard' represent varied breeding methods, diverse germplasm and different markets.

Both crop management and breeding may bring about a shift in the partitioning of resources between the organs of a plant. Even the relative amount of assimilate partitioned between compounds (such as non-structural carbohydrates), within organs, may be modified (Halford 1999). In sweetpotato, the relationship between the assimilate source (photosynthetic tissue) and main assimilate sink (storage roots) and their relative importance to crop yield have been studied. Source potential (leaf area and photosynthetic rate) and sink capacity (mean storage root weight and number) vary amongst sweetpotato cultivars (Hahn 1977, 1982; Bouwkamp & Hassam 1988). The rate of photosynthate translocation between source and sink is important to storage root yield (Austin & Aung 1973; Kays et al. 1982; Bhagsari & Ashley 1990). The source potential and sink capacity interact in that an increasing sink capacity may stimulate an increase in photosynthetic rate (Zhong 1991). During plant growth the relative importance of source potential to sink capacity varies, as a strong storage root sink during early growth competes with the development of further source leaves. Thus in efficient plant establishment, early development of an adequate LAI (Leaf area index or leaf area per unit area of land) and LAR (Leaf area ratio or ratio of leaf area to total plant dry weight) before storage root initiation, is important. However, in the later stages of plant growth a relatively strong root sink is required to maximise storage root yield (Bhagsari 1990).

The sweetpotato crop is normally harvested when the production of premium-sized roots is maximised. Sweetpotato growers select a harvest date based on experience (cultivar by season effects), the number of days from transplanting and by digging random plant samples. While at present the primary criterion for selecting a harvest point is root yield, in the future root quality will become increasingly important. Root quality, as defined by product size, appearance, flavour, texture, nutritional attributes, health benefits and suitability for processing, will play an important part in determining the crop's market share.

Commercially significant changes in root quality are more difficult to assess in the field than root yield alone. The detection of such changes requires either sophisticated equipment for direct measurement or the development of models to approximate the direction and magnitude of changes against a baseline, such as time. An increasing amount of international research is being directed at studying biochemical changes in

sweetpotato storage roots during plant growth, including the topics of tissue colour, texture and flavour.

Two root tissue colours are currently of particular interest, purple high anthocyanic roots and orange high carotenoid roots. Root anthocyanin content and composition have been studied during development with a view to maximising the concentration of sweetpotato anthocyanins (Miyazaki 1992; Yoshinaga et al. 2000) both for their radical-scavenging and antimutagenic activities (Yoshimoto et al. 1999) as well as for their use as natural food additives and colourants. While anthocyanins are synthesized throughout storage root development the rate of accumulation is not constant (Yoshinaga et al. 2000). The consumption of carotenoids has been associated with various health benefits and β-carotene is a major precursor of vitamin A. Although orange-fleshed sweetpotatoes have higher total carotenoid concentrations than light coloured cultivars, for any given cultivar the rate of carotenoid accumulation varies during root development (Hagenimana et al. 1999).

Sweetpotato root texture is an important aspect of eating and processing quality. An examination of the physicochemical properties of sweetpotato starches (Noda et al. 1995) demonstrated that while sweetpotato starch grains are of consistent shape (spherical and polygonal) throughout root development, they increase in size as roots age. Starch pasting properties such as peak viscosity and breakdown tend to increase with root physiological age, while pasting temperatures decrease. Storage root dry matter content and sugar concentration are fundamental elements of sweetpotato texture and flavour that also vary over the period of root development (Takahata et al. 1996; Katayama & Tamiya 1999; La Bonte et al. 2000).

While the main sugars in raw roots are sucrose, glucose and fructose, maltose is produced during cooking through the conversion of starch (Picha 1985). The concentrations of sugar components may differ markedly between cultivars at any given harvest date, or even as trends over time (La Bonte et al. 2000). However, the ratio of fructose to glucose appears to be relatively stable across cultivars (Lewthwaite et al. 1997) and harvest dates (La Bonte et al. 2000). There is a significant linear correlation between %DW and starch content throughout storage root development (Brabet et al. 1999; La Bonte et al. 2000). The %DW may, therefore, estimate the amount of starch available for conversion to maltose, as well as aspects of textural quality. In general,

increasing the sugar concentration and %DW of sweetpotato roots significantly improves their palatability (Katayama & Tamiya 1999), with the exception of the North American preference for sweet low dry matter cultivars.

The sweetpotato crop has a high level of intra-clonal genetic heterogeneity, as demonstrated by experiment in chapter 3 and supported by international literature. One method for limiting the potential heterogeneity is to propagate the crop using nodal shoot cuttings as they have pre-formed meristems (Villordon & La Bonte 1996). However nodal cuttings are both small and without roots, so are not directly viable for field establishment. Nodal cuttings that are inserted into a plug based rooting system, as demonstrated by experiment in chapter 4 and supported by previous research (Lewthwaite & Triggs 1999), are capable of robust field establishment. Building on the work in previous chapters, the plant establishment system used for this experiment was based on nodal cuttings rooted in plug trays to reduce inter-plant variation. The next major crop management decision is to estimate time of crop harvest. This experiment evaluates the hypothesis that plant growth attributes change in a steady and continuous manner throughout the period of storage root enlargement.

5.2 Materials and methods

5.2.1 Root production

Three sweetpotato cultivars were selected for the experiment, 'Owairaka Red' (Lewthwaite 1998) from New Zealand, 'Beauregard' (Rolston et al. 1987) from the United States and 'Beniazuma' (Shiga et al. 1985) from Japan. All three sweetpotato cultivars were propagated by inserting 1 node of 3 node apical cuttings into 45 ml plugs containing commercial peat/pumice bedding mix, 30 days before transplanting (Lewthwaite, 1999; Lewthwaite & Triggs, 1999). The plugs were hand transplanted (29 November 1995) into the field (Patumahoe clay loam soil) at the Pukekohe Research Centre (Lat. 36° 57' S) New Zealand. A base fertiliser of 30% potassic superphosphate (1 t/ha) was broadcast and incorporated prior to planting. General cultural practice followed commercial recommendations (Coleman 1972). Water was in good supply throughout the season as rainfall was supplemented by overhead irrigation as required, based on tensiometer readings. The experiment consisted of the three cultivars by 10

harvest dates, with three replicates of each. Spatially, the trial was arranged in a modified alpha row and column design (Williams & John 1989) 6 plots wide by 15 plots long, giving a total of 90 plots. All plots were fully surrounded by buffer plants of the cultivar 'Northland Rose', formerly clone '93N9/2' (Lewthwaite et al. 1997). The plots were hand harvested on 10 occasions over the period of storage root development, 71, 78, 85, 91, 99, 105, 112, 120, 127 and 134 days after transplanting (DAT). The harvested portion of each plot contained a total of 20 plants, arrayed in two rows of ten plants. Each row was 0.75 m wide and within-row plant spacing was 0.30 m. So the harvested area of each plot was 1.5 m wide by 3.0 m long.

At harvest, plant tops were removed at soil level. For each plot, all leaves and stems were removed and weighed before sub-samples (10% by fresh weight) were taken at random. Leaves were separated at the juncture of the petiole and lamina. The stem and leaf sub-samples were oven-dried at 80°C for 5 days before weighing. The individual fresh leaf area and oven dry weight of 20 randomly selected leaves with open lamina were recorded for each cultivar at each of the ten harvests. Leaf area was obtained using a Delta-T leaf area meter (Delta-T Devices Ltd, Cambridge, England). The leaf shape of all three cultivars examined here is primarily cordate.

Roots with a diameter greater than 5 mm were harvested. Roots were graded into size categories by diameter at harvest (cm); Cull < 2.5, Canner 2.5 to 5, Number 1 (N1) 5 to 9, Jumbo > 9, adapted from USA categories (Sterrett et al. 1987).

Three roots (diameter 2.5 – 5.0 cm) were selected at random from each harvested plot and cut in half longitudinally. Three halves were bulked and used to determine %DW by drying at 80°C for five days. The remaining three halves were bulked to determine sugar content by high performance liquid chromatography (HPLC). The samples for HPLC were frozen at -30°C until all harvesting was completed.

5.2.2 Growth analysis

The growth curves fitted for plant (Fig. 5.5), root (Fig. 5.6), stem (Fig. 5.7) and leaf (Fig. 5.8) dry weight over time are a modified form of the generalised logistic function originally proposed by Richards (1959), adapted for computer by Causton (1969) and presented in Hunt (1978). The equation (Richards 1959) was used previously to

examine growth in sweetpotato, by Huett & O'Neill (1976). The equation of the fitted curves is:

$$Y = a + b \times r^t \qquad \text{(equation 5.1)}$$

Where 'a', 'b' and 'r' are constants, 't' is time and 'Y' is the resulting fitted value. This equation is included in the GENSTAT®: Standard Curves computer software package, as supplied by the Statistics Department, Rothamsted Experimental Station, Hertfordshire, England. Each of the fitted curves was fitted to observed data following transformation by natural logarithm to account for increasing variance with time, so fitted curves are given in the form:

$$\log_e Y = a + b \times r^t \qquad \text{(equation 5.2)}$$

The maximum likelihood estimates of the parameters a, b and r were found by nonlinear least squares regression. A single equation was fitted to the pooled data simultaneously.

The relative growth rate (RGR), as derived from this equation (equation 5.2), is given by:

$$RGR = b \times \log_e r \times r^t \qquad \text{(equation 5.3)}$$

The leaf area ratio (LAR) and net assimilation rate (NAR) were calculated according to the method of Hunt (1990), where $\log_e A$ are the fitted values for leaf area over time and $\log_e W$ are the fitted values for plant dry weight over time.

$$LAR = \exp(\log_e A - \log_e W) \qquad \text{(equation 5.4)}$$

$$NAR = {}^{RGR}/_{LAR} \qquad \text{(equation 5.5)}$$

5.2.3 HPLC analysis

The HPLC method used (Lewthwaite et al. 1997) was a modification of that of Picha (1985). Exactly 5.00 g of randomly selected tissue were homogenised in 80% (v/v) ethanol for 1 minute at high speed using a Waring 801/G blender (model 91-358). The resulting slurry was agitated gently for 18 hours at 20°C and filtered through Whatman #4 paper. The residue and original container were washed with additional 80% (v/v) ethanol and filtered through Whatman #4 paper. The first and second filtrates were combined and made up to a final volume of 50 ml with 80% (v/v) ethanol.

Approximately 5 ml of the combined filtrate was clarified by centrifugation (10,000 *g*, 10 min) prior to injection into the HPLC.

Sugar standards were prepared by dissolving appropriate amounts of the sugar in 80% (v/v) ethanol and making up the solution to an appropriate total volume in a standard flask. Standard sugar solutions were clarified by centrifugation (10,000 *g*., 10 min) prior to injection into the HPLC.

A Waters liquid chromatograph, consisting of a model 626 pump and controller, model 717-plus autosampler and a model 410 refractive index detector, was used. The detector signal (output at attenuation setting 64) was stored, integrated and manipulated using a personal computer running Waters 'Millennium' software. Sugars were separated with a 220 x 4.6 mm Applied Biosystems Brownlee AMINO column fitted with a 15 x 3.2 mm Applied Biosystems Brownlee NewGuard AMINO guard column thermostatted to 30°C using a Waters column heater. The mobile phase was degassed HPLC-grade acetonitrile:water (80:20 v/v). Solvent flow rate was 1.5 ml min^{-1}. Injection volume for both sugar standards and root extracts was 10 μL. Identification of each sugar was based on HPLC retention time. Detector response to all sugars was linear over the concentration range 0-20% (w/v). Standard sugars exhibited less than 2% variability in individual sugar concentrations between triplicate injections of the same sample.

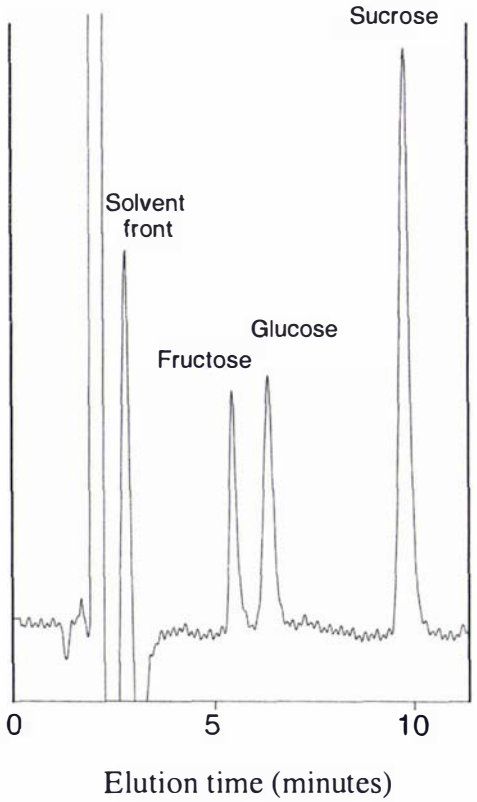


Figure 5.1: Typical chromatograph of sugar separation against elution time in extracts of sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beauregard' storage roots. Based on an Applied Biosystems Brownlee AMINO column at 30°C using a mobile phase of acetonitrile:water (80:20 v/v) at 1.5 ml min^{-1}.

5.2.4 Statistical analysis

Data were analysed using the statistical software GENSTAT®: REML, ANOVA, Linear regression and Non-linear regression procedures. Ternary diagrams are commonly used in science disciplines such as chemistry (Daniels & Alberty 1961) and petrology (Shepard 1954). Ternary diagrams (triangle plots) were constructed from sets of three variables (stem, leaf and root or fructose, glucose and sucrose) that summed to 100%, using the graphics software SigmaPlot®: Ternary diagram procedure. Cubic spline interpolation curves were fitted using the graphics software SigmaPlot®: Spline fitting procedure.

5.3 Results

5.3.1 Dry matter partitioning amongst plant organs

Plant dry matter distributions amongst roots, stems and leaves over time were plotted for cultivars 'Owairaka Red' (Fig. 5.2), 'Beniazuma' (Fig. 5.3) and 'Beauregard' (Fig. 5.4). Similar growth patterns were observed in all three cultivars, with steady increases in root dry matter while stem and leaf dry matter showed little change. The data was then subject to formal statistical analysis.

The field trial was constructed as a row and column design, but analysis by the residual maximum likelihood (GENSTAT®: REML) method showed no spatial effects at row, column or replicate level. The partitioning data required $\log_e$ transformation to stabilise the variance, then analysis was completed using analysis of variance (GENSTAT®: ANOVA). Growth curves were fitted for each cultivar (GENSTAT®: Standard curves procedure) and all data were presented on a dry weight basis.

Averaged over all harvests, there were large differences in total plant dry weight between the cultivars (Fig. 5.5), with 'Owairaka Red' producing the largest plants at 261 g ($\log_e$, 5.6), then 'Beniazuma' at 220 g ($\log_e$, 5.4) and 'Beauregard' at 176 g ($\log_e$, 5.2) per plant ($P<0.001$). The pattern of total plant weight increase over sequential harvests did not differ significantly between cultivars ($P=0.32$).

The cultivars showed different responses over harvests for root weight ($P=0.016$). However, pairwise analysis demonstrated that the pattern of root dry weight accumulation did not differ for 'Beniazuma' and 'Beauregard' ($P=0.74$), while 'Owairaka Red' differed (Fig. 5.6) from each of the other cultivars ($P=0.011$ and 0.013, respectively). As can be observed from the fitted curves (Fig. 5.6), 'Owairaka Red' had a higher rate of root dry matter accumulation than the other two cultivars. 'Owairaka Red' and 'Beniazuma' produced the same mean root dry weight (Table 5.1), while 'Beauregard' produced significantly less ($P<0.001$). The coefficients of determination (R^2) for the fitted curves in each of the cultivars were 96.9% or greater.

The harvest index (root weight/total plant weight) averaged across all harvest dates was significantly lower in 'Owairaka Red' at 57.9% ($P<0.001$) than both 'Beniazuma' (66.2%) and 'Beauregard' (65.8%), which did not differ ($P>0.05$).

The patterns of dry matter accumulation for either stem or leaf weight did not differ across harvests for the cultivars (P=0.54 and 0.59, respectively). On average, 'Owairaka Red' partitioned significantly more dry matter into stems (Table 5.1) than the other two cultivars (P<0.001). 'Beniazuma' partitioned significantly more dry matter to stem tissue than 'Beauregard' (P<0.05). While all the growth curves for stem dry matter accumulation showed a significant fit, 'Beniazuma' was more variable than the other two cultivars (Fig. 5.7). 'Beauregard' produced significantly (P<0.001) less leaf dry matter (Table 5.1) than 'Owairaka Red' or 'Beniazuma', which did not differ (P>0.05). Fitted curves for leaf dry matter accumulation were significant (P<0.001) for each cultivar, but were poor in describing the data (Fig. 5.8), with 42.8% as the highest coefficient of determination. For all three cultivars, total leaf dry weight showed little change throughout the period of storage root growth.

A ternary diagram for each cultivar (Figs. 5.9, 5.10 and 5.11) shows the relative proportion (%) of dry matter partitioned into root, leaf and stem tissue across harvest dates. As plants develop, the relative amount of root tissue increases for all cultivars. The proportional increase in root tissue over time is well approximated by a line for each cultivar, which demonstrates a relatively stable ratio between the proportion of leaf and stem tissue. The ratio for each cultivar is 'Owairaka Red' (leaf 0.44: stem 0.56), 'Beniazuma' (leaf 0.65: stem 0.35) and 'Beauregard' (leaf 0.52: stem 0.48). Of these cultivars, 'Owairaka Red' is the only one that partitions more into stem than leaf tissue.

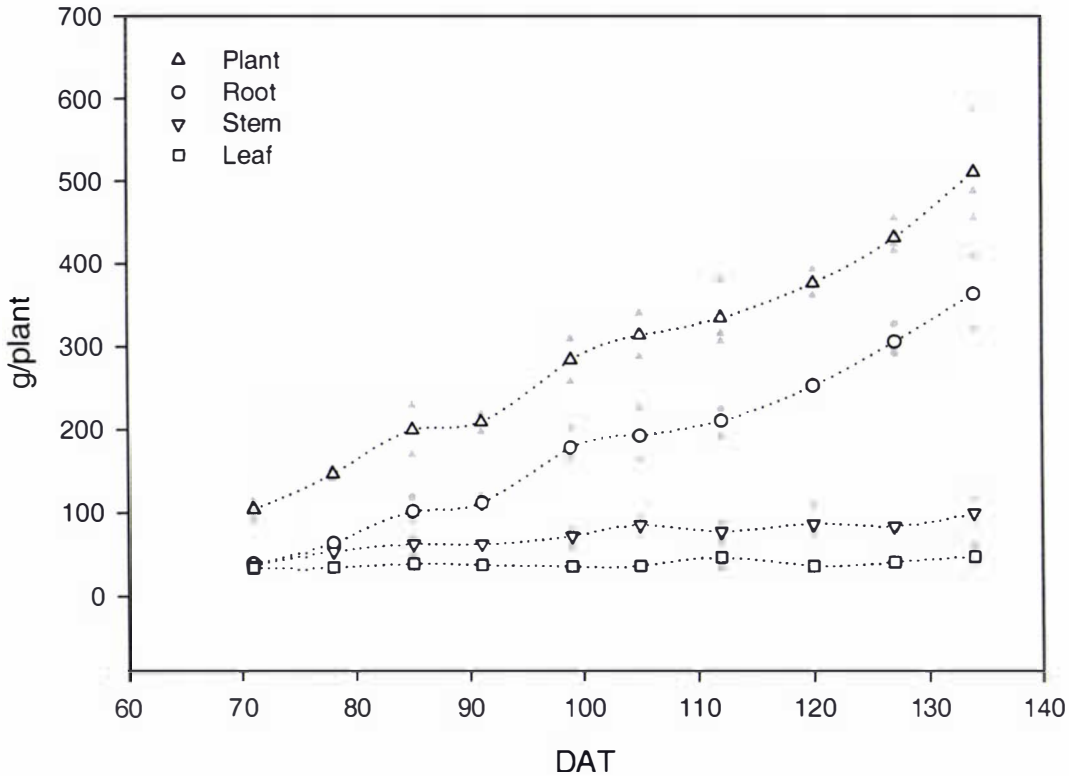


Figure 5.2: Observed (grey symbols) and mean (open symbols) harvest values with cubic spline interpolation curves illustrating the relationship between the principal plant organs (g/plant, dry matter basis) against days after transplanting (DAT) for commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red'.

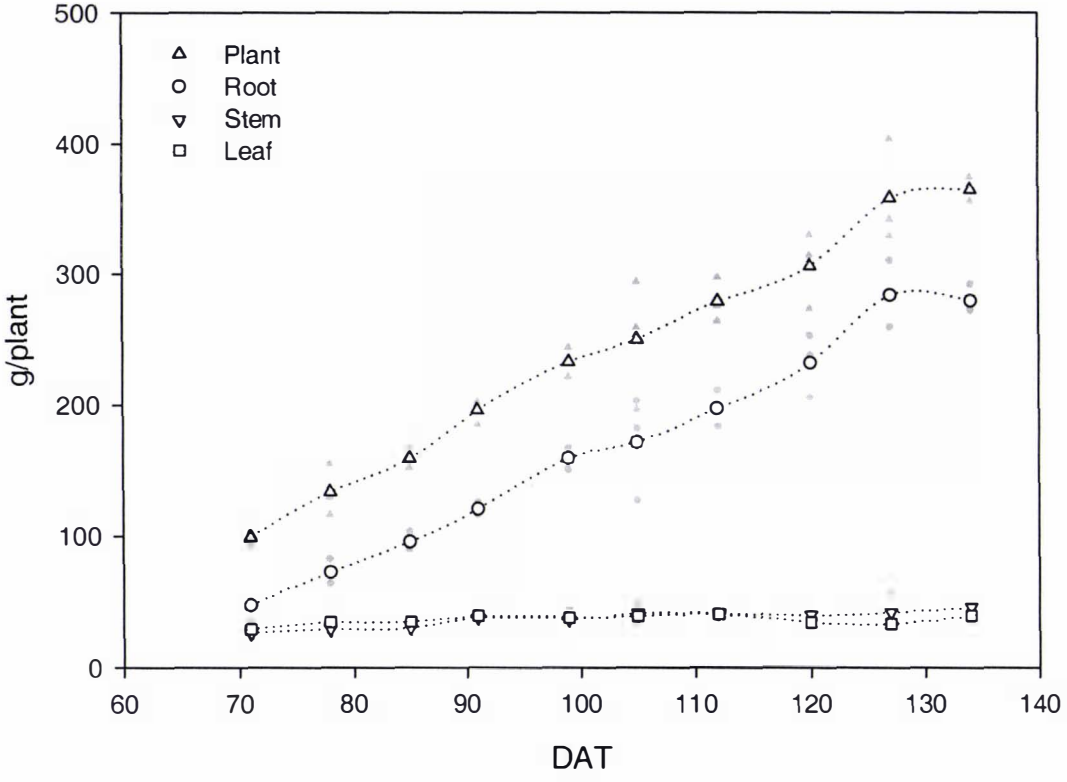


Figure 5.3: Observed (grey symbols) and mean (open symbols) harvest values with cubic spline interpolation curves illustrating the relationship between the principal plant organs (g/plant, dry matter basis) against days after transplanting (DAT) for commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beniazuma'.

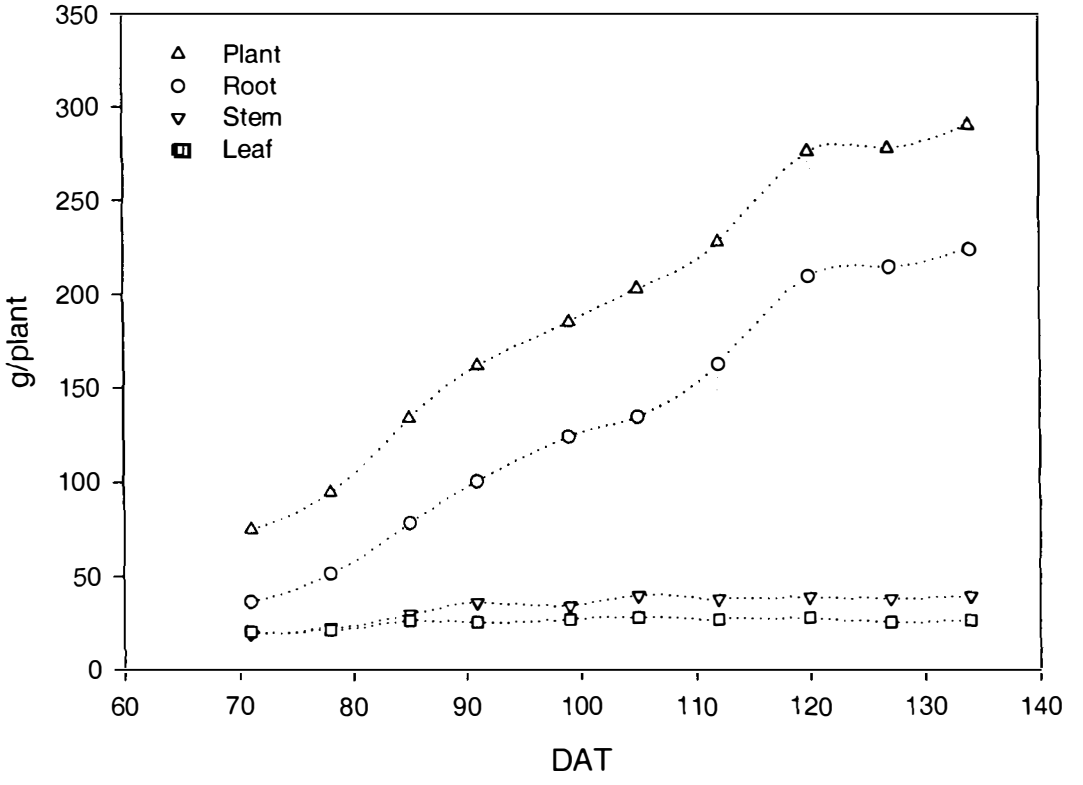


Figure 5.4: Observed (grey symbols) and mean (open symbols) harvest values with cubic spline interpolation curves illustrating the relationship between the principal plant organs (g/plant, dry matter basis) against days after transplanting (DAT) for commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beauregard'.

Table 5.1: The mean dry weight of sweetpotato (*Ipomoea batatas* (L.) Lam.) plants and plant components, averaged across 10 harvest dates. Transformed (log_e) and back-transformed data for three cultivars. Growth curves were fitted and coefficients of determination (R^2) are shown. P values of <0.001 were obtained for all fitted curves, apart from leaf tissue of cv. 'Owairaka Red' (P=0.008).

Cultivar	Plant		Root		Stem		Leaf	
	g/plant	R^2 (%)	g/plant	R^2 (%)	g/plant	R^2 (%)	g/plant	R^2 (%)
'Owairaka Red'	261 (5.6)	96.0	148 (5.0)	97.3	68 (4.2)	81.7	37 (3.6)	25.1
'Beniazuma'	220 (5.4)	95.5	148 (5.0)	96.9	35 (3.6)	59.5	35 (3.6)	40.2
'Beauregard'	176 (5.2)	97.3	114 (4.7)	97.8	32 (3.5)	83.6	25 (3.2)	42.8
$LSD_{(0.05)}$	(0.05)		(0.05)		(0.07)		(0.07)	

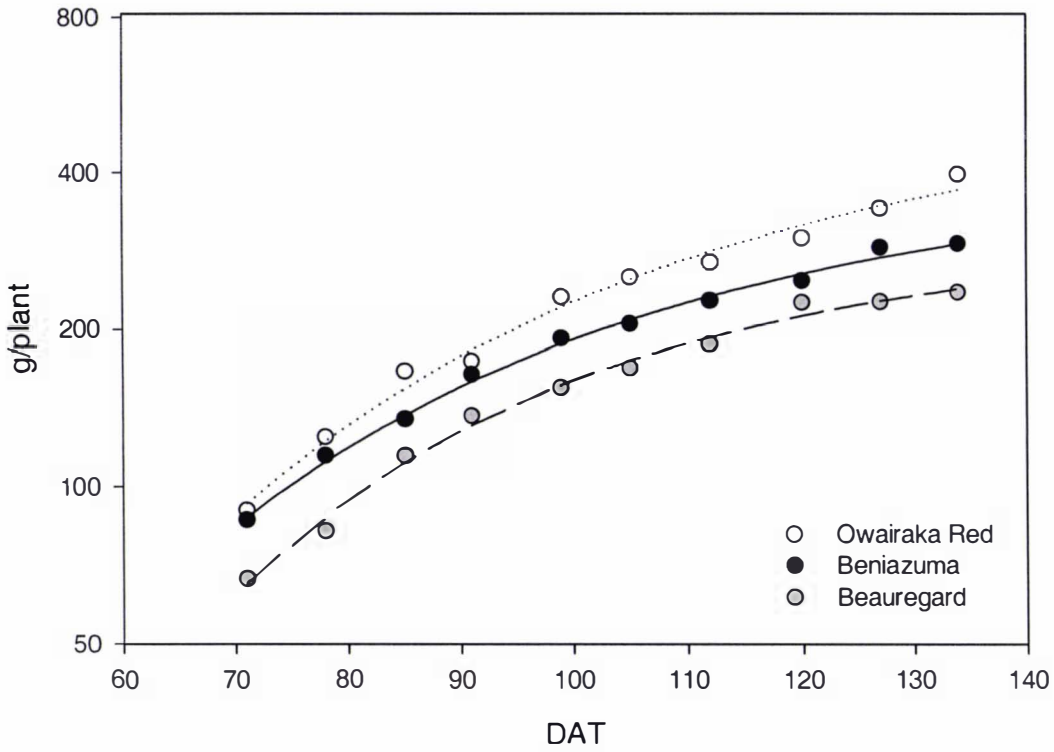


Figure 5.5: Mean observed values and fitted curves showing the relationship between total plant dry matter (TDM) accumulation (g/plant, $\log_e$ scale) and days after transplanting (DAT), for three commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars.

Fitted curves:

'Owairaka Red'	$\text{Log}_e\text{TDM} =$	6.6	-	10	x	0.976^{DAT}
(s.e.)		(0.19)		(2.6)		(0.0044)
'Beniazuma'	$\text{Log}_e\text{TDM} =$	6.2	-	10	x	0.975^{DAT}
(s.e.)		(0.16)		(2.6)		(0.0047)
'Beauregard'	$\text{Log}_e\text{TDM} =$	5.9	-	14	x	0.970^{DAT}
(s.e.)		(0.09)		(3.4)		(0.0037)

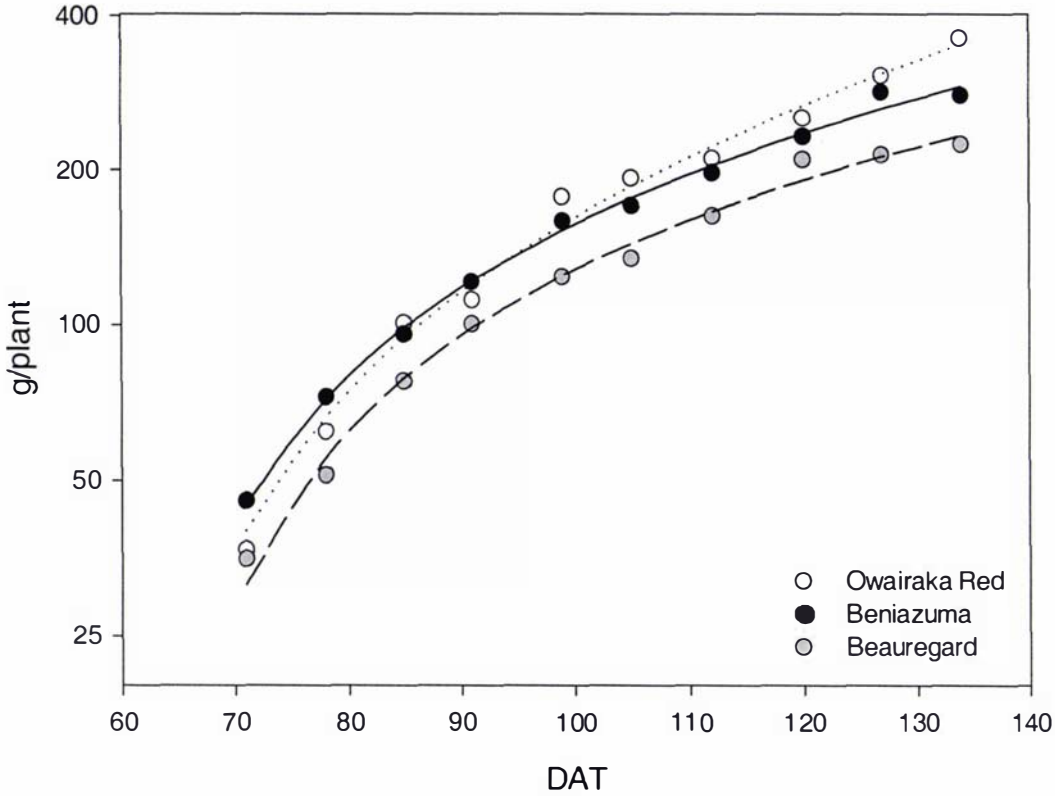


Figure 5.6: Mean observed values and fitted curves showing the relationship between storage root dry matter (RDM) accumulation (g/plant, $\log_e$ scale) and days after transplanting (DAT), for three commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars.

Fitted curves:

'Owairaka Red'	$Log_eRDM=$	6.2	-	22	x	0.970^{DAT}
(s.e.)		(0.15)		(5.1)		(0.0037)
'Beniazuma'	$Log_eRDM=$	6.0	-	16	x	0.972^{DAT}
(s.e.)		(0.15)		(3.8)		(0.004)
'Beauregard'	$Log_eRDM=$	5.8	-	18	x	0.971^{DAT}
(s.e.)		(0.12)		(3.8)		(0.0033)

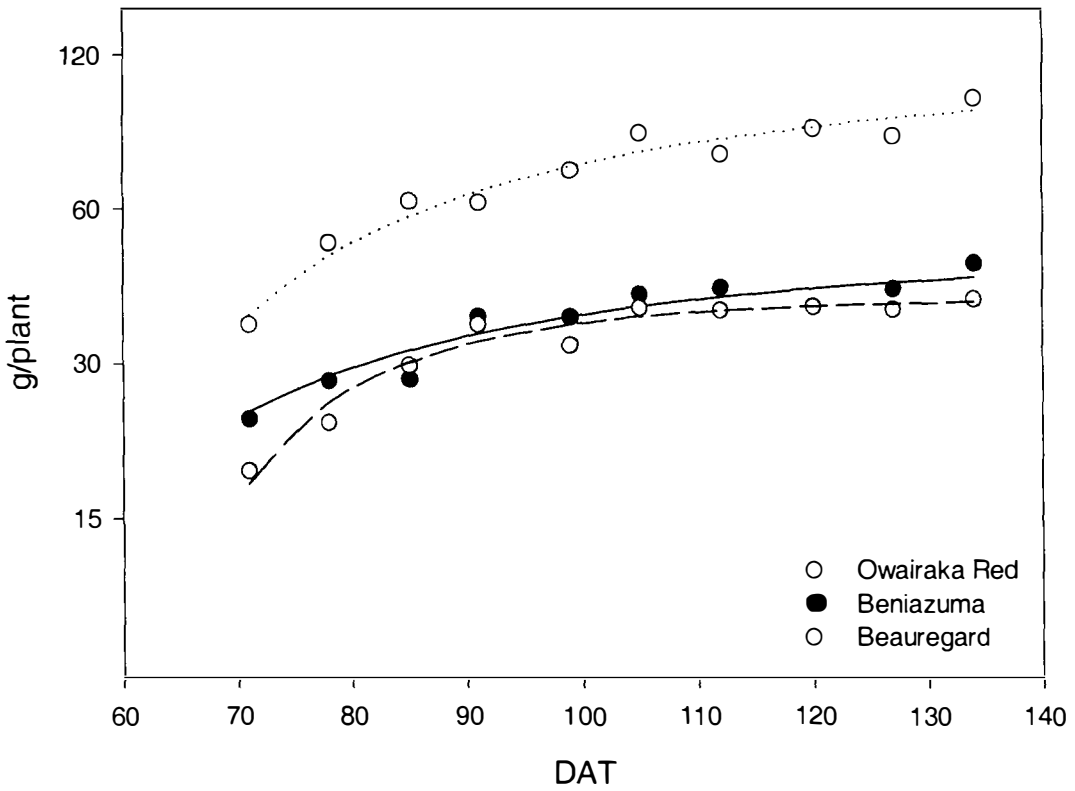


Figure 5.7: Mean observed values and fitted curves showing the relationship between stem dry matter (SDM) accumulation (g/plant, $\log_e$ scale) and days after transplanting (DAT), for three commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars.

Fitted curves:

'Owairaka Red'	$Log_eSDM=$	4.55 -	27 x	0.954^{DAT}
(s.e.)		(0.084)	(22.9)	(0.0118)
'Beniazuma'	$Log_eSDM=$	3.8 -	11 x	0.961^{DAT}
(s.e.)		(0.12)	(14.5)	(0.0192)
'Beauregard'	$Log_eSDM=$	3.69 -	95 x	0.935^{DAT}
(s.e.)		(0.043)	(93.8)	(0.013)

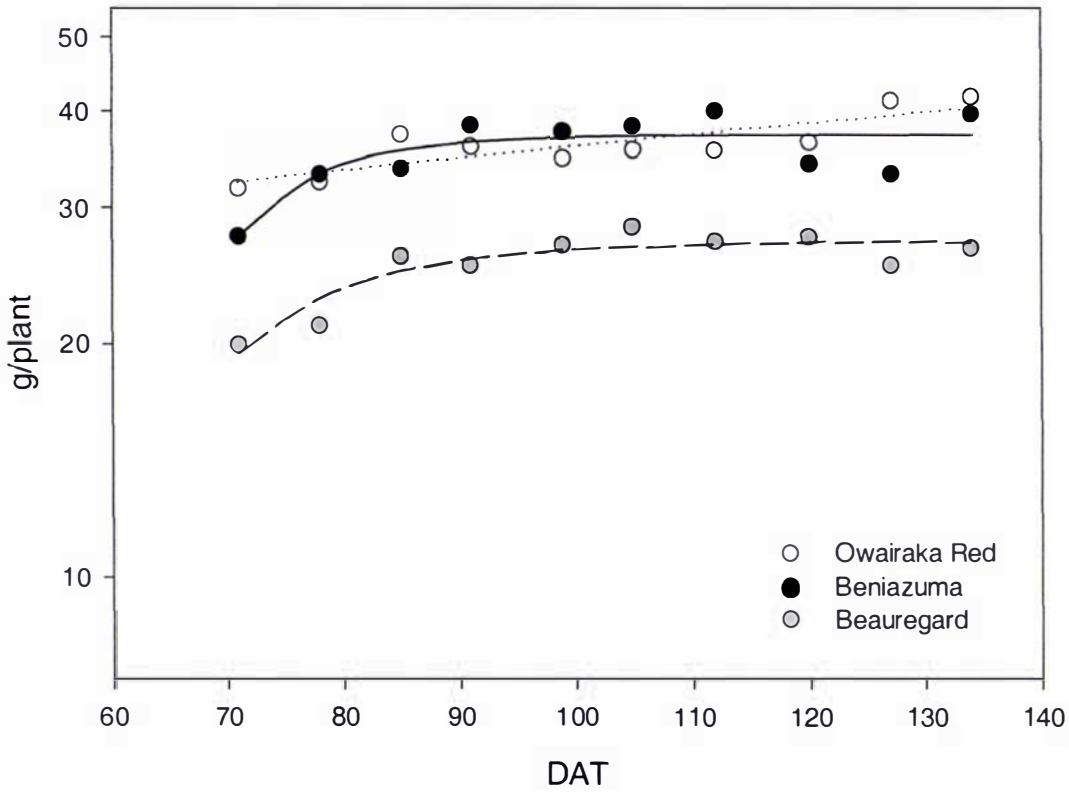


Figure 5.8: Mean observed values and fitted curves showing the relationship between leaf dry matter (LDM) accumulation (g/plant, $\log_e$ scale) and days after transplanting (DAT), for three commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars.

Fitted curves:

'Owairaka Red'	$Log_eLDM=$	3.4	+ 0.027 x	1.021^{DAT}
(s.e.)		(0.41)	(0.145)	(0.0356)
'Beniazuma'	$Log_eLDM=$	3.61	- 3954 x	0.875^{DAT}
(s.e.)		(0.027)	(19507)	(0.0605)
'Beauregard'	$Log_eLDM=$	3.29	- 215 x	0.913^{DAT}
(s.e.)		(0.034)	(682)	(0.0405)

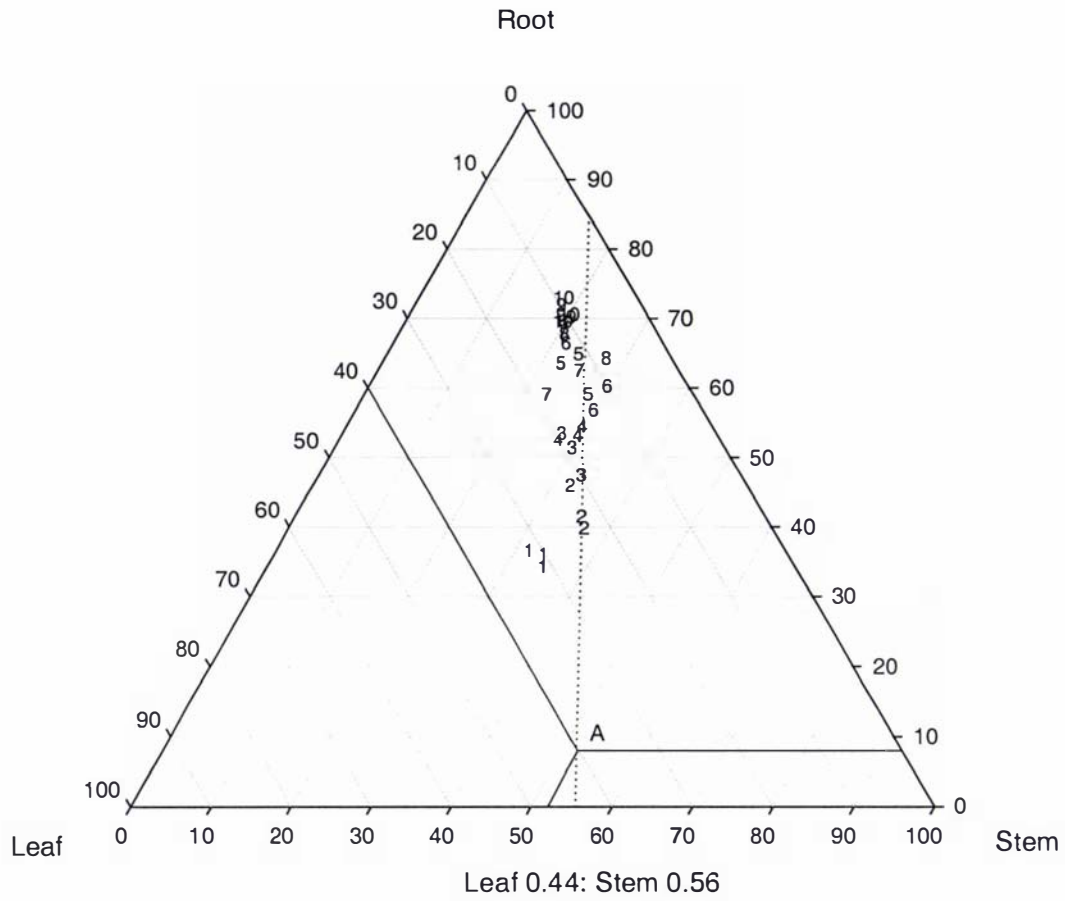


Figure 5.9: Ternary diagram of the dry matter distribution (%) between the main plant organs (leaves, stems and roots) for sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red'. Data were recorded over 10 harvests 71, 78, 85, 91, 99, 105, 112, 120, 127 and 134 days after transplanting (labelled 1 to 10 respectively), with three replicates. The relationship between leaf and stem dry weight over harvests is approximated by a dotted line (Leaf 0.44:Stem 0.56).

Note: Each axis is labelled at its highest point (100%), so the base axis tick labels represent 'stem' dry matter. Any point on the basal axis itself is the intersection of a leaf and stem component only, there is no root contribution (0%). If the inserted trend line (dotted) is extended, it can be directly read in the two dimensions of the basal axis as demonstrating an approximately constant leaf to stem ratio (0.44:0.56) throughout harvest dates. As the Leaf:Stem trend line does not pass symmetrically through the root vertex, its coordinates are calculated in two dimensional space then translated to three dimensions for the body of the diagram. However actual plotted data points can be read directly from any position in the diagram, and theoretical point 'A' has been included to illustrate a point representing all three components. Relative percentages at 'A' are read from drop lines to the three respective axes (Leaf 40%, Stem 52%, Root 8%). Three-dimensional points can be accurately represented on the two-dimensional space of a printed page as they are compositions of three proportions, i.e. Root = 100 - Leaf - Stem.

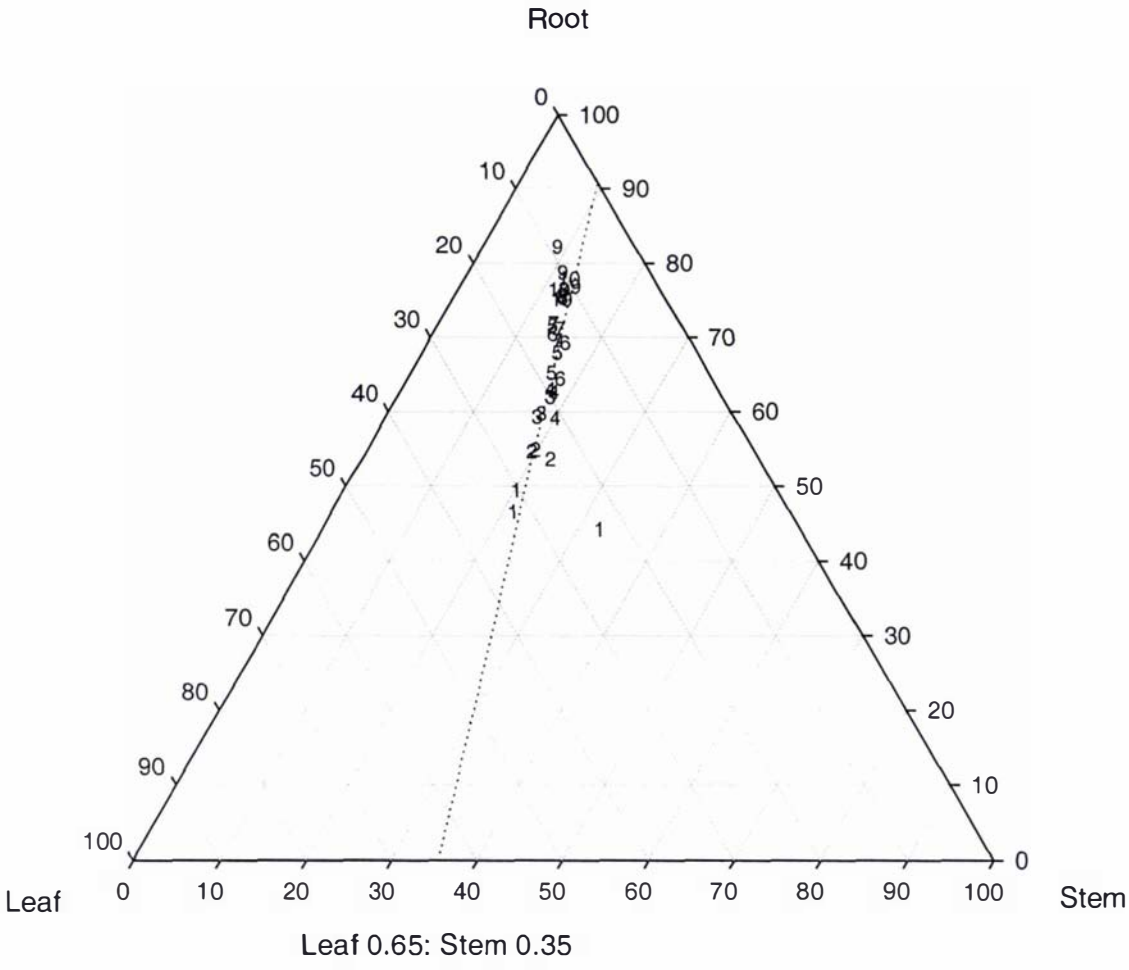


Figure 5.10: Ternary diagram of the dry matter distribution (%) between the main plant organs (leaves, stems and roots) for sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beniazuma'. Data were recorded over 10 harvests 71, 78, 85, 91, 99, 105, 112, 120, 127 and 134 days after transplanting (labelled 1 to 10 respectively), with three replicates. The relationship between leaf and stem dry weight over harvests is approximated by a dotted line (Leaf 0.65:Stem 0.35).

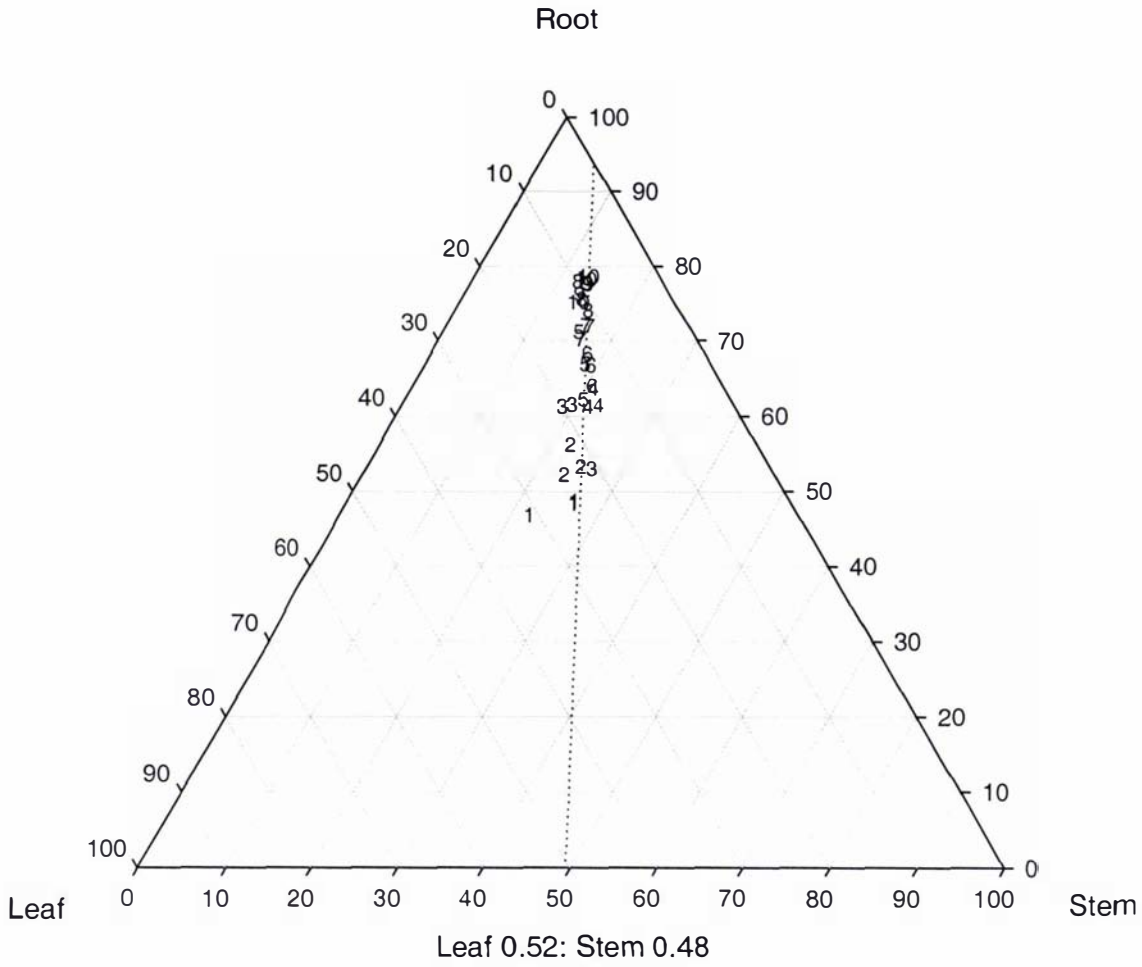


Figure 5.11: Ternary diagram of the dry matter distribution (%) between the main plant organs (leaves, stems and roots) for sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beauregard'. Data were recorded over 10 harvests 71, 78, 85, 91, 99, 105, 112, 120, 127 and 134 days after transplanting (labelled 1 to 10 respectively), with three replicates. The relationship between leaf and stem dry weight over harvests is approximated by a dotted line (Leaf 0.52:Stem 0.48).

5.3.2 Growth analysis

In each cultivar, RGR (Fig. 5.12) declined throughout the storage root bulking period ($P<0.001$). There were no significant cultivar by DAT interactions ($P=0.415$). Mean RGR did not differ significantly between 'Owairaka Red' at 0.024 g $g^{-1}day^{-1}$ and 'Beauregard' at 0.023 g $g^{-1}day^{-1}$ ($P>0.05$). 'Beniazuma' (0.021 g $g^{-1}day^{-1}$) had a significantly lower mean RGR than both 'Beauregard' and 'Owairaka Red' ($P<0.05$).

The NAR steadily declined with increasing growing period (Fig. 5.13) for all three cultivars ($P<0.001$). However, there was a significant cultivar by DAT interaction for NAR ($P<0.001$). While the patterns of NAR decline for 'Owairaka Red' and 'Beniazuma' were similar, 'Beauregard' showed a more rapid decline. NAR at the initial harvest for 'Beauregard' was 10.68 x 10^{-4} g cm^{-2} day^{-1}, almost double that of 'Beniazuma' (6.16 x 10^{-4} g cm^{-2} day^{-1}) or 'Owairaka Red' (6.28 x 10^{-4} g cm^{-2} day^{-1}). By 99 DAT the three cultivars did not differ for NAR ($P>0.05$). The mean NAR for cultivar 'Beauregard' (5.91 x 10^{-4} g cm^{-2} day^{-1}) was significantly higher than that of 'Owairaka Red' (4.74 x 10^{-4} g cm^{-2} day^{-1}) and 'Beniazuma' (4.65 x 10^{-4} g cm^{-2} day^{-1}), which did not differ $P(>0.05)$.

The data for individual leaf areas (LA) and leaf dry weights (LDW) were pooled across harvests for each of the three cultivars. The fitted lines for the relationships between leaf area (cm^2) and dry weight (g) were:

Owairaka Red' LA = 147.7LDW + 33.3 ($P<0.001$, R^2 = 56.6%, s.e. (slope)= 9.15);

'Beniazuma' LA = 175.9LDW + 21.1 ($P<0.001$, R^2 = 76.2%, s.e. (slope)= 6.97);

'Beauregard' LA = 142.2LDW + 23.1 ($P<0.001$, R^2 = 53.9%, s.e. (slope)= 9.30).

As the leaf data was variable, the relationship between SLA (specific leaf area or leaf area/leaf dry weight) and time was explored using the S-Plus®: loess curve fitting procedure (Venables & Ripley 2002). Cultivar specific loess fitted curves approximated a linear relationship, so the Genstat®: linear regression procedure was used to produce fitted lines.

There were significant differences in mean SLA between 'Owairaka Red' at 330.5 cm^2 g^{-1} and the other two cultivars, 'Beauregard' at 260.9 cm^2 g^{-1} and 'Beniazuma' at 268.9 cm^2 g^{-1} ($P< 0.01$). The fitted lines for SLA (cm^2 g^{-1}) over the harvest dates were:
'Owairaka Red' SLA = 7.1DAT + 291.7 ($P=0.004$, R^2 = 3.6%, s.e. (slope)= 2.43);
'Beniazuma' SLA = 3.6DAT + 249.1 ($P=0.009$, R^2 = 2.9%, s.e. (slope)= 1.36);
'Beauregard' SLA = 13.5DAT + 186.6 ($P<0.001$, R^2 = 29.2%, s.e. (slope)= 1.48).
While the coefficients of determination were particularly small for 'Owairaka Red' and 'Beniazuma', all three cultivars showed a significant increase in SLA as the season progressed. 'Beauregard' had significantly smaller leaves (mean 51.6 cm^2) than Beniazuma' (64.4 cm^2) or 'Owairaka Red' (62.7 cm^2) ($P<0.05$, based on $\log_e$ transformed data).

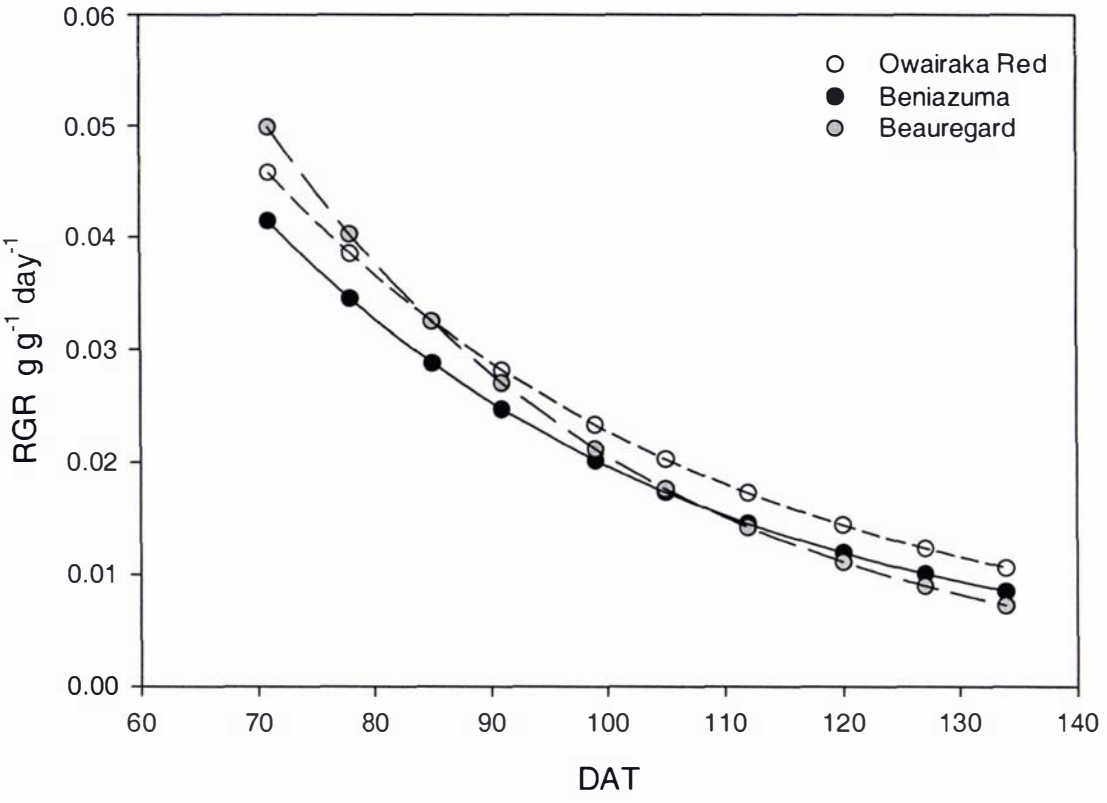


Figure 5.12: Mean values and fitted curves showing the relationship between plant relative growth rate (RGR) and days after transplanting (DAT) for three commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars.

Fitted curves:

'Owairaka Red'	$RGR = 0.00193 + 0.271 \times 0.98^{DAT}$			
(s.e.)		(0.00440)	(0.079)	(0.005)
'Beniazuma'	$RGR = 0.00104 + 0.269 \times 0.97^{DAT}$			
(s.e.)		(0.00408)	(0.087)	(0.005)
'Beauregard'	$RGR = 0.00002 + 0.439 \times 0.97^{DAT}$			
(s.e.)		(0.00320)	(0.119)	(0.004)

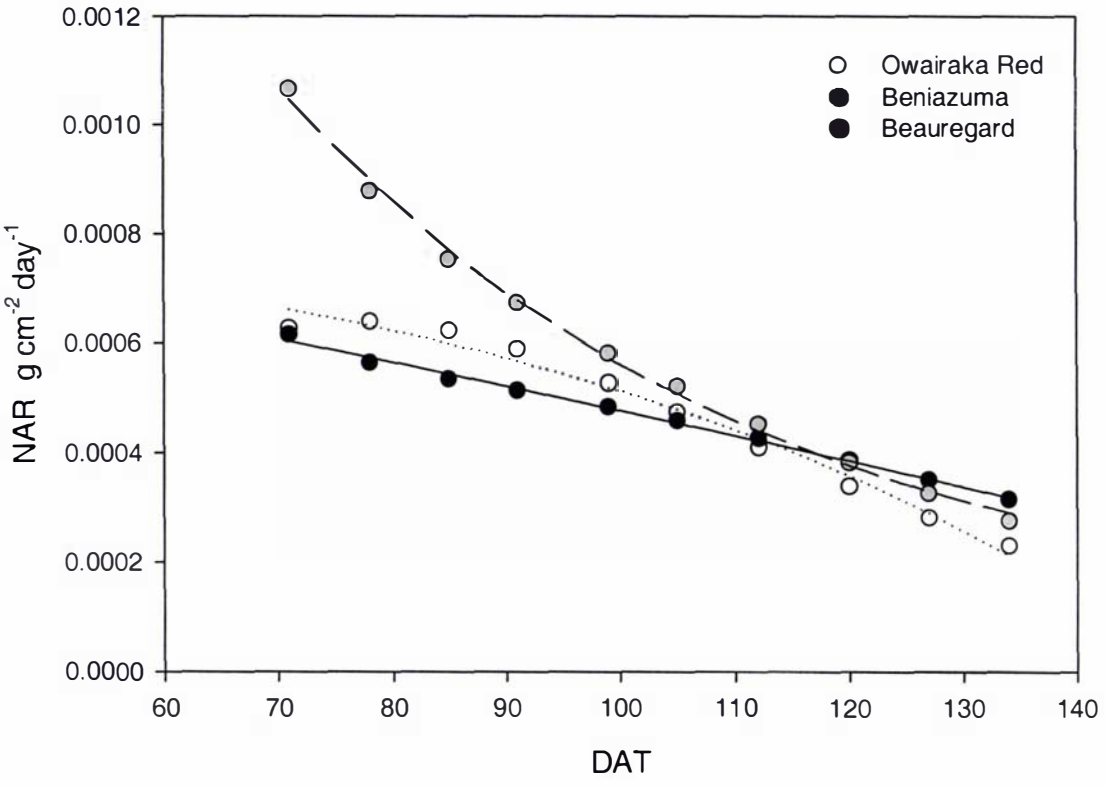


Figure 5.13: Mean values and fitted curves showing the relationship between net assimilation rate (NAR) and days after transplanting (DAT) for three commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars.

Fitted curves:

'Owairaka Red'	NAR= 0.00089 - 0.00007 x 1.02^{DAT}	
(s.e.)	(0.00018)	(0.00008) (0.007)
'Beniazuma'	NAR= 0.00280 - 0.00190 x 1.00^{DAT}	
(s.e.)	(0.01270)	(0.01250) (0.011)
'Beauregard'	NAR= 0.00007 + 0.00527 x 0.98^{DAT}	
(s.e.)	(0.00009)	(0.00125) (0.004)

5.3.3 Storage root size grades

The relative contributions of storage root grade categories to total root mass over harvest dates were compared for each of the three cultivars. The mean observed values of each root grade were joined across harvests with cubic spline interpolation curves. In 'Owairaka Red' (Fig. 5.14) and 'Beauregard' (Fig. 5.15) the cull and canner grades maintained a relatively consistent root mass throughout the growing period. By the fifth harvest (99 DAT), both cultivars had storage roots entering the jumbo grade, while root mass in the N1 grade still increased. Following the eighth harvest (120 DAT) for 'Beauregard' and the ninth harvest (127 DAT) for 'Owairaka Red', the jumbo grade increased at the expense of the N1 grade. The cultivar 'Beniazuma' had particularly long thin roots and performed differently from the former two cultivars. While the cull grade in 'Beniazuma' was of relatively consistent mass throughout the growing season, the canner grade continued to increase. The N1 grade also increased throughout the growing season, apart from the final harvest (134 DAT) when storage roots of sufficient diameter to be placed in the jumbo grade were found. For all three cultivars, the average storage root dry weight (>2.5 cm diameter) showed a steady increase from the second (78 DAT) to the eighth harvest (120 DAT). Averaged across all ten harvests, 'Owairaka Red' had a mean storage root (>2.5 cm diameter) dry weight of 31.7 g ($\log_e$, 3.5) which did not differ from that of 'Beauregard' at 31.8 g ($\log_e$, 3.5), while 'Beniazuma' had a significantly higher average storage root weight of 38.4 g ($\log_e$, 3.7) ($P< 0.001$).

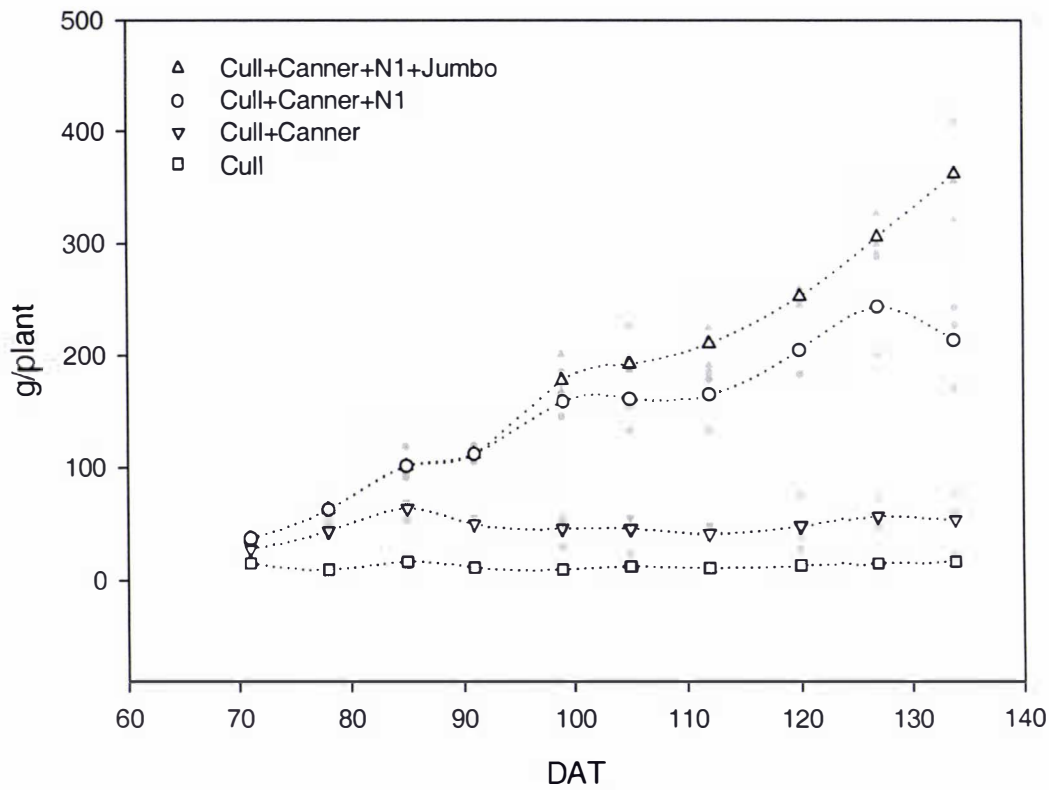


Figure 5.14: Observed (grey symbols) and mean (open symbols) harvest values with cubic spline interpolation curves illustrating the relationship between root grades (g/plant, dry matter basis) against days after transplanting (DAT) for commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red'. Graded by root diameter (cm) at harvest; Cull < 2.5, Canner 2.5 to 5, N1 5 to 9, Jumbo > 9.

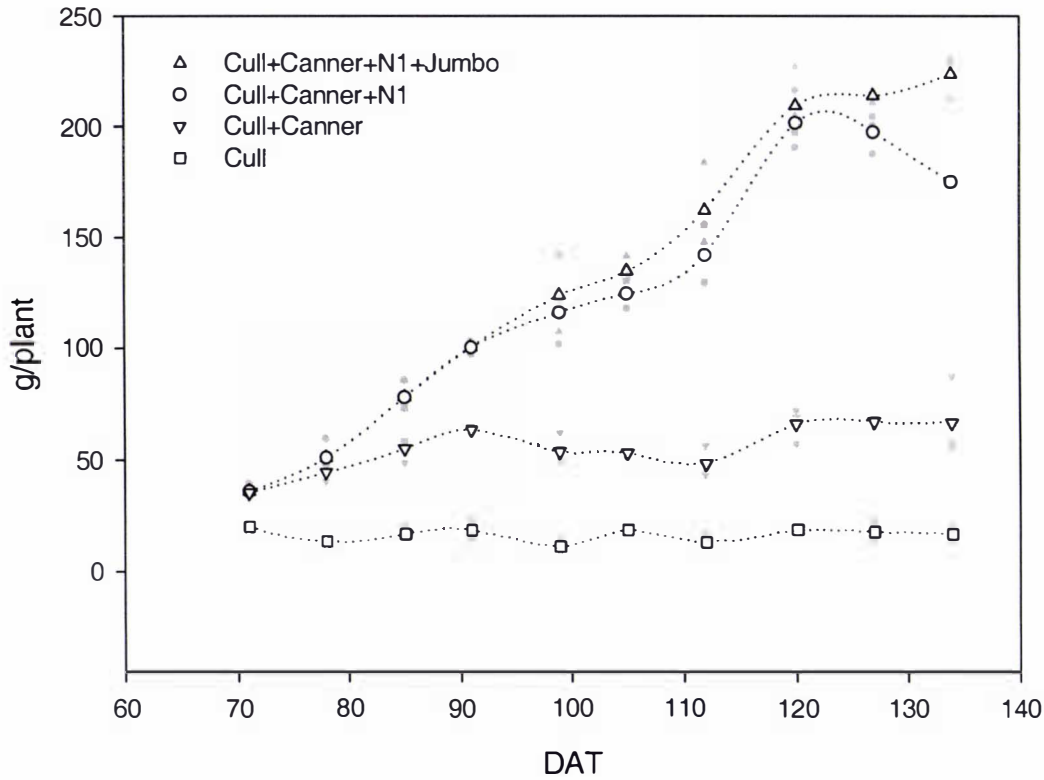


Figure 5.15: Observed (grey symbols) and mean harvest (open symbols) values with cubic spline interpolation curves illustrating the relationship between root grades (g/plant, dry matter basis) against days after transplanting (DAT) for commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beauregard'. Graded by root diameter (cm) at harvest; Cull < 2.5, Canner 2.5 to 5, N1 5 to 9, Jumbo > 9.

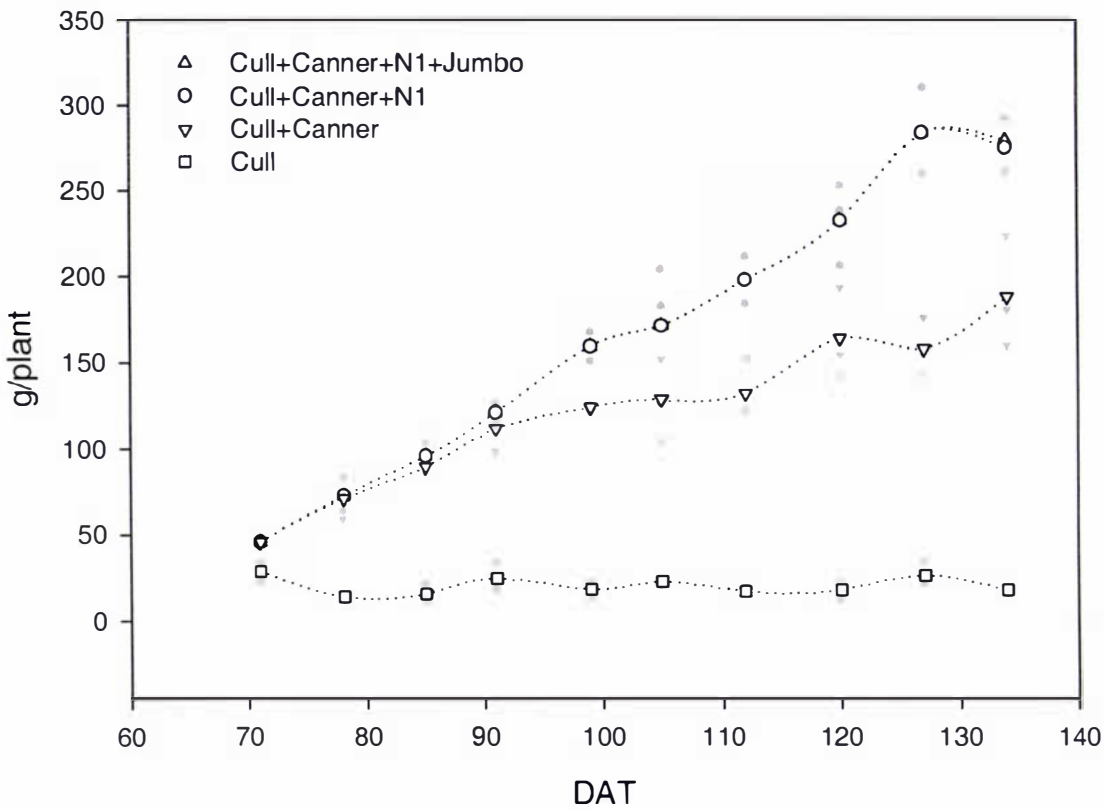


Figure 5.16: Observed (grey symbols) and mean (open symbols) harvest values with cubic spline interpolation curves illustrating the relationship between root grades (g/plant, dry matter basis) against days after transplanting (DAT) for commercial sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Beniazuma'. Graded by root diameter (cm) at harvest; Cull < 2.5, Canner 2.5 to 5, N1 5 to 9, Jumbo > 9.

5.3.4 Carbohydrate composition within storage roots

REML analysis of data for storage root %DW and sugar concentration did not show spatial effects at row, column or replicate level so analysis was completed using GENSTAT®: ANOVA. Storage root %DW data did not require transformation to stabilise the variance, and simple linear regression models were fitted for each cultivar. The total free sugar concentration (sucrose + glucose + fructose) data required a $\log_e$ transformation to stabilise the variance before analysis.

Averaged over all harvests, there was a large difference in mean storage root %DW between the cultivars, from 'Beniazuma' at 28.1%, 'Owairaka Red' at 22.0%, to 'Beauregard' at 19.5% ($P<0.001$). The pattern of differences between the cultivars was not consistent at each harvest ($P<0.001$). 'Beniazuma' roots had higher %DW than the other two cultivars at every harvest date ($P<0.001$). At the first two harvests (71 and 78 DAT), the %DW of 'Owairaka Red' and 'Beauregard' did not differ significantly ($P>0.05$). However, the %DW in 'Owairaka Red' increased at a faster rate, as shown by subsequent harvests. In all three cultivars the storage root %DW increased with extensions to the growing period (Fig. 5.17). The rate of %DW accumulation in 'Owairaka Red' did not differ from 'Beniazuma', where a 10-day extension to growing period increased storage root dry matter content by 1.4% (s.e. 0.09%), (R^2=90.7%, $P<0.001$). For 'Beauregard', %DW increased more slowly 0.8% (s.e. 0.07%), (R^2=78.5%, $P<0.001$).

The sucrose concentrations at the third harvest (85 DAT) for 'Owairaka Red' and 'Beniazuma' were anomalous due to a processing delay, so were removed from analysis. The pattern of increase in total free sugar concentration with later harvest dates was not consistent across cultivars ($P<0.001$) and simple linear regression models were fitted for each cultivar (Fig. 5.18). Total free sugar concentration did not increase with increasing DAT for 'Owairaka Red' roots ($P=0.086$). However, a significant linear increase in total free sugar concentration was found with increasing DAT for 'Beniazuma' and 'Beauregard' ($P<0.001$). There was no evidence that the total free sugar accumulation rate of these two cultivars differed ($P=0.755$) and a 10-day extension to the growing period increased total free sugar concentration by 9.5% (s.e. 0.098%), (R^2=80.1%, $P<0.001$).

Sucrose was the predominant sugar in each cultivar and the mean sucrose concentration differed significantly ($P<0.001$), with 'Beniazuma' at 1.143 g/100 g fresh weight, 'Beauregard' at 0.951 g/100 g and 'Owairaka Red' at 0.518 g/100 g. 'Beniazuma' had significantly ($P<0.001$) lower mean fructose (0.065 g/100 g) and glucose (0.061 g/100 g) concentrations than either 'Beauregard' (fructose, 0.481 g/100 g; glucose, 0.428 g/100 g) or 'Owairaka Red' (fructose, 0.375 g/100 g; glucose, 0.275 g/100 g). A ternary diagram for each cultivar (Fig. 5.19) describes the relative contribution (%) of sucrose, glucose and fructose to total free sugar concentration during the period of storage root development, without regard to trends in time. 'Owairaka Red' and 'Beauregard' demonstrated a similar range of sugar composition ratios. The mean ratio of glucose + fructose to total free sugar concentration is 0.54 (s.e. 0.021) for 'Owairaka Red', 0.50 (s.e. 0.016) for 'Beauregard' and 0.103 (s.e. 0.0045) for 'Beniazuma'. 'Beniazuma' maintained relatively high sucrose levels, but low fructose and glucose levels throughout the period of storage root development. The ternary diagrams (Fig. 5.19) suggest that throughout plant growth, regardless of sucrose contribution and whilst making allowances for any inherent variability between replicates, all three cultivars produce a relatively stable fructose:glucose ratio of approximately 0.44:0.56.

The fructose:glucose ratio for each cultivar was then further examined for trends across harvest dates (Fig. 5.20). 'Owairaka Red' and 'Beniazuma' showed no significant change in fructose:glucose ratio with increasing DAT ($P=0.66$, 0.16 respectively). However, 'Beauregard' showed a significant linear trend ($P<0.001$), with each 10-day increase in DAT reducing the fructose:glucose ratio by 0.15 (s.e. 0.028). The mean cultivar fructose:glucose ratios were compared with the standard 0.786 ratio (Lewthwaite et al. 1997) seen in harvested and stored sweetpotato roots (fructose at 44% and glucose at 56%, giving a ratio of 0.786). 'Owairaka Red' gave a mean ratio of 0.74 (s.e. 0.015), which was similar to the standard, and 'Beniazuma' produced a mean ratio of 0.96 (s.e. 0.038), which was higher than the standard. However the low absolute levels of fructose and glucose gave less consistent data (Fig. 5.20). 'Beauregard' produced a mean fructose:glucose ratio of 0.89 (s.e. 0.011), somewhat higher than the standard 0.786, but exhibited a linear reduction in fructose:glucose with increasing DAT.

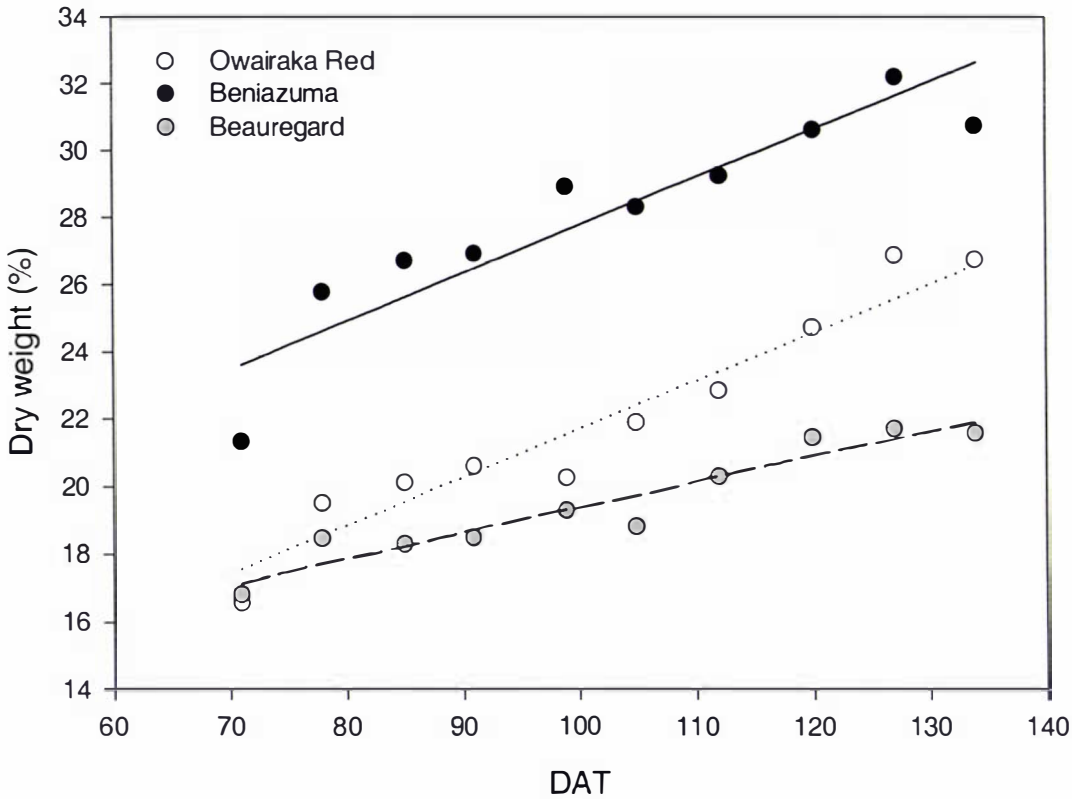


Figure 5.17: Mean observed storage root dry matter content (%) over sequential harvests and fitted regression lines for three sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars with days after transplanting (DAT).

Fitted lines:

'Owairaka Red'	%DW=	0.14	x	DAT	+	7
(s.e.)		(0.009)				(0.9)
'Beniazuma'	%DW=	0.14	x	DAT	+	13
(s.e.)		(0.009)				(0.9)
'Beauregard'	%DW=	0.076	x	DAT	+	12
(s.e.)		(0.0073)				(0.8)

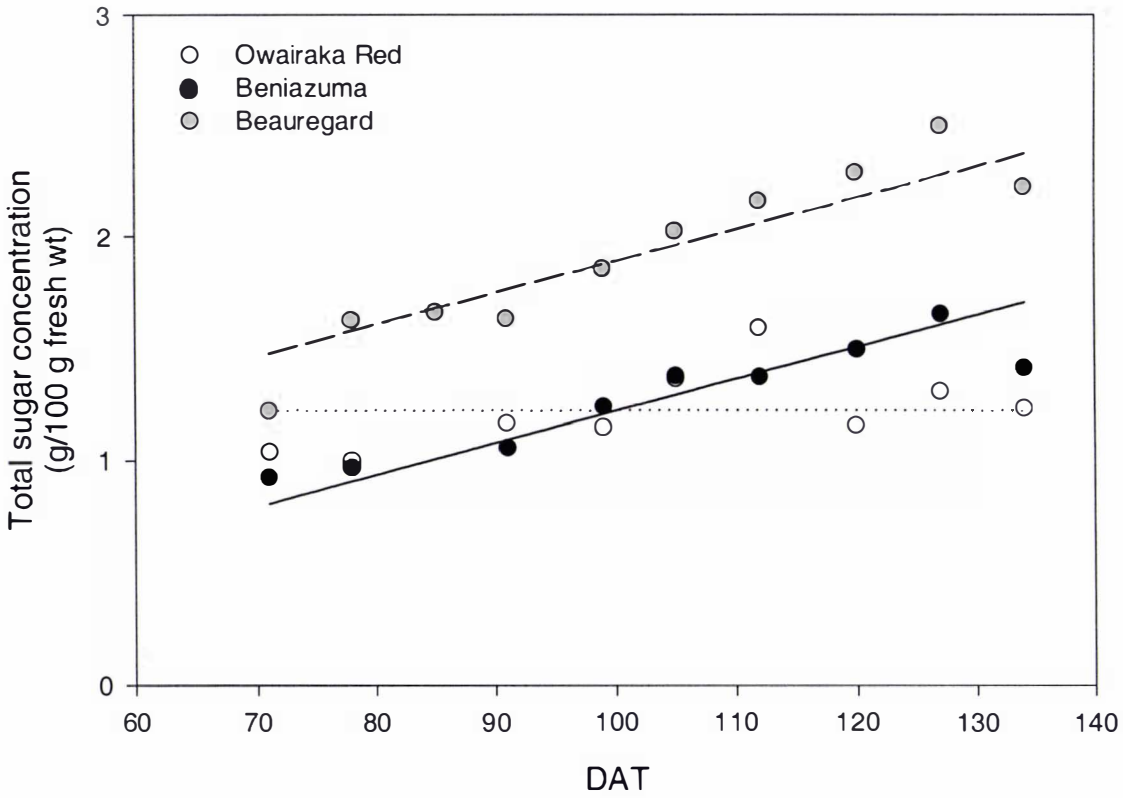


Figure 5.18: Mean observed total free sugar (sucrose + glucose + fructose) concentration (g/100 g fresh weight) within the storage roots of three sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars over sequential harvests. Fitted regression lines of total free sugar (TS) concentration with days after transplanting (DAT). Total free sugar concentration showed no significant trend over time for 'Owairaka Red'.

Fitted lines:

'Beniazuma'	$Log_eTS =$	0.0091	x DAT	- 0.723
(s.e.)		(0.00089)		(0.0967)
'Beauregard'	$Log_eTS =$	0.0091	x DAT	- 0.303
(s.e.)		(0.00089)		(0.0947)

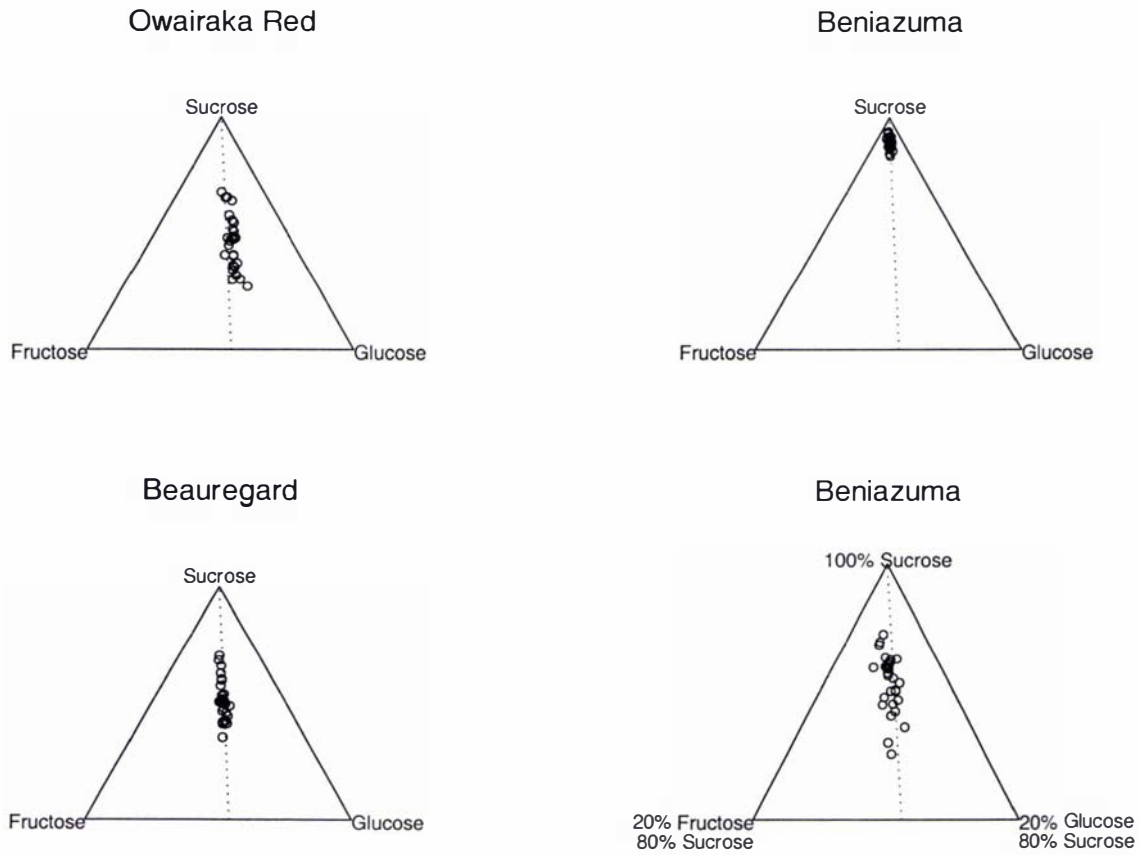


Figure 5.19: Ternary diagrams of relative sucrose, glucose and fructose concentrations (%) within the storage roots of three sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars during plant growth. The cultivar 'Beniazuma' had relatively low levels of glucose and fructose, so a diagram for high sucrose (%) is included. The fructose:glucose ratio 0.44:0.56 is indicated by a dotted line.

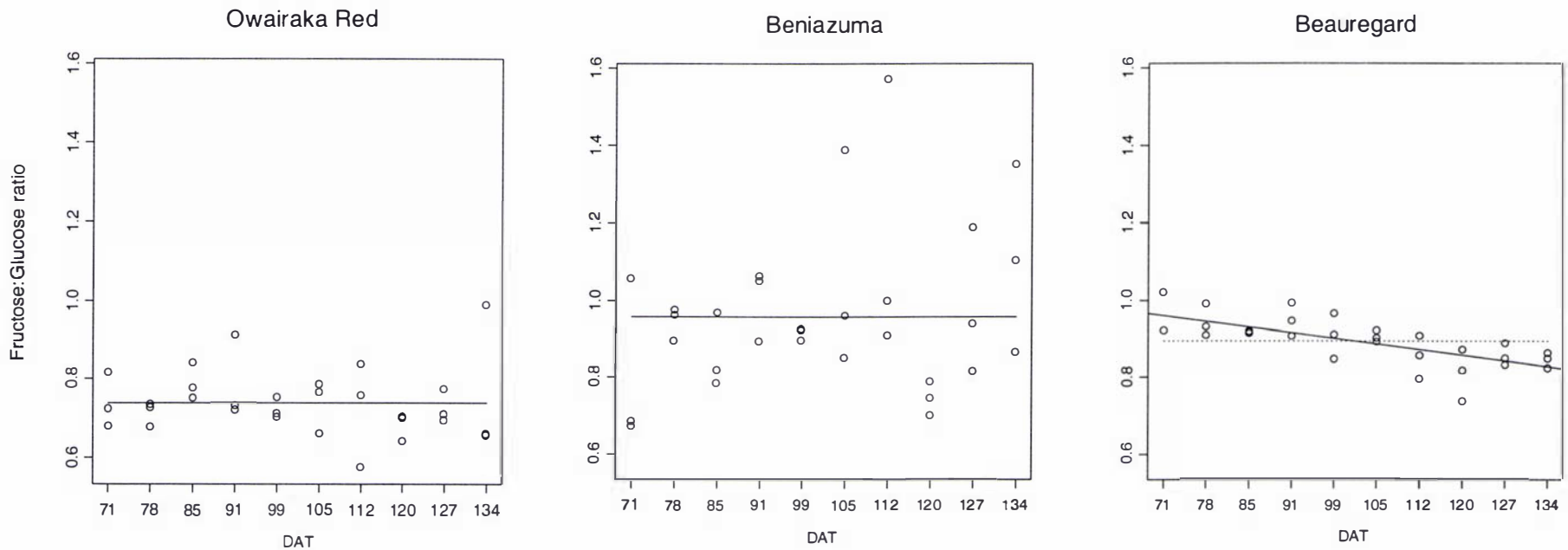


Figure 5.20: Changes in the fructose:glucose ratio with harvest date (days after transplanting or DAT) for the storage roots of three sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars, 'Owairaka Red, 'Beniazuma', 'Beauregard'.

5.4 Discussion

5.4.1 Dry matter partitioning amongst plant organs

'Owairaka Red' represents a commercial cultivar selected by mutation from early germplasm of limited range, under a cropping system dependent on vigorous vine and sprout growth for the production of propagation material with minimal inputs. Although 'Owairaka Red' produced the largest plants it had the lowest harvest index, indicating that despite a high biological yield it was not efficient at partitioning dry matter into storage roots (Bhagsari & Harmon 1982). While 'Owairaka Red' had a similar amount of leaf tissue to 'Beniazuma', it placed a greater dry matter investment into stem tissue. 'Owairaka Red' was the only cultivar to produce proportionally more stem than leaf tissue. Dry matter accumulation in 'Owairaka Red' stems continued throughout storage root development. This suggests that 'Owairaka Red' yields might be increased through cultural practices that discourage vine growth late in the season (Nair & Nair 1992). The vines of 'Owairaka Red' show relatively vigorous spreading growth, which may be considered advantageous for reducing early weed competition (Lewthwaite & Triggs 2000). The rate of dry matter accumulation in the roots of 'Owairaka Red' was higher than in the other cultivars, suggesting that in this cultivar root yield might be relatively more sensitive to harvest date. Even a small delay in harvest for 'Owairaka Red' may have a significant effect by increasing root yield.

'Beauregard' and 'Beniazuma' are examples of modern sweetpotato breeding and it is noteworthy that despite their different origins and growth habits, they produced similar high harvest indices. 'Beauregard' partitioned significantly less dry matter into leaf and stem tissue than either of the other cultivars. However, the fresh weight yield of 'Beauregard' is relatively high, with a proportionally low root dry matter content as required for the North American market. 'Beauregard' partitioned equivalent amounts of photosynthate into leaf and stem tissue while 'Beniazuma' allocated almost twice as much dry matter into leaves than stems. In these two cultivars, the total amount of dry matter in leaf tissue remained almost constant throughout a considerable period of storage root development. The maintenance of leaf area is dynamic, as leaves age and become shaded by surrounding leaves, they abscise. While older leaves are shed, new leaves continue to form toward the vine apex throughout crop growth (Enyi 1977).

The ternary diagrams allowed the relationship between all three plant components to be interpreted simultaneously. Because the plotted points followed a time course, the growth of the whole plant could be shown as it progressed. Throughout the period of storage root enlargement, carbohydrate partitioning maintained relatively constant cultivar specific ratios of leaf to stem tissue (dry weight). Should further research confirm these results, that cultivar specific ratios remain constant within seasons and sites, a minimum of two robust field assessments would be required to approximate the respective ratios of leaf, stem and root mass throughout the storage root bulking period. However, across sites and seasons relative ratios between plant organs would be expected to vary with environmental conditions and crop management decisions, so each set of growing conditions would require a minimum of two calibrating assessments. Producing such ratios would minimise the work required to characterise plant responses to differing growing environments and assist in developing models that optimise crop growth.

5.4.2 Growth analysis

There have been a number of studies to determine if sweetpotato growth is generally source or sink limited, with varying results from different experimental approaches, cultivars, stages of development and growing conditions (Hahn 1977; Bouwkamp & Hassam 1988; Bhagsari 1990; Bhagsari & Ashley 1990; Keutgen et al. 2002). Storage root yield is more dependent on current photosynthate production rather than the redistribution of carbohydrate stored in stems (Oswald et al. 1995b), somewhat simplifying the source/sink relationship. In general it is thought that dry matter accumulation is limited by sink strength, however this relationship changes throughout the season (Hahn & Hozyo 1983). It is important that the canopy develops well before the storage roots compete for photosynthates. In this early phase, there is no correlation between photosynthetic rate and root dry matter accumulation until total storage root dry weight reaches about 10 g per plant (Nakatani et al. 1988). In later growth, photosynthetic rate correlates with storage root weight, as storage roots represent the dominant sink for photosynthates and quantitatively influence source activity (Nakatani et al. 1988; Keutgen et al. 2002). In the three cultivars examined here the storage root sink appears to strongly compete with the canopy for photosynthate, as in each case the SLA increases with growth suggesting carbohydrates are not accumulating within the leaves. Further, partitioning of dry matter to the canopy appears to plateau, yet storage

roots continue to show a steady accumulation of photosynthate throughout the bulking period.

The RGR values presented here are similar to those reported for various sweetpotato cultivars grown in Australia (Huett & O'Neill 1976) and Trinidad (Roberts-Nkrumah et al. 1986), which also demonstrated a relatively steady decline throughout the storage root bulking period. Absolute growth increased throughout the season (Fig. 5.5). However, as an increasing proportion of the plant's carbohydrates are accumulated in storage roots rather than used in producing more photosynthetic tissue, the rate of dry matter production relative to total plant mass declined (Fig. 5.12). 'Owairaka Red' on average maintained a similar RGR to 'Beauregard', both cultivars having a slightly higher rate than 'Beniazuma'.

NAR is an estimate of the rate in which carbohydrate is accumulated per unit area of leaf tissue, assuming negligible photosynthate contribution from stem and petiole tissue or weight contribution from mineral elements (Hunt 1978). Across plant species, NAR generally declines with time, as photosynthetic efficiency decreases through aging and mutually shaded leaves, while respiratory loss increases with a larger plant mass. The three sweetpotato cultivars examined here declined in NAR as the season progressed, but at different rates. It is reported that individual leaf net photosynthetic rates decrease as sweetpotato leaves age, contributing to the general decline in NAR (Bhagsari 1988). Artificial shading has also been shown to reduce NAR and increase LAR and SLA in sweetpotato (Roberts-Nkrumah et al. 1986). The NARs reported here are similar to those found using various cultivars in Australia (Huett & O'Neill 1976) and Japan (Tsuno & Fujise 1965). While 'Owairaka Red' and 'Beniazuma' showed comparable changes in NAR throughout the season, 'Beauregard' proved more efficient in accumulating carbohydrate with a high initial NAR followed by a more rapid rate of decline. For individual sweetpotato leaves, there is a negative correlation between net photosynthetic rate and leaf size, which perhaps contributed to the high initial NAR in 'Beauregard' (Bhagsari & Brown 1986). There may also have been less mutual shading amongst 'Beauregard' leaves, as it partitions less dry matter to the canopy than the other two cultivars.

Typically, reported sweetpotato SLA varies from approximately 230 to 500 cm^2 g^{-1} (Bhagsari & Harmon 1982; Roberts-Nkrumah et al. 1986; Indira & Kabeerathumma 1990), in keeping with the values observed in this trial. In other plant species, SLA is considered extremely variable, depending on the environmental conditions (Penning de Vries et al. 1989) and season length (Kropff et al. 1994), however increasing the number of leaves sampled at each harvest may have improved SLA estimation in this study. All three cultivars showed a significant increase in SLA as the season progressed. Essentially the leaf area/weight ratio increased with growing period, largely through the translocation of dry matter from leaves to the storage roots (Somda & Kays 1990; Somda et al. 1991).

5.4.3 Storage root size grades

An examination of the respective contributions of different commercial root grades to total root mass over time, shows differences between the cultivars. Under non-limiting growing conditions, harvest decisions are made on the basis of average root diameter. Both 'Owairaka Red' and 'Beauregard' had produced oversized (jumbo grade) roots by the fifth harvest (99 DAT). Following the eighth harvest (120 DAT) for 'Beauregard' and the ninth harvest (127 DAT) for 'Owairaka Red', the premium grade (N1) decreased at the expense of increasing oversized (jumbo grade) roots. In both these cultivars there were distinct harvest windows, delimited by the ratio of premium to oversized roots. The cull and canner grades remained relatively constant, so would not influence harvest timing decisions. The cultivar 'Beniazuma' showed a different grade response, due to the development of typically long narrow storage roots. In contrast to the previous two cultivars, the canner grade increased throughout the season. Only by the tenth harvest (134 DAT) were oversized roots developing. In 'Beniazuma', commercial harvesting decisions would predominantly rest on the requirement to increase root size for maximum yield, rather than be limited by the production of oversized roots in an extended season.

5.4.4 Carbohydrate composition within storage roots

Sugars and %DW within the swollen storage roots of each cultivar were examined further. The three sweetpotato cultivars showed considerable differences in mean root dry matter content, representing the different market requirements of their countries of

origin. Traditionally the market in New Zealand has used cream-fleshed sweetpotatoes of moderately dry texture and high to medium sweetness, while the USA requires orange-fleshed sweetpotato with a moist mouth feel and very sweet taste (Martin & Jones 1986). In Asia, cultivars with white or yellow flesh, a dry to moderately dry texture and a sweet to moderately sweet taste are traditionally used (Lin et al. 1985). A dry texture similar to that of potato (*Solanum tuberosum*) is preferred when the crop is commonly used as a staple, while the moist texture seen in North American cultivars is more suitable for a dessert sweetpotato (Villareal 1982). Moist or dry texture is due to the amounts of starch, sugar and types of dextrins left after cooking, which are in turn influenced by the activity of amylolytic enzymes (Walter et al. 1975). Orange flesh, as seen in 'Beauregard', is a trait genetically linked with low storage root dry matter content (Jones 1977), although the International Potato Centre (CIP) has recently broken the linkage to produce true seed populations combining orange flesh and high dry matter for increased β-carotene production (Dr. D. Zhang, pers. comm.).

As the growing season progressed the storage root dry matter content increased in a linear manner, at a cultivar dependent rate. While some cultivars (La Bonte et al. 2000) do not show significant changes in root dry matter content through time, that was not true of the cultivars in this experiment. An early planting and/or later harvest date could be used to manipulate storage root dry matter content for all three cultivars, within the constraints of optimising the root size distribution and seasonal limitations. The cultivar 'Beniazuma' produced roots with a relatively higher dry matter content throughout the harvested period. The rates of root dry matter content accumulation were similar for 'Beniazuma' and 'Owairaka Red'. While 'Owairaka Red' and 'Beauregard' had similar root dry matter contents in the early harvests, 'Owairaka Red' demonstrated a more rapid increase in dry matter content. Previous work (Brabet et al. 1999; La Bonte et al. 2000) demonstrated a significant linear correlation between dry matter content and starch levels. Root dry matter content has a significant influence on root quality for both the fresh and processing markets. The levels of starch available for maltose production radically affect sweetness in cooked roots (Lewthwaite et al. 1997). During processing, dry matter content may directly influence product yield, production costs (cartage, water removal, oil absorption) and health benefits (nutrient and oil content).

In contrast to the other cultivars, the total free sugar concentration of 'Owairaka Red' roots did not show a significant trend over time. This suggests that in 'Owairaka Red' total free sugar concentration is not an important determinant of optimum harvest date. Early measurements of total free sugar concentration within 'Owairaka Red' provide a good estimate of final concentration. This result is consistent with previous work on white-fleshed cultivars (La Bonte et al. 2000), which showed no significant quantitative changes through time. 'Beniazuma' and 'Beauregard' both showed significant linear increases in total free sugar concentration with time, at a common rate. 'Beniazuma' had lower sugar levels than 'Beauregard', but a particularly high dry matter content makes maltose a significant contributor to sweetness in cooked products of this cultivar (Lewthwaite et al. 1997). Total free sugar concentration in 'Beauregard' increased rapidly, so this attribute is an important determinant of root quality with harvest date. In 'Beauregard', low dry matter content coupled with a low rate of increase contrasts with the relatively high, rapidly increasing, total free sugar concentration. The contribution of total free sugar concentration to sweetness appears relatively more important in 'Beauregard' than in the other cultivars studied here, as 'Beauregard's relatively low dry matter content limits the potential amount of maltose produced on cooking.

The sugar components (sucrose, glucose and fructose) in raw roots provide important information on quality, even though cooking may contribute a considerable increase in sweetening through maltose. Sugar components at the same concentration vary significantly in sweetness level. The component sugar profiles for 'Owairaka Red' and 'Beauregard' were comparable throughout their growth. Sucrose was the predominant sugar in all three cultivars. 'Beniazuma' was unusual in producing very little fructose and glucose, while maintaining high levels of sucrose. This may indicate that 'Beniazuma' is low in invertase activity (Huang et al. 1999). The levels of fructose and glucose were consistently low throughout the growth of 'Beniazuma' so they were not important indicators of optimum harvest date. However, the consistently low level of reducing sugars is of particular interest for processing sweetpotato into fried products, as high levels of fructose and glucose can cause excessive darkening.

The ratio of fructose to glucose appears to be relatively stable across cultivars and harvest dates, so that one can be used to estimate the other (Lewthwaite et al. 1997; La

Bonte et al. 2000). In 'Owairaka Red' the ratio remained approximately stable at 0.74 (s.e. 0.015) over the entire growth period, differing little from the mean ratio found in stored roots of 0.79 (Lewthwaite et al. 1997). The ratio for 'Beniazuma' was relatively stable at a mean of 0.96, but as the fructose and glucose levels were low, plot variability was high (s.e. 0.038) and more evaluation is required. "Beauregard' also had a higher mean amount of fructose (0.89) than anticipated, but showed a significant linear reduction with harvest date, contributing a temporal change to aspects of root sweetness.

The hypothesis, that plant growth attributes change in a steady and continuous manner throughout the period of storage root enlargement, was supported by this study. As the sweetpotato crop becomes more specialized, simultaneously optimizing a number of attributes for any given product will become increasingly important. Storage root yields and size distribution will always be important economic parameters for sweetpotato production, but future markets will give increasing weight to aspects of root quality. Parallel to the development of novel non-traditional sweetpotato products is the need to select new cultivars with specific groups of quality characteristics (Collins & Hall 1992; La Bonte et al. 2000). Knowledge of the direction and rate of assimilate investment amongst plant components and the biochemical changes within developing storage roots creates an opportunity to optimise root quality in any given cultivar.

5.4.5 Chapter summary

Three indicative cultivars, 'Owairaka Red', 'Beniazuma' and 'Beauregard' were examined in this study of carbohydrate partitioning over time. Estimation of leaf to stem dry matter ratios showed that approximately stable cultivar specific proportions were maintained over the period of storage root enlargement. 'Owairaka Red' invested more dry matter into stems than the other two cultivars and had the lowest harvest index. 'Beauregard' allocated carbohydrate in equivalent amounts to leaf and stem tissue, but partitioned significantly less dry matter into the total canopy than the other two cultivars. 'Beniazuma' allocated more dry matter into leaves than stem tissue. 'Beauregard' and 'Beniazuma' produced similar high harvest indices. Storage root development based on commercial size grades showed similar patterns for 'Beauregard' and 'Owairaka Red', with harvesting delays increasing the production of oversized

roots. Oversized roots were not a primary consideration in harvesting point decisions for 'Beniazuma', due to its production of typically long narrow storage roots.

Partitioning of assimilate between compounds within the storage root has been modified through plant breeding and selection, to produce country specific organoleptic qualities. Storage root dry matter content increased in a linear manner, at a cultivar specific rate, as the season progressed. 'Beniazuma' produced roots with a particularly high dry matter content. The rates of dry matter accumulation for 'Beniazuma' and 'Owairaka Red' were similar. 'Beniazuma' and 'Beauregard' showed linear increases in total free sugar concentration with time, while no significant time trend was observed for 'Owairaka Red'. 'Beniazuma' produced very little fructose and glucose compared to 'Owairaka Red' and 'Beauregard'. Fructose to glucose ratios pooled across harvests appear to show relatively similar proportions in each cultivar, as has been observed previously in stored roots. However trend analysis over harvest dates showed cultivar specific patterns of fructose to glucose ratios. The patterns of assimilate partitioning in sweetpotato have important consequences for crop management and root quality.

Chapter 6

Season's end: storage root necrosis

6.1 Introduction

The sweetpotato is a perennial plant of tropical or subtropical origin. The storage roots of sweetpotatoes do not mature in the sense of reaching some final size or stage of development nor do they naturally pass through a resting or dormant phase (Jones et al. 1986). If sweetpotato are grown without environmental restrictions, the storage roots continue to increase in size. As demonstrated in the previous chapter, storage root attributes change in a steady manner throughout plant growth. In temperate climates where cool temperatures may cause tissue chilling injury, the sweetpotato is cropped seasonally as an annual. Sweetpotato roots may suffer chilling injury at temperatures below 12 ºC (Lewis & Morris 1956). However storage roots produced under temperate conditions may exhibit an internal disorder that limits the growing season, before temperatures likely to induce chilling injury are experienced. The nature of this internal disorder is the subject of this investigation, as it brings the sweetpotato production season to an abrupt end.

The New Zealand sweetpotato industry is dominated by the cv. 'Owairaka Red', which was released as a commercial cultivar in 1954 (Lewthwaite 1998). The release of 'Owairaka Red' allowed the crop's rapid development into the present sweetpotato industry (Coleman 1969). The storage root disorder brown centre (BC) was first recorded in New Zealand in 1955 when it was described as 'brown heart' (Gillard 1955). The timing of 'Owairaka Red's release, the industry's rapid growth phase and the first reported incidence of the disorder is striking. It was noted that the disorder was not a disease, but occurred particularly in sweetpotato crops from heavy, wet soils where harvesting had been delayed. The flesh of affected roots turned brown, beginning from the centre but sometimes extending throughout the root, rendering it inedible. It was recommended that harvesting should be completed by the end of April in order to avoid the problem. The disorder occurred in the field prior to harvest and had no external symptoms.

Research into BC was first conducted in 1964 when root samples from a crop with the disorder were examined over a five-month storage period without showing any increase in symptoms (Nielsen & Harrow 1966). Samples of root tissue with BC were grafted into unaffected roots to test the pathology of the disorder, but failed to transmit the symptoms. These researchers described BC as an internal necrosis, with tan to light brown tissue occurring in the pith region, but not in the cortical phloem tissue. They also observed one root where the necrotic tissue had disintegrated, leaving a cavity. The cause of the disorder was not determined, but it was concluded that internal cork virus was not involved.

BC continued to be a problem and in 1979 a three-year field study was initiated at Pukekohe (Wood & Schappi 1984). The disorder was then described as a light to dark brown internal staining, evident if the storage roots were cut open. The disorder was present at harvest and sound roots did not develop the disorder in storage. The degree of BC in plants produced from sound nursery material was the same as that produced from BC-affected parent roots. Even if only a small part of the root showed BC symptoms, the whole root could be 'woody' when cooked and developed objectionable flavours. There was no evidence of a relationship between the occurrence of BC and plant yield components. No specific cause of the 'brown centre' disorder could be identified, but it was recommended that harvesting be completed by mid-April.

The disorder still remains a problem to the industry, and was especially severe in the Dargaville region during the 1996/97 season. It is estimated that about 2% of the season's entire crop was affected, but the disorder was disproportionately found in crops harvested from mid April (A. de Bruin, President - Northern Wairoa Vegetable Growers' Association pers. comm.). Much of the damaged crop was rejected at washing, but many affected roots are thought to have reached the market. When people purchase damaged roots, perceptions of produce quality are lowered considerably and market expansion is hindered.

BC-like symptoms are seen internationally in sweetpotato and are induced by a wide range of agents, including pathogens, stress and nutrition. Early research demonstrated that a pathogen was not associated with the occurrence of BC in New Zealand (Nielsen & Harrow 1966; Wood & Schappi 1984), although international experience shows that

infection by viral and fungal pathogens may produce similar symptoms (Clark & Moyer 1988). With regard to stress, previous research has not associated any particular soil temperatures or soil moisture levels with initiation of the BC disorder (Wood & Schappi 1984), but internationally it is well documented that low temperatures produce tissue browning and other symptoms of chilling injury in sweetpotato roots (Lewis & Morris 1956). There have been no observed nutrient deficiencies or fertiliser programmes associated with BC in New Zealand (Wood & Schappi 1984). However, in other countries BC-like symptoms have been produced in sweetpotato grown in nutrient deficient conditions (O'Sullivan et al. 1997).

In summary, research to date indicates that BC occurs at the same time of year across different locations, is present at harvest, is not pathological and, unless severe, does not produce external lesions. Affected roots contain various degrees of brown necrotic tissue, which may remain hard on cooking and usually have an objectionable flavour. Commercial growers have also observed that BC is more common in ground in which sweetpotato has not previously been grown. In New Zealand there may be a number of different disorders collectively labelled BC. This investigation examines the hypothesis that an assessment of the growing environments and germplasm that produce BC, set against the background of published research, will provide a line of inquiry for further detailed study.

6.2 Materials and methods

6.2.1 Dargaville site evaluation

As there was no direct evidence as to what initiated the BC disorder, the initial requirement was to isolate the potential causes. The first phase of the investigation was to look for diagnostic factors by collecting samples from the 1996/1997 season at Dargaville (Lat. 35° 55' S), in which BC was particularly common (A. de Bruin pers. comm.). Plant tissue and soil analyses were used to determine the possible presence or nature of any nutrient disorders.

Stored BC-affected and sound roots were obtained from adjacent sweetpotato plants. These roots were produced at a commercial Dargaville site and harvested in May 1997.

Following a short storage period they were cut open (June 1997) and found to include roots exhibiting BC. Five of the sound roots and five BC affected roots were analysed for tissue macro- and micro-nutrient levels using established procedures (R. J. Hill Laboratories Ltd, Hamilton, New Zealand). For roots showing BC symptoms, tissue was divided into sound and necrotic portions then analysed as separate samples. All root tissue was oven-dried at 60°C prior to analysis. For BC affected roots, the dry weights of both healthy and necrotic portions were recorded, to allow calculation of whole root mineral levels.

Soil samples were collected at random to a depth of 10 cm from two Dargaville sites where BC affected roots were consistently produced (Site 1 sampled June 1997; Site 2 sampled December 1997). Site 1 produced the root samples used for tissue analysis (above). Site 2 also produced roots with BC, as established by a commercial pack house during processing of stored roots in December 1997. Almost all of the domestic sweetpotato market is supplied with produce that has been stored, to ensure a year long supply. As BC shows no external symptoms and for economic reasons destructive sampling of produce is limited, the industry assessment of BC occurs following storage and at the point produce enters the market. These two sites were selected for soil testing as the BC roots could be clearly traced back to their origin and the fields had not been modified since harvest.

Average rainfall and air temperatures at Dargaville for the 1996/1997 season were compared with historical records, from the National Institute of Water and Atmospheric Research Ltd (NIWA) meteorological site at Dargaville. As soil temperatures have not been recorded at Dargaville since 1988, it was important to understand the relationship between air and local soil temperatures. Hourly temperature recordings were made in the 1997/98 and 1998/99 seasons by placing three programmed data loggers (SAPAC temprecord^{TM}) within a Dargaville sweetpotato crop. Air temperature was recorded within a screen at 1.3 m above ground level, soil surface temperature was recorded beneath the crop's canopy and soil temperature was recorded 10 cm beneath the mould crest.

6.2.2 Pukekohe site evaluation

A cultivar trial was established at the Pukekohe Research Centre (Lat. 37° 13' S) in the 1997/98 season to evaluate the incidence of BC in sweetpotato germplasm from diverse international sources. Healthy storage roots retained from the previous season were sprouted in trays of peat/pumice bedding soil mix, to provide planting material. Transplants consisted of 30 cm long sprouts, with three nodes hand inserted into the soil. The trial sites were fallow over the winter period, without any cover crop. A base fertilizer of 30% potassic superphosphate (N:P:K 0:7:14) was broadcast at 1 t/ha and incorporated prior to planting. General cultural practice followed commercial recommendations (Coleman 1972). The transplants were well watered during establishment and rainfall was supplemented by overhead irrigation during the growing season. The first trial was planted on 16 December 1997, with 16 clones arranged in a four column modified alpha row and column design containing three replicates (Williams & John 1989). The 16 clones included commercial cultivars 'Owairaka Red', 'Toka Toka Gold', 'Beauregard', 'Landtec', 'Northland Rose', and eleven advanced breeding selections. The eleven clonal selections had previously been chosen for storage root yield and shape from segregating imported botanical seed, sourced from the Louisiana State University breeding program and the Asian Vegetable Research and Development Centre breeding program in Taiwan. Rows were 0.75 m wide and within row plant spacing was 0.30 m. Each plot consisted of 20 plants, arranged in two rows. The trial was lifted by a two row potato harvester on 16 April 1998. On the day of harvest, all roots of commercial size (greater than 2.5 cm diameter) were cut lengthwise, to estimate the incidence of BC (presence/absence). The trial design ensured a random distribution of the clones across the site, however the BC incidence was so low that only the total percent incidence for each clone was reported.

The trial was repeated the following season using a similar protocol and including the same cultivars. The trial was planted on 7 December 1998, with a delayed harvest on 24 May 1999 to enhance the development of BC. A block of 1000 'Owairaka Red' plants was transplanted into the field on 7 December 1998, using the same protocol, and hand harvested on 16 April 1999. Hand harvested storage roots were examined *in situ* for BC symptoms by cutting along their main axis. An observation plot of 20 'Waina' plants was also established, 'Owairaka Red' is derived from 'Waina' by mutation.

Hourly temperature recordings were made in the 1998/99 season, by placing a programmed data logger (SAPAC temprecord^{TM}) 10 cm deep within a mould in the BC sweetpotato trial. The NIWA meteorological site located at the Pukekohe Research Centre also supplied recorded soil temperatures from the 1998 and 1999 seasons. Soil temperatures were recorded daily (9 am) by NIWA under established pasture at depths of 0.1, 0.2 and 1.0 m.

6.3 Results

6.3.1 Dargaville site evaluation

Root tissue analysis (Table 6.1) indicated that only potassium concentrations showed significant differences between BC and sound roots. Potassium is the most common mineral in sweetpotato roots. The mean potassium level in roots with BC was 2.04% (dry weight basis), while in healthy roots it was 1.80% ($P = 0.040$). Nitrogen levels in necrotic tissue (mean 0.88%) tended to be slightly higher ($P > 0.05$) than in healthy tissue (mean 0.8%) of the same root (Table 6.2).

Table 6.1: Mineral analysis of sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage roots grown at Dargaville in the 1996/97 season, comparing sound and necrotic roots (dry weight basis). Necrotic tissue was symptomatic of the BC disorder.

		Roots without BC symptoms						Roots with BC symptoms					
		Root number						Root number					
Element	Unit	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Nitrogen	%	0.8	0.8	1.0	0.8	1.0	0.9	0.9	0.8	0.8	0.8	0.9	0.8
Phosphorus	%	0.17	0.19	0.20	0.20	0.21	0.19	0.18	0.18	0.18	0.19	0.21	0.19
Potassium	%	1.8	1.8	1.7	1.9	1.8	1.8	1.8	2.1	2.0	2.2	2.1	2.0
Sulphur	%	0.06	0.08	0.09	0.08	0.08	0.08	0.07	0.08	0.09	0.09	0.08	0.08
Calcium	%	0.07	0.07	0.07	0.11	0.07	0.08	0.08	0.06	0.05	0.07	0.10	0.07
Magnesium	%	0.10	0.11	0.12	0.12	0.10	0.11	0.09	0.11	0.10	0.09	0.11	0.10
Sodium	%	0.05	0.05	0.07	0.07	0.09	0.07	0.07	0.05	0.06	0.05	0.07	0.06
Iron	µg/g	30	31	35	32	38	33	40	31	31	27	35	33
Manganese	µg/g	22	39	39	38	27	33	21	33	27	26	26	27
Zinc	µg/g	12	13	16	16	15	9	13	28	14	15	14	17
Copper	µg/g	8	8	9	9	9	9	9	7	8	8	9	8
Boron	µg/g	6	9	9	9	7	8	5	8	7	8	7	7

Table 6.2: Mineral analysis of sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage roots grown at Dargaville in the 1996/97 season, comparing sound and necrotic tissue within roots (dry weight basis). Necrotic tissue was due to brown centre (BC) disorder.

		Tissue without BC symptoms						Tissue with BC symptoms					
		Root number						Root number					
Element	Unit	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Nitrogen	%	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.8	1.0	0.8	0.9	0.9
Phosphorus	%	0.20	0.19	0.18	0.18	0.19	0.15	0.17	0.17	0.17	0.20	0.22	0.19
Potassium	%	1.7	2.0	2.0	2.2	1.7	1.9	1.8	2.2	2.3	2.3	2.3	2.2
Sulphur	%	0.07	0.08	0.09	0.08	0.07	0.08	0.07	0.08	0.08	0.10	0.09	0.08
Calcium	%	0.11	0.06	0.05	0.06	0.06	0.07	0.05	0.06	0.04	0.08	0.12	0.07
Magnesium	%	0.11	0.11	0.10	0.07	0.09	0.10	0.08	0.10	0.08	0.12	0.12	0.10
Sodium	%	0.04	0.05	0.06	0.05	0.09	0.06	0.09	0.06	0.05	0.06	0.05	0.06
Iron	µg/g	53	34	31	24	27	34	28	26	28	30	40	30
Manganese	µg/g	29	35	29	14	18	25	15	28	9	39	32	25
Zinc	µg/g	15	36	14	11	12	18	11	12	10	19	15	13
Copper	µg/g	8	7	8	7	8	8	9	8	7	10	9	9
Boron	µg/g	6	10	7	8	7	8	4	5	6	8	7	6

Table 6.3: Soil analysis of two Dargaville sites producing the brown centre disorder in storage roots of sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', during the 1996/97 season (analysis methods in Appendix 1). Site one was sampled in June 1997, site two was sampled in December 1997.

Analysis	Units	Site 1	Site 2
pH		5.8	6.1
Olsen P	μg/ml	37	59
Potassium	me/100 g	1.08	1.80
Calcium	me/100 g	18.1	21.6
Magnesium	me/100 g	5.01	8.88
Sodium	me/100 g	0.25	0.35
Exchangable cations	me/100 g	32.8	38.7
Base saturation	%	75	84
Volume weight	g/ml	0.84	0.90
K/Mg ratio		0.22	0.20
Available nitrogen	kg/ha	374	190
Boron	μg/g	1.6	1.8

Soil analysis (Table 6.3) indicated no mineral deficiencies in fields producing BC and generally nutrients were in good to ample supply. There was no shortage of boron, a mineral that has been associated with the production of brown necrotic tissue in some sweetpotato crops (Wilson & Averre 1989; O'Sullivan et al. 1997). However, available nitrogen levels appeared particularly high at these BC-producing sites. Under typical conditions of repeated annual crop production, an available nitrogen range of 50 to 100 kg/ha would be expected (J. Turner, R. J. Hill Laboratories Ltd. pers. comm.).

The monthly rainfall over the harvest period (March, April, May) at Dargaville in the 1996/97 season (Fig. 6.1) was similar in quantity and distribution to the long term average. The mean monthly air temperature (Fig. 6.2) and the mean daily minimum air temperature (Fig. 6.3) were also comparable to the long term average. The extreme minimum temperatures recorded over the 1996/97 harvest period (5.6°C, March; 3.9°C,

April and 4.1°C, May 1997) were not dissimilar to equivalent long term averages (6.1°C, March; 3.7°C, April and 1.6°C, May - recorded since 1943).

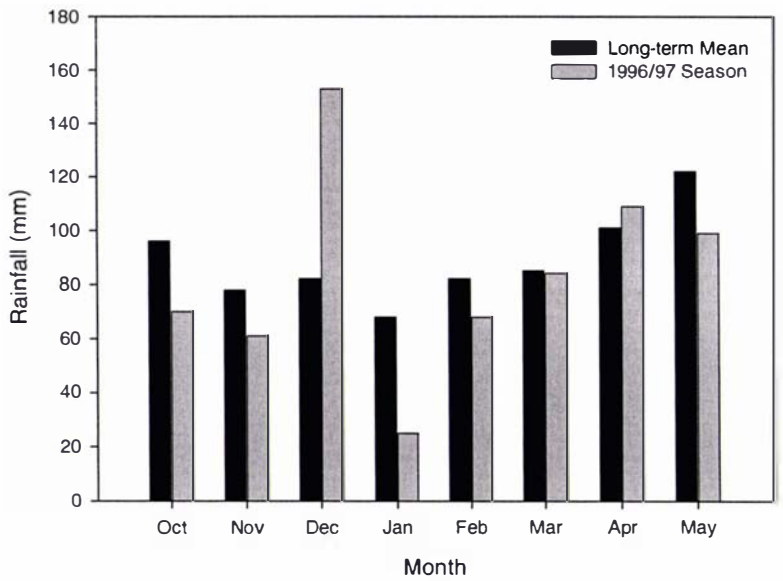


Figure 6.1: Monthly rainfall (mm) at Dargaville over the 1996/97 growing season compared with the long term mean (recorded since 1943). Data supplied by NIWA.

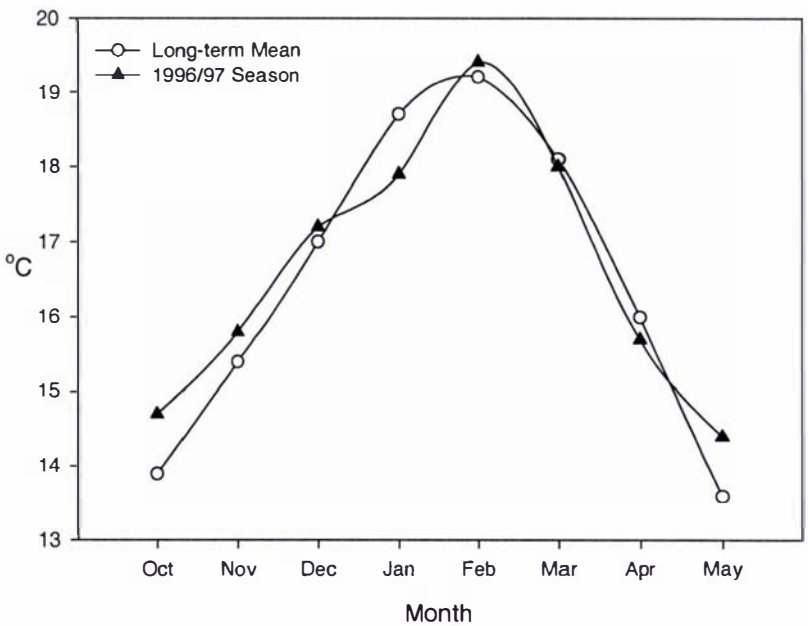


Figure 6.2: Mean monthly air temperature (°C) at Dargaville over the 1996/97 growing season compared with the long term mean (recorded since 1943). Data supplied by NIWA. Data points connected by a cubic spline interpolation curve, using graphics software SigmaPlot® 2000.

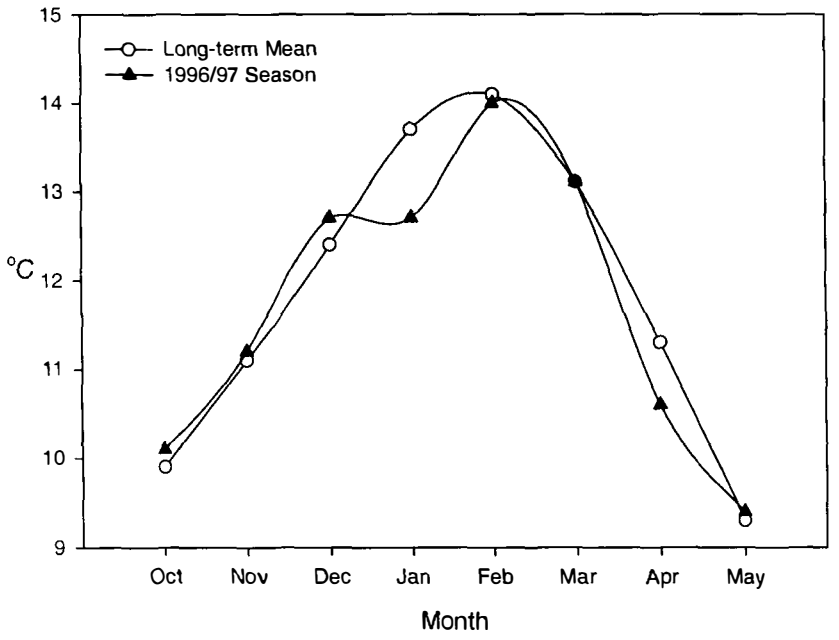


Figure 6.3: Mean daily minimum air temperature (°C) each month at Dargaville over the 1996/97 growing season compared with the long term mean (recorded since 1943). Data supplied by NIWA. Data points connected by a cubic spline interpolation curve, using graphics software SigmaPlot® 2000.

The average monthly soil temperature approximated air temperatures early in the 1998/99 season when the soil surface was bare (Fig. 6.4), but as the leaf canopy developed soil temperatures more closely followed those at the soil surface. Beneath the soil, temperatures were much more stable than in the air or at the soil surface and showed little diurnal fluctuation (Fig. 6.5).

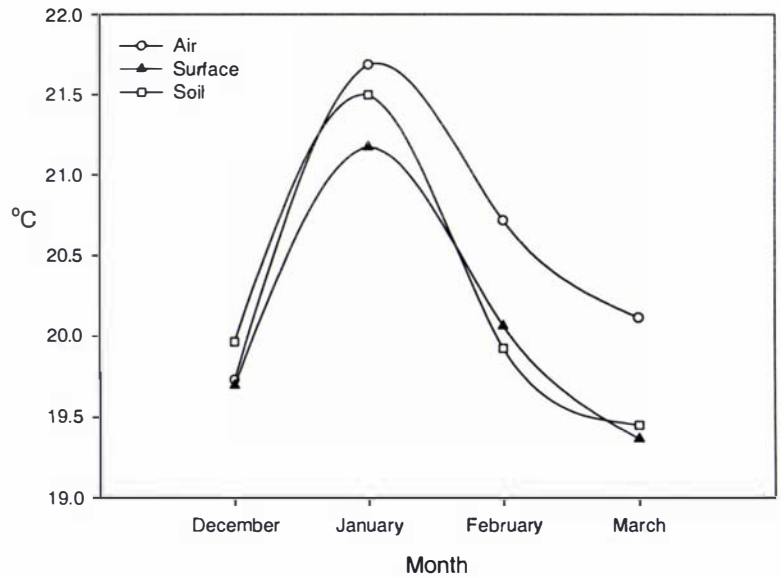


Figure 6.4: Mean monthly temperatures (°C) at Dargaville over the 1998/99 growing season. Air, soil surface (0 cm depth) and soil (10 cm depth) temperatures were recorded at one-hour intervals within a sweetpotato crop. Data points connected by a cubic spline interpolation curve, using graphics software SigmaPlot® 2000.

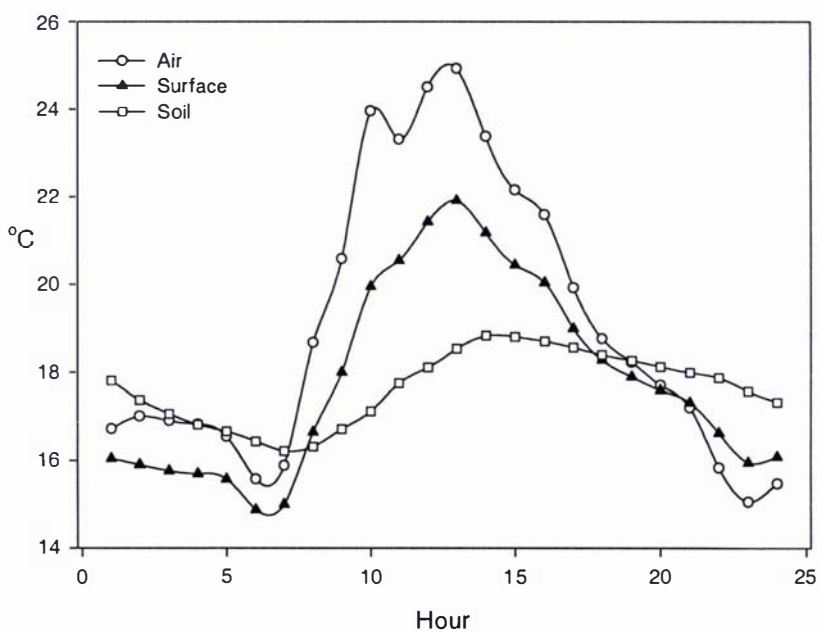


Figure 6.5: Diurnal air, soil surface (0 cm depth) and soil (10 cm depth) temperature changes within a Dargaville sweetpotato crop, recordings at one hour intervals on 10 April 1999, just prior to harvest. Data points connected by a cubic spline interpolation curve, using graphics software SigmaPlot® 2000.

6.3.2 Pukekohe site evaluation

In the 1997/98 season, three of the 16 clones evaluated developed BC symptoms at the Pukekohe Research Center. The BC incidence level in these roots was; cv. 'Owairaka Red' (5.7%), cv. 'Northland Rose' (3.8%) and clone 'LSU-1' (10.0%). Roots of the commercial cultivars 'Toka Toka Gold', 'Beauregard', and 'Landtec' did not contain any BC symptoms, nor did LSU-2 to 6 and AVRDC-1 to 5. In the 1998/99 season only cv. 'Owairaka Red' produced any BC symptoms (2.4%); none were found in any of the other clones apart from in two roots from the 'Waina' observation block.

Plate 6.1: Longitudinal section of a sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage root showing the distribution of brown center disorder symptoms.

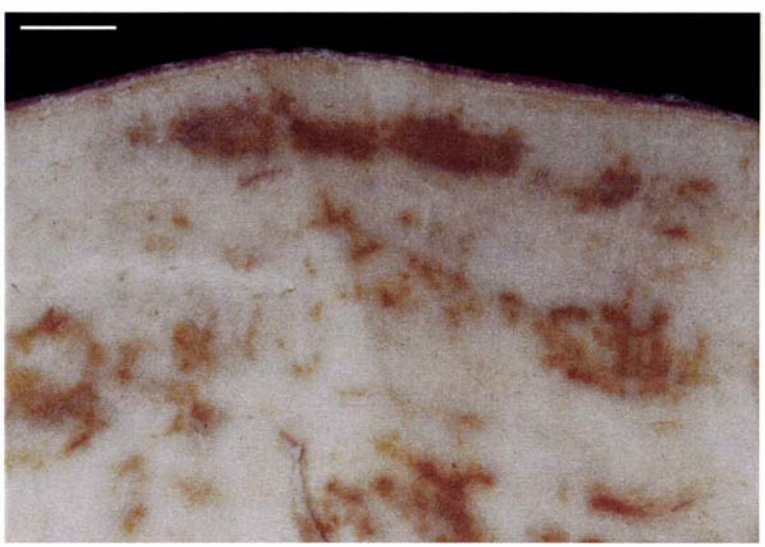


Plate 6.2: Longitudinal section of a sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage root, with a close view of brown center disorder symptoms (scale bar: 1.0 cm).

Hand harvested cv. 'Owairaka Red' plants grown enmasse during the 1998/99 season, demonstrated that individual plants with BC appeared sporadically across the field, rather than in discernable groups or spatial gradients. For any individual plant affected by BC, some storage roots showed the symptoms while others on the same plant remained completely sound. However, there was no clear pattern to the distribution of necrotic and sound roots within plants.

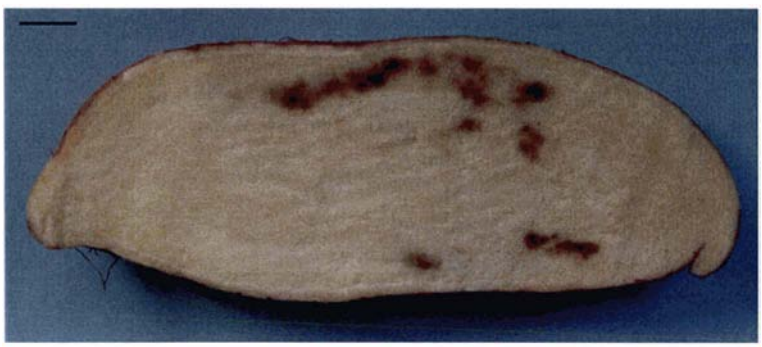


Plate 6.3: Longitudinal section of a sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Northland Rose' storage root showing the distribution of brown center disorder symptoms (scale bar: 1.0 cm).

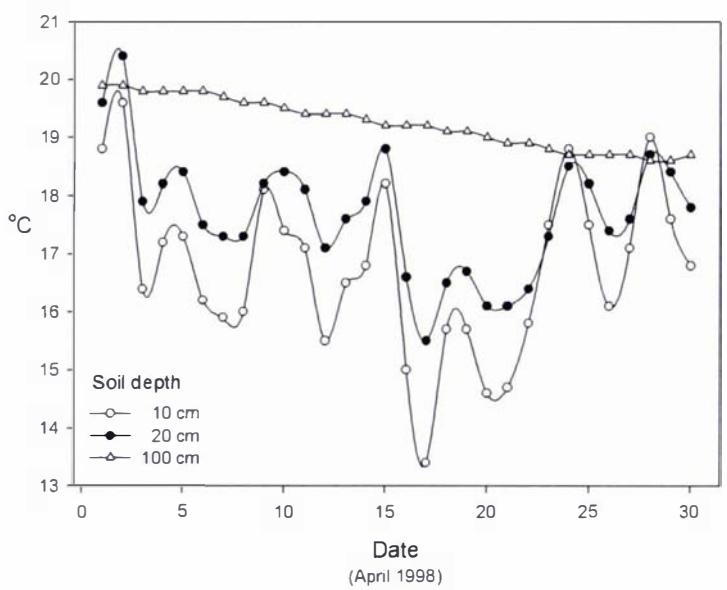


Figure 6.6: Daily soil temperatures (9 am) from various depths; 10, 20 and 100 cm below established pasture at the Pukekohe Research Centre, April 1998. Sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' was harvested at this location on 16 April 1998 with a 5.7% incidence of BC. Temperature data supplied by NIWA. Data points connected by a cubic spline interpolation curve, using graphics software SigmaPlot® 2000.

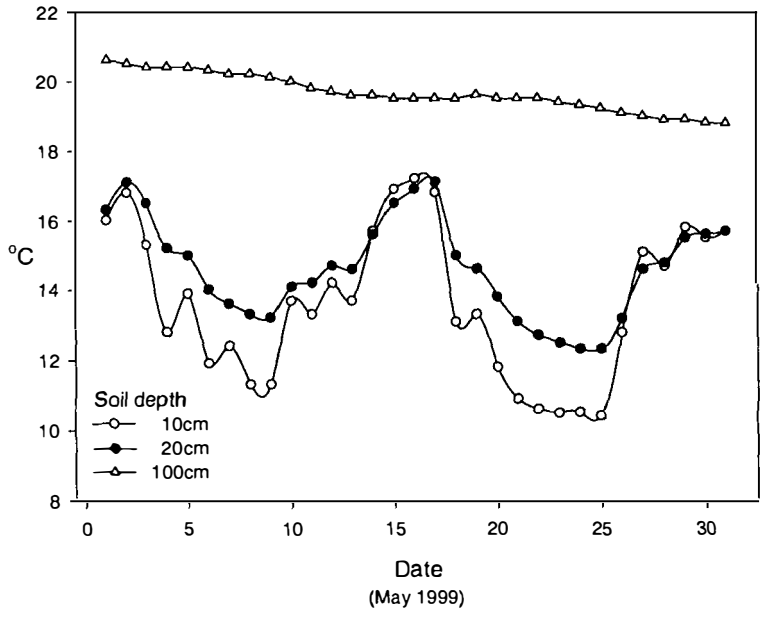


Figure 6.7: Daily soil temperatures (9 am) from various depths; 10, 20 and 100 cm below established pasture at the Pukekohe Research Centre, May 1999. Sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' was harvested at this location on 24 May 1999 with a 2.4% incidence of BC. Temperature data supplied by NIWA. Data points connected by a cubic spline interpolation curve, using graphics software SigmaPlot® 2000.

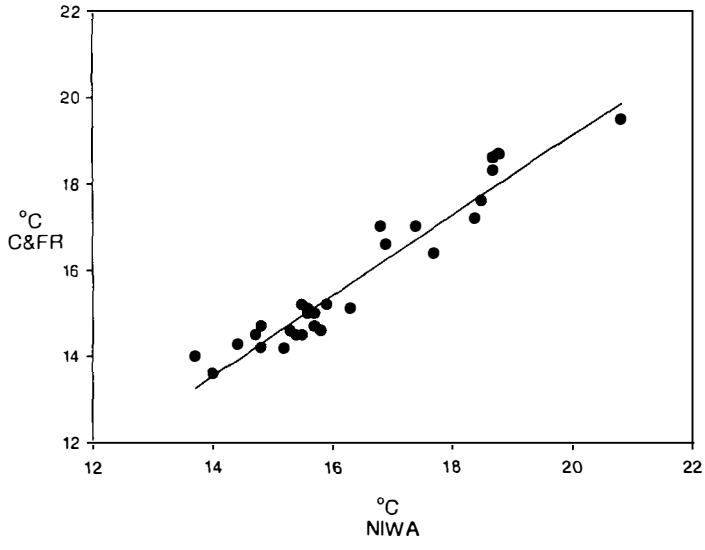


Figure 6.8: Comparison of NIWA (pasture) and C&FR (sweetpotato crop) sources of daily soil temperature recordings, from the Pukekohe Research Centre, April 1999. Fitted line, C&FR = 0.93(NIWA) + 0.54 (R^2 = 92.8%, se (slope)= 0.05).

6.4 Discussion

6.4.1 Dargaville site evaluation

6.4.1.1 Plant nutrition

Having established that the BC disorder in New Zealand is not associated with a pathogen (Nielsen & Harrow 1966; Wood & Schappi 1984), physiological factors were examined. The 1996/97 season in the Dargaville region produced particularly high levels of BC, especially in harvests after mid April. The area planted in sweetpotato has been steadily increasing over recent years, so much of the 1996/97 season production was from newly converted pasture land (Lewthwaite 1998).

Root samples collected from Dargaville were analysed (Table 6.1), but indicated that BC and sound roots differed significantly only in potassium levels. Potassium was the most common mineral present in the sweetpotato roots, but this element has not previously been linked with the incidence of BC-like disorders. While not statistically significant, nitrogen levels in necrotic tissue tended to be slightly higher than in dried healthy tissue of the same root (Table 6.2).

Soil samples (Table 6.3) from fields that produced high levels of BC affected roots indicated that there were no mineral deficiencies and generally nutrients were readily available. However soil available nitrogen levels appeared particularly high, especially considering that the sites were sampled well after crop removal. There is no record of high nitrogen levels causing BC-like symptoms, but there is support for an interactive effect with boron. Increasingly high applications of nitrogen may reduce plant boron uptake, and the association of boron deficiency with the production of brown necrotic tissue in a range of crops is well known (Gupta 1979). Boron levels in the sampled fields appeared adequate.

Although the information obtained from tissue and soil analysis was inconclusive, soil available nitrogen levels were of interest. The higher levels of BC experienced on new cropping land, freshly converted from pasture, also suggested a link with high nitrogen levels.

6.4.1.2 Rainfall and temperature

As already established, sweetpotatoes are perennial plants with steady development throughout the season. The sweetpotato cultivars currently grown in New Zealand rarely flower under commercial conditions, so there is a lack of distinctive biological milestones in their development. However, despite the steady state of sweetpotato growth, the BC disorder appears to develop from the same time each year (Gillard 1955; Wood & Schappi 1984), suggesting that onset of BC is a response to seasonal change.

Rainfall over the harvest period at Dargaville in the 1996/97 season (Fig. 6.1) was similar in quantity and distribution to the long term average. Mean air temperatures (Fig. 6.2) were also comparable to the long term average (Fig. 6.3), as were extreme low air temperatures. There was no definitive seasonal event in 1996/97, with respect to air temperature, that could be directly interpreted as contributory to the season's particularly high BC incidence.

Soil temperatures were not available for the 1996/97 season, so data loggers were placed at Dargaville in the 1998/99 season to establish the relationship between air and soil temperatures. Beneath the soil, temperatures were much more stable than in the air or at the soil surface and showed little diurnal fluctuation (Fig. 6.5). While mean soil temperatures dropped steadily during the harvesting window and approached chilling levels, temperatures did not appear particularly low for extended periods. Growers have reported that storage roots missed at harvest may pass through an entire winter and be dug up in the field during ground preparation the following season, without internal defects.

Storage roots of the cv. 'Owairaka Red' are generally produced well within the soil profile at approximately 10 cm depth and remain covered with soil until harvest. Harvesting conditions in the 1996/97 season were sometimes too wet for mechanized harvesters, so many crops were ploughed out and lifted by hand. Roots should never be left in the field overnight after digging, even during apparently warm conditions, due to the risk of chilling injury (Clark & Moyer 1988). However, there was no evidence of extraordinary post harvest chilling due to field exposure in the 1996/97 season. In the

1996/97 harvest, BC symptoms were only found in the cv. 'Owairaka Red', not in the two other dominant cultivars grown; cv. 'Toka Toka Gold' and cv. 'Beauregard', which were produced under the same conditions. This pattern of susceptibility in commercial cultivars was also seen in trials at the Pukekohe Research Centre.

6.4.1.3 Dargaville summary

The 1996/97 season produced storage roots of the sweetpotato cv. 'Owairaka Red' with abnormally high levels of BC (*c.* 2%). While the results of soil and plant nutrient analysis were inconclusive, the role of high soil nitrogen levels is an area for further research. Data from the Dargaville meteorological site did not suggest that the region received rainfall or air temperatures that differed from the long term average. It is unfortunate that soil temperatures were not available from Dargaville in the 1996/97 season. However, data from a Dargaville sweetpotato crop (1998/99) showed large diurnal fluctuations in air temperature while soil temperature beneath the leaf canopy remained relatively steady, removing the likelihood of short windows of rapid chilling temperatures around storage roots. The mean monthly soil temperature for March 1999 was less than 1°C below the equivalent air temperature Although 1996/97 air temperatures dropped during the harvest period, soil temperatures would have barely reached chilling levels.

6.4.2 Pukekohe site evaluation

6.4.2.1 Germplasm

Sweetpotato germplasm from Asia, North America and the Pacific was included in this study. The cultivars 'Owairaka Red' and 'Toka Toka Gold' are unique New Zealand selections, putatively from Pacific sources. The cv. 'Landtec' was selected by a New Zealand company from segregating seed supplied by the University of North Carolina (Lewthwaite 1998). The cv. 'Beauregard' was bred at Louisiana State University (LSU). Six breeding lines, also sourced from LSU were included in the trial (LSU-1 to 6). The cultivar 'Northland Rose' (formerly breeding line 93N9/2) was selected in New Zealand from segregating seed supplied by the Asian Vegetable Research and Development Centre (AVRDC), Taiwan. Five breeding lines, also sourced from AVRDC were included in the trial (AVRDC-1 to 5).

In the 1997/98 season, three clones grown at the Pukekohe Research Centre developed BC symptoms (Plate 6.1), namely 'Owairaka Red' (Plate 6.2), 'Northland Rose' (Plate 6.3) and 'LSU-1'. BC susceptibility appeared to be present in a wide range of germplasm, sourced from different countries. Commercial cultivars 'Toka Toka Gold', 'Beauregard' and 'Landtec' did not produce any BC symptoms, as found in the 1996/97 Dargaville season. None of the other breeding lines selected from Asian or North American material produced BC symptoms.

In the 1998/99 season only 'Owairaka Red' had any BC symptoms, at approximately half the 1997/98 incidence rate. The clone 'LSU-1' had the highest rate in 1997/98 but developed none at all in 1998/99. While a range of germplasm appears susceptible to BC, 'Owairaka Red' appears to develop the disorder more consistently. Sporadic BC in hand dug 'Owairaka Red' plants from the 1998/99 season demonstrated that BC effects were not only localized to individual plants, but affected only some of the plant's storage roots. An observation plot of cv. 'Waina' showed that it is also prone to developing BC. As 'Waina' was originally introduced to New Zealand in the 1850's and 'Owairaka Red' is derived from it by clonal selection, BC may well have affected the New Zealand sweetpotato industry for over 100 years.

6.4.2.2 Temperature

Aside from chilling injury, cool temperatures may affect the dynamics of growing plants, by modifying storage root development through assimilate supply or respiration rate. Soil temperatures were recorded at the Pukekohe Research Center for the two seasons in which sweetpotato crops developed BC, 1997/98 and 1998/99.

Soil temperatures at increasing depths showed less fluctuation and greater heat retention than those recorded near the soil surface (Fig. 6.6; 6.7). In 1998, the lowest daily (9 am) 10 cm soil temperature recorded in March was 17.9°C and the lowest in April (prior to harvest; 16 April) was 15.0°C (Fig. 6.6), based on NIWA data. In 1999 the lowest daily (9 am) 10 cm soil temperature recorded in April was 13.7°C and the lowest in May (prior to harvest; 24 May) was 10.5°C (Fig. 6.7), based on NIWA data. The NIWA and Crop & Food Research 10 cm soil data were highly correlated based on a comparison of data for April 1999 (Fig. 6.8). NIWA site temperatures were good predictors of temperatures within the local sweetpotato crop.

The 1997/98 crop did not experience chilling temperatures prior to harvest (Fig. 6.6), yet 'Owairaka Red' developed 5.7% BC, 'Northland Rose' 3.8% and clone 'LSU-1' 10.0%. In the 1998/1999 crop, soil temperatures dropped as low as 10.5 °C (Fig. 6.7) just prior to harvest, but 'Owairaka Red' developed only 2.4% BC, while 'Northland Rose' and 'LSU-1' developed none. Temperatures experienced in both of these seasons were not low enough to explain the occurrence of the BC disorder by typical chilling injury alone, unless 'Owairaka Red' was particularly temperature sensitive.

6.4.2.3 Pukekohe summary

The BC disorder was found to occur in sweetpotato sourced from diverse international sources; Asia, North America and the Pacific. The New Zealand cv. 'Owairaka Red' appeared to be particularly consistent in developing the disorder. BC was found in both seasons in which germplasm evaluations were made, allowing a comparison of the temperatures to which they were exposed. Crops grown in neither season appeared to experience particularly low chilling temperatures. Contrary to expectations with chilling, BC levels in the cooler season were lower than those seen under warmer conditions.

6.4.3 Chapter summary

In this investigation, the BC disorder was observed to occur over three consecutive seasons; 1996/97, 1997/98 and 1998/99. This disorder has had significant consequences for the New Zealand sweetpotato industry, causing changes in crop management and lowering product quality. While predominantly found in cv. 'Owairaka Red', BC can be found in a range of germplasm sourced internationally. Although the disorder has been studied by both national (Gillard 1955; Wood & Schappi 1984) and international researchers (Nielsen & Harrow 1966), the casual agents have not been identified. This investigation suggests the need for further detailed study of the susceptibility of 'Owairaka Red' to chilling injury, and the effect of high soil nitrogen levels on the occurrence of BC.

Chapter 7

Storage root: chilling and nutrition responses

7.1 Introduction

Brown centre (BC) is an important limitation to sweetpotato storage root production. The BC disorder frequently occurs in susceptible sweetpotato storage roots, but its underlying causes have not been identified. In the previous chapter it was suggested that the susceptibility of 'Owairaka Red' to chilling injury, and the effect of high soil nitrogen levels might be implicated in the development of BC.

Sweetpotato cv. 'Owairaka Red' appears to consistently produce BC symptoms, so was retained for all experiments. While seasonal changes during autumn include a number of interacting environmental factors, it is well documented that sweetpotato storage roots are prone to chilling injury (Clark & Moyer 1988). As field temperatures recorded during the development of BC did not appear particularly low, a series of experiments examined the chilling sensitivity of cv. 'Owairaka Red' storage roots. Field evidence for the role of high nitrogen levels is circumstantial but substantiated by growers' experience. A possible mechanism for BC induction by high nitrogen levels was investigated.

Chilling injury is a physiological defect in plants of tropical and subtropical origin that results in reduced produce quality through exposure to low but non freezing temperatures (Parkin et al. 1989). About one-third of all fruits and vegetables on the USA market are susceptible to chilling injury (Wang 1996), which is generally caused by temperatures between 0 and 15°C (Markhart 1986). Plant tissues that have been chilled typically develop symptoms such as pitting and browning. They are also more susceptible to damage by pathogens and sometimes water logging (Wills et al. 1989). The warming of produce after exposure to chilling temperatures generally hastens the development of chilling injury symptoms (Whitaker 1994).

Sweetpotato is particularly susceptible to chilling injury. The leaves of some sweetpotato cultivars show chilling injury, as expressed by electrolyte leakage, after exposure to 5°C for 6 hours (Woods et al. 1991). Sweetpotato roots are generally maintained at 13-16°C for long term storage (Walter & Schadel 1982), but are subject to chilling injury at temperatures below 12 °C (Lewis & Morris 1956). The severity of chilling injury in roots depends on the temperature and length of exposure below 12°C. Important symptoms of chilling injury in sweetpotato roots are hardcore and tissue browning.

Hardcore, in which parts of the root remain hard after cooking, was initially thought to be caused by pathogenic organisms such as viruses (Hammond et al. 1974; Daines et al. 1974). It is now known that hardcore is a physiological disorder of chilled sweetpotato (Broadus et al. 1980). Cultivars differ in their susceptibility to hardcore, but susceptibility is not correlated with root dry matter content (Lewis & Morris 1956; Daines et al. 1976; Porter et al. 1976; Hammett et al. 1978). One day of chilling at 1.7°C followed by a day at 21°C may be sufficient to produce severe hardcore (Buescher et al. 1975). Temperatures of 10°C for at least three days may also produce hardcore (Clark & Moyer 1988), sensitivity being cultivar dependent. Roots that are cured prior to chilling are less susceptible to hardcore than uncured roots (Daines et al. 1976). Chilled roots that are subsequently stored in non chilling temperatures develop greater levels of hardcore than roots cooked immediately after chilling. Sweetpotatoes must be cooked to express hardcore (Buescher et al. 1976; Daines et al. 1976). There is some evidence that high ethylene levels encourage hardcore and may be involved in normal hardcore development (Timbie & Haard 1977).

The browning of sweetpotato root tissue as a consequence of chilling has been known for some time (Lauritzen 1931; Lewis & Morris 1956; Lieberman et al. 1959; Porter et al. 1976). The darkening (brown to black in colour) of internal tissues is a chilling injury symptom that is most pronounced near the cambium and vascular bundles. Darkening of the central pith tissue can be observed in severely injured roots (Picha 1987). This discolouring may be due to oxidized phenols, which can increase during chilling. Histochemical tests indicate phenolics are located in the periderm, cambium, latex of laticifers and vascular bundles, but not in the parenchymatous cells which contain many starch granules (Schadel & Walter 1981). Variations in levels of chilling

discoloration between cultivars may be due to differences in tissue pH and chlorogenic acid content (Porter et al. 1976).

The BC disorder seen in 'Owairaka Red' appears in mid to late April (Gillard 1955; Wood & Schappi 1984), coincident with cooling air temperatures. When roots with BC are cooked, areas of the tissue remain woody and develop objectionable flavours (Wood & Schappi 1984), symptoms also seen in chilling injury.

Nutritional disorders develop in many crops, through either nutrient excess or deficiency, and sweetpotato is no exception (O'Sullivan et al. 1997). Boron (B) deficiency commonly produces disorders with brown or discoloured tissue, in a diverse range of crops including sweetpotato. The production of brown tissue as a result of B deficiency is thought to be due to the accumulation of polyphenolic compounds (Gupta 1979). Sweetpotato, like many latex producing plants (Mengel & Kirkby 1982), is particularly susceptible to B deficiency. Boron deficiency causes the development of brown tissue in sweetpotato storage roots, a disorder that appears similar to BC.

A disorder very similar to BC was found in Japan (Fujita & Kouzuma 1997) and was investigated over a four year period. Like BC, the disorder had no external symptoms, did not appear due to particularly chilling temperatures or disease, and its incidence varied between cultivars and across fields. The cultivar 'Beni-Otome' produced the most severe symptoms, that were very similar (colour, size and distribution) to the necrotic areas seen with BC. In Japan, the addition of B fertiliser (borax) reduced the incidence of the disorder, but did not always eliminate it in sensitive cultivars.

The foliar symptoms of B deficiency in sweetpotato first appear late in the growing season (Clark & Moyer 1988). Early signs of B deficiency are usually found in the modified growth of young leaves (O'Sullivan et al. 1997), as B is required for the maintenance of meristematic activity (Gupta 1979). Initially, the effect of B deficiency on sweetpotato root tissue was demonstrated when discoloured tissue, initially thought to be due to chilling injury, was eliminated or reduced by the use of borax fertiliser (Willis 1943). Further research described the disorder, referred to as 'internal brown spot', as being characterized by brown necrotic spots in the root tissue. These spots were variable in size with indistinct margins and occurred throughout the flesh, but

were more common in the cambial zone near the periphery of the root (Nusbaum 1946). Another symptom of B deficiency in sweetpotato was later described as 'blister', a disorder that was seldom present at harvest but developed on sweetpotato roots after about 30 days in storage. The root surface became brown or black, with small raised bumps or blisters. The frequency of blister symptoms varied with cultivar, cv. 'Jewel' being particularly prone to the disorder (Nielsen 1965; Miller & Nielsen 1970; Wilson et al. 1989).

The availability of B to plants is influenced by the soil B concentration, soil acidity, moisture supply, temperature, lime content, organic matter, leaching and the purity of chemical fertilisers applied. Boron is readily leached from soils. A considerable portion of the soil's B content is held in organic matter, from which it is gradually released by microorganisms. During periods of drought, when microbial activity in the soil slows, B availability decreases whereas when soil moisture is adequate more B is released and supplied to plants (Sauchelli 1969). Cold conditions also increase B deficiency. In sweetpotato, B is required at approximately 0.5-1 kg/ha (Shorrocks 1974). There is some suggestion that B is not readily transported within the sweetpotato plant (O'Sullivan et al. 1997). However, foliar B (Solubor) is commonly applied to commercial sweetpotato crops in the USA (M. Cannon, pers. comm.) where it reduces blister symptoms, indicating that B deficient conditions have been overcome (Paterson & Speights 1971).

In general, soil nitrogen (N) levels have an important effect on B uptake and photosynthate partitioning within plants. Increasing levels of applied N reduce B uptake (Gupta 1979). This relationship has been demonstrated in a number of crops and has been used to reduce toxic levels of B in citrus. Conversely, when B is already deficient, the addition of N may further depress yield. If N levels are high late in the growing season, photosynthate in both sugar beet and sugar cane are diverted into vegetative leaf growth rather than the synthesis and storage of sugars (Mengel & Kirkby 1982). Under high N conditions sweetpotato dry matter distribution is also modified and vines may grow at the expense of root yield, although the degree of yield loss is cultivar dependent (O'Sullivan et al. 1997). The period just prior to sweetpotato harvest is characterized by the rapid accumulation of photosynthate in storage roots, which can be affected by high N levels (Hartemink et al. 2000). In New Zealand, commercial

sweetpotato growers report that BC is more common in fields out of pasture. Land cultivated from pasture commonly has higher levels of N than those previously used to grow sweetpotato crops.

This investigation initially examined the hypothesis that the development of BC in 'Owairaka Red' roots is due to their particular susceptibility to developing chilling injury. A further experiment was also conducted to evaluate the hypothesis that levels of available minerals, particularly N, modify the incidence of BC.

7.2 Materials and methods

7.2.1 Chilling injury

Four experiments were performed in this study, to examine the sensitivity of harvested cv. 'Owairaka Red' sweetpotato roots to low temperatures and to compare chilling symptoms with those of BC.

7.2.1.1 Experiment 1: Sound and BC affected storage roots

Eight sound 'Owairaka Red' roots and eight roots with BC symptoms were selected from the same field at Dargaville in the 1996/97 season. All roots were cut in half along their length. Roots with BC were scored for the percentage of brown necrotic tissue observable on one cut face (0, BC absent; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC). All 16 roots were weighed and placed in individual nylon mesh bags before being boiled for 60 minutes. Tissue samples of five sound roots from the same crop were oven-dried at 80°C to establish the general % dry matter content. On cooling, all cooked roots were individually mashed by hand and any hard tissue was retained. This hard material fitted the description of hardcore tissue evident in roots with chilling injuries. The hardcore material was oven-dried at 80°C then weighed. Hardcore occurrence was expressed as the proportion of hardcore tissue in each whole root, on a dry weight basis.

7.2.1.2 Experiment 2: Chilling temperatures and durations

Approximately 500 healthy storage roots of cv. 'Owairaka Red' were harvested at Dargaville on 23 March 1998. The % dry matter content was calculated from ten randomly sampled roots, oven-dried at 80°C. These ten roots contained no field derived BC. All remaining roots were assigned to one of four temperature controlled storage rooms at either 1, 5, 10 or 15°C, on the day after harvest. Each storage room contained a total of 120 roots, assigned at random into four samples. One sample was removed from each temperature regime after four storage times, 5, 13, 20 and 27 days from the commencement of chilling. Of the 30 roots in each sample, 15 roots were placed in 20°C storage for a further 7 days before assessment, while the other 15 roots were assessed immediately. Chilling injury was estimated by cutting each root in half along its length, then scoring the proportion of cut surface showing brown discoloured tissue (BT). The BT scale was: 0, no BT; 1, up to 25% BT; 2, up to 50% BT; 3, up to 75% BT; 4, up to 100% BT. Each root was identified, placed in individual nylon mesh bags, then boiled for 60 minutes. On cooling, all roots were individually mashed by hand and any hard tissue was retained. This hardcore material was oven-dried at 80°C, then weighed. Roots that underwent further storage at 20°C were assessed in the same manner. Roots that developed rots in storage were removed from the experiment.

7.2.1.3 Experiment 3: Medium chilling temperatures

Storage roots of the sweetpotato cv. 'Owairaka Red' were harvested at Dargaville on 1 April 1998. The uncured roots were stored in an enclosed room within multi-walled paper bags, in ambient conditions at the Pukekohe Research Centre. On 2 June the stored roots were assigned to temperature controlled storage rooms at either 5, 7 or 9°C. Randomly selected samples of roots were removed from each temperature regime at eight time intervals; 8, 15, 22, 30, 37, 44, 50 and 58 days from the commencement of chilling. On removal from the chilling treatments the samples were placed in 20°C storage for a further 7 days, before assessment. Each sample consisted of 22 roots, of which 2 were used to calculate the dry matter content while the remainder were assessed for chilling injury. Root dry matter content was calculated from root samples oven-

dried at 80°C. Chilling injury was assessed by cutting each root in half along its length, then scoring the proportion of cut surface with brown discoloured tissue (as for experiment 2). The cut surfaces were also scored for the degree of pithiness: 0, solid tissue; 1, pithy; 2, cavities formed. The roots were identified, placed in individual nylon mesh bags, then boiled for 60 minutes. On cooling, all roots were individually mashed by hand and any hard tissue was retained. This hardcore material was oven-dried at 80°C, then weighed. Roots that developed rots in storage were removed from the experiment.

7.2.1.4 Experiment 4: Intermittent chilling

Storage roots of the sweetpotato cv. 'Owairaka Red' were harvested at Dargaville on 1 April 1998. The uncured roots were stored in an enclosed room, within multi-walled paper bags, under ambient conditions at the Pukekohe Research Centre. Dry matter content was calculated from 12 roots, oven-dried at 80°C. On each of seven dates (24, 26, 28, 30 April and 2, 4, 6 May), 60 roots were randomly selected and divided into three treatments of 20 roots. One of the three samples was placed in a temperature controlled storage room at 5°C, another in a temperature controlled storage room at 20°C, while the third sample alternated between the two temperatures. The alternating sample was placed in the 20°C room for 8 hours and in the 5°C room for 16 hours of each day. The samples were chilled in this manner for 14, 12, 10, 8, 6, 4 and 2 days respectively. On 8 May all samples were removed from the chilling treatments and stored at 20°C for nine days, before assessment for tissue injury. Chilling injury was assessed by cutting each root in half along its length, then scoring the proportion of the cut surface with brown discoloured tissue (as for experiment 2). The cut surfaces were also scored for the degree of pithiness (as for experiment 3). The roots were identified, placed in individual nylon mesh bags, then boiled for 60 minutes. On cooling, all roots were individually mashed by hand and any hard tissue was retained. This hardcore material was oven-dried at 80°C, then weighed. As chilling treatment differences were generally large and there was a high degree of loss due to rot as experiments progressed, formal statistical analysis was not applied to the chilling experiments.

7.2.2 Nutrition trial

A Pukekohe Research Centre field site was prepared by broadcasting one t/ha of 30% potassic superphosphate (N:P:K 0:7:14), prior to moulding. Cultivar 'Owairaka Red' sweetpotato plants produced commercially as sprouts were transplanted into the field on 4 December 1998 and watered in with overhead irrigation. The soil consisted of Patumahoe clay loam, which was randomly sampled (11 February 1999) just prior to commencement of the treatments, with the following analysis (techniques in Appendix 1): phosphorus 37 μg/ml, potassium 0.72 me/100 g, calcium 12.3 me/100 g, magnesium 0.75 me/100 g, sodium 0.20 me/100 g, exchangeable cations 15.0 me/100 g, boron 0.6 μg/g, available nitrogen 60 kg/ha, pH 6.8 and a volume/weight ratio of 0.97 for dried ground soil. The site was selected as levels of both available N and boron were relatively low. Available N was assessed by anaerobic incubation followed by ammonium-N extraction using 2M KCl, then determined by Berthelot colorimetry (R. J. Hill Laboratories Ltd).

The trial consisted of 14 treatments, including three nitrogen (N) rates and four boron (B) rates in all combinations, and two defoliation treatments. The trial was arranged in a modified alpha row and column design (Williams & John 1989) with four replicates. The entire trial was seven plots wide by eight plots long. Each plot was four rows wide by 7 m long, with only the two middle rows used for BC assessment. Each row was 0.75 m wide and within-row plant spacing was 0.30 m. The portion of each plot harvested and assessed contained a total of 40 plants, arranged in two rows. The cultivar 'Jewel' is particularly sensitive to low B levels (Nielson 1965; Miller & Nielsen 1970), so a double row of 'Jewel' plants was transplanted immediately adjacent to and along one entire length of the trial block, as a B level bio-indicator.

Nitrogen was broadcast by hand using urea (46% N) on three dates (18 February, 5 March and 22 March 1999). On each date the N was applied at 0, 100 or 200 kg/ha. The urea was well watered in by overhead irrigation (*c.* 20 mm) after each application. Boron was applied in four foliar applications (19 February, 8 March, 23 March and 4 April 1999) using Solubor (17.5% B). On each date B was applied at 0, 0.5, 1.0 or 1.5 kg/ha. The Solubor was applied over the foliage using a hand-operated backpack

sprayer. The spray boom extended over the entire plot width (3 m) and delivered 307 l/ha (Hardi 4110-12 spray nozzles). Each defoliation treatment was applied once, on 4 April 1999, and did not receive any supplementary N or B. There were two defoliation treatments, in the first (designated 50%D) the vines were completely cut along the hollow between adjacent moulds (38 cm from the crown of the plant), in the second (designated 75%D) the vines were completely cut along the shoulders of the mould (19 cm from the crown of the plant).

Plant tops were mown off on 18 May 1999. The trial was harvested by a two-row, tractor-drawn harvester on 21 May 1999. Following harvest, storage roots of marketable size (greater than 2.5 cm diameter) were individually cut in half along their length and assessed for BC. The assessment scale was based on the proportion of exposed flesh affected by BC: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, above 50% BC. The incidence and severity (score) of BC was recorded for each root. The data were analysed using the statistical software package GENSTAT^{tm}: ANOVA procedure. A logit transformation was used to equalise the variance across the data set prior to analysis.

7.3 Results

7.3.1 Chilling injury

7.3.1.1 Experiment 1: Sound and BC affected storage roots

When cooked, BC roots obtained from the field produced hardcore tissue similar to that found in chilled sweetpotato roots (Table 7.1). Sound roots did not contain any hardcore tissue. There was a general increase in the percentage of hardcore as BC symptoms (BC score) increased.

Table 7.1: Cooked sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage roots from a commercial Dargaville field (1997/98 season). Levels of hardcore in roots exhibiting the brown centre (BC) disorder.

Root number	1	2	3	4	5	6	7	8
BC score[1]	1	1	1	1	2	2	3	4
% HC [2]	0.44	0.59	1.05	0.85	4.88	0.75	6.97	5.13

[1] BC score: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC.

[2] Hardcore (HC) is presented on a dry weight basis (%).

7.3.1.2 Experiment 2: Chilling temperatures and durations

Chilling induced brown tissue and hardcore within freshly harvested cv. 'Owairaka Red' storage roots. Roots that were assessed immediately after chilling showed either no symptoms or more subtle injury symptoms than those stored in a warm environment just prior to assessment. The amount of brown tissue (BT score) increased as the chilling temperature decreased and the chilling period was prolonged (Table 7.2). Storage at 15°C produced no chilling symptoms.

Storage at 10°C for 13 days produced injury symptoms (hardcore) when assessed immediately after chilling, but hardcore occurrence was erratic and not observed following any other storage periods at this temperature. The amount of hardcore tissue assessed post warming from 10°C (Table 7.4) was also very small and erratic in occurrence (1% after 5 chilling days, but none at 13, 20 or 27 days), as was the number of roots affected (7% after 5 chilling days).

At 5°C hardcore chilling symptoms were more consistent. Hardcore tissue was found within samples that had been chilled at 5°C and warmed (Table 7.5), following both 5

and 13 days of chilling, while rots developed with longer chilling periods. At 1°C no chilling symptoms were seen until the 13 day, unless the sample was warmed post chilling. If post chilling warming was applied, the 5 day treatment at 1°C showed chilling symptoms but roots collapsed if chilled for longer periods.

The level of brown tissue (BT score) in roots that had been stored at 10°C and then warmed was very slight, but occurred consistently after 5 chilling days (Table 7.4), and the incidence of BT (Table 7.3) increased with the chilling period. The level of brown tissue measured post warming from 5°C was relatively severe and by 13 chilling days 93% of the roots were affected with brown tissue with a mean BT score of 2.4. However, even the most extreme treatment, 1°C with warming, while producing high levels of severely discoloured tissue did not match BC symptoms in necrotic tissue colour and distribution.

Table 7.2: Mean brown tissue (BT) scores in sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage roots after exposure to low temperatures for various durations. Roots were cut along their main axis and the proportion of BT on cut surfaces scored: 0, no BT; 1, up to 25% BT; 2, up to 50% BT; 3, up to 75% BT; 4, up to 100% BT.

Chilling duration (days)	Post chilling assessment[1] Chilling temperature (°C)				Post warming assessment[2] Chilling temperature (°C)			
	1	5	10	15	1	5	10	15
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	1.3	0.3	0.1	0.0
13	0.7	0.2	0.0	0.0	†	2.4	0.1	0.0
20	1.9	0.8	0.1	0.0	†	†	0.1	0.0
27	†	†	0.0	0.0	†	†	0.2	0.0

[1] Roots assessed for BT immediately after chilling.

[2] Roots assessed for BT following chilling, then storage at 20°C for 7 days.

† Roots that developed rots in storage were removed from analysis.

Table 7.3: The percentage of roots affected with brown tissue (BT) in sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage roots after exposure to low temperatures for various durations.

Chilling duration (days)	Post chilling assessment[1] Chilling temperature (°C)				Post warming assessment[2] Chilling temperature (°C)			
	1	5	10	15	1	5	10	15
0	0	0	0	0	0	0	0	0
5	0	0	0	0	53	31	7	0
13	67	20	0	0	†	93	7	0
20	100	80	13	0	†	†	13	0
27	†	†	0	0	†	†	21	0

[1] Roots assessed for BT immediately after chilling.

[2] Roots assessed for BT following chilling, then storage at 20°C for 7 days.

† Roots that developed rots in storage were removed from analysis.

Table 7.4: The percentage of hardcore tissue in cooked storage roots of the sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', after exposure to low temperatures for various durations. (Percentage of hardcore tissue within roots exhibiting hardcore symptoms on a dry weight basis.)

Chilling duration (days)	Post chilling assessment[1] Chilling temperature (°C)				Post warming assessment2 Chilling temperature (°C)			
	1	5	10	15	1	5	10	15
0	0	0	0	0	0	0	0	0
5	0	0	0	0	12	15	1	0
13	51	4	2	0	†	7	0	0
20	65	7	0	0	†	†	0	0
27	†	†	0	0	†	†	0	0

[1] Roots assessed for hardcore immediately after chilling.

[2] Roots assessed for hardcore following chilling, then storage at 20°C for 7 days.

† Roots that developed rots in storage were removed from analysis.

Table 7.5: The incidence of hardcore in cooked roots of the sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', after exposure to low temperatures for various durations. The percentage of roots affected with hardcore.

Chilling duration (days)	Post chilling assessment[1] Chilling temperature (°C)				Post warming assessment[2] Chilling temperature (°C)			
	1	5	10	15	1	5	10	15
0	0	0	0	0	0	0	0	0
5	0	0	0	0	13	54	7	0
13	10	13	17	0	†	20	0	0
20	10	13	0	0	†	†	0	0
27	†	†	0	0	†	†	0	0

[1]Roots assessed for hardcore immediately after chilling.

[2]Roots assessed for hardcore following chilling, then storage at 20°C for 7 days.

†Roots that developed rots in storage were removed from analysis.

7.3.1.3 Experiment 3: Medium chilling temperatures

Brown tissue (BT) appeared after 15 days of chilling for both 5 and 7°C treatments (Table 7.6, Plate 7.1), and after 30 days for 9°C. Thus, brown tissue development was delayed compared to the 5°C treatment with warming in Experiment 2, but was similar to the Experiment 2 brown tissue levels assessed without a warming period. Over half the roots contained brown tissue after 37 days of chilling at 7°C, with a mean BT score of 1.0. Chilling at 9°C produced an erratic response, with low levels of brown tissue development and variable numbers of roots affected. Hardcore was found for all assessment times after 5°C storage (Table 7.7). The proportion of the root tissue affected and hardcore incidence amongst roots increased with the duration of chilling. A small amount of hardcore was found after 8 days of chilling at 7 and 9°C, possibly due to damage in the pre-treatment storage period, but a regular response to chilling shows from 30 days at 7°C and 50 days at 9°C. In this experiment, pithy tissue and cavities within the root were recorded (Table 7.8). Using this measure of injury, the first signs of damage at 9°C storage were after 37 days, while 5 and 7°C storage showed damage at the first assessment (8 chilling days).

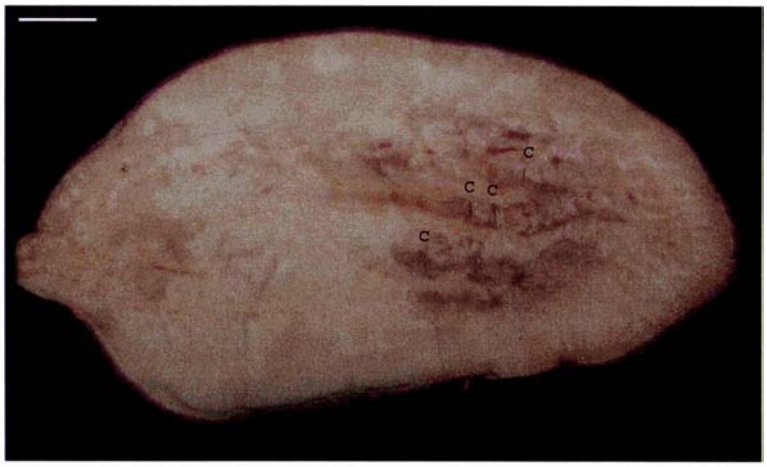


Plate 7.1: Longitudinal section of a sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage root showing tissue chilling injury after storage at 5° C for 15 days followed by 7 days at 20 °C (scale bar: 1.0 cm). The brown tissue at the distal end shows some cavity formation (C).

Plate 7.2: Longitudinal section of a sweetpotato (*Ipomoea batatas* (L.) Lam. cv. 'Owairaka Red' storage root showing tissue cavitation associated with long term storage at non-chilling temperatures (scale bar: 1.0 cm). Note absence of tissue browning.

Table 7.6: Mean BT scores and the percent incidence of BT after chilling sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage root at low temperatures for various durations. Roots were cut along their main axis and the proportion of BT on cut surfaces scored: 0, no BT; 1, up to 25% BT; 2, up to 50% BT; 3, up to 75% BT; 4, up to 100% BT.

Chilling period (days)[1]	Mean BT score Chilling temperature (°C)			BT incidence (%) Chilling temperature (°C)		
	5	7	9	5	7	9
8	0.0	0.0	0.0	0	0	0
15	0.2	0.2	0.0	10	15	0
22	1.3	0.1	0.0	80	10	0
30	1.3	0.2	0.1	75	10	5
37	†	1.0	0.2	†	55	15
44	†	0.4	0.0	†	35	0
50	†	†	0.1	†	†	10
58	†	†	0.0	†	†	0

[1] Excluding post chilling period at 20°C.
† Roots that developed rots in storage were removed from analysis.

Table 7.7: The percentage of hardcore tissue and the incidence of hardcore-affected roots in cooked roots of the sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', after exposure to low temperatures for various durations. (Percentage of hardcore tissue within roots exhibiting hardcore symptoms, on a dry weight basis).

Chilling period (days)[1]	Hardcore tissue (%) Chilling temperature (°C)			Hardcore incidence (%) Chilling temperature (°C)		
	5	7	9	5	7	9
8	1	1	3	20	10	5
15	11	0	0	80	0	0
22	23	0	0	95	0	0
30	50	4	0	95	30	0
37	†	4	0	†	20	0
44	†	10	0	†	80	0
50	†	†	2	†	†	20
58	†	†	2	†	†	5

[1] Excluding post chilling period at 20°C.
† Roots that developed rots in storage were removed from analysis.

Table 7.8: The percentage of storage roots at each cavity score for sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', after exposure to low temperatures for various durations. Cavity score: 0, solid tissue; 1, pithy; 2, cavities formed.

Chilling period (days)[1]	5 °C storage			7 °C storage			9 °C storage		
	0	1	2	0	1	2	0	1	2
8	80	5	15	85	0	15	100	0	0
15	25	15	60	95	0	5	100	0	0
22	50	10	40	100	0	0	100	0	0
30	20	15	65	55	20	25	100	0	0
37	†	†	†	55	10	35	95	0	5
44	†	†	†	5	25	70	85	10	5
50	†	†	†	†	†	†	65	20	15
58	†	†	†	†	†	†	75	0	25

[1] Excluding post chilling period at 20°C.

† Roots that developed rots in storage were removed from analysis.

7.3.1.4 Experiment 4: Intermittent chilling

Under steady chilling at 5°C low levels of brown tissue developed from 6 chilling days, then generally increased in both level and incidence as the chilling period was prolonged (Table 7.9). Hardcore was seen after 8 days of chilling, as was the development of tissue cavities (Table 7.10). The chilling injury seen here occurred after a similar period to that of Experiment 2 at 5°C, but at reduced levels possibly due to physiological changes during the storage period prior to chilling. Constant storage at 20°C produced no brown tissue, hardcore or cavities. Under intermittent chilling, no hardcore or cavities were formed, while brown tissue was seen at a low level after 12 days in storage but not at 14 days.

Table 7.9: Mean BT scores and the percent incidence of BT after chilling sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage root at low temperatures for various durations. Roots were cut along their main axis and the proportion of BT on cut surfaces scored: 0, no BT; 1, up to 25% BT; 2, up to 50% BT; 3, up to 75% BT; 4, up to 100% BT.

	Mean BT score		BT incidence (%)	
Chilling period (days)[1]	Storage at 5°C	Intermittent chilling	Storage at 5°C	Intermittent chilling
2	0.00	0.00	0	0
4	0.00	0.00	0	0
6	0.05	0.00	5	0
8	0.35	0.00	35	0
10	0.70	0.00	40	0
12	1.60	0.10	60	10
14	1.25	0.00	75	0

[1] Excluding post chilling period at 20°C.

Table 7.10: The percentage of hardcore tissue in cooked storage roots and incidence of roots with tissue cavities in the sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red', following exposure to 5°C for various durations. (Percentage of hardcore tissue within roots exhibiting hardcore symptoms, on a dry weight basis.)

Chilling period (days)[1]	Hardcore tissue (%)	Cavity incidence (%)
2	0	0
4	0	0
6	0	0
8	3	25
10	12	20
12	9	40
14	3	20

[1] Excluding post chilling period at 20°C.

Plate 7.3: Transverse section of a sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. 'Owairaka Red' storage root showing tissue chilling injury and hardcore after storage at 1°C for 13 days (scale bar: 1.0 cm).

7.3.2 Nutrition trial

Brown centre was found in 91% of the plots assessed in the trial. Significant differences (P=0.028) in the incidence of BC relative to the control were found in treated plots (Table 7.11). Harvested storage roots of the cultivar 'Jewel' did not develop any symptoms of blister after 5 months in storage, indicating an adequate supply of B, although soil analysis suggested critically low B levels. While neither the defoliation nor the B treatments changed the incidence rate, adding N effectively doubled the levels of BC (Table 7.12). The incidence of BC with N application was similar whether all levels of BC were compared or only the more severe cases (> 25% of the flesh affected).

Following the application of N, BC incidence increased significantly($P < 0.001$), while there was no evidence that B had a significant effect ($P = 0.77$) or of an N x B interaction ($P = 0.65$). Nitrogen also had a significant effect on BC severity ($P =$

0.005). There was no evidence of a B application effect (P = 0.89) or of an N x B interaction (P = 0.85) as the effect of N was the same at each level of B.

Table 7.11: Incidence of brown centre in storage roots (%) of the sweetpotato (*Ipomoea batatas* (L.) Lam. cv. 'Owairaka Red' harvested at the Pukekohe Research Centre in the 1998/99 season. Incomplete factorial design, with mean percent incidence by back transformation (logit), LSD based on 39 degrees of freedom.

Total B applied	Defoliation (%)	Total N applied (kg/ha)					
		0		300		600	
0	0	1.9[a]	(-3.9)	5.0[a]	(-2.9)	6.2[a]	(-2.7)
0	50	2.6	(-3.6)	-		-	
0	75	1.6	(-4.1)	-		-	
2	0	2.3	(-3.8)	4.1	(-3.1)	4.4	(-3.1)
4	0	4.0	(-3.2)	6.1	(-2.7)	3.9	(-3.2)
6	0	2.5	(-3.7)	6.5	(-2.7)	4.7	(-3.0)
$LSD_{0.05}$			(0.90)				

[a]Back-transformed.

Table 7.12: The effect of applied nitrogen (N) on the general percent incidence of brown centre (BC) and the incidence of severe BC in storage roots of the sweetpotato (*Ipomoea batatas* (L.) Lam. cv. 'Owairaka Red' harvested at the Pukekohe Research Centre in the 1998/99 season. Roots were considered to have severe BC when more than 25% of the exposed flesh was affected. The data were transformed (logit) to stabilize the variance, LSD based on 39 degrees of freedom.

Total N applied (kg/ha)	Total BC incidence[1] (%)		Severe BC incidence[1] (%)	
0	2.4[a]	(-3.7)	1.6[a]	(-4.1)
300	5.4	(-2.9)	3.0	(-3.5)
600	4.7	(-3.0)	2.7	(-3.5)
$LSD_{0.05}$		(0.45)		(0.50)

[a]Back-transformed.

7.4 Discussion

7.4.1 Chilling injury

7.4.1.1 Experiment 1: Sound and BC affected storage roots

Tissue browning and the production of hardcore within storage roots are symptomatic of chilling injury. The BC disorder produces brown tissue, but while BC roots have been describe as 'woody' when cooked (Wood & Schappi 1984) there has not been a comparison with chill induced hardcore. Hardcore is only expressed after cooking (Buesher et al. 1976) and BC affected roots are normally eliminated during food preparation. The BC roots obtained from the field and cooked, produced hardcore tissue similar to that of chilled sweetpotato roots (Table 7.1). The hardcore from BC roots was slightly more granular in texture than that from chilled roots. Sound roots did not contain any hardcore tissue. There was a general increase in the percentage of hardcore as BC symptoms (BC score) increased.

7.4.1.2 Experiment 2: Chilling temperatures and durations

As anticipated, chilling temperatures induced brown tissue and hardcore within cv. 'Owairaka Red' storage roots. However, 'Owairaka Red' roots were not unusually sensitive to chilling, as the temperatures and periods required to produce symptoms were similar to those reported for sweetpotato (Picha 1987; Clark & Moyer 1988). Roots that were assessed immediately after chilling showed either no symptoms or more subtle injury symptoms than those that passed through a warm environment before cooking (Buescher et al. 1976; Daines et al. 1976).

At 15°C there were no chilling symptoms, even if warm treatments were applied. The minimum chilling treatment, 5 days at 10°C, was enough to elicit subtle chilling symptoms in 7% of the roots, provided that they were exposed to a warm period post chilling. This supports the results of Lewis & Morris (1956), who suggested that temperatures below 12°C can produce tissue injury. The amount of brown tissue (BT score) increased as the chilling temperature decreased and the chilling period was prolonged.

The amount of hardcore tissue assessed post warming from 10°C (Table 7.4) was very low and erratic in occurrence. While exposure to temperatures of 10°C for 5 days produced chilling symptoms, the degree of tissue browning within roots was very limited and poorly defined. In general, the brown colouration within artificially chilled roots was not as prominent as that of BC in roots from the field, nor did tissue damaged by chilling have such pronounced margins with sound tissue. As the severity of chilling increased, the root tissue became brown, then increasingly pithy until cavities formed, finally entering a state of general collapse. Even most extreme treatment, 1°C with warming, which produced high levels of severely discoloured tissue did not produce symptoms similar in colour and distribution to that seen in BC.

7.4.1.3 Experiment 3: Medium chilling temperatures

It was established in Experiment 2, that while 'Owairaka Red' roots are susceptible to chilling injury they are not unusually sensitive compared to other sweetpotato cultivars. Chilling produced brown tissue and hardcore symptoms, which were similar but not identical to those of field BC. This experiment tested if prolonged chilling at medium chilling temperatures produced symptoms more similar to those seen in BC. The temperatures 7 and 9°C were chosen to induce low level injury and avoid total tissue collapse. A post chilling warm period was used to develop injury symptoms in all treatments.

The roots in this experiment were not heat cured, but as they were stored indoors in ambient conditions for 61 days after harvest, a 5°C treatment was included for direct comparison with the responses to treatments in Experiment 2. Cured roots are considered to be less susceptible to chill induced hardcore than uncured roots (Daines et al. 1976). In this experiment, longer exposures to chilling temperatures were required to produce levels of brown tissue comparable to those in freshly harvested roots of experiment 2.

The 9°C treatment took 30 days to produce even low levels of tissue browning but by 37 days cavities were beginning to form within the root tissue. At 7°C various levels of

tissue browning were seen over chilling durations of 15 to 44 days, before tissue collapse. Although a range of BT and hardcore levels were induced within otherwise sound roots by these treatments, the BC symptoms could not be reproduced. The chilling symptoms differed from BC in distribution, colour, definition and texture. While cultivar differences in sensitivity to chilling temperatures have been reported (Daines et al. 1976; Porter et al. 1976), this experiment confirmed that cv. 'Owairaka Red' was not atypical.

7.4.1.4 Experiment 4: Intermittent chilling

The relationship between cool and warm temperatures is important in the development of chilling injury symptoms (Daines et al. 1976). While experiments 1, 2 and 3 demonstrated that chilling injury produced similar symptoms to those seen in field BC, the symptoms do not correspond precisely in distribution, colour or general appearance. From experiments 2 and 3, the cv. 'Owairaka Red' is not unusually sensitive to chilling injury, so it is difficult to correlate typical temperature levels during late harvest with the level of field BC tissue injury. In this experiment intermittent cooling, as might occur diurnally, was examined to see if it exacerbated the chilling symptoms.

The constant 5°C treatment produced the levels and incidence of chilling injury expected from previous experiments. No chilling injury was seen in the 20°C treatment. The intermittent treatment produced no chilling symptoms apart from the appearance of low levels of brown tissue after 12 days of storage. Thus intermittent cooling at this level reduced chilling injury rather than exacerbating the symptoms. The symptoms seen under a constant 5°C regime and as appeared at 12 days of intermittent chilling were again similar to those of BC but not identical.

7.4.1.5 Chilling summary

There were four experiments in this study, comparing chilling injury to field BC symptoms in the sweetpotato cv. 'Owairaka Red'. Both chilling temperatures and the BC disorder delimit the temperate season, producing brown necrotic tissue within

storage roots. As cooling temperatures and the occurrence of BC were coincident, it was unknown if the onset of BC was a simple temperature effect.

In experiment 1 it was shown that BC roots harvested from the field contained not only brown tissue but also hardcore while sound roots from the same field contained neither. The amount of hardcore tissue within a root generally increased with increasing proportions of brown tissue. However, the hardcore from BC roots was more granular in texture than that from chilled roots. Experiment 2 demonstrated that tissue browning and hardcore also occurred as a direct result of chilling injury. As temperatures decreased and chilling periods lengthened, the symptoms of tissue browning and hardcore increased in severity. Roots stored in warm conditions after chilling developed more pronounced chilling symptoms than those assessed immediately after chilling. While chilling produced the same kind of symptoms as BC, the colour and definition of the brown tissue was not identical. In experiment 3 medium chilling temperatures were applied for prolonged periods, followed by warm storage. A combination of constant chilling temperatures (7 or 9°C) with chilling periods of up to 30 days failed to produce brown tissue that matched BC symptoms in appearance and severity. Experiments 2 and 3 demonstrated that cv. 'Owairaka Red' is not atypical in its sensitivity to chilling temperatures, which are generally reported to occur below 12°C for sweetpotato (Lewis & Morris 1956). A further experiment was conducted to evaluate the effect of intermittent chilling. This experiment demonstrated that intermittent chilling alleviated chilling symptoms rather than enhancing them. On the basis of these experiments and the field trials presented in the previous chapter, it was concluded that BC in sweetpotato storage roots is not caused by simple chilling injury.

7.4.2 Nutrition trial

The BC disorder was widespread throughout the trial (91% of the plots), although the mean incidence across treatments was relatively low (1.6 to 6.5 %). Disorders very similar to BC are found in sweetpotato crops stressed by either nutrient excess or deficiency (O'Sullivan et al. 1997). The effects of supplementary applications of nitrogen and boron were assessed in this trial, along with partial defoliation.

The role of nitrogen in producing the BC disorder was suggested by the increase in BC incidence on sites out of pasture, along with soil and plant tissue nitrogen levels associated with the disorder (previous chapter). However previous evidence for the involvement of high nitrogen levels was inconclusive and no reports of similar effects have been published. If nitrogen is involved in BC, a mechanism is required by which it induces the storage root disorder. High nitrogen levels must effectively induce brown necrotic tissue in storage roots of susceptible cultivars within a narrow seasonal window.

Sweetpotato is very efficient in obtaining N from soils even when N fertilizer is not provided (Yoneyama et al. 1998). High levels of N may reduce B uptake (Gupta 1979). Sweetpotato is highly susceptible to B deficiency (O'Sullivan et al. 1997), which produces 'internal brown spot' within the root tissue. The B deficiency root symptoms were initially thought to be due to chilling injury (Willis 1943), so both are symptomatically similar. As much of the soil's B is held in organic matter, cold temperatures can increase B deficiency (Sauchelli 1969).

In this trial, the addition of N effectively doubled the incidence ($P < 0.001$) and increased the severity of the BC disorder ($P = 0.005$). The increase in BC incidence with added N occurred across the severity grades. So whether all BC grades were compared or only the most severe grade (> 25% flesh affected), the incidence with added N was similarly increased (Table 7.12). The increase in BC with high N levels as seen in this trial is consistent with growers' observations and the evidence considered earlier. High fertility in general may also play a part, as suggested by the significantly higher potassium levels in BC roots (previous chapter).

Boron treatments were included in the trial to evaluate B deficiency as a possible mechanism by which N might induce BC. However the addition of B did not have a significant effect on the incidence of BC ($P = 0.77$) nor was there a N x B interaction ($P = 0.95$). There was also no evidence of a B effect on BC severity ($P = 0.89$) or of a N x B severity interaction ($P = 0.85$).

The supply of both N and B are important in shoot growth. As already established, high levels of N may encourage excessive shoot growth, while an adequate supply of B is

important for maintaining meristematic activity such as in shoot tips and new leaf development (Mengel & Kirkby 1982). As plant nitrogen content increases, LAD (Leaf area duration or leaf area persistance) increases (Tsuno & Fujise 1965). Removing a portion of the plant canopy provides a source/sink relationship which contrasts with the excessive canopy of the high nitrogen regimes. Renewed canopy growth during the later part of the season, particularly after nutrient removal in cuttage and release of apical dominance, might place greater meristematic demand on available B supplies. However defoliation did not affect the incidence of BC.

Soil analysis indicated B levels were very low, so the sweetpotato 'Jewel' was planted alongside the field trial as a B-sensitive indicator (Wilson et al. 1989). However, given that 'Jewel' storage roots did not develop any symptoms of blister after 5 months in storage, B could not be considered in short supply. While there is good evidence that high N levels are implicated in BC development, there is no evidence of a B involvement. Standard or artificially raised B levels had no significant effects on the incidence or severity of BC.

7.4.3 Chapter discussion

In chapter 6 it was established that while cv. 'Owairaka Red' is particularly susceptible to BC, intemationally sourced germplasm is also prone to the disorder. It was also demonstrated from the field that while temperatures were dropping during the time at which BC occurs, low temperature levels and durations did not appear to be enough to induce the level of tissue necrosis seen in BC. This investigation initially examined the hypothesis that the development of BC in 'Owairaka Red' roots is due to their particular susceptibility to developing chilling injury.

Chilling injury is well recognised as directly inducing brown tissue within sweetpotato storage roots and that sweetpotato cultivars differ in chilling susceptibility. If the BC disorder was simply localised chilling, the differential cultivar response, timing and symptoms of the disorder could be explained. One of the chilling injury symptoms in sweetpotato is the development of tissue called hardcore, which does not soften on cooking. Roots affected by the BC disorder were compared with sound roots, and while

the BC roots produced hardcore the sound roots did not. Storage roots of the cv. 'Owairaka Red' were further evaluated for chilling sensitivity. A wide range of temperatures (1 to 15°C) were applied to harvested roots for various durations. No chilling was experienced at 15°C but injury was induced at 10°C, demonstrating that cv. 'Owairaka Red' was not atypical in sweetpotato chilling sensitivity. While chilling produced symptoms similar to BC, none of the range of chilling temperatures or exposure periods induced identical symptoms. Two further experiments were conducted, the first evaluated the effect of exposure to prolonged medium chilling temperatures, while the second assessed intermittent chilling temperatures. Neither experiment produced storage root tissue necrosis that symptomatically matched that seen in the BC disorder. Based on the temperatures recorded in field crops and supported by laboratory evaluation of induced chilling injury, it is concluded that the BC disorder is not the result of tissue chilling injury. There appear to be two independent season limiting factors, the BC disorder and seasonal chilling.

Also in chapter 6, there was some circumstantial evidence that high N levels were involved in the development of BC. A field experiment was conducted to evaluate the hypothesis that levels of available minerals, particularly N, modify the incidence of BC. Applied N fertilizer produced significant increases in the incidence and severity of BC. The mechanism by which high N induces BC is unknown, however B deficiency is well known to produce necrotic brown tissue within sweetpotato storage roots. Sensitivity to B deficiency differs amongst cultivars, while high N decreases the uptake of B from the soil and cool temperatures reduce the availability of B. However, the foliar application of B under various N regimes did not affect the incidence of BC. It can be concluded that while N levels have a role inducing BC, modifying the level of available B has no effect.

However, the mechanism by which high N levels induce BC symptoms and the nature of its timing requires further investigation and a novel mechanism is suggested. It is well established that sweetpotatoes differ in their nitrogen fertilizer requirements (Bouwkamp 1985), and can also be differentiated based on the vigour of their vine and leaf production. In cultivars, such as cv. 'Owairaka Red', that naturally produce disproportionately high vine and leaf growth (refer chapter 5), application of high nitrogen levels encourage excessive vine growth and reduce storage root yields

(Bouwkamp 1985; Hartemink et al. 2000). In cultivars, such as cv. 'Beauregard', that produce relatively little vine and leaf (refer chapter 5), the application of N produces higher yields of both roots and vines (Haynes et al. 1967; Jett et al 1996). During the autumn harvest period, both temperature and light levels are falling. Over the harvesting months, March, April and May, average incident monthly radiation (years 1986 to 2001) at the Pukekohe Research Centre decreased from 15.6, to 11.0 and 7.7 MJ m^{-2} respectively. The mechanism suggested would involve BC susceptible cultivars having naturally vigorous vine and leaf growth, further promoted by excessive nitrogen. Under falling radiation levels, as seen during harvest, carbohydrate within the storage root could be re-mobilized to sustain the canopy. Similar responses to stimulated canopy growth have been observed as 'jelly-end rot' in potatoes (Snowdon 1991) and the diversion of photosynthate into vegetative leaf growth rather than storage under late season high N conditions in sugar beet (Mengel & Kirkby 1982). The occurrence of a similar mechanism in sweetpotato is also supported by research suggesting that in some cultivars, when the supply of photosynthates is limiting, a greater proportion is used in stems (Austin & Aung 1973; Martin 1985), especially under shade treatments where canopy growth may be favoured (Oswald et al. 1995a). As seen in chapter 5, 'Owairaka Red' invests more carbohydrate in stem tissue than the other two cultivars studied. Further investigation of sweetpotato BC is required, to evaluate the contributions and relationship of the three factors; susceptible germplasm, high soil nitrogen levels and incident radiation.

Chapter 8

General summary and conclusions

This chapter presents a general summary and conclusions of the research undertaken and reported in preceding chapters. Further research areas are also suggested, extending both from this study and from that reported in scientific literature.

8.1 Germplasm

The sweetpotato is currently represented by a wide variety of land-race, clonally selected and artificially hybridised cultivars, which in turn exhibit a broad range of phenotypes (Plate 3.1). A fundamental premise of modem crop production is that a cultivar specific monoculture allows yield and quality to be maximised through the use of uniform management techniques. In sweetpotato all cultivars are propagated vegetatively, so by definition a cultivar is a clone. A cloned plant is generally considered a faithful genetic duplicate of its source plant. However observations of commercial sweetpotato cultivars at harvest showed a wide range of storage root skin and flesh mutations (Plate 3.2), indicating a high level of intra-clonal genetic heterogeneity. A lack of genetic uniformity and the inability to maintain clear cultivar definition has consequences for efficient sweetpotato crop management and product consistency.

Heterogeneity was ascertained at the genomic level using the AFLP analytical technique (Plate 3.6; 3.7). The predominant New Zealand commercial cultivar is 'Owairaka Red', which is itself reported as being derived by mutation from the historic cultivar 'Waina'. This alleged relationship was supported by the DNA analysis reported here (Fig. 3.1). The historic genetic differentiation of further cultivars by mutation from 'Waina' and more recently from 'Owairaka Red' was also indicated (Fig. 3.3). Under current commercial practice 'Owairaka Red' continues to show intra-cultivar differentiation into distinct genotypes, although they are neither recognised commercially nor distinguished by name.

Statistical analysis of the data showed that the genotypes evaluated could be divided into eight distinct and robust groups or clusters, on the basis of the similarity of their DNA profiles (Fig. 3.1). The *I. plummerae* seedling (Cluster I) and the recently imported genotypes 'Beniazuma' (II) and 'Beauregard' (III) were clearly distinct from other germplasm. The New Zealand cultivar 'Toka Toka Gold' of unknown origin was also clearly distinct, within its own cluster (VII). Of particular interest was the cultivar 'Tauranga Red', putatively a bridging mutant between 'Waina' and 'Owairaka Red'. 'Tauranga Red' formed its own distinctive cluster (VIII) in this analysis, appearing closer to 'Toka Toka Gold' than to any other genotype represented. This degree of dissimilarity from 'Waina' and 'Owairaka Red' would suggest that either historical records of their relationship are incorrect or the source of 'Tauranga Red' germplasm has been compromised. Of further interest are the observed similarities between 'Toka Toka Gold' and 'Tauranga Red' storage root production in the field.

Examining the 'Owairaka Red' clones more thoroughly, a further distinct cluster (IV) included 'Owairaka Pink' and 'Owairaka Red' strain ORC0 (ex Japan curation). On the basis of the AFLP results, these two clones appeared more similar to the recently imported 'Beniazuma' and 'Beauregard' than to commercial 'Owairaka Red'. Another distinct cluster (VI) included two commercial 'Owairaka Red' strains, ORC1 and ORC5. The marked separation of these 'Owairaka Red' clones clearly indicates the high level of heterogeneity currently within the cultivar.

The remaining cluster (V) included a wide range of material related to 'Owairaka Red'. While further statistically robust differentiation of these genotypes was beyond the level of resolution applied in this analysis, differences were observed down to sprouts sourced from the same storage root. If the recognized cultivars 'Gisbome Red' and 'Waina' are used as a standard for cultivar differentiation within this cluster (Fig 3.1), the 'Owairaka Red' strains, ORC4 and ORC8 appear to separate out as a group at the same level of similarity (Fig 3.2).

While it has been established that significant genetic drift has occurred, the natural unimpeded rate of drift is uncertain without further high resolution analysis of sprouts sourced from the same root. Banding differences were observed amongst the small number of sprouts included here (S1 to S5), however four years of deliberately applied

field selection pressure (C1 to C5) failed to produce strongly differentiated clones (Fig 3.1).

In the USA, 'Beauregard' is considered to be relatively stable. Locally, samples of 'Beauregard' from the same initial source were maintained separately in either continuous tissue culture or commercial field production for ten years. Within the limited sample examined here, 'Beauregard' from tissue culture differed little from that propagated within a commercial production system. However, storage root mutants are commonly observed within commercial 'Beauregard' crops, but are removed at harvest.

8.2 Plant establishment

A sweetpotato cultivar is primarily selected for storage root production based on market requirements, the crop management tools available and the environment in which it will be grown. Once selected, the initial stages of production are plant propagation and field establishment, which have the potential to introduce high levels of variability into the crop. If the propagules are morphologically, physiologically, and genetically consistent throughout the establishment phase, a more uniform crop may result.

A number of treatments were applied to evaluate their effect on plant establishment (Table 4.1). Plants treated with anti-transpirants or fertilizer starter solutions did not differ in plant weight from the commercial control (Table 4.2). At the initial harvest, small four node transplants produced significantly reduced total plant weights, with lower leaf weights, pencil root weights and pencil root numbers relative to commercial six node sprouts (Table 4.2). Varying the number of buried nodes made no significant difference to the plant growth rate, relative to the commercial control. These smaller plants never caught up with the growth of the commercial control plants (Table 4.3).

At the first harvest, Plug and Held-2 (6 day holding) treatments both produced total plant weights significantly greater than the commercial control (Table 4.2). The greater total root weight in the Plug, Held-2 and Sand-2 (6 day) treatments, relative to the Control, demonstrated efficient plant establishment. The significantly higher storage root weights in the Sand-2 and Sand-3 (9 day) treatments showed an improvement in

plant establishment, but the Plug and Held-2 treatments produced increases in both storage root weight and number, demonstrating a greater storage root sink potential. The significant growth increases in both of the 6-day storage treatments (Held-2 and Sand-2), but not for the 3 or 9-day storage periods, suggests an optimal holding period. While these treatments showed growth gains relative to the commercial control at the first harvest, they had lost those gains by the second harvest (Table 4.3), possibly due to seasonal conditions.

The use of plugs for transplanting potentially allows the integration of material with low genetic variance through nodal propagation, incorporation of virus free material and robust mechanical field establishment. However, if roots formed within the plug are forced to coil, it is important to produce storage roots outside the plug volume following transplanting (Plate 4.2). By lignification of the coiled plug roots (Plate 4.3), only unlignified extensions from these roots (Plate 4.4) developing outside the plug volume have the capacity for a storage function.

8.3 Carbohydrate partitioning

The sweetpotato crop is normally harvested when the production of premium-sized roots is maximised, however in the future root quality will become increasingly important. The dry matter content and sugar concentration of storage roots are fundamental elements of sweetpotato texture and flavour that vary during root development. Storage root quality was assessed over the root bulking period, against the background of carbohydrate partitioning amongst plant organs and changing root size distribution. Based on previous work, the field trial was established using plug transplants to maximise plant uniformity.

Although 'Owairaka Red' produced the largest plants it had the lowest harvest index. 'Beauregard' and 'Beniazuma' produced similar high harvest indices. While 'Owairaka Red' had a similar amount of leaf tissue to 'Beniazuma', it placed a greater dry matter investment into stem tissue (Fig. 5.7). 'Owairaka Red' was the only cultivar to produce proportionally more stem than leaf tissue. Dry matter accumulation in 'Owairaka Red' stems continued throughout storage root development. 'Beauregard' partitioned

equivalent amounts of photosynthate into leaf and stem tissue (Fig. 5.11) while 'Beniazuma' allocated almost twice as much dry matter into leaves than stems (Fig. 5.10). 'Beauregard' partitioned significantly less dry matter into the canopy (leaf and stem tissue) than either of the other cultivars. For 'Beniazuma' and 'Beauregard' the total amount of dry matter in leaf tissue remained almost constant throughout a considerable period of storage root development (Fig. 5.8). Relatively stable cultivar specific ratios of leaf to stem tissue (dry weight) were maintained throughout storage root enlargement.

The rate of dry matter accumulation in the roots of 'Owairaka Red' was higher than in the other cultivars. While 'Beauregard' had a relatively low root dry matter content (Fig. 5.17), fresh weight yield was high. Cultivar commercial root grade distributions differed over time. In 'Beauregard' and 'Owairaka Red', oversized roots were initially produced by 99 DAT (Fig. 5.14, 5.15) and the premium grade decreased with increasing oversized roots from 120 to 127 DAT. In contrast, 'Beniazuma' (Fig. 5.16) produced long thin storage roots and oversized roots only appeared by the last harvest (134 DAT).

As the growing season progressed the storage root dry matter content increased in a linear manner, at a cultivar dependent rate (Fig. 5.17). The cultivar 'Beniazuma' produced roots with a relatively higher dry matter content throughout the harvested period. The rates of root dry matter content accumulation were similar for 'Beniazuma' and 'Owairaka Red'. While 'Owairaka Red' and 'Beauregard' had similar root dry matter contents in the early harvests, 'Owairaka Red' demonstrated a more rapid increase in dry matter content.

In contrast to the other cultivars, the total sugar concentration of 'Owairaka Red' roots did not show a significant trend over time (Fig. 5.18). 'Beniazuma' and 'Beauregard' both showed significant linear increases in total sugar concentration with time, at a common rate. The sugar components (sucrose, glucose, and fructose) in raw 'Owairaka Red' and 'Beauregard' roots were comparable throughout their growth. Sucrose was the predominant sugar in all three cultivars. 'Beniazuma' was unusual in producing very little fructose and glucose, while maintaining high levels of sucrose (Fig. 5.19).

8.4 Season limitations

The sweetpotato is a tropical or subtropical perennial plant. In tropical climates sweetpotato may be grown throughout the entire season, so that vine cuttings from a well established crop may be used to plant a new crop concurrently. As shown previously, plant and storage root attributes appear to change in a steady manner throughout root bulking. Sweetpotato is cropped seasonally as an annual in temperate climates so as to maximise yield while avoiding chilling temperatures. However roots may develop an internal physiological disorder called 'brown centre' (Plate 6.1), that restricts the length of the growing season before the onset of theoretically chilling temperatures. In New Zealand, the onset of the disorder appears to occur regularly from mid-April.

Brown centre disorder was found to occur in sweetpotato sourced from diverse international sources; Asia, North America and the Pacific. However the New Zealand cultivar 'Owairaka Red' was particularly consistent in developing the disorder. Temperatures recorded in the field gave no evidence of a relationship between chilling and the occurrence of brown centre (Fig. 6.6, 6.7).

Harvested 'Owairaka Red' storage roots were chilled to test if their tissue was particularly sensitive to low temperature (Table 7.2), however the chilling reaction occurred at temperatures similar to those reported elsewhere (Table 7.3) and the symptoms did not entirely match those seen in brown centre.

Evidence from commercial fields suggested that brown centre could be associated with high levels of soil nitrogen (Table 6.3). Previously low boron levels have been associated with symptoms similar to brown centre. The effect of supplying high levels of nitrogen and boron were tested in the field. While boron did not produce a response (Table 7.11), the addition of nitrogen significantly increased the incidence of brown centre (Table 7.12).

It was suggested that in susceptible cultivars a combination of falling autumn light levels and excessive canopy growth (stimulated by high soil nitrogen levels) combine to

change the storage root's role from a net carbohydrate sink to that of a carbohydrate source.

8.5 Integrated storage root production

Plant growth and development, as it is realized, represents the interplay of genetic and environmental factors. Because the components of storage root production are highly integrated, optimisation of the process requires a holistic approach encompassing sequences of temporal events and spatial differences. As established, sweetpotato has a level of genetic ambiguity that contributes to the complexity of accurately interpreting the process.

The 'Owairaka Red' strains represent a genetic pool selected for both high vine production to assist propagation as well as for storage root production. The distinctiveness of 'Owairaka Red' established at a genetic level, was confirmed by the development of unusually high dry matter partitioning into stem tissue throughout the season and a relatively low harvest index. The development of potentially competing sinks, stems and storage roots, appeared to contribute to the occurrence of the brown centre disorder under conditions favourable to vine growth.

The cultivar 'Beauregard' was selected for the USA market and produced distinctive sweet orange flesh roots, that while globose in shape were of relatively low dry matter content. 'Beauregard' had a particularly high net assimilation rate during early storage root bulking, possibly due to the reported correlation between small leaf size and high net photosynthetic rate. 'Beauregard's canopy was also fairly shallow relative to the other cultivars, so there may have also been less mutual leaf shading. While the amount of dry matter invested in leaves plateaued over most of the storage root bulking period, net assimilation rate declined, suggesting an aging leaf population with lower photosynthetic efficiency under conditions of reducing incident radiation.

'Beniazuma' was a distinctive Japanese selection with high storage root dry matter content. The roots were uniformly long and slender in shape compared to those of 'Owairaka Red' and 'Beauregard'. Roots shape is determined by the activity of the

cambia, with uniformly thickened roots increasing in girth primarily through the activity of the normal vascular cambium rather than that of the anomalous cambia.

8.6 Future research

8.6.1 Germplasm

It has been well established that sweetpotato has a high mutation rate, as observed through changing phenotype (Edmond & Ammerman 1971). In the work reported here, the level of underlying genetic heterogeneity within a cultivar was assessed at the genomic level. This work concurred with reports suggesting that somatic mutations are continually appearing within any given sweetpotato cultivar (Villordon & La Bonte 1995) but modified attributes are not always discernable in the phenotype.

Further work is required to establish the economic consequences of this genetic drift, to gauge the value of investing in its resistance. The sweetpotato vegetative propagation system tends to promote the accumulation of both mutations and virus titre. These two confounded effects need to be separated in order to study the contribution of mutation to clonal decline (La Bonte et al. 2000). Recent surveys of New Zealand sweetpotato virus incidence and level have been reported elsewhere (Fletcher et al. 2000), however the direct effect of viral load on crop yield and quality has yet to be assessed. A further complication for this work is the potential interaction of virus titre with mutation rate, through increased plant stress. Although the use of careful seed root selection (Wilson & Averre 1989) and sweetpotato nodal cuttings (Villordon & La Bonte 1996) have been suggested as a means to slow genetic drift, research into more definitive methods of genetic conservation are required.

8.6.2 Plant establishment

Plant establishment is a crucial area for further international sweetpotato research (Kozai et al. 2000) due its disproportionate contribution to production costs and the need for integrated genetic, pathological, and physiological studies. Plant establishment is particularly important under the seasonal time constraints of temperate conditions (Ahn et al. 2000).

Delayed planting of sprouts and use of plugs appear to offer two areas for further investigation. In both treatments, the rate of root lignification with varying water availability should be explored further (Nakatani 1993). For delayed planting, the optimum sprout storage time and conditions are yet unrefined, with the suggestion that storage in the dark may be more effective than under light conditions (Dr. L. Zhang, Shandong Academy of Agricultural Sciences, pers. comm.). For plug grown plants, the optimum growing period, conditions, plug shape and volume (Islam et al. 2002) require further investigation. Although the ability to maintain early growth advantages appears to vary with the season, the underlying cause of this variation has yet to be understood.

8.6.3 Carbohydrate partitioning

Sweetpotato carbohydrate partitioning over the period of storage root bulking appears to be adequately described by cultivar specific mathematical functions. The plant's contribution to storage root growth is maintained throughout bulking while stem and leaf growth plateau. Relative carbohydrate partitioning between the leaf and stem components remain constant during bulking. As storage roots continue to accumulate photosynthate, their diameter increases in a cultivar specific manner, producing changes in root grade distribution. Root quality was assessed by measuring dry matter content and sugar concentration. Root dry matter content increased in a linear manner over time, at a cultivar dependent rate. While root total sugar (sum of sucrose, glucose, and fructose) concentration increased over time for two cultivars, it remained constant in 'Owairaka Red' cultivar. The ratio of fructose to glucose remained relatively constant across cultivars, but a closer examination showed cultivar specific differences in how this ratio was achieved.

While the work reported here contributes to knowledge on predicting general aspects of storage root development, further investigation is required to produce improved growth models capable of forecasting root yield and quality across management practices and environments (Hill & Bonsi 1992). Initial requirements are for data that allow prediction of storage root development under extremes of water stress, flood and drought conditions. As much sweetpotato production occurs in low lying locations

without a ready supplementary water supply, the ability to anticipate crop responses would assist in crop management decisions.

8.6.4 Season limitations

The brown centre disorder has a significant impact on sweetpotato production in New Zealand. Produce affected by the disorder show no external symptoms, so can not be removed during grading. To allow scheduling of harvest dates prior to mid-April, crops of susceptible cultivars must be established early in the season. It is also recommended that susceptible cultivars are not grown under high nitrogen levels.

Further investigation is required to test the relative contributions of the three factors; susceptible germplasm, high soil nitrogen levels and incident radiation. While commercially significant, brown centre symptoms usually occur at relatively low levels throughout a given crop. Large field trials are therefore still required to ensure brown centre occurs at statistically significant levels. The initial requirement is for a replicated field trial of cultivar 'Owairaka Red' with factorial combinations of supplied nitrogen and artificial shading treatments. The level of brown centre incidence and changes in carbohydrate partitioning under these treatment combinations would further our understanding of the disorder.

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Appendix 1

Soil analysis

Soil analysis methodology courtesy of R. J. Hill Laboratories Ltd, Hamilton, New Zealand, who conducted the various tests. Samples were prepared for analysis by being air dried at 35°C for two days (residual moisture typically 4%) and crushed through a 2 mm screen.

Test	Extraction/Digestion	Determination
pH	1:2 (v/v) soil:water slurry	Potentiometrically using a pH electrode.
Phosphorus	Olsen extraction	Molybdenum Blue colorimetry.
Extractable cations - Mg, Ca, K, Na	1 M Neutral ammonium acetate extraction	Atomic absorption (Mg, Ca) and atomic emission (K, Na).
Exchangeable cations	None	Summation of extractable cations (K, Ca, Mg, Na) and the acidity determined from the change in pH of the cation extraction solution.
Base saturation	None	Calculated from extractable cations and exchangeable cations.
Volume weight	None	The weight/volume of dried, ground soil.
Available nitrogen	Anaerobic incubation, followed by ammonium-N extraction using 2M KCl.	Berthelot colorimetry
Boron (hot water soluble)	0.01 M Calcium chloride extraction.	Azomethine-H colorimetry

Chapter 6 Season's end: storage root necrosis
Chapter 7 Storage root: chilling and nutrition responses

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