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Investigation into the causes of brown centre in sweetpotato

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1 Executive summary

The objective of this investigation was to identify the conditions that produce the disorder brown centre (BC) in sweetpotato. The disorder has been on record in New Zealand for almost 50 years, but previous efforts to isolate the cause have been unsuccessful. Further, there appears to be no international experience of this disorder.

BC occurs predominantly in the most commonly grown New Zealand sweetpotato cultivar, Owairaka Red. It is found within sweetpotato roots at harvest, but the disorder does not progress during storage. There is generally little external sign that a root has BC, and the symptoms are only seen when the root is cut open. When looking for BC, the root should be cut along its main axis, as transverse sections may miss damaged tissue. Roots with BC contain areas of brown necrotic tissue, which vary in size and distribution. This brown tissue does not soften on cooking. It has been established by previous researchers that harvesting the crop before mid April may avoid the disorder.

The incidence of the brown centre disorder appears to increase at high fertility sites. Evidence suggests that it is related to plant nutrition and possibly growth rate.

This investigation included eight studies.

- It was established that BC is not pathological (Study 1).
- Under conditions that induce BC, its occurrence and severity are cultivar dependent (Study 2).
- Analysis of root tissue with BC symptoms and soils that produce BC suggest that the disorder is associated with sites of high fertility (Study 3).
- The 1996/97 season produced particularly high levels of BC at Dargaville. However, air temperatures and rainfall within that season were similar to the long term mean (Study 4).
- The effect of chilling temperatures on harvested sweetpotato roots was investigated. While similar symptoms to BC were produced they did not exactly match those of field BC in magnitude or appearance (Study 5).
- Sweetpotato plants grown in the field at the Pukekohe Research Centre produced roots that exhibited BC without exposure to chilling temperatures (Study 6).
- A glasshouse experiment tested various sources of plant stress, including water-logged soil, but did not produce BC symptoms (Study 7).
- A field trial established that applications of boron fertiliser did not mitigate the symptoms. However, adding nitrogen late in the season doubled the incidence of BC (Study 8).

This project produced evidence based on tissue and soil analysis, as well as field experimentation, that the incidence of BC is exacerbated by fertile growing conditions. Further research is required to confirm this relationship and to define critical nutrient levels.

Based on current evidence it is suggested that in highly fertile fields the risk of BC might be lowered by initially growing crops other than sweetpotato or by growing a sweetpotato cultivar resistant to BC. If the sweetpotato cultivar Owairaka Red is planted in fertile fields, it should be harvested before mid April. Once the fertility is lowered, Owairaka Red crops grown in subsequent years should be less subject to BC. While it cannot be assumed at this stage that BC occurrence is solely the product of nutrition and the plant's growth rate, inter plant spacing should not be increased on fields at risk.

2 Introduction

This study examined the pathology of the disorder, and the effects of plant physiological stress and nutrient status on its occurrence and severity.

The New Zealand sweetpotato (kumara) industry is dominated by the clone Owairaka Red, which was released as a commercial cultivar in 1954 (Lewthwaite 1997). 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 mentioned in New Zealand in 1955 when it was described as 'brown heart' (Gillard 1955). 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 (Plate 1). 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. No specific cause of the 'brown centre' disorder could be identified, but it was recommended that harvesting be completed by mid-April.

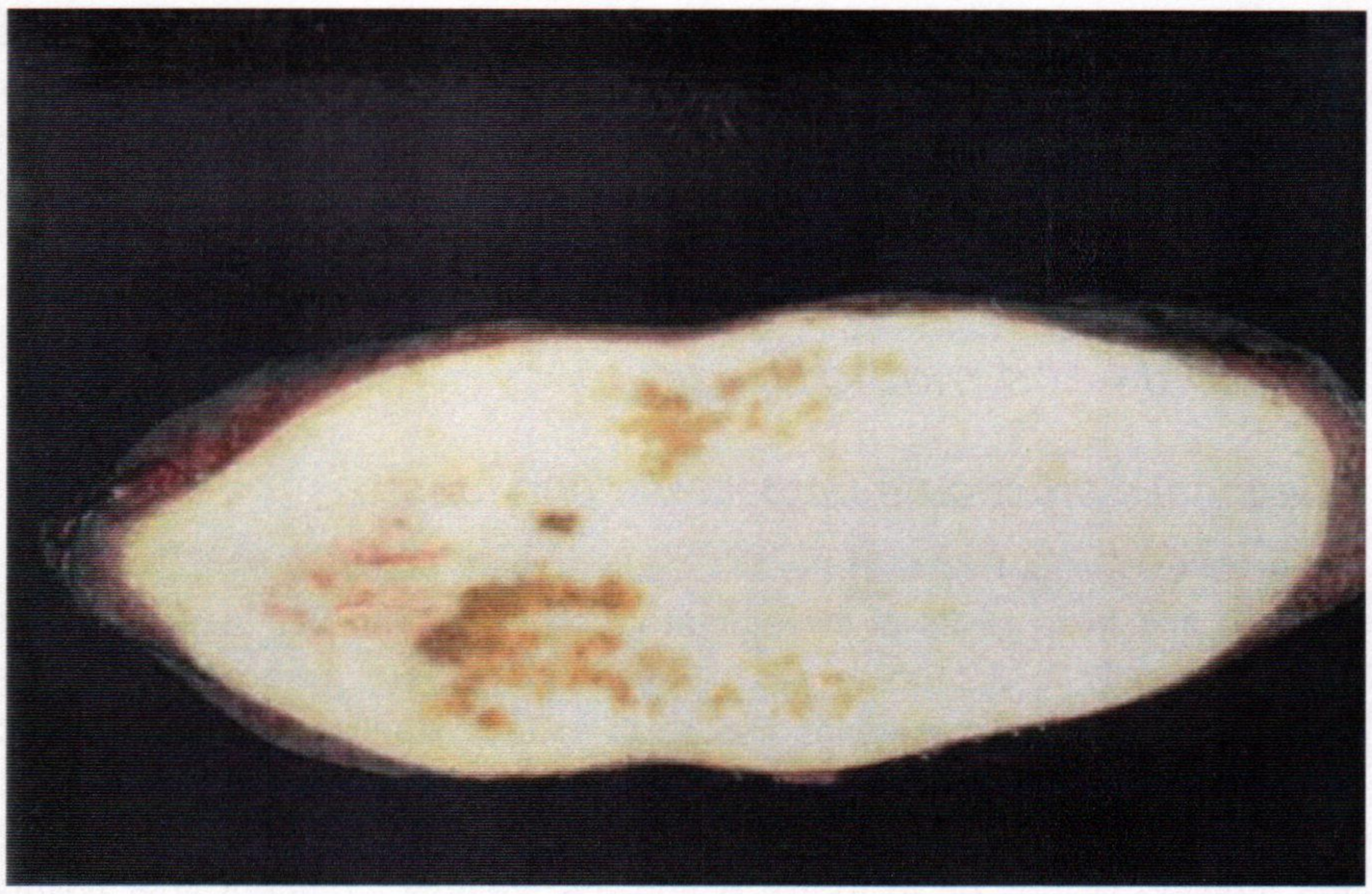

Plate 1: Brown centre symptoms within a sweetpotato storage root, cv. Owairaka Red (longitudinal section).

The disorder still remains a problem to the industry, and was especially severe in the 1996/97 season. It is estimated that about 2% of the crop was affected in that season (A de Bruin 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. In summary, New Zealand 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 BC in the Owairaka Red sweetpotato from three main perspectives: pathological, physiological stress and plant nutrition.

3 Study 1: Pathogen analysis

3.1 Introduction

Although early research did not find a pathogen associated with the occurrence of BC in New Zealand (Nielsen & Harrow 1966; Wood & Schappi 1984), international experience shows that infection by pathogens may produce symptoms similar to BC in sweetpotato roots (Clark & Moyer 1988). The pathogens require favourable environmental conditions and a suitable substrate to produce such symptoms. The interaction of these factors might

explain the consistent timing (mid to late April) of BC development in the sweetpotato crop, when temperatures are cooling and storage root development is well advanced. It is also important to establish whether the BC described before the 1996/97 season is consistent with the type seen in recent years.

3.2 Materials and methods

3.2.1 Diagnostic procedure for bacteria and fungi

Roots of Owairaka Red with the BC disorder were obtained from Dargaville. Stained and fresh slices of sweetpotato root tissue with BC were examined microscopically to determine if bacteria or fungi were associated with the areas of brown, corky material. Pieces of surface sterilised and unsterilised sweetpotato tissue were placed on Prune agar to culture any fungi from the affected tissues. Bacterial cultures were prepared by streaking exudates from the affected tissues on to King's B and 5% sucrose - nutrient agar culture medium.

3.2.2 Diagnostic procedure for viruses

Storage roots with BC were examined, then samples of necrotic tissue were mechanically inoculated onto *Chenopodium quinoa*, *Pisum sativum, Cucumis sativa*, and *Gomphrena globosa*. The sweetpotato roots were then sprouted and growing plant tissue was serologically tested for a range of sweetpotato viruses using a serological DIBA (dot immuno-binding assay).

3.3 Results and discussion

A pathogen was not found to be associated with the occurrence of brown centre so it is not considered to be a disease.

The microscopic examination for bacteria or fungi associated with the necrotic material was inconclusive. None of the cultures for fungi or bacteria yielded any disease organisms. No virus symptoms were detected by inoculation onto indicator plants. Sweetpotato latent potyvirus (SPLV) was found with the DIBA. However, this virus is generally considered symptomless in sweetpotato.

There was no direct evidence of the involvement of a fungi, bacteria or virus in the development of BC in the New Zealand sweetpotato cultivar Owairaka Red. As no pathogen was detected and the physical description of the disorder and its time of occurrence matches that recorded by Gillard (1955), we conclude that the BC disorder seen in recent years is consistent with that found prior to 1996/97.

4 Study 2: Genetic effects

4.1 Introduction

The sweetpotato cultivar Owairaka Red is susceptible to BC, but it is not known if other cultivars develop the symptoms. Commercial New Zealand sweetpotato production has been very reliant on cultivars closely related to Owairaka Red. The cultivar Waina was introduced into New Zealand in the 1850s, and by 1913 was grown more widely than any other cultivar (Berridge 1913). By 1925 Waina was almost the only cultivar used for a general crop (Best 1925). By 1948 the two main sweetpotato cultivars grown in New Zealand were the Waina mutants, Tauranga Red and New Zealand Pink (Gillard 1948). In 1954 the cultivar Owairaka Red, a Tauranga Red mutant, was released and by 1969 commercial production consisted entirely of Owairaka Red. At present, about 80% of the crop consists of Owairaka Red (Lewthwaite 1997). Waina and its mutants are important cultivars that have been grown commercially for over 100 years, but it is not known if all early versions of the cultivar developed similar levels of BC. It seems likely that they were all susceptible because a selection of Owairaka Red strains from commercial growers in different districts all developed BC (Wood & Schappi 1984). In this study we evaluate sweetpotato clones unrelated to Waina or its derivatives for susceptibility to BC.

Under conditions that induce brown centre, its occurrence and severity are cultivar dependent.

4.2 Materials and methods

Crop & Food Research conducts a sweetpotato breeding program from the Pukekohe Research Centre. A cultivar trial was planted at Pukekohe on 16 December 1997, with 16 cultivars arranged in a modified alpha design containing three replicates (Williams & John 1989). The trial was harvested on 16 April 1998, when roots were cut lengthwise to estimate the incidence of BC (presence/absence). The following season a cultivar trial was planted on 7 December 1998 and harvested on 24 May 1999. This trial was harvested late in the season to optimise the development of BC. Harvested roots of the three cultivars Owairaka Red, Northland Rose and clone 11/19 were similarly cut lengthwise to estimate the incidence of BC.

4.3 Results and discussion

In the 1997/98 season, three clones developed BC symptoms. The incidence of BC in these roots was, Owairaka Red (5.7%), Northland Rose (3.8%) and clone 11/19 (10.0%). Roots of the commercial cultivars Toka Toka Gold, Beauregard and Landtec did not contain any BC symptoms. In the 1998/99 season only Owairaka Red had any BC symptoms (2.4%); none were found in Northland Rose or clone 11/19. Northland Rose (formerly 93N9/2) is currently undergoing evaluation for commercial release and was selected from true seed supplied by the Asian Vegetable Research and Development Centre, Taiwan. Clone 11/19 was selected from true seed supplied by the University of Louisiana, USA. Hand dug plants of Owairaka Red in the

1998/99 season demonstrated that plants with BC appeared sporadically across the field, rather than in groups. For any individual plant affected by BC, some storage roots showed the symptoms while others on the same plant remained completely sound.

BC develops in sweetpotato clones sourced internationally and unrelated to the most widely grown New Zealand cultivar, Owairaka Red. However, Owairaka Red seems particularly susceptible to BC, which may explain the local industry's problem with BC.

5 Study 3: Root tissue and soil analysis

5.1 Introduction

Analysis of root tissue with brown centre symptoms and soils that produce brown centre suggests that the disorder is associated with sites of high fertility.

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). Plant tissue and soil tests are important diagnostic tools in establishing nutrient disorders, so samples were collected from Dargaville for analysis.

Materials and methods

BC-affected roots and sound roots from adjacent sweetpotato plants were obtained from Dargaville (courtesy of A de Bruin). Five sound roots and five affected roots were analysed for tissue macro and micro nutrient levels using established procedures. The tissue of roots with BC symptoms was further divided into sound and discoloured portions for separate analysis. All root tissue was oven-dried at 60°C. The relative weights of the oven-dried portions of BC roots were recorded to allow the nutritional composition of whole roots to be calculated. Soil samples were collected from two Dargaville sites where BC roots were consistently produced (Site 1 sampled June 1998; Site 2 sampled December 1998).

5.3 Results and discussion

Table 1: The nutritional analysis of sweetpotato cultivar Owairaka Red storage roots grown at Dargaville in the 1997/98 season (dry weight basis).

		Roots without BC symptoms					Roots with BC symptoms				
		Root number					Root number				
Element	Units	1	2	3	4	5	1	2	3	4	5
Nitrogen	%	0.8	0.8	1.0	0.8	1.0	0.9	0.8	0.8	0.8	0.9
Phosphorus	%	0.17	0.19	0.20	0.20	0.21	0.18	0.18	0.18	0.19	0.21
Potassium	%	1.8	1.8	1.7	1.9	1.8	1.8	2.1	2.0	2.2	2.1
Sulphur	%	0.06	0.08	0.09	0.08	0.08	0.07	0.08	0.09	0.09	0.08
Calcium	%	0.07	0.07	0.07	0.11	0.07	0.08	0.06	0.05	0.07	0.10
Magnesium	%	0.1	0.11	0.12	0.12	0.1	0.09	0.11	0.10	0.09	0.11
Sodium	%	0.05	0.05	0.07	0.07	0.09	0.07	0.05	0.06	0.05	0.07
Iron	μg/g	30	31	35	32	38	40	31	31	27	35
Manganese	μg/g	22	39	39	38	27	21	33	27	26	26
Zinc	μg/g	12	13	16	16	15	13	28	14	15	14
Copper	μg/g	8	8	9	9	9	9	7	8	8	9
Boron	μg/g	6	9	9	9	7	5	8	7	8	7

Table 2: Soil characteristics of two BC-producing sites near Dargaville. Site 1 sampled in June 1998, Site 2 sampled in December 1998.

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
Cation exchange capacity	me/100 g	32.8	38.7
Base saturation	%	75	84
Volume weight	g/ml	0.84	0.90
K/Mg ratio		0.2	0.2
Available nitrogen	kg/ha	374	190
Boron	µg/g	1.6	1.8

In the root tissue tests (Table 1), only potassium showed significant differences between BC and sound roots. Potassium is the most common nutrient 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% ($P = 0.040$). The soil tests (Table 2) showed no deficiencies 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. However, available nitrogen levels seem particularly high at these BC-producing sites compared to non BC-producing sites.

6 Study 4: The 1996/97 season - Dargaville

6.1 Introduction

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. The 1996/97 season at Dargaville produced particularly high levels of BC, so average rainfall and air temperatures for that season were compared with historical records.

6.2 Materials and methods

The 1996/97 season produced particularly high levels of brown centre at Dargaville. However, air temperatures and rainfall within that season were similar to the long term mean.

The National Institute of Water and Atmospheric Research Ltd (NIWA) supplied rainfall and air temperature data from the Dargaville meteorological site. However, soil temperatures have not been recorded there since 1988. In the 1997/98 and 1998/99 seasons Crop & Food Research staff recorded air, soil surface and sub soil temperatures at hourly intervals within Dargaville sweetpotato crops to establish the relationship between air and soil temperatures.

6.3 Results and discussion

The monthly rainfall over the harvest period at Dargaville in the 1996/97 season (Fig. 1) was similar in quantity and distribution to the long term average. The mean monthly air temperature (Fig. 2) and the mean daily minimum air temperature (Fig. 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 the long term average (6.1°C, March; 3.7°C, April and 1.6°C, May - recorded since 1943).

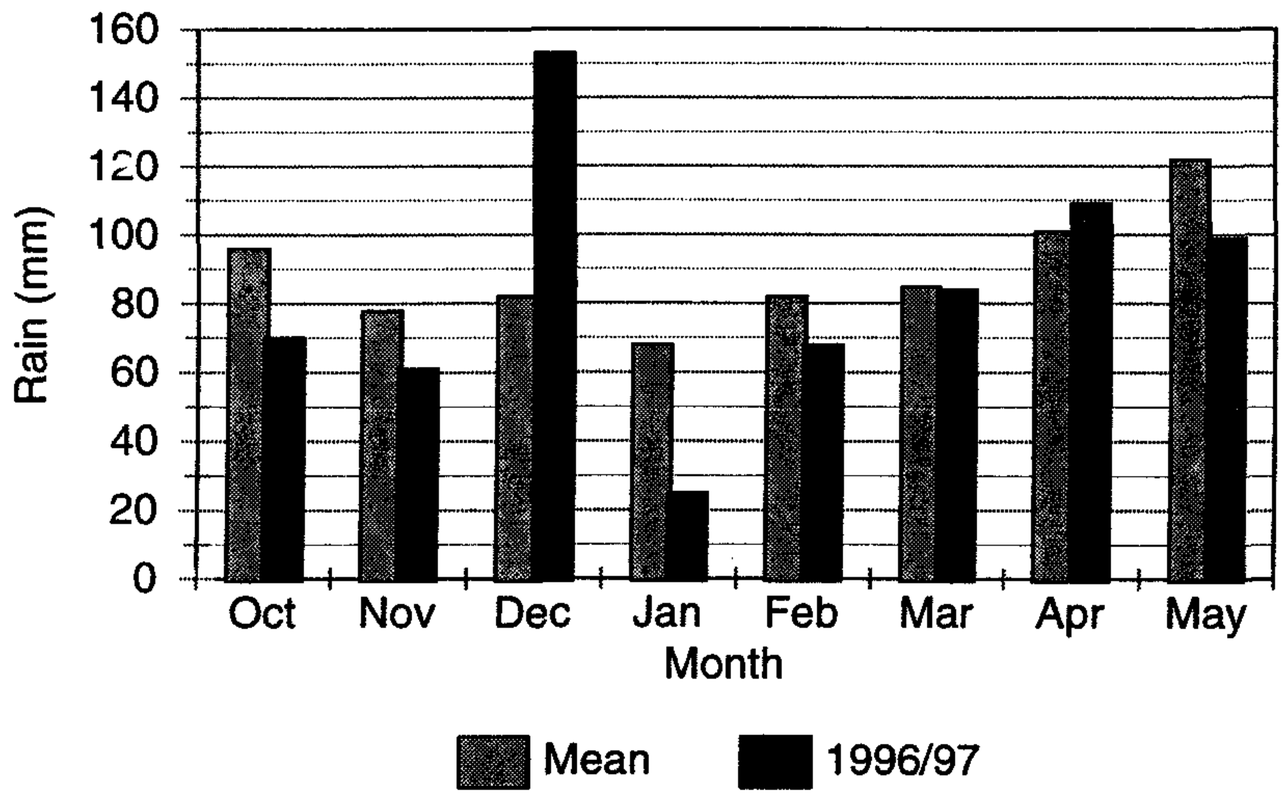

Figure 1: Monthly rainfall (mm) at Dargaville over the 1996/97 growing season compared with the long term mean (recorded since 1943). Data supplied by NIWA.

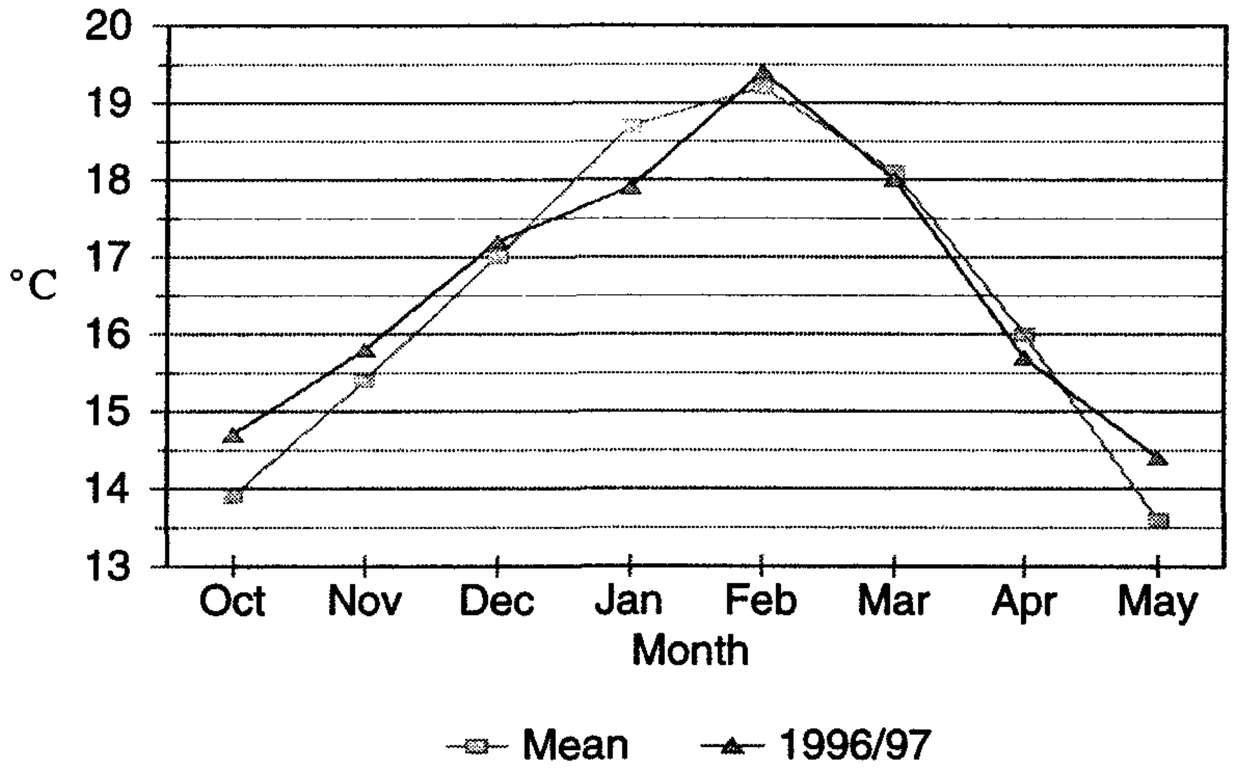

Figure 2: Monthly mean air temperature (°C) at Dargaville over the 1996/97 growing season compared with the long term mean (recorded since 1943). Data supplied by NIWA.

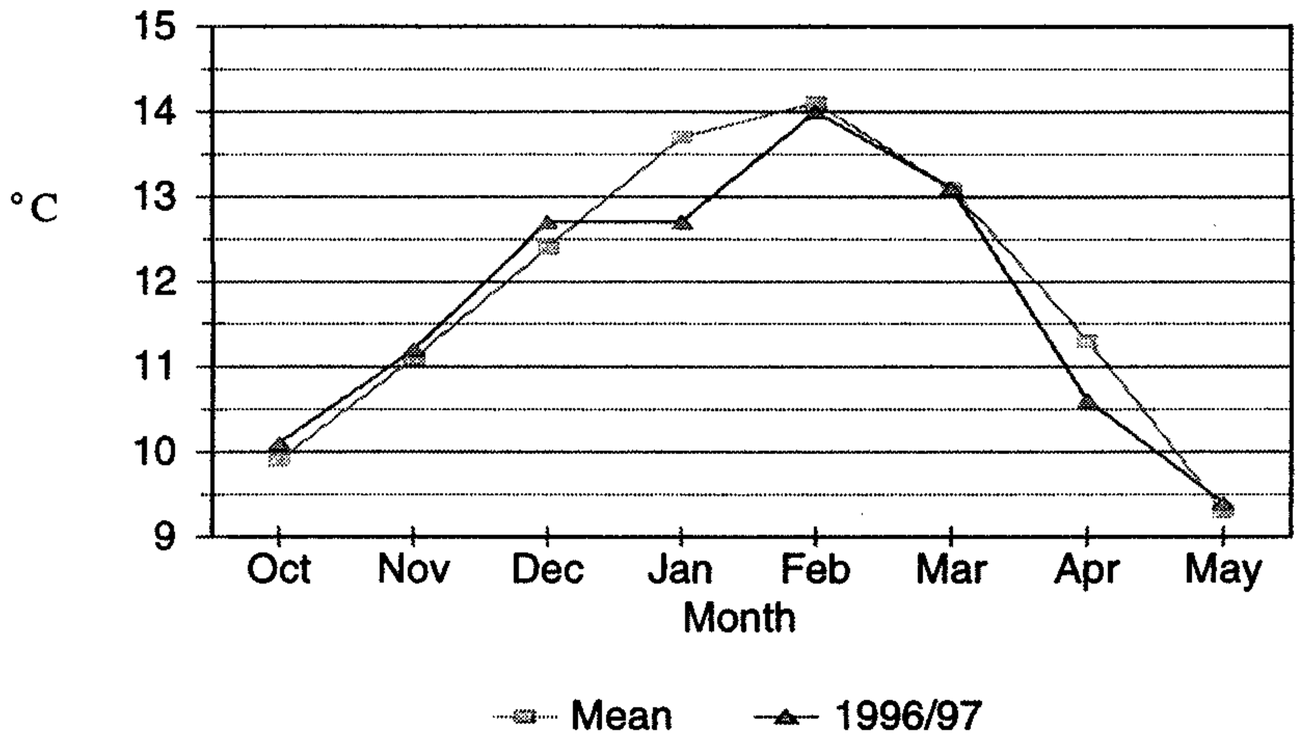

Figure 3: Mean daily minimum air temperature (°C) each month at Dargaville over the 1996/97 growing season compared with the long term mean (recorded since 1943). Data supplied by NIWA.

The average Dargaville monthly soil temperatures approximated air temperatures early in the 1998/99 season when the soil surface was bare (Fig. 4), but as the leaf canopy developed soil temperatures more closely followed those at the soil surface. The leaf canopy had a buffering effect on temperatures at the soil surface (Fig. 5). Beneath the soil, temperatures were much more stable than in the air or at the soil surface and showed little diurnal fluctuation.

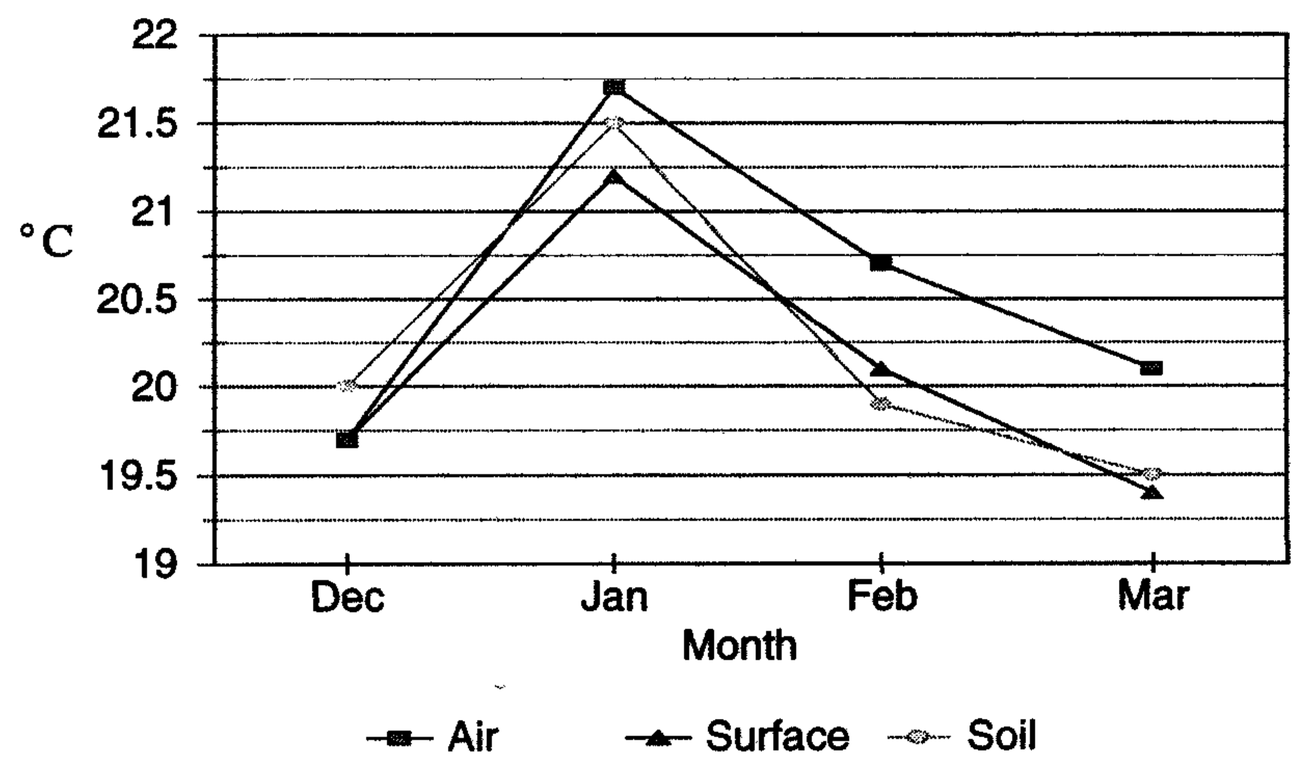

Figure 4: Monthly mean temperatures (°C) at Dargaville over the 1998/99 growing season. Temperatures were recorded at one-hour intervals within a sweetpotato crop, in the air (1.3 m above the ground), on the soil surface (sub canopy) and in the soil (10 cm beneath the crest of the mould).

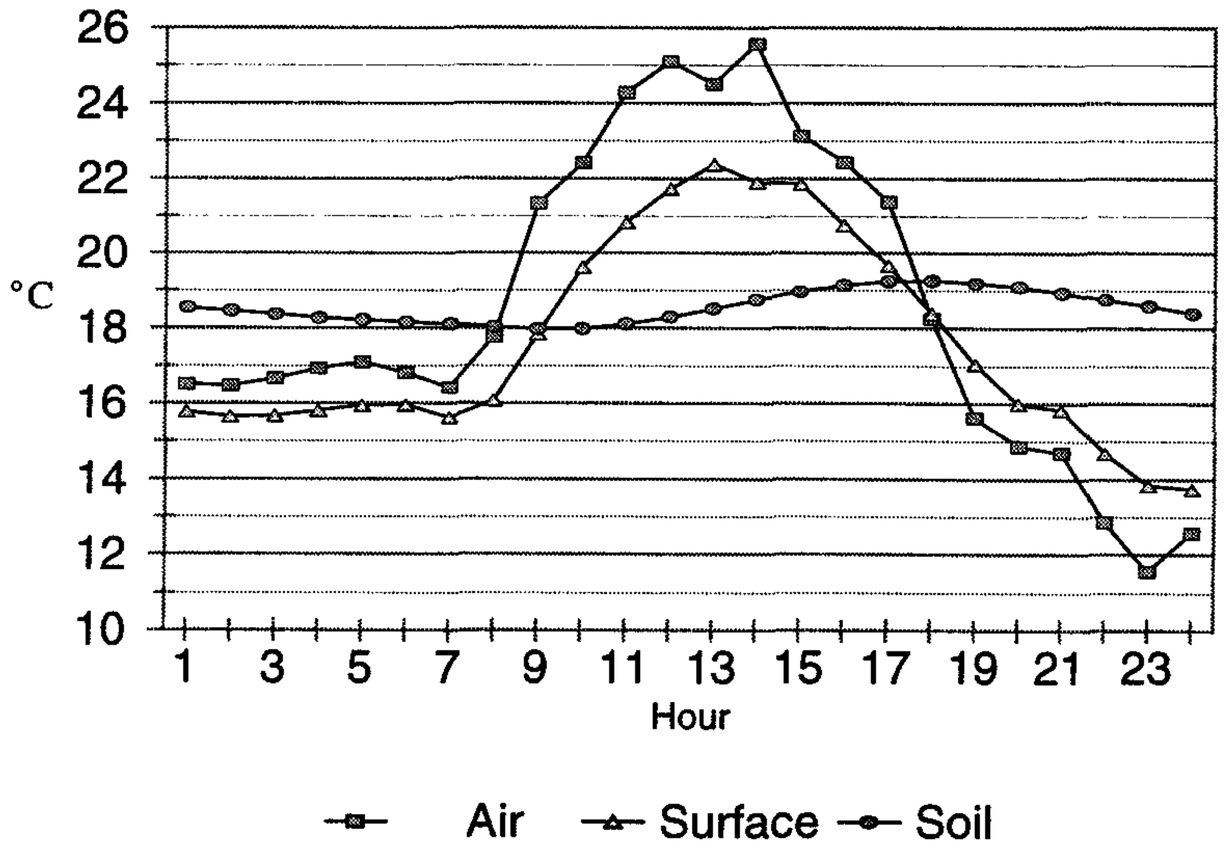

Figure 5: The temperature at one-hour intervals within a sweetpotato crop over a 24-hour period, in air (1. 3 m above the ground), on the soil surface (sub canopy) and in the soil (10 cm beneath the crest of the mould) at Dargaville on 10 April 1999.

The 1996/97 season produced storage roots of the sweetpotato Owairaka Red with abnormally high levels of BC (*c.* 2%). However, data from the Dargaville meteorological site do not suggest that the region underwent 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. Data from a sweetpotato crop (1998/99) showed large diurnal fluctuations in air temperatures while soil temperatures beneath the leaf canopy remained relatively steady. Storage roots of the cultivar Owairaka Red are generally produced well within the soil 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).

7 Study 5: Chilling injury

7.1 Introduction

Chilling injury is a physiological defect in plants of tropical and subtropical origin that results in reduced produce quality though exposure to low but nonfreezing 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). Horticultural products that are sensitive to low temperatures include avocado, banana, citrus, cucumber, mango, papaya, peach, pepper, pineapple, squash, sweetpotato and tomato. 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).

While similar symptoms to brown centre were produced following periods of chilling they did not exactly match those of field brown centre in magnitude or appearance.

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 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).

This study identifies the temperatures and exposure times that cause chilling injury in harvested roots of the sweetpotato cultivar Owairaka Red. 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.

7.2 *Materials and methods*

Four experiments were performed in this study to investigate the effect of chilling on harvested Owairaka Red sweetpotato roots:

- **Experiment 1:** Comparison of hardcore levels in field-grown sound roots and those with BC,
- **Experiment 2:** Evaluation of BC production under a range of chilling temperatures and durations,
- **Experiment 3:** Evaluation of BC production under medium chilling temperatures for long durations,
- **Experiment 4:** Evaluation of BC production under conditions of intermittent chilling.

7.2.1 *Experiment 1 - hardcore levels in field-grown BC and BC-free roots*

Eight sound Owairaka Red roots and eight roots with BC symptoms were selected from the same field at Dargaville in the 1997/98 season (courtesy of A de Bruin). The roots were cut in half along their length. Roots with BC were scored for the percentage of their cut surface showing brown tissue (0, no BC; 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 then placed in individual nylon mesh bags before being boiled for 60 minutes. On cooling, all 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. Tissue samples of sound roots from the same crop were oven-dried at 80°C to establish the dry matter content. Hardcore was expressed as the percentage of hardcore tissue in each root, on a dry weight basis.

7.2.2 *Experiment 2 - a range of chilling temperatures and durations*

Storage roots of the sweetpotato cultivar Owairaka Red were harvested at Dargaville on 23 March 1998. The root dry matter content was calculated from roots oven-dried at 80°C. On 24 March samples of the roots were assigned to temperature controlled storage rooms at either 1, 5, 10 or 15°C. Four samples were stored at each temperature; a sample consisted of 30 randomly selected roots. One sample removed from each temperature regime at four time intervals: 5, 13, 20 and 27 days from the commencement of chilling storage. From each 30 root sample removed from storage, 15 roots were immediately placed in 20°C storage for a further 7 days before assessment, while the other 15 roots were immediately assessed. Chilling injury was assessed by cutting each root in half along its length, then scoring the proportion of the cut surface with brown tissue (BC). The BC scale was: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC. 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 underwent further storage at 20°C were assessed in the same manner. Roots that developed rots in storage were removed from the analysis.

7.2.3 Experiment 3 - medium chilling temperatures and long durations

Storage roots of the sweetpotato cultivar Owairaka Red were harvested at Dargaville on 1 April 1998. The uncured roots were stored indoors, 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. Dry matter content was calculated from roots oven-dried at 80°C. Chilling injury was assessed by cutting each root in half along its length, then scoring the proportion of the cut surface with brown tissue (BC). The BC scale was: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC. 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 analysis.

7.2.4 Experiment 4 - intermittent chilling

Storage roots of the sweetpotato cultivar Owairaka Red were harvested at Dargaville on 1 April 1998. The uncured roots were stored indoors, within multi-walled paper bags, under ambient conditions at the Pukekohe Research Centre. On 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. On each of these dates, 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 way for 14, 12, 10, 8, 6, 4 and 2 days respectively. On 8 May all samples were stored at 20°C for nine days, then were assessed for chilling injury. Dry matter content was calculated from 12 roots, oven-dried at 80°C. Chilling injury was assessed by cutting each root in half along its length, then scoring the proportion of the cut surface with brown tissue (BC). The BC scale was: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC. 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.

7.3 Results and discussion

7.3.1 Experiment 1

The BC roots obtained from the field produced hardcore tissue similar to that of chilled sweetpotato 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 (Table 3).

Table 3: Sweetpotato cultivar Owairaka Red roots with the BC disorder compared to sound roots from the same field at Dargaville.

	Sound roots								Brown centre roots							
Root number	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
BC score[1]	0	0	0	0	0	0	0	0	1	1	1	1	2	2	3	4
% Hardcore[2]	0	0	0	0	0	0	0	0	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 (%) is presented on a dry weight basis.

7.3.2 Experiment 2

Chilling induced brown tissue and hardcore within freshly harvested Owairaka Red storage roots. 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 in other countries (Lewis & Morris 1956; Picha 1987; Clark & Moyer 1988). Roots that were assessed immediately after chilling showed either no symptoms or more subtle injury symptoms than those stored in a warm environment following chilling but prior to assessment. The amount of brown tissue (BC score) increased as the chilling temperature decreased and the chilling period was prolonged (Table 4). Storage at 15°C produced no chilling symptoms while 10°C storage for 13 days produced injury symptoms (hardcore) when assessed immediately after chilling, as did a 5-day chilling period followed by 7 days at 20°C (see Table 6). The level of brown tissue in roots that had been stored at 10°C and then warmed was very slight, but occurred consistently after 5 chilling days (Table 4), as did the incidence of BC (Table 5) which increased with the chilling period. The level of brown tissue assessed 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 BC score of 2.4. The amount of hardcore tissue assessed post warming from 10°C (Table 6) was very small 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). Hardcore tissue was found within samples that had been chilled at 5°C and warmed (Table 7), following both 5 and 13 days of chilling, while rots developed with longer chilling periods. The brown colouration within chilled roots was generally not as prominent as that of BC in roots from the field, nor did tissue damaged by chilling have such pronounced boundaries with sound

tissue. As the severity of chilling increased, the root tissue became increasingly pithy until cavities formed.

Table 4: Mean BC scores after chilling freshly harvested roots of the sweetpotato cultivar Owairaka Red at low temperatures over time. Roots were cut along their main axis and the proportion of BC on cut surfaces scored: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC.

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 BC immediately after chilling.
[2] Roots assessed for BC following chilling and a further storage period of 7 days at 20°C.
† Roots that developed rots in storage were removed from analysis.

Table 5: The incidence of BC after chilling freshly harvested roots of the sweetpotato cultivar Owairaka Red at low temperatures over time. The percentage of roots affected with BC.

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 BC immediately after chilling.
[2] Roots assessed for BC following chilling and a further storage period of 7 days at 20°C.
† Roots that developed rots in storage were removed from analysis.

Table 6: The percentage of hardcore tissue in cooked roots of the sweetpotato cultivar Owairaka Red, after chilling freshly harvested roots at low temperatures over time. (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 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	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 and a further storage period of 7 days at 20°C.
† Roots that developed rots in storage were removed from analysis

Table 7: The incidence of hardcore in cooked roots of the sweetpotato cultivar Owairaka Red after chilling freshly harvested roots at low temperatures over time. 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	100	13	17	0	†	20	0	0
20	100	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 and a further storage period of 7 days at 20°C.
† Roots that developed rots in storage were removed from analysis.

7.3.3 *Experiment 3*

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 reported in the literature. Chilling injury symptoms became more obvious if the roots were stored in warm conditions after chilling. Chilling produced brown tissue and hardcore symptoms, which were similar but not identical to those of field BC. In this experiment temperatures known to induce chilling injury (from Experiment 2) were applied, but those chosen were more likely to be seen in nature during April and May. A post chilling warm period was used to develop chilling symptoms in all treatments.

The roots in this experiment were not heat cured, but as they were stored 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.

Brown tissue (BC score) appeared after 15 days of chilling for both 5 and 7°C treatments (Table 8, Plate 2), 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 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 BC 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 9). 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 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 10). 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). Despite these long storage periods at medium chilling temperatures, the chilling symptoms did not precisely match field BC symptoms in either magnitude, colour or general appearance.

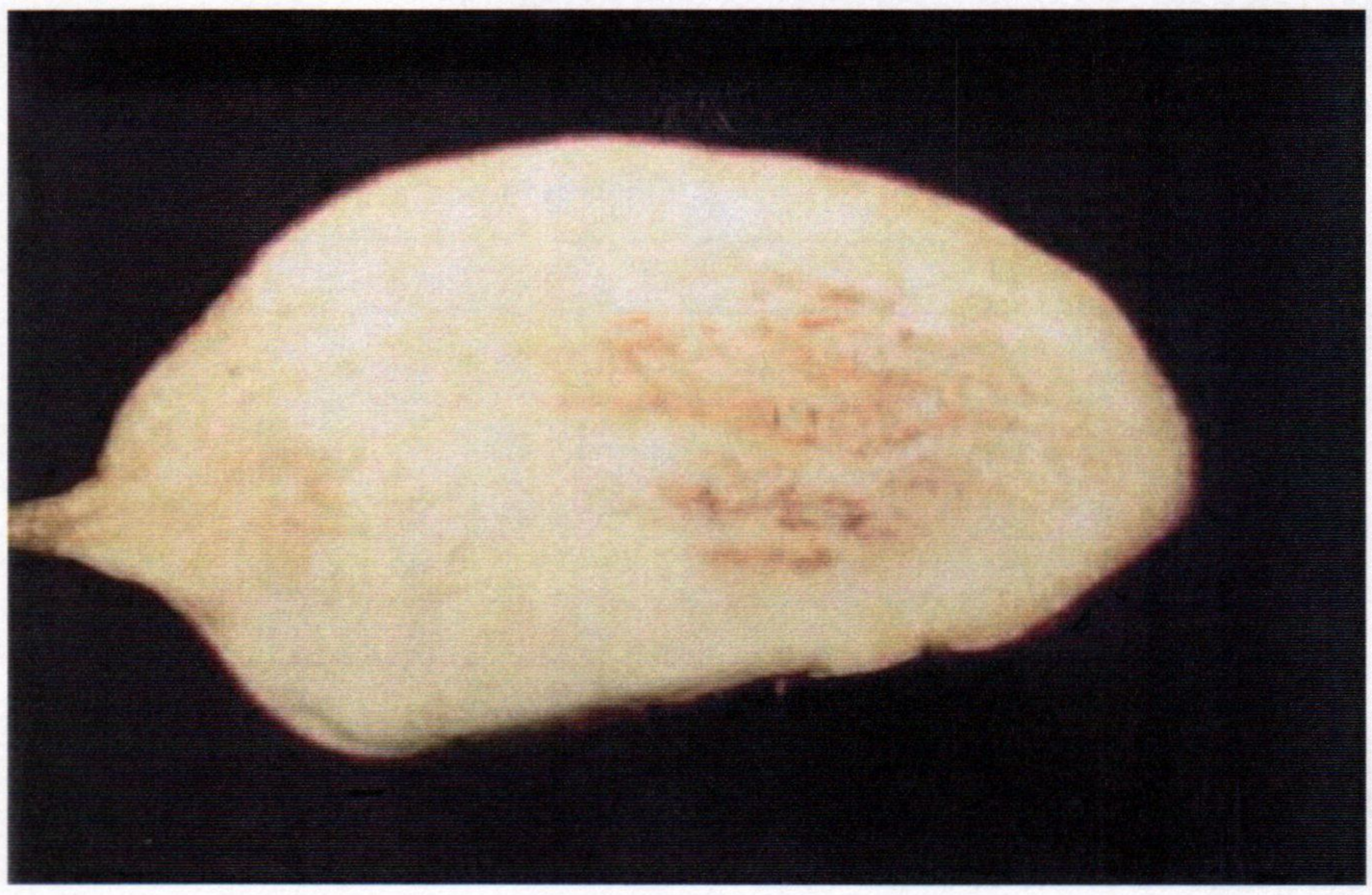

Plate 2: Chilling injury induced within a raw sweetpotato storage root, cv. Owairaka Red, stored at 5°C for 15 days followed by 7 days at 20°C (longitudinal section).

Table 8: Mean BC scores and the percent incidence of BC after chilling roots of the sweetpotato cultivar Owairaka Red at low temperatures over time. Roots were cut along their main axis and the proportion of BC on cut surfaces scored: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC.

Chilling period (days)[1]	Mean BC score			BC incidence (%)		
	Chilling temperature (°C)			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 9: The percentage of hardcore tissue and the incidence of hardcore-affected roots in cooked roots of the sweetpotato cultivar Owairaka Red, after chilling roots at low temperatures over time. (Percentage of hardcore tissue within roots exhibiting hardcore symptoms, on a dry weight basis).

Chilling period (days)[1]	Hardcore tissue (%)			Hardcore incidence (%)		
	Chilling temperature (°C)			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 10: The percentage of roots at each cavity score after chilling roots of the sweetpotato cultivar Owairaka Red at low temperatures over time. Cavity scale: 0, solid tissue; 1, pithy; 2, cavities formed.

	Cavity scale								
Chilling period	5°C storage			7°C storage			9°C storage		
(days)[1]	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.

7.3.4 Experiment 4

The relationship between cool and warm temperatures is important in the development of chilling injury symptoms. While Experiments 2 and 3 demonstrated that chilling injury produces similar symptoms to those seen in field BC, the symptoms do not correspond precisely in magnitude, colour or general appearance. In this experiment intermittent cooling was examined to see if it exacerbated the chilling symptoms.

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 11). Hardcore was seen after 8 days of chilling, as was the development of tissue cavities (Table 12). 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. So intermittent cooling reduced the level of chilling injury rather than exacerbating the symptoms.

Table 11: Mean BC scores and the percent incidence of BC after chilling roots of the sweetpotato cultivar Owairaka Red at low temperature over time. The roots were cut along their main axis and the proportion of BC on cut surfaces scored: 0, no BC; 1, up to 25% BC; 2, up to 50% BC; 3, up to 75% BC; 4, up to 100% BC.

	Mean BC score			BC incidence (%)		
Chilling period (days)[1]	Storage at 5°C	Intermittent chilling	Storage at 20°C	Storage at 5°C	Intermittent chilling	Storage at 20°C
2	0.00	0.00	0.00	0	0	0
4	0.00	0.00	0.00	0	0	0
6	0.05	0.00	0.00	5	0	0
8	0.35	0.00	0.00	35	0	0
10	0.70	0.00	0.00	40	0	0
12	1.60	0.10	0.00	60	10	0
14	1.25	0.00	0.00	75	0	0

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

Table 12: The percentage of hardcore tissue in cooked roots and the incidence of roots with tissue cavities in the sweetpotato cultivar Owairaka Red, after chilling roots at low temperature over time. (Percentage of hardcore tissue within roots exhibiting hardcore symptoms, on a dry weight basis.)

	Hardcore tissue (%)			Cavity incidence (%)		
Chilling period (days)[1]	Storage at 5°C	Intermittent chilling	Storage at 20°C	Storage at 5°C	Intermittent chilling	Storage at 20°C
2	0	0	0	0	0	0
4	0	0	0	0	0	0
6	0	0	0	0	0	0
8	3	0	0	25	0	0
10	12	0	0	20	0	0
12	9	0	0	40	0	0
14	3	0	0	20	0	0

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

7.4 Summary

There were four experiments in this study, comparing chilling injury in the sweetpotato cultivar Owairaka Red to field BC symptoms. In Experiment 1 it was shown that BC roots harvested from the field not only contained 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. Experiment 2 demonstrated that tissue browning and hardcore also occured as a direct result of chilling injury. As temperatures reduce and chilling periods lengthen, the symptoms of tissue browning and hardcore increase 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 were not identical. In Experiment 3 chilling temperatures more likely to occur in nature were applied for prolonged periods, followed by a warm period. 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. A further experiment was conducted to evaluate the effect of intermittent chilling. This experiment demonstrated that intermittent chilling alleviated chilling symptoms rather than enhanced them.

7.5 Conclusion

These experiments demonstrate that Owairaka Red storage roots are not unusually sensitive to chilling injury when compared to others described in the literature. Relatively extreme chilling temperatures and durations are required to produce symptoms approaching the magnitude of BC symptoms observed in the field. None of the combinations of chilling temperature and duration produced brown tissue with an appearance exactly like that of field BC. It is concluded that BC is not caused by the chilling of storage roots.

8 Study 6: Pukekohe temperatures

8.1 Introduction

Chilling temperatures adversely affect harvested sweetpotato roots, but evidence (Study 5) shows that this does not produce typical BC symptoms. Cool temperatures also affect the dynamics of growing plants, so may modify storage root development and produce BC. This study examines the soil temperatures experienced by two sweetpotato crops that developed BC in the field.

8.2 Materials and methods

Field BC was found in sweetpotato roots grown at the Pukekohe Research Centre in both the 1997/98 and 1998/99 seasons (Study 2). The sites of the 1997/98 and the 1998/99 crops were adjacent level fields. In the 1997/98 season the sweetpotatoes were harvested on 16 April 1998, and 5.7% of the

Owairaka Red roots developed BC. The cultivar Northland Rose had 3.8% BC and the clone 11/19 had 10.0% BC. In the 1998/99 season the sweetpotatoes were harvested on 24 May 1999 and 2.4% of the Owairaka Red roots developed BC. There was no BC in either Northland Rose or clone 11/19.

Soil temperatures for March and April 1998 and March, April and May 1999 were obtained from the NIWA meteorological site at the Pukekohe Research Centre. Soil temperatures had been recorded daily at 9 am under established pasture at depths of 10, 20 and 100 cm. In the 1998/99 season Crop & Food Research recorded hourly temperatures in the soil (10 cm beneath the crest of the mould) within the sweetpotato crop that yielded BC-affected roots.

Sweetpotato plants grown in the field at the Pukekohe Research Centre produced roots that exhibited brown centre without exposure to chilling temperatures.

8.3 *Results and discussion*

At deeper soil depths temperatures showed less fluctuation and greater heat retention than those recorded near the soil surface (Figs 6 and 7). In 1998, the lowest daily (9 am) 10 cm soil temperature recorded in March was 17.9°C and the lowest in April (up to harvest; 16 April) was 15.0°C (Fig. 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 (up to harvest; 24 May) was 10.5°C (Fig. 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. 8). The regression line for Crop & Food Research soil temperatures on NIWA soil temperatures (°C) in April 1999 is: CFR = 0.93(NIWA) + 0.54 (R^2 = 92.8%, se = 0.44).

The 1997/98 crop did not experience chilling temperatures prior to harvest (Fig. 6), yet Owairaka Red developed 5.7% BC, Northland Rose 3.8% and clone 11/19 10.0%. In the 1998/1999 crop, soil temperatures dropped as low as 10.5°C just prior to harvest, but Owairaka Red developed only 2.4% BC, while Northland Rose and 11/19 developed none. It is concluded from this study that cool temperatures are not required to produce the BC disorder within growing plants.

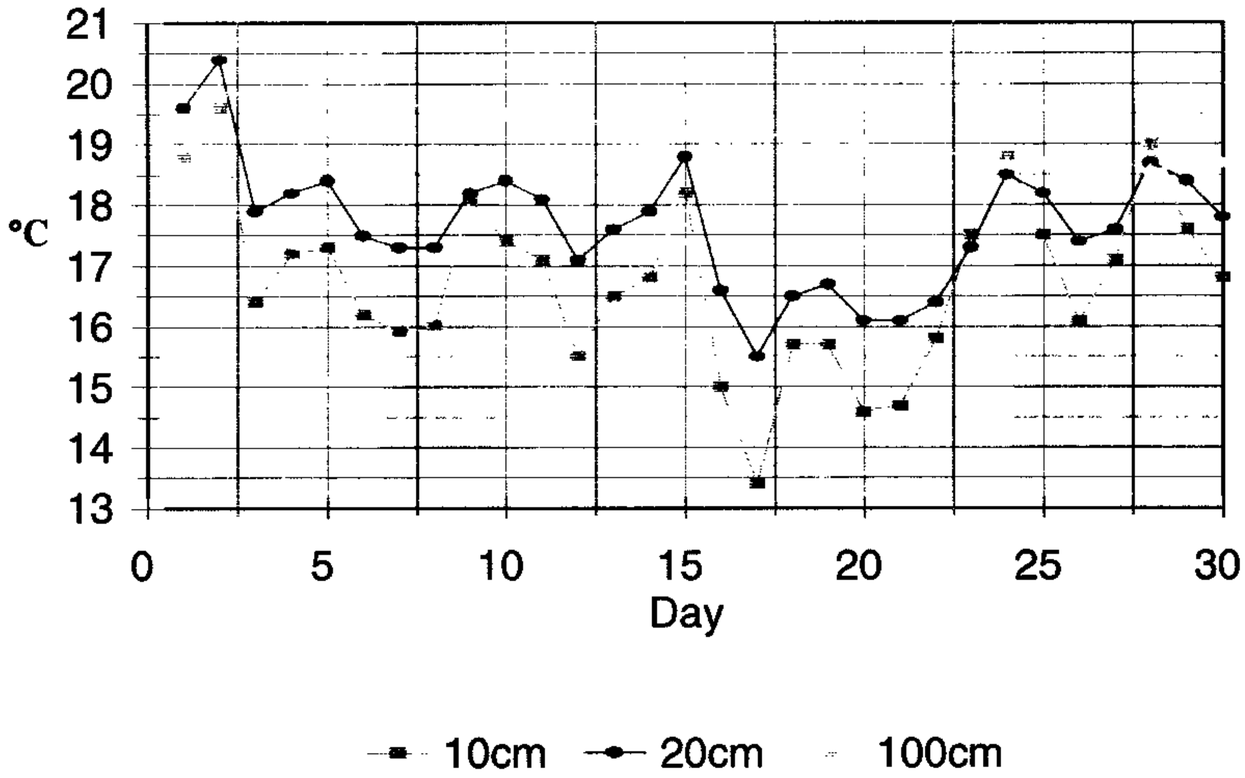

Figure 6: Daily soil temperatures (9 am) from various depths (10, 20 and 100 cm) below established pasture at the Pukekohe Research Centre, April 1998. Sweetpotato cultivar Owairaka Red was harvested at this location on 16 April 1998 with a 5.7% incidence of BC. Temperature data supplied by NIWA.

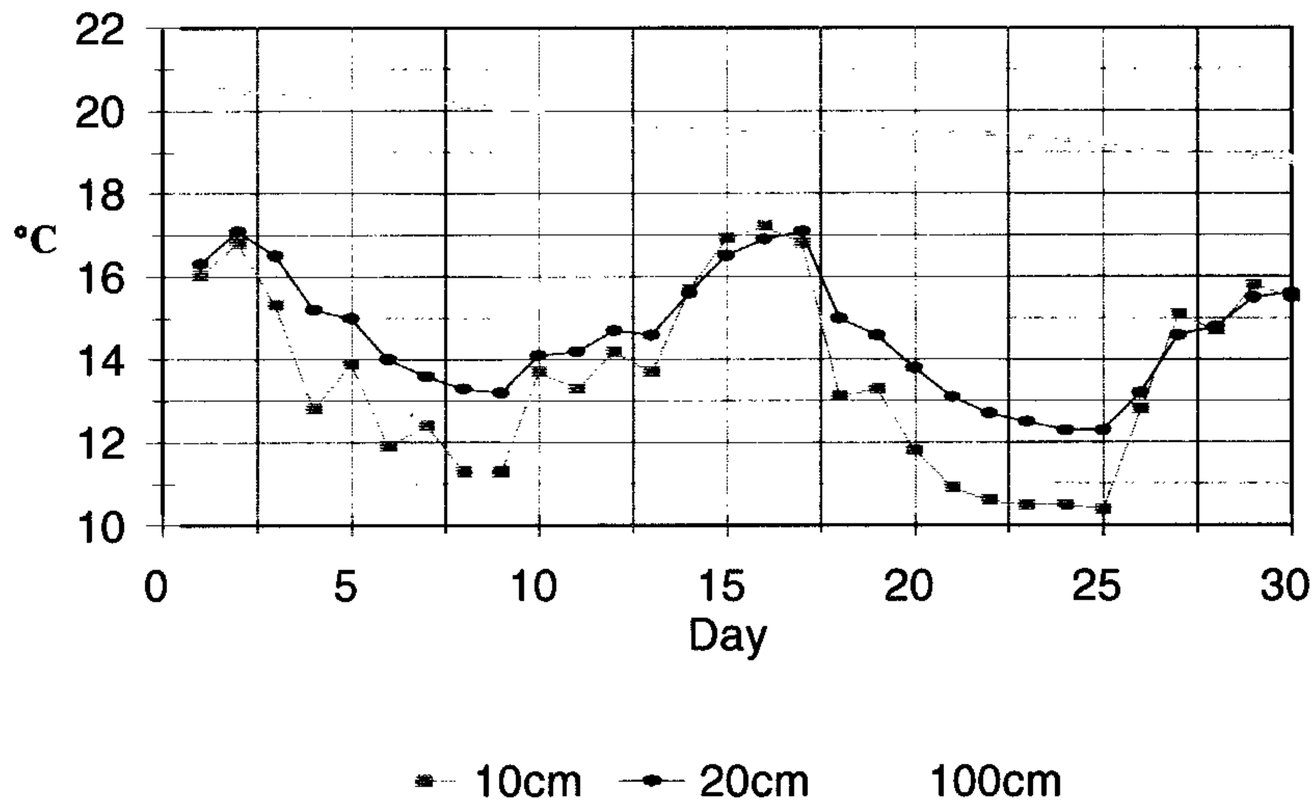

Figure 7: Daily soil temperatures (9 am) from various depths (10, 20 and 100 cm) below established pasture at the Pukekohe Research Centre, May 1999. Sweetpotato cultivar Owairaka Red was harvested at this location on 24 May 1999 with a 2.4% incidence of BC. Temperature data supplied by NIWA.

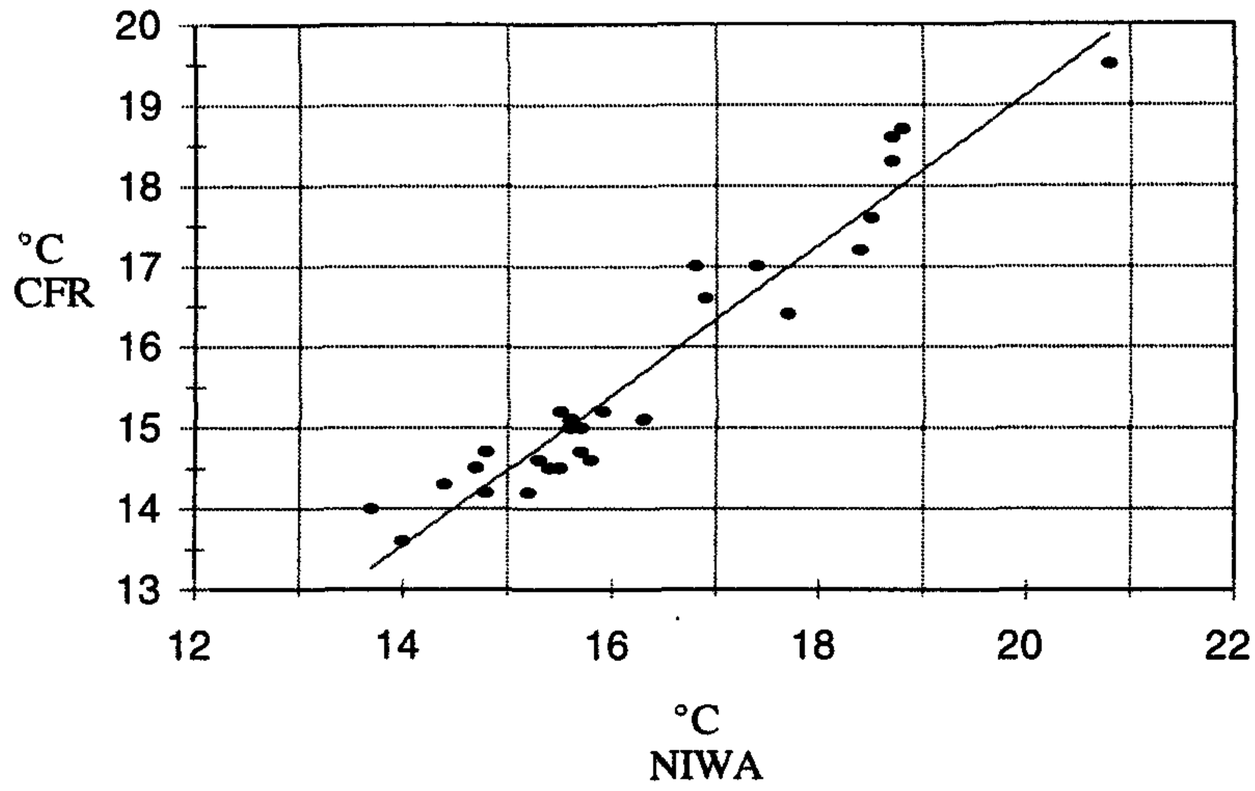

Figure 8: Comparison of two sources of daily soil temperatures, both from the Pukekohe Research Centre, April 1999. The NIWA soil temperatures were recorded once daily (9 am) at 10 cm below established pasture. The CFR data points are daily means from soil temperatures recorded hourly beneath a sweetpotato crop with full leaf cover, 10 cm beneath the crest of the mould. The regression line for CFR soil temperatures on NIWA soil temperatures (°C) in April 1999 is: CFR = 0.93(NIWA) + 0.54 (R^2 = 92.8%, se = 0.44).

9 Study 7: Plant stress

9.1 Introduction

Brown centre occurs late in the season (Gillard 1955; Wood & Schappi 1984), coincident with falling temperatures. However, previous studies suggest it is unlikely that cold temperatures are the only factor involved. A combination of plant stresses may be required to produce BC, possibly involving cool temperatures (Lewis & Morris 1956). Such plant stresses could include effects on the roots' assimilate supply or respiration rate, like those seen in the development of fleck in potato tubers (Anderson & Triggs 1998). Anaerobic conditions may be involved, as seen in waterlogged soils (Corey & Collins 1982). Low boron (B) levels in the soil may also be associated with the disorder (Fujita & Kouzuma 1997) and stress may amplify its effect.

At present the environmental factors, if any, associated with the disorder are poorly understood. Thus, the present research examined the effects of a broad range of plant stresses applied to container-grown plants close to harvest.

9.2 Materials and methods

The stress conditions we applied (various temperature regimes, high nitrogen conditions and water-logged soils) did not produce symptoms of brown centre.

Unrooted cuttings of the sweetpotato cultivar Owairaka Red were planted into 15 litre plastic buckets containing bark media on 9 December 1998. The bark media contained base fertiliser and trace elements, while additional nitrogen (N) and potassium (K) were applied by fertigation using drip irrigation (Appendix I). The plants were grown in an unheated 6 x 15 m glasshouse at the Plant Growth Unit, Massey University. A row spacing of 1.5 m was used. Plants were spaced 40 cm apart in the rows on alternate sides of the drip irrigation line. There were four rows across the width of the house. A RCB design was used with 4 replicates and 3 plant plots. A total of 144 experimental plants were grown. The condition of the plants 36 and 67 days after planting is illustrated in Plates 3 and 4 respectively.

The treatments applied were broad based and likely to stress the plant and directly affect assimilate production and distribution or root respiration. Six treatments were combined factorially with 2 levels of B (low and standard) to give 12 treatments.

The stress treatments were:

1. control,
2. low temperatures at night - plants shifted to cool store (5°C),
3. high day temperatures - plants shifted to another greenhouse during day (30+°C),
4. high N - 200 ppm liquid feed,
5. wet soil - bottom 25-30% of the container flooded,
6. dry soil - plants wilted prior to each watering.

These six treatments were applied late in the life of the crop, from 24 March until 14 April 1999, a period of three weeks.

Trace elements were applied using a standard NFT trace element solution. The low B treatment received no B via fertigation, although B was available to these plants via impurities in the base fertiliser. The water supply used was very low in B. Daily fertigation ceased on 2 February and from then until the end of the experiment trace elements only were applied via drip irrigation and then only once or twice weekly. Plants were watered daily. From 8 February there were 3 waterings per day. The plants were trained up stakes from 28 January to prevent damage to the foliage from staff walking along the rows. This procedure also kept the plants separate so that plants receiving the low and high temperature treatments could be shifted during the last three weeks of the experiment.

Plate 3: Glasshouse-grown potted Owairaka Red sweetpotato plants 36 days after planting (Study 7 – Plant stress).

Plate 4: Glasshouse-grown potted Owairaka Red sweetpotato plants 67 days after planting – supported by stakes (Study 7 – Plant stress).

Leaf nutrient analysis was determined on a number of occasions during the season. The seventh to ninth opened leaf blade was sampled. At the final harvest data were collected on the fresh weight of plant top, root number and weight, then cut roots were assessed for BC. Roots were graded as small or large (marketable). The assessment of the cut roots for BC was carried out 2 weeks after the roots had been harvested.

9.3 *Results*

No root discolorations of any type (including BC) were noted when the roots were cut 2 weeks after they were harvested. Foliage growth showed an interaction between B level and the treatment. This was due to the low foliage weight of the control B x high temperature treatment and the high foliage weight of the control B x high N treatment (Table 13). The high N treatment

(4) provided the greatest amount of foliage and the cold and dry treatments (2 and 6) the least.

Table 13: Fresh foliage weight of sweetpotato cultivar Owairaka Red (kg/plant), SED=0.19.

Boron	Stress treatment					
	Control	Dry	Wet	Cold	Hot	High N
Low	1.9	1.6	1.9	1.3	1.9	2.1
Control	1.6	1.5	2.0	1.6	1.4	2.4

The dry treatment had a greater number and yield of small storage roots (< 5 cm diameter) than the cold and wet treatments (Table 14). There were no significant differences in the mean weight of small roots. The high temperature and control treatments had a greater mean weight and yield of large roots than the cold and wet treatments. There were no significant differences in the number of large roots.

Table 14: Treatment effects on storage root number, mean root weight and total root weight in the sweetpotato Owairaka Red.

Root grade	Control	Dry	Wet	Hot	Cold	High N	SED
Number/plant							
Small	4.7	6.1	2.2	4.3	2.9	3.9	1.18
Large	3.8	3.4	3.1	4.3	3.0	3.3	0.54
Weight (g/plant)							
Small (mean)	27.9	27.2	30.6	26.2	29.7	26.8	4.22
Small (total)	124	169	65	94	85	116	30.8
Large (mean)	263	205	157	266	211	244	23.6
Large (total)	994	709	505	1139	600	838	140.8

Nutrient data in leaf samples taken on 15 January (37 days after planting) can be compared with the adequate concentration ranges suggested by O'Sullivan et al. (1997) at 28 days after planting (Table 15). The levels for the present experiment were higher for N, phosphorus (P), calcium and iron (Fe), but lower for B. The mean for the four samples incorporated data for a significant part of the growing season. When these data were compared with those of Scott & Boukamp (1974), which were means for samples taken at 2-weekly intervals during the growing season with the range covering four cultivars, the N and P levels are still high and the Fe and B levels are low for the low B treatment. The B levels (mg/g) increased throughout the experiment, but in the low B treatment were always lower than in the control.

Table 15: Leaf nutrient data for the sweetpotato Owairaka Red, compared to nutrient levels observed internationally.

Sample date	Boron level	N	P	K	S	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
					(%)						(mg/g)		
15 Jan.	Low	6.0	0.79	4.9	0.49	1.37	0.50	0.05	202	128	32	<2	28
	Control	6.3	0.77	4.9	0.51	1.43	0.49	0.06	167	137	32	<2	34
2 Feb.	Low	5.3	0.42	3.9	0.44	0.83	0.38	0.02	165	190	39	3	23
	Control	5.7	0.43	3.8	0.45	0.99	0.41	0.02	198	282	37	2	27
14 Mar.	Low	4.4	0.28	3.3	0.39	1.02	0.41	0.01	383	368	31	3	37
	Control	4.2	0.27	3.2	0.38	1.20	0.46	0.02	369	274	37	3	69
14 Apr.	Low	3.0	0.20	2.3	0.28	1.07	0.42	0.01	123	255	21	3	45
	Control	3.3	0.21	2.5	0.29	1.07	0.42	0.02	229	233	29	3	71
Seasonal	Low	4.7	0.43	3.6	0.40	1.07	0.43	0.02	218	235	31	3	33
mean	Control	4.9	0.42	3.6	0.41	1.17	0.45	0.03	241	232	34	3	50
International leaf nutrient levels:													
Normal range[1] (28 days)													
	Max.	5.0	0.45	6.0		1.2	0.70		80	500	60	14	200
	Min.	4.2	0.26	2.8		0.9	0.25		45	26	30	5	50
Seasonal mean[2]													
	Max.	2.3	0.29	3.4		1.5	0.43		289	269			51
	Min.	2.0	0.24	2.9		1.0	0.27		256	201			49

[1] O'Sullivan et al. (1997).
[2] Scott and Boukamp (1974).

9.4 *Discussion*

Roots produced were stored at room temperature in a field laboratory prior to BC assessment, two weeks after harvest. Brown centre or any other root discoloration were not observed, apart from roots that had started to rot in the wet treatment. All of the plants were adequately supplied with nutrients and the levels of B in roots from the low B treatment at maturity were not extreme. There was, therefore, no evidence that the stresses applied in this experiment caused BC. However, at field sites where BC occurs these stresses might enhance the disorder.

No explanation can be offered for the production of less foliage in the control B x high temperature treatment than in the low B x high temperature treatment. The cold and dry treatments restricted growth while the high N treatment encouraged foliage growth. The wet treatment did not depress foliage growth.

The stresses were applied during the 3 weeks prior to harvest - a period when significant storage root growth could be expected to take place under satisfactory growing conditions (Kays 1985). There were no significant effects on the number of large roots, possibly because the number of roots in this category had largely been determined when the treatments were applied.

The wet and cold treatments had the lowest mean weight and total weight of large roots, and the high temperature and control treatments had the highest. Thus, the treatments influenced the yield of large roots by producing larger rather than more roots. The warm temperature treatment clearly did not stress the plants as this was the highest yielding treatment. This is perhaps not surprising as the sweetpotato is of subtropical origin and the daily maximum temperature in the greenhouse was 30-36°C. The high N treatment encouraged foliage growth. Consequently, the yield of large roots was below that of the control, but this difference was not significant.

High N in the field (Study 8) was associated with BC. This did not occur in the present study, possibly because the plants were grown in containers which may have constrained root growth. Such an explanation is consistent with the suggestion made in Study 8 that plant spacing may be a factor in BC expression. For example, at wider spacings competition is less likely to constrain root growth so BC expression may be enhanced.

The number and weight of small roots was low for the cold and wet treatments and high for the dry treatment. Small roots were those that had shown some thickening, up to about 5 cm in diameter. It is suggested that the cold and wet treatments affected the root environment more than the other treatments, so fewer roots thickened up past the pencil root stage. On the other hand the dry regime in the 15 litre buckets appeared to encourage a greater number of roots to swell late in the season.

The mean B level over the season for the low B treatment was below the 40 mg/kg reported by O'Sullivan et al. (1997) as the critical concentration for B deficiency. Despite this observation, this experiment was unable to adequately test the response of the stress treatments to low B. Leaf nutrient analysis showed that, although the low B treatment produced the lower B leaf concentrations, there were no marked differences in B levels between the low B treatment and the control.

Throughout the experiment the N and P levels of the plants were high, This may have been an artefact of the base fertiliser dressing and nutrient feeding programme used. The control plants grew and yielded well.

9.5 *Summary*

1. In this plant stress experiment the treatments applied during the last 3 weeks of cropping failed to induce BC or any other root discolorations.
2. The cold and wet treatments markedly reduced the yield of marketable storage roots by reducing root size.
3. The dry treatment may have encouraged storage root formation.
4. The warm temperature treatment produced the highest yield of marketable roots.
5. The high N treatment encouraged foliage growth and may have decreased yields.
6. This experiment did not adequately test the response of plants to low B.

10 Study 8: Nutrient disorders

10.1 Introduction

Crops may develop disorders through either nutrient excess or deficiency, and sweetpotato is no exception (O'Sullivan et al. 1997). Boron deficiency commonly produces disorders with brown or discoloured tissue that are seen in crops as diverse as almond (*Prunus amygdalus*), apple (*Malus sylvestris*), apricot (*Prunus armeniaca*), cauliflower and broccoli (*Brassica oleracea* var. *botrytis*), celery (*Apium graveolens* var. *dulce*), kale (*Brassica oleracea* var. *acephala*), kohlrabi (*Brassica oleracea* var. *gongylodes*), mangold (*Beta vulgaris* var. *vulgaris*), peanut (*Arachis hypogea*), pear (*Pyrus communis*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*), red beet (*Beta vulgaris*), rutabaga (*Brassica napobrassica*), sugar and fodder beet (*Beta vulgaris*), swede (*Brassica rutabaga*), sweetpotato (*Ipomoea batatas*) and turnip (*Brassica rapa*). 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.

Applications of boron fertiliser did not mitigate the symptoms of brown centre. However, adding nitrogen late in the season doubled the incidence of this disorder.

A disorder very similar to BC was found in Japan (Fujita & Kouzuma 1997). It was subsequently investigated for four years. Like BC, the disorder had no external symptoms, was not due to 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). 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, Jewel being particularly prone to the disorder (Nielson 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 (Dr M Cannon, pers. comm.) where it reduces blister symptoms, indicating that B deficient conditions have been overcome (Paterson & Speights 1971).

Soil N levels have an important effect on B uptake and photosynthate partitioning within the plant. 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). 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. 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 used to grow sweetpotato crops, which may have undergone years of cropping with little applied N fertiliser (refer: Study 3). The present study examines the interaction of N and B levels, as well as partial plant defoliation, on the incidence of BC.

10.2 Materials and methods

One t/ha of 30% potassic superphosphate was broadcast over a Pukekohe Research Centre field site, prior to moulding. Owairaka Red sweetpotato plants were transplanted into the field on 4 December 1998 and watered in with overhead irrigation. The soil at the site was a Patumahoe clay loam, which was sampled (11 February 1999) just prior to commencement of the treatments with the following analysis: 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, cation exchange capacity 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. Available N was assessed by anaerobic incubation followed by ammonium-N extraction using 2M KCl, then determined by Berthelot colorimetry.

The trial consisted of 14 treatments (three N rates and four B rates in all combinations, two defoliation treatments and an unmodified control). The trial was arranged in a modified alpha 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 (including inter-plot spacing), 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 harvested portion of each plot, therefore, contained a total of 40 plants within a 10.5 m^2 area.

Nitrogen was applied 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 watered in by irrigation (approximately 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). The defoliation treatments were applied once, on 4 April 1999, and these treatments 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 GENSTAT^{tm} statistical software package.

10.3 Results and discussion

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 16). While neither the defoliation nor the B treatments changed the incidence rate, adding N effectively doubled the levels of BC (Table 17). 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), while again there was no evidence of a similar effect following B application (P = 0.89) or of an N x B interaction (P = 0.85).

The N used in this trial was applied late in the season and at artificially high levels. The plant canopies in plots with applied N were noticeably greener and higher than those without added N. However, a reduction in the canopy size through artificial defoliation did not significantly reduce or increase the incidence of BC.

The effect of N on BC incidence may be through the promotion of plant growth, causing a shortfall in the balanced supply of micro elements such as B. However, in this trial there was no evidence of any effects of B on BC incidence. The sweetpotato cultivar Jewel was planted alongside this field

trial as a B-sensitive indicator, but did not develop any symptoms of blister after 5 months in storage, suggesting B was not in short supply.

The effect of N seen in this trial is consistent with growers' observations that BC is more common in ground that is out of pasture. This may be due to generally high fertility rather than N itself, as suggested by the significantly higher K levels in BC roots (Study 3). Some similarities may be found in other crops growing in high N conditions. High N levels are known to cause hollow stem in broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis*), without any evidence of B deficiency (Scaife & Wurr 1990). In these plants the hollow stem condition develops following the initiation of the central inflorescence. While there is generally no discoloration of the hollows at harvest, discoloration and pith breakdown may occur soon after harvest. The incidence of hollow stem is influenced by the level of N, plant spacing and cultivar (Cutcliffe 1972; Cutcliffe 1975). It is suggested (Scaife & Wurr 1990) that hollow stem in these crops is caused by the curd growth rate in the final stages of formation. The N and cultivar effects seen in sweetpotato suggest it would be worthwhile conducting further experiments at a range of inter-plant spacings to examine the effect of varying plant growth rates over the later stages of root development.

Table 16: Incidence of brown centre in roots of the sweetpotato cultivar Owairaka Red harvested at the Pukekohe Research Centre in the 1998/99 season. Mean percent incidence by back transformation (logit) [1].

Total B applied (kg/ha)	Defoliation (%)	Total N applied (kg/ha)					
		0		300		600	
0	0	1.9	(-3.94)	5.0	(-2.94)	6.2	(-2.72)
0	50	2.6	(-3.61)	-		-	
0	75	1.6	(-4.09)	-		-	
2	0	2.3	(-3.77)	4.1	(-3.14)	4.4	(-3.07)
4	0	4.0	(-3.19)	6.1	(-2.73)	3.9	(-3.21)
6	0	2.5	(-3.65)	6.5	(-2.67)	4.7	(-3.01)
LSD $P<0.05$ (0.90)							

[1] Treatment means with associated LSDs in brackets

Table 17: The effect of applied N on the general incidence of brown centre (BC) and the incidence of severe BC in roots of the sweetpotato cultivar 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[1].

Total N applied (kg/ha)	Total BC incidence[2] (%)		Severe BC incidence[2] (%)	
0	2.4	(-3.71)	1.6	(-4.1)
300	5.4	(-2.87)	3.0	(-3.5)
600	4.7	(-3.02)	2.7	(-3.5)
LSD $P<0.05$		(0.45)		(0.50)

[1] Transformed means with associated LSDs in brackets.
[2] Back-transformed.

11 General project summary

Brown centre has been a problem for the New Zealand sweetpotato industry for many years. However, its commercial importance varies from season to season. The disorder was recognised soon after commercial release of the most widespread cultivar, Owairaka Red, almost 50 years ago. Despite several previous attempts to isolate the cause of the disorder, little progress has been made. Harvesting the crop by the end of April was suggested as an appropriate strategy for avoiding the disorder (Gillard 1955) – a suggestion that was later confirmed following scientific inquiry (Wood & Schappi 1984). A pathology study in 1966 (Nielsen & Harrow) found no evidence of infection by internal cork virus, which produces BC-like symptoms in the USA sweetpotato crops.

A number of studies were conducted in this investigation. By testing for bacteria, fungi and viruses it was demonstrated that BC does not have a pathological source (Study 1). This conclusion is reinforced by a previous study comparing the incidence of BC in plants derived from BC-affected and unaffected roots (Wood & Schappi 1984). While Owairaka Red appears to be more susceptible to BC than most cultivars, other clones sourced internationally also develop BC. The natural incidence of BC in plants across a field appears to be sporadic. While some storage roots on individual plants show the disorder, others are healthy (Study 2). Nutrient analysis of root tissue from plants with BC and from soil at sites producing BC indicated that roots with BC had higher levels of K and soils in which they were grown appeared high in available N (Study 3).

A comparison of the 1996/97 season, which produced high levels of BC around Dargaville, with historic weather data did not show any abnormal rainfall or temperature patterns (Study 4). However, meteorological data

available from Dargaville were limited in that they did not include soil temperatures. Rather, they were a generalised daily record for the district based on one site. Sweetpotatoes are known to be subject to chilling injury at temperatures below 12°C, but the chilling sensitivity of the Owairaka Red cultivar was unknown. An examination of harvested Owairaka Red roots chilled under various temperatures for different periods produced symptoms approximating those observed in field BC, but not identical in either incidence or severity (Study 5). It was concluded that the chilling of sweetpotato root tissue was not the cause of the disorder. However, cool temperatures may affect the growth of the sweetpotato plant without causing direct tissue injury. BC occurred naturally at the Pukekohe Research Centre during the 1997/98 and 1998/99 seasons (Study 6). Pukekohe soil temperature recordings indicated that while the 1997/98 season produced a greater incidence of BC than the 1998/99 season, soil temperatures (10 cm depth) did not drop below 15°C prior to harvest in 1998. Therefore, BC can develop in the absence of cool temperatures.

As BC does not appear to arise from chilling injury, combinations of environmental stress and varying nutrition were tested in a glasshouse experiment (Study 7). Environmental stress included wet and dry soil conditions and low and high temperatures. Nutrient variations included low and high B levels and medium and high N levels. No BC developed in this glasshouse study. A field trial was established at the Pukekohe Research Centre to test the effects of varying B and N rates. Vine cutting treatments were also included to investigate modifications to the root/shoot ratio (Study 8). The field trial developed BC, even in the control plots. The B and cutting treatments did not affect the incidence of BC, but adding N doubled it.

12 Conclusion

In conclusion, the BC seen in New Zealand occurs late in the sweetpotato growing season, is not pathological, does not require chilling temperatures or water-logged soils, and is not mitigated by applications of B fertiliser. It is, however, cultivar dependent. There is evidence from tissue and soil analysis, as well as field experimentation, that the incidence of BC is exacerbated by fertile growing conditions.

13 Recommendations

To lower the risk of BC we recommend that a crop other than sweetpotato or a sweetpotato cultivar resistant to BC is planted in highly fertile fields, especially fields just out of established pasture. If the sweetpotato cultivar Owairaka Red is planted in fertile fields, it should be harvested before mid April. Once the fertility is lowered, Owairaka Red crops grown in subsequent years should be less subject to BC. While it cannot be assumed at this stage that BC occurrence is solely the product of nutrition and the plant's growth

rate, inter plant spacings should not be increased on fields at risk as wider spacings may increase the growth rate.

Further research is required to establish the critical nutrient levels and stages of plant development that affect the incidence rate of BC.

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Appendix I

Study 7: Plant stress

The base dressing supplied all or most of the calcium (some in liquid feed), magnesium and phosphorus plus some nitrogen and potassium. A liquid feed (fertigation) supplied the remaining nitrogen and potassium and all the trace elements. The trace elements were as for NFT with control boron at 0.3 ppm from boric acid with low level boron concentration initially at zero. Boron will, however, be a contaminant in the superphosphate, lime and dolomite lime.

The media used was:

100% bark plus the following fertiliser:

- 3 kg ground limestone (Ca))
- 3 kg dolomite lime (Ca, Mg)) per m^3
- 3 kg superphosphate (P))
- 0.3 kg potassium nitrate (K, N))

Liquid feeds: There were two tank solutions each diluted 1:100

Control (C): 100 ppm N and 120 ppm K plus NFT trace elements

	grams or ml per litre of tank solution
■ potassium nitrate (KNO_3)	31.6 g
■ ammonium nitrate (NH_4NO_3)	16.9 g
■ trace elements A	100 ml
■ trace elements B	100 ml

Low boron (B): 100 ppm N and 120 ppm K plus NFT trace elements less boron

	grams or ml per litre of tank solution
■ potassium nitrate (KNO_3)	31.6 g
■ ammonium nitrate (NH_4NO_3)	16.9 g
■ trace elements A	100 ml
■ trace elements C	100 ml

Trace element stock solutions A-C

Use 100 ml for each 1 litre of tank solution which will then be diluted 1:100

Grams per 1 litre of <u>trace elements A</u> (both treatments)		
chelated iron (FeNa EDTA)	78.88	(12 ppm Fe)
Grams per 1 litre of <u>trace elements B</u> (control)		
manganous sulphate ($MnSO_4.H_2O$)	6.154	(2 ppm Mn)
boric acid (H_3BO_3)	1.714	(0.3 ppm B)
copper sulphate ($CuSO_4.5H_2O$)	0.275	(0.07 ppm Cu)
ammonium molybdate ($(NH_4)_6M_7O_{24}.4H_2O$)	0.092	(0.05 ppm Mo)
zinc sulphate ($ZnSO_4.7H_2O$)	0.308	(0.07 ppm Zn)
Grams per 1 litre of <u>trace elements C</u> (low boron)		
manganous sulphate ($MnSO_4.H_2O$)	6.154	(2 ppm Mn)
copper sulphate ($CuSO_4.5H_2O$)	0.275	(0.07 ppm Cu)
ammonium molybdate ($(NH_4)_6M_7O_{24}.4H_2O$)	0.092	(0.05 ppm Mo)
zinc sulphate ($ZnSO_4.7H_2O$)	0.308	(0.07 ppm Zn)

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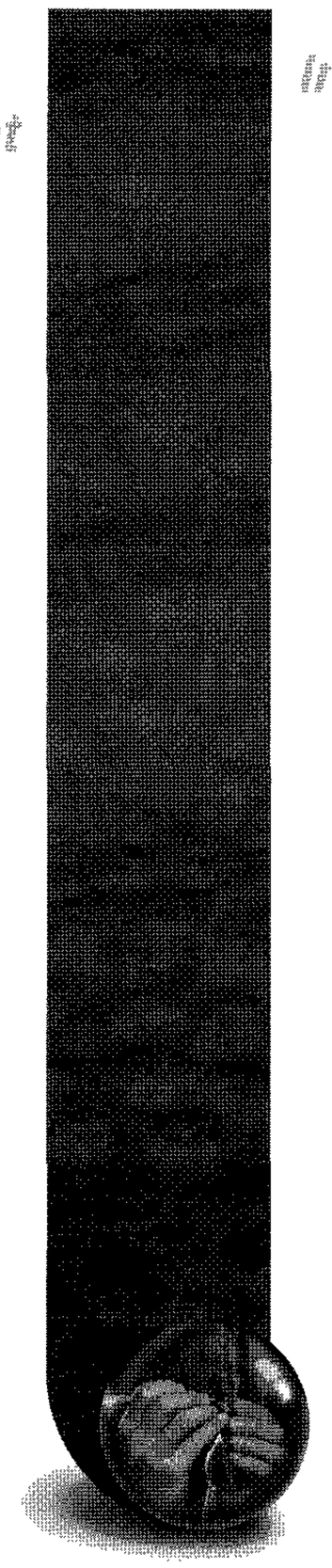

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