Cultivar decline in sweetpotato (*Ipomoea batatas*)

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Abstract The sweetpotato (*Ipomoea batatas*) crop is propagated vegetatively by field transplanting adventitious sprouts produced on storage roots retained from the previous season's harvest. This system promotes the persistence and accumulation of both viruses and spontaneous mutations. A phenomenon known as cultivar decline has been reported internationally, where the root yield and appearance of commercially grown sweetpotato cultivars appear to deteriorate over successive growing seasons. The relative contributions of virus infection and plant mutation to cultivar decline are uncertain, but both issues are addressed through the use of virus-tested tissue cultured propagation systems. This study assessed the degree of decline for cultivars 'Owairaka Red' and 'Beauregard' within the New Zealand biophysical production environment. Storage root yield decreased significantly with increasing field exposure, for both cultivars ($P < 0.001$). The general appearance of 'Beauregard' roots deteriorated with greater field exposure, but the appearance of 'Owairaka Red' showed no significant change ($P < 0.001$).

Keywords kumara, disease, virus, mutation, micro-propagation.

INTRODUCTION

The term 'cultivar decline' has been applied to an internationally observed phenomenon of the sweetpotato (*Ipomoea batatas* (L.) Lam.) or kumara crop (Clark et al. 2002). In this syndrome the yield and/or quality of a sweetpotato cultivar gradually declines in a broadly accumulative manner over succeeding years. This paper examines the significance of sweetpotato cultivar decline to production under New Zealand's crop management systems and biophysical environment.

The sweetpotato crop is generally propagated by transplanting un-rooted sprouts into the field. Sprout production is a carefully managed operation, in which storage roots retained from the previous season's harvest are placed in beds of freely draining soil and induced to sprout by applying warm humid conditions. Prior to transplanting, the sprouts are severed above soil level to reduce the incidence of fungal disease transfer from the bedded roots into the new crop (Lebot 2009). Alternatively, the vine tips of plants

already established in the field may be gathered and used for further propagation. Vine tips are only used to a limited extent due to the labour involved in collecting them and the resulting delay in growth of the plant from which they are removed. However, storage roots derived from vine tips are considered particularly disease free and useful in producing good quality storage roots for further propagation. As the sweetpotato exhibits high rates of natural somatic mutation, sprouts or vine cuttings for propagation are only sourced from storage roots or plants that appear either 'true to type' or an improvement on the original clone.

At establishment the sweetpotato propagule has no significant energy reserves so is immediately reliant on growth for survival. This growth is subject to localised genotype by environment interactions. The relatively high plant to plant variation within a sweetpotato crop is thought to be due to comparatively small modifications in the components of this interaction. Despite the performance variability observed at plant, cultivar, season, geographic and crop management levels, the general trend known as cultivar decline has remained perceptible. The relative importance of individual factors contributing to cultivar decline may vary within genotype and environmental settings but the common feature is that the agents of cultivar decline appear to accumulate within the propagating material (Clark et al. 2002). Sweetpotato viral infection and somatic mutation are considered significant contributors to cultivar decline (La Bonte et al. 2001), as both introduce detrimental effects that are proliferated by the vegetative propagation system.

The presence of sweetpotato viruses in New Zealand has been acknowledged for some time (Over de Linden & Elliot 1971), but it is only in recent years that viruses have been studied in an industry-wide setting. The first extensive sweetpotato survey, examining the distribution of specific viruses within the local crop, was published in 2001 (Fletcher et al. 2001). A thorough review of New Zealand plant virus literature records provided a comprehensive list of the viruses detected by 2006 (Pearson et

al. 2006). The results of a further comprehensive sweetpotato field survey were published in 2009 (Perez-Egusquiza et al. 2009), and subsequently an updated list of sweetpotato viruses recorded in New Zealand was published in 2010 (Lewthwaite & Fletcher 2010). It is considered that the most effective method of minimising sweetpotato viral disease is through the use of virus-tested propagation material derived from meristem shoot tip culture (Loebenstein et al. 2009), but this approach has not been commonly practiced within the New Zealand commercial crop.

Generally clonal material is defined as having strict genetic fidelity to its source, but the level of natural somatic mutation in sweetpotato necessitates annual selection to maintain cultivar consistency (Edmond & Ammerman 1971; Huett 1982). Adventitious sprouts and roots, produced from tissues containing somatic mutations, tend to preserve the mutation in subsequent plants, an occurrence that is promoted by the current commercial propagation system. However, there is evidence that 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).

The issues of accumulative viral load and somatic mutation may be mitigated through the use of virus-tested tissue culture propagation systems. This study examined the performance of two sweetpotato cultivars sourced from such a micro-propagation system, but maintained using standard commercial practice for varying numbers of seasons.

MATERIALS AND METHODS

The sweetpotato cultivars 'Owairaka Red' (Lewthwaite 1998) and 'Beauregard' (Rolston et al. 1987) were selected for this study as they currently dominate the New Zealand market.

'Owairaka Red' was released commercially in 1954. This cultivar was derived by selection of a naturally occurring mutant form of an earlier cultivar named 'Waina'. An 'Owairaka Red' clonal selection programme was initiated in the 1998/99 season, from which time an

independent population of 'Owairaka Red' clones has been maintained in the field, using standard commercial practice coupled with systematic single hill selection. Two clones were chosen from this population by single hill selection, cleared of virus by meristem shoot tip culture and maintained *in vitro*. 'Owairaka Red' plants of clone one (OR-1) were sourced from tissue culture and grown in the field at the Pukekohe Research Centre. OR-1 plants with exposure to one, two, three or four field growing cycles, as calculated at crop harvest, were used in this trial. 'Owairaka Red' plants of clone two (OR-2) that had either only been maintained using standard commercial practice or were sourced from virus-tested tissue culture, were also included in the trial.

'Beauregard' was imported into New Zealand from the USA in 1991, as virus-indexed tissue cultured plants. The first 'Beauregard' field evaluation was conducted at the Pukekohe Research Centre in the 1992/93 growing season. A sample of the original material has been continuously maintained in tissue culture since importation, while field propagated stock has also been maintained using standard commercial practice for a total of 18 growing cycles. In recent years further 'Beauregard' plants have been sourced from tissue culture and grown in the field at the Pukekohe Research Centre. 'Beauregard' plants with exposure to 1, 2, 3, 4 or 18 field growing cycles, as calculated at crop harvest, were used in this trial. The area of sweetpotato planted in the immediate vicinity of the trial, and to which the trial plants were exposed in preceding seasons, was composed of approximately 0.5 ha of sweetpotato from a wide range of sources.

The field experiment was laid out in a row and column design (Williams & John 1989), comprising 180 plots arranged in a rectangular array of 9 rows by 20 columns. Each plot was two ridges wide and 4 m long. The plots were separated within columns by a 1 m gap, to allow for mechanical harvesting. Individual ridges were 75 cm wide and inter-plant spacing along ridges was 40 cm. Therefore, every plot contained a total of 20 plants, arranged within two ridges each containing 10 plants. There were 12 treatments,

each with 15 replicates. The 12 treatments were: OR-1 plants with exposure to 1, 2, 3 or 4 field growing cycles; OR-2 plants with 1 or at least 12 cycles; 'Beauregard plants' with exposure to 1, 2, 3, 4 or 18 cycles; and an 'Owairaka Red' dummy treatment to balance the trial structure.

The trial was transplanted into the field on 9 November 2009, and watered in with overhead irrigation. Rainfall was supplemented with overhead irrigation as required throughout the season. Weeds were controlled with the herbicides acetochlor and alachlor, supported by mechanical scarification and hand weeding. Plant survival was recorded on a per plot basis on 5 January 2010. A soil test was taken on 9 February 2010, with the following analysis: pH 6.9, phosphorus 74 mg/litre (Olsen), potassium 0.64 me/100 g, calcium 12.6 me/100 g, magnesium 1.75 me/100 g, sodium 0.28 me/100 g, cation exchange capacity 20 me/100 g, total base saturation 78%, volume weight 1.04 g/ml, available nitrogen 40 kg/ha and organic matter 3.9%.

The trial was mown off on 13 April 2010 and harvested with a two row tractor-mounted lifter. Following harvest the storage roots from each plot were graded based on their width (using modified USA categories, Sterrett et al. 1987), with all roots less than 2.5 cm in diameter discarded, roots from 2.5 to 5 cm placed in 'Canner' grade, roots greater than 5 up to 9 cm placed in 'Number-1' grade, and those above 9 cm placed in 'Jumbo' grade. The roots in each grade were counted and weighed. Four Number-1 grade roots from each plot were cut into pieces, combined, and oven-dried at 80°C to calculate storage root dry matter content. A score was assigned to each plot as an assessment of root quality. The score was based on overall root appearance, including root size distribution. A four point scale was used, with a score of three representing a generally acceptable commercial standard, whereas a score of four represented a particularly attractive root sample.

The data were analysed using the REML procedure of the statistical software GENSTAT® 2008. Curves were fitted using the statistical package's regression procedure for exponential

functions and plotted using the graphical software SigmaPlot® 2006.

RESULTS AND DISCUSSION

There were no significant differences between treatments in plant survival following field establishment ($P=0.214$). Since 'Owairaka Red' clones (OR-1 and OR-2) did not significantly differ in root yield for treatments immediately sourced from tissue culture – i.e. those with exposure to one field growing cycle – the data from both clones were combined (Figure 1). Both 'Beauregard' and 'Owairaka Red' showed a significant decline in total storage root yield with increasing numbers of field growing cycles ($P<0.001$). Decreasing exponential curves were fitted to the data sets of each cultivar, producing similarly shaped curves (Figure 1) with an initial rapid yield decline followed by a diminishing reduction with increasing iterations of field growing cycles. Sourcing propagation material of sweetpotato cultivars 'Beauregard' and 'Owairaka Red' from virus-tested tissue culture systems provided a significant yield increase of 35.7 and 29.8% respectively for the immediately subsequent crop, relative to crops propagated from long-term field-maintained material ($P<0.001$). A similar comparison of 'Beauregard' yields in Australia, found that propagation material from a virus-tested tissue culture system provided a 148% increase, relative to material field propagated for 10 years. However the average storage root yield increase, across all 14 cultivars in the Australian trial, was 38% (Okpul et al. 2011).

The cultivars 'Beauregard' and 'Owairaka Red' produce storage roots with differing water contents. In this trial 'Beauregard' produced roots containing an average of 17.8% dry matter, whereas 'Owairaka Red' produced roots with 21.8% dry matter content. When storage root dry matter content was compared within cultivars for different lengths of field exposure, none differed significantly except for some 'Beauregard' values. While 'Beauregard' appeared to show a trend of decreasing dry matter with length of field exposure, only the extremes, samples grown in the field for 1 and 18 years respectively, differed significantly ($P<0.001$). When root yield data were

Figure 1 Sweetpotato (*Ipomoea batatas* (L.) Lam.) storage root yield on a fresh weight basis in relation to the number of field production cycles to which the crop has been exposed subsequent to maintenance in virus-tested tissue culture. Data presented are fitted curves and mean observed yields for cultivars 'Beauregard' (closed circles) and 'Owairaka Red' (open circles). The LSD ($P=0.05$) of 3.44 t/ha is indicated by a vertical bar.

Figure 2 Sweetpotato (*Ipomoea batatas* (L.) Lam.) storage root yield on a dry weight basis in relation to the number of field production cycles to which the crop has been exposed subsequent to maintenance in virus-tested tissue culture. Combined data for cultivars 'Beauregard' and 'Owairaka Red', showing mean observed yields, fitted curve and the LSD ($P=0.05$) of 0.86 t/ha (vertical bar).

expressed on a dry matter basis, as opposed to the fresh weight basis shown in Figure 1, a common curve (Figure 2) could be fitted across the data sets of both cultivars ($P<0.001$). The two cultivars are shown to have a comparable level of efficiency in root carbohydrate storage and share a similar response pattern to increasing field exposure.

The yield components underlying cultivar decline were examined. ‘Beauregard’ plants grown in the field for only one season had significantly more storage roots per plant than those exposed for longer periods ($P<0.001$). The same was true for OR-1, but OR-2 showed no significant change in root numbers due to field exposure (Table 1). When mean root weights were compared within cultivars, there was no significant response to increased field exposure for ‘Beauregard’ or OR-1, but OR-2 showed a significant decrease in mean root weight following long-term field exposure ($P<0.001$). The clone OR-2 was selected for its blocky root shape so relatively fewer roots were discarded due to diameters of less than 2.5 cm; however the retained smaller roots lowered the derived mean root weight.

The yield and number of ‘Beauregard’ roots in the Canner and Jumbo grades did not significantly differ with increasing field exposure. In both cultivars, the yield and number of Number-1 grade roots significantly decreased with long-term field exposure ($P<0.001$). ‘Owairaka Red’ (OR-1 and OR-2) showed a significant decrease in the yield and number of

Jumbo grade roots with increasing field exposure ($P<0.001$). A comparison of clones OR-1 and OR-2 after 1 year of field exposure showed OR-2 to have a significant increase in yield and number of Jumbo grade roots, due to its shape-based selection criterion ($P<0.001$).

The root quality score for ‘Beauregard’ roots after one season of field exposure (mean score of 3.7) was significantly higher than those with greater periods of exposure, particularly roots from plants with 18 seasons (mean score of 2.8) of field maintenance ($P<0.001$). The root quality score for ‘Owairaka Red’ did not significantly differ with increasing field exposure, but roots of this cultivar are particularly irregular in appearance.

This study demonstrated that sweetpotato cultivar decline occurs in New Zealand, although high levels of treatment replication are required to robustly quantify its effect. It has also shown that the two cultivars dominating the local market have a similar pattern and degree of response, as measured by root carbohydrate accumulation. There is some evidence that under this syndrome, yield is primarily reduced through a decline in storage root numbers, as has been observed in China (Feng et al. 2000). There is also support for the suggestion (Bryan et al. 2003) that root quality may decline with increasing numbers of field growth cycles, based on the general impression scores observed for cultivar ‘Beauregard’. The relatively rapid yield decline over the first three to four field maintenance cycles, subsequent

Table 1 Mean number of storage roots per plant for tissue cultured sweetpotato (*Ipomoea batatas* (L.) Lam.) clones following various iterations of field growing cycles.

Cultivar	Field exposure (seasons)				
	1	2	3	4	>10
Beauregard					
Clone 0	6.8	6.2	5.8	5.9	5.5 ¹
Owairaka Red					
Clone 1	6.5	5.6	5.4	5.6	
Clone 2	5.3				5.4 ²

P<0.001, LSD (P=0.05) = 0.64

¹18 growing seasons in the field following the initial introduction from tissue culture.
²12 growing seasons in the field since the initiation of a single hill clonal selection programme.

to sourcing material from virus-tested tissue culture, is similar to that observed in the USA (Bryan et al. 2003) and China (Zhang et al. 2006).

While this study provides a broad outline of the effects of cultivar decline, it does not identify the specific causal agents. Interpretation of the possible underlying issues and determining their relative contributions is dependent on research conducted offshore, which is still not definitive.

The majority of mutational events that lead to phenotypic change produce undesirable sweetpotato plants (La Bonte et al. 2001). Some adverse effects are easily observed due to altered colouration, as in leaves unable to synthesise chlorophyll or plant tissues that have lost the capacity to produce either anthocyanin or carotene. Other effects, such as lower root yield or reduced quality, are more subtle and are less easily determined within a varying environmental context. Occasionally the phenotypic variation produced by mutation may be beneficial, allowing selection of cultivars such as 'Owairaka Red' (Lewthwaite 1998) or newly derived versions such as the clone OR-2 used in this study. While some phenotypic changes are readily observed, molecular tools are required to provide a broad measure of genomic variability. However, the level of molecular variability does not necessarily reflect the degree of phenotypic variability (Villordon & La Bonte 1995). When 12 virus-tested mericlones of the cultivar 'Beauregard' were evaluated in field trials, a comparison of the highest and lowest yielding mericlones showed a 40% difference in total marketable yield (Villordon et al. 2003).

While mutations were once considered the primary issue for cultivar maintenance, recently acquired knowledge has identified virus infection as an important factor in cultivar decline (La Bonte et al. 2001). The physical symptoms of viral infection in sweetpotato can be relatively subtle, so the subject has been under-researched for many years. Sweetpotato plants are often co-infected with a number of viruses, producing more severe disorders through their synergistic interaction. A further complication to research has been the difficulty in reproducing typical field symptoms in artificially inoculated plants (Clark & Valverde 2001). Aphid and whitefly have been

identified as important vectors, but the methods of transmission for some viruses remain unknown (Loebenstein et al. 2009). Average yield losses due to viral diseases in China, the world's largest sweetpotato producer, are estimated at over 20% (Gao et al. 2000), while losses of 98% have been reported in some African cultivars (Njeru et al. 2004). Responses to viral infection are cultivar-specific, as demonstrated by a recent Australian study in which virus-tested tissue-cultured and field-derived plants were compared. Amongst the 14 cultivars evaluated, 'Beauregard' showed the greatest gain with a 148% increase in total storage root yield when grown from tissue cultured plants, whilst 'Wanum' showed the greatest loss, decreasing in yield by 23% under the same experimental regime (Okpul et al. 2011).

An interaction may occur between the biotic stress caused by viral infection and the high levels of mutation observed in sweetpotato. It has been suggested that as the transcriptional activity of sweetpotato retrotransposons may be modified by stress, the relative levels of transcription and therefore mutation could be increased by viral infection (Kokkinos 2002).

Although there is still much to learn about the factors contributing to cultivar decline, in recent years the local industry has begun to examine the benefits of introducing micro-propagated plants as source material within the commercial growing cycle.

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