Sweetpotato cultivar susceptibility to postharvest soft rot caused by *Rhizopus stolonifer*

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Abstract Infection by the fungal pathogen *Rhizopus stolonifer* causes a postharvest disease in sweetpotato (*Ipomoea batatas*) roots known as soft rot. In recent years, due to changes in legislation prescribing acceptable agrichemical residues, post-wash applications of the fungicide dicloran can no longer be used on exported sweetpotato roots. An important component of any alternate disease control system is cultivar resistance. This study examined the range of responses within artificially inoculated roots of various cultivars, under different wounding regimes. While none of the cultivars evaluated was immune to infection, they differed in their degree of susceptibility ($P < 0.001$). Cultivars also differed in their response to the type of wound they received at inoculation ($P < 0.001$). The internationally recognised cultivar, 'Beauregard', was vulnerable to infection through piercing wounds, but showed relatively less susceptibility when the wound was a bruise. Disease evaluation using both piercing and bruising wounds appears necessary in characterising sweetpotato germplasm.

Keywords kumara, fungus, disease, resistance, wound.

INTRODUCTION

The fungal pathogen *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. causes a disease in sweetpotato or kumara (*Ipomoea batatas* (L.) Lam.) storage roots known as rhizopus soft rot. Internationally, soft rot is considered one of the crop's most important postharvest diseases (Clark et al. 2009), but it may also cause preharvest losses in production beds and the field (Coleman 1972). In New Zealand, the first formal record of *R. stolonifer* on sweetpotato was published in 1959 (Brien & Dingley 1959). Thirteen years later, soft rot was described as a particularly prevalent and destructive disease of stored sweetpotato roots (Coleman 1972).

Rhizopus stolonifer is a ubiquitous fungus with a wide range of host plants. For infection to occur, the pathogen requires a wound associated with necrotic tissue (Clark & Moyer 1988). Once established, the disease progresses into healthy tissue, causing a soft watery rot throughout the root's parenchymatous tissue. The root's periderm and fibrous material remain largely intact. Long whiskery mycelia typically grow out through lenticels and other openings in the periderm, producing sporangia (Clark & Moyer 1988).

As the pathogen requires a wound to establish itself within plant tissue, a key control measure is to minimise wounds and encourage their

healing. However, new injuries are inevitable during washing, packing, shipping and marketing operations (Snowdon 1991). New Zealand's first commercial sweetpotato washing plants were established in the Dargaville region in 1989 (Lewthwaite 1998). As almost all of the nation's crop is now washed before packing and shipping, soft rot has become a major issue for commercial-scale production. This problem has largely been resolved by adding the fungicide dicloran to washing systems.

Dicloran has been in common international use for postharvest *R. stolonifer* control for several decades (Clark et al. 2009). However from a lifestyle and marketing perspective, there has been increasing interest in minimally modified food products, including reduced agrichemical applications. This consumer focus has resulted in both voluntary and legislated changes to production and marketing requirements with regard to agrichemical usage. The European Union, and the United Kingdom (UK) in particular, represent this market, where consumers expect the highest quality produce with minimal inputs from synthetic sources. The UK imports significant quantities of sweetpotatoes, but has recently introduced legislation that lowers its accepted agrichemical residue levels. As a consequence, dicloran can no longer be used on exported sweetpotato, and New Zealand's international trade in fresh roots has ceased. It is expected that consumer interest in reduced agrichemical use will become more pronounced and increasingly widespread throughout international markets, as well as within New Zealand (Lewthwaite 2006).

Alternate multi-factorial or 'multi-hurdle' systems of soft rot control are required, as opposed to reliance on a single effective fungicide. Any new approaches must also enhance consumer acceptance of sweetpotato as a product. Sweetpotato roots shipped to the UK from New Zealand have to remain in good market condition for 1 to 2 months after being washed and packed, a period that includes transit and market display. Potential approaches to reduce disease include sanitation of the washing-plant and sweetpotato

root surfaces (Brash et al. 2010), minimised root damage during bulk handling operations, application of market acceptable compounds that form a barrier to disease development, and sweetpotato cultivar resistance.

While sweetpotato cultivars vary in their susceptibility to soft rot (Clark & Hoy 1994), no cultivars are considered immune. Previous researchers have also indicated that the disease response varies with the duration of the storage period (Holmes & Stange 2002) and the type of wound that provides pathogen entry (Clark & Hoy 1994; Holmes & Stange 2002). This study evaluated soft rot disease development in sweetpotato storage roots of different cultivars, using varying wounding methods.

MATERIALS AND METHODS

The *R. stolonifer* strain used in this study was isolated from a sweetpotato storage root grown in a commercial crop at Dargaville, New Zealand. This fungal strain was deposited in the International Collection of Micro-organisms from Plants (ICMP), where it is maintained as ICMP number 10436 (Young & Fletcher 1997). Sterile Petri dishes containing potato dextrose agar (Oxoid Ltd, Hampshire, England) were inoculated with pure cultures of this fungal strain and incubated at 20°C for 1 day, to provide actively growing cultures. A sterilised cork borer was used to take 5-mm diameter agar plugs from culture margins, and these plugs were used as inoculum sources throughout the study.

Six distinctive sweetpotato cultivars were selected for assessment, namely 'Owairaka Red' (Lewthwaite 1998), 'Beauregard' (Rolston et al. 1987), 'Toka Toka Gold' (Lewthwaite 1998), 'Northland Rose' (Broadhurst et al. 1997), 'Radical' (Philpott et al. 2003) and 'S1819' (Lewthwaite et al. 2011). 'Beauregard' was included as a control, as it is considered relatively soft rot resistant (Clark et al. 2009); it is also grown internationally and is New Zealand's second most widely grown commercial cultivar. Four experiments were conducted in this study; procedures common to all experiments are as follows. Just prior to treatment, healthy storage

roots of commercial size were selected for each sweetpotato cultivar. The roots were hand-washed in running tap water and their surfaces air-dried over a 4 hour period. At the time of treatment, the roots were wounded or left intact, inoculated with *R. stolonifer*-infected or sterile agar plugs, lightly misted with sterile distilled water and then sealed in high-humidity storage chambers at 20°C for 2 days. A chamber consisted of a fully enclosed polystyrene box prepared by lining the base with corrugated cardboard soaked with distilled water. Each experiment was arranged in a randomised complete block design with chambers as blocks. Following incubation, the roots were individually weighed, cut open through the point of inoculation, and any rotted tissue washed out under a stream of water. The remaining healthy root tissue was patted surface dry with paper towels and re-weighed. The difference between the initial root weight and the weight after rot removal provided a measure of rot development. Control treatments of the cultivar 'Beauregard', which were either wounded and had a sterile agar plug applied or were left intact with an infected agar plug applied, were included in the storage chambers of all four experiments.

Sweetpotato storage roots that appeared healthy were harvested from a commercial field at Dargaville on 4 April 2011 and stored under controlled conditions in multi-walled paper bags at 15°C and 85% relative humidity. All six cultivars were examined in the initial three experiments, which differed primarily in the type of wound applied. In the first experiment, a sterile 5-mm diameter cork borer was driven 5 mm into each root, half way along the root's length and at right angles to the root surface. The core of root tissue was removed and a *R. stolonifer*-infected agar plug was inserted into the cavity. The agar plug was placed in an upright position, so that fungal growth was level with the root surface. Roots used in this experiment had been stored for 42 weeks from harvest. In the second experiment, the sterile 5-mm diameter cork borer was similarly driven 5 mm into each root, but the borer was drawn straight out to leave the core of tissue still attached at its base,

providing a puncture wound. At inoculation, the infected agar plug was positioned inside the circular puncture wound, in an inverted position so that fungal growth was in direct contact with the root's periderm. Roots used in this experiment had been stored for 44 weeks from harvest. The wounding method for the third experiment consisted of dropping a 100-g dome-headed bolt down a vertical 1-m tube onto the mid section of each root, causing a bruise. An inverted infected agar plug was placed on the bruised tissue. Roots in this experiment had been stored for 46 weeks from harvest. For these three experiments, two roots of each cultivar were included in each of the ten storage chambers. The mean weight of rotted tissue per cultivar in each chamber was used for analysis.

A fourth trial was undertaken to compare cultivar wounding responses in one common experiment. Sweetpotato storage roots harvested on 13 April 2012 from a Pukekohe field were stored under controlled conditions, as for the previous three experiments. Apparently healthy roots from five cultivars were selected ('Beauregard', 'Toka Toka Gold', 'Northland Rose', 'Radical' and 'S1819') and hand-washed. However, for this experiment, because of space constraints, all roots were trimmed at either end to provide a 7-cm length. Roots used in this experiment had been stored for 25 weeks from harvest. Each root was wounded with one of the three previous methods (cavity, puncture or bruise) prior to inoculation. In total 17 chambers were used in this experiment, each chamber containing one root for each treatment combination (cultivar by wounding method) and the control treatments. The weight of rotted tissue for each individual root was used for analysis, providing 17 replicates of each treatment. Data from all experiments were subject to analysis of variance, and means were compared with Fisher's least significant difference (LSD) test, using the Genstat® statistical package (2011).

RESULTS AND DISCUSSION

No disease symptoms developed on unwounded roots inoculated with *R. stolonifer* or on wounded

roots with sterile agar plugs applied. None of the cultivars was immune to infection by *R. stolonifer*, but the cultivars differed in their degree of susceptibility. The interface between rotted and healthy tissue was well defined in all infected roots, allowing a simple weight assessment by removal of the necrotic tissue. In some cultivars, the disease symptoms developed in distinctive ways. In cultivar 'S1819' rots developed in specific tissue types, so lesions became slightly more dendritic, whilst the rotted material of 'Radical' was of a drier texture than that of other cultivars.

The wounding applied in the first experiment was relatively severe, but similar cavities can be observed when well-established sprouts are removed from stored roots during their preparation for market. The amounts of rotted tissue in 'S1819' (57.5 g), 'Owairaka Red' (70.1 g) and 'Northland Rose' (72.0 g) did not significantly differ from that of 'Beauregard' (64.5 g; $P>0.05$; Figure 1). There was, however, a significantly lower degree of rotting in 'Toka Toka Gold' (49.6 g; $P<0.01$), with a highly significant reduction in 'Radical' (31.6 g; $P<0.001$).

Figure 1 Mean weights (g/root) of rotted tissue in storage roots of sweetpotato (*Ipomoea batatas*) cultivars inoculated with *Rhizopus stolonifer*. Three independent experiments investigated the effects of wounding method (cavity, puncture or bruise). Vertical bars indicate least significant differences ($P=0.05$) for each experiment.

Piercing or cutting wounds break the root periderm without removing tissue, as simulated by the puncture wound of the second experiment. Under this wounding system, amounts of rotted tissue were similar for 'Owairaka Red' (46.7 g), 'Toka Toka Gold' (44.6 g) and 'Beauregard' (44.6 g; $P>0.05$; Figure 1). 'Northland Rose' (56.3 g) developed significantly larger rots ($P<0.01$), while 'Radical' (26.4 g) and 'S1819' (26.9 g) had highly significant reductions in rot development ($P<0.001$).

There are opportunities for bruising injuries to occur throughout packhouse/marketing operations, via accidental falls or impact with processing equipment, produce containers and other roots. New Zealand sweetpotato growers have indicated that in their experience the traditional cultivar 'Owairaka Red' develops soft rot more readily than does the cultivar 'Beauregard'. Overseas-derived results also suggest that 'Beauregard' is relatively less prone to soft rot than other cultivars (Clark & Hoy 1994; Clark et al. 2009), but the comparison of cultivar responses to cavity and puncture wounds conducted here does not support this conclusion. A third investigation, based on bruising injury, was carried out to test the relative contribution of this wounding method to cultivar sensitivity. Following bruise injury, rotted tissue weights for cultivars 'S1819' (1.3 g) and 'Radical' (12.3 g) did not differ significantly from that of 'Beauregard' (5.8 g; $P>0.05$; Figure 1). However, significantly increased rotting was observed in 'Toka Toka Gold' (17.0 g; $P<0.05$), 'Owairaka Red' (19.8 g; $P<0.01$) and 'Northland Rose' (23.0 g; $P<0.001$).

The first three experiments were independent investigations, so the final experiment included all three wounding methods, to confirm the presence and nature of cultivar by wound interaction effects. Because of limited space, cultivar 'Owairaka Red' was not included in this experiment. In this fourth experiment, the cultivar, wound, and cultivar by wound interaction effects were all highly significant ($P<0.001$). The different wounding methods showed the same general pattern and magnitude of rotting seen across the three independent

experiments. The cavity wounding method produced the greatest weight of rotted tissue, across all cultivars (Figure 2), while the puncture wound produced significantly less and the bruising wound produced less again ($P < 0.001$). Across all wounding methods, 'Northland Rose' (64.0 g) had a significantly greater weight of rotted tissue than 'Beauregard' (42.6 g; $P < 0.001$), while 'S1819' (37.8 g; $P < 0.01$), 'Radical' (14.8 g) and 'Toka Toka Gold' (33.5 g) had significantly less ($P < 0.001$).

In the cavity wounded portion of the fourth experiment, 'S1819' (60.4 g; $P < 0.05$), 'Toka Toka Gold' (45.5 g) and 'Radical' (20.3 g; $P < 0.001$) had significantly less rotting than 'Beauregard' (70.0 g). For the puncture wound, only 'Radical' (15.4 g) and 'Toka Toka Gold' (32.3 g) had significantly less rotting than 'Beauregard' (52.3 g; $P < 0.001$). When the wound was a bruise, 'Northland Rose' (57.5 g) and 'Toka Toka Gold' (22.6 g) produced significantly ($P < 0.001$) greater rots than 'Beauregard' (5.5 g; Figure 2).

A cultivar by wound interaction effect can be seen in the fourth experiment, which appears to be supported by the results of the proceeding three experiments. Comparison of the weight of rotted tissue produced with a puncture wound against that of a cavity wound, for each cultivar, shows a difference varying from 71% ('Toka Toka Gold') to 90% ('Northland Rose'). However, comparing the weight of rotted tissue produced with a bruising wound against that of a cavity wound, for each cultivar, shows differences of 42% ('Radical'), 50% ('Toka Toka Gold') and 81% ('Northland Rose'); whilst bruising in both 'S1819' and 'Beauregard' produced only 8% of the cavity rot. This suggests that 'S1819' and 'Beauregard' are particularly resistant to soft rot infection from bruising wounds, even though disease progress via cavity and puncture wounds demonstrates that their tissues are not generally resistant. If it is assumed that the most common wounds in commercial production are bruises, then resistance to soft rot infection through bruising wounds may explain commercial growers' observation that 'Owairaka Red' appears more susceptible to soft rot than 'Beauregard'.

Figure 2 Mean weights (g/root) of rotted tissue in storage roots of sweetpotato (*Ipomoea batatas*) cultivars inoculated with *Rhizopus stolonifer*. In experiment 4, the effects of three wounding methods (cavity, puncture and bruise) were investigated for each cultivar. A vertical bar shows the least significant difference ($P = 0.05$).

Amongst the cultivars examined here, 'Northland Rose' appears particularly prone to soft rot infection. However, a comparison of the infection response (mean of 23.0 g) in the third experiment (Figure 1), with that of the bruising wound (mean of 57.5 g) in the fourth experiment (Figure 2), shows an elevated amount of rotted tissue for 'Northland Rose'. While the results of the first three experiments are broadly in accord with that of the fourth experiment, the difference in degree of response for bruising in 'Northland Rose' may be due to the different sources and storage durations of roots used in the two experiments. Other researchers have shown a temporal change in root susceptibility to soft rot infection, with high resistance to infection for the first month or two after harvest, followed by increasing susceptibility for approximately 3–6 months, then a decline in susceptibility as storage is prolonged (Holmes & Stange 2002).

The cultivar 'Radical' appears to develop relatively low levels of soft rot infection, regardless of wounding method. Further work is required to understand what plant characteristics underlie this response and for those cultivars that are relatively robust to bruising effects.

The bioassay systems developed here provide a useful comparison of cultivar responses to soft rot infection. It appears that at least two wounding methods are required to characterise germplasm within a sweetpotato breeding programme. The availability of sweetpotato cultivars with a range of soft rot susceptibilities may provide the basis for developing cultivars that make a meaningful contribution to soft rot management.

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