

## Laboratory evaluation of sweetpotato (*Ipomoea batatas*) resistance to sclerotinia rot

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**Dedication** This paper is dedicated to Philip G. Broadhurst (1963–99), who initiated the research that this paper describes.

**Abstract** Sweetpotato (*Ipomoea batatas*) resistance to sclerotinia rot (pink rot), caused by the fungus *Sclerotinia sclerotiorum*, was evaluated using a laboratory technique. Excised stem lengths of four commercial sweetpotato cultivars were pierced, then inoculated with mycelial disks from *S. sclerotiorum* cultures. The inoculated stems were incubated in moist chambers for 48 h at 20°C and then assessed for rot severity. Of the four cultivars, ‘Toka Toka

Gold’ was most susceptible to the fungus and ‘Beauregard’ most resistant. The cultivars ‘Owairaka Red’ and ‘Northland Rose’ had moderate levels of resistance. Correlations between laboratory and field results were good for ‘Toka Toka Gold’ and ‘Beauregard’. Although plant growth habit affected the disease responses of ‘Owairaka Red’ and ‘Northland Rose’, laboratory and field results for these cultivars were similar. Laboratory tests will prove useful in determining the level of disease resistance in diverse sweetpotato germplasm.

**Keywords** sweetpotato; *Ipomoea batatas*; pink rot; sclerotinia rot; *Sclerotinia sclerotiorum*; disease resistance; cultivars

### INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L.) Lam.) or kumara is a vegetable crop of economic importance in New Zealand and throughout the world. The history of sweetpotato cultivation in New Zealand was described by Lewthwaite (1997). New Zealand’s sweetpotato production area for the year ending 30 June 2000 was 1200 ha, with the Northland district accounting for 94% of production (Statistics New Zealand). Almost all sweetpotatoes grown in New Zealand are sold fresh for the domestic market.

Several varieties of sweetpotato are grown commercially in New Zealand. The cultivar ‘Owairaka Red’ (an early post-European introduction) accounts for 80–90% of New Zealand production (Lewthwaite 1991). More recently, other cultivars have been grown commercially, including ‘Toka Toka Gold’ (a New Zealand selection), ‘Beauregard’ (an introduced cultivar from the United States), and ‘Northland Rose’ (formerly 93N9/2, a New Zealand selection from seed supplied from Taiwan).

Sclerotinia rot (commonly referred to as “pink rot”), caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is an economically important disease of commercial sweetpotato crops in New Zealand (Lewthwaite 1997). *S. sclerotiorum* causes

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destructive diseases of many important crops worldwide. The pathogen has been recorded on 408 hosts in many regions of the world (Workneh & Yang 2000), and on 101 hosts in New Zealand (Pennycook 1989). The disease occurs in sweetpotato propagation beds and in the field (Broadhurst et al. 1997). The fungus survives as resting bodies (sclerotia) in soil and crop debris. Infection usually takes place at or near soil level, causing rotting of plant tissue and leading to collapse of vines and sometimes death of entire plants. Storage roots may also be infected, causing wet rots that may be encrusted with black sclerotia (Broadhurst et al. 1997; Lewthwaite 1997). The predominant cultivar, 'Owairaka Red', has storage roots with deep red/purple skin that turn bright pink when infected with *S. sclerotiorum*, hence the common name for the disease, pink rot.

The most noticeable symptom of sclerotinia rot in sweetpotato plants is characteristic wilting of the leaves, followed by rotting of the stems (Coleman 1978). Irregular-shaped whitish masses of mycelium are produced on infected plant tissue and these structures, after exuding liquid, continue development into sclerotia (Coleman 1978; Broadhurst et al. 1997). The sclerotia enable the fungus to survive in the soil for several years (Chupp & Sherf 1960). Optimum conditions for infection of plants by *S. sclerotiorum* are abundant moisture and moderate temperatures between 15 and 21°C (Chupp & Sherf 1960).

The use of resistant varieties in conjunction with crop rotation are effective means for controlling diseases caused by *Sclerotinia* (Chupp & Sherf 1960; Steadman 1983). During field evaluation of local and international sweetpotato clones Broadhurst et al. (1997) found considerable variation in plant susceptibility to sclerotinia rot.

There has been no published systematic screening of sweetpotato for resistance to *S. sclerotiorum*, apart from Broadhurst et al. (1997). However, several workers have screened sweetpotato breeding lines for resistance to other fungal pathogens, including *Alternaria* spp. (Anginyah et al. 2001), *Ceratocystis fimbriata* (Cheo 1953), *Fusarium* spp. (Clark et al. 1986), *Monilochaetes infuscans* (Nielsen & Yen 1966), and *Rhizopus* spp. (Clark & Hoy 1994). Many crops have been evaluated for resistance to *S. sclerotiorum* using laboratory inoculation methods, including soybean (Cline & Jacobsen 1983; Wegulo et al. 1998), bean (Hunter et al. 1981), sunflower (Robert et al. 1987), cabbage (Dickson et al. 1996), and alfalfa (Pratt & Rowe 1995).

*Sclerotinia sclerotiorum* is a particularly important disease in New Zealand sweetpotato crops as the established cultivar 'Toka Toka Gold' is highly susceptible to the pathogen. Also, little rotation is practised in the main sweetpotato production area, as fresh land and alternative crops are limited. Sweetpotato clones and seed from around the world are being evaluated to broaden the genetic base of the crop. It is important that new sweetpotato material is robust when challenged by diseases that are already of economic importance under New Zealand's current crop management regimes. The development of an economic and reliable test to assess sweetpotato germplasm for resistance to *S. sclerotiorum* would be of considerable benefit in the selection of clones suited to New Zealand conditions.

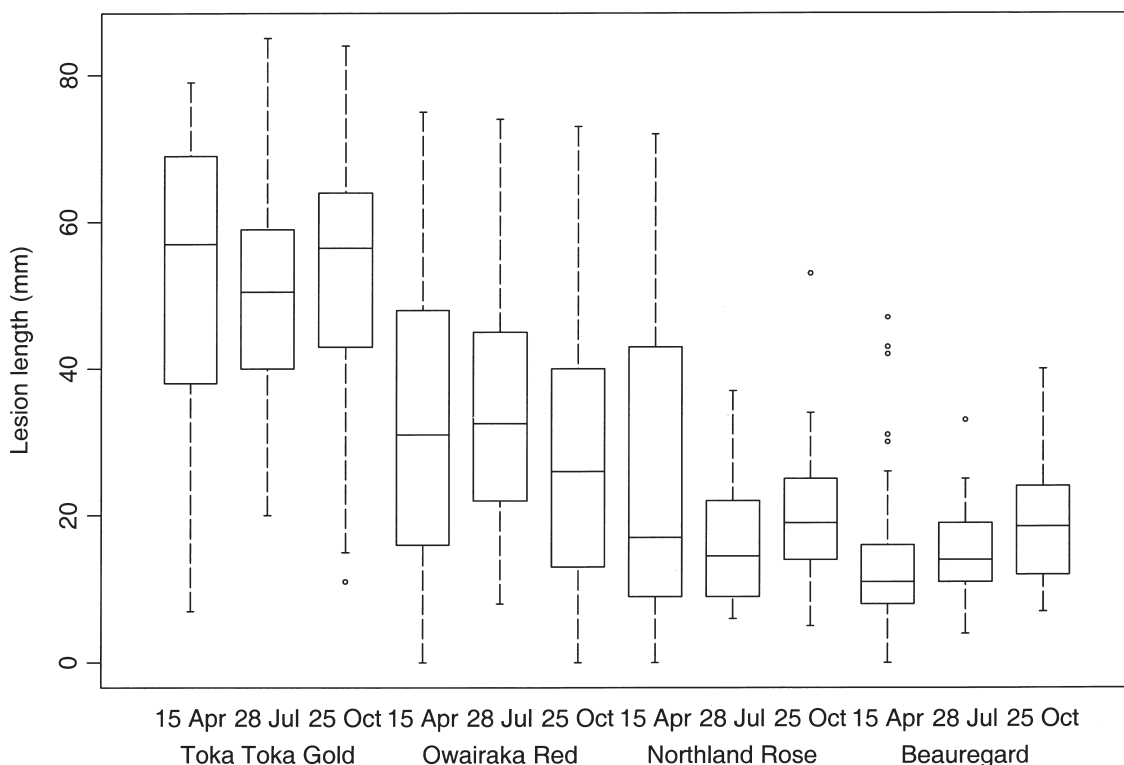
This paper describes a laboratory screening method for evaluating sweetpotato cultivar resistance to *S. sclerotiorum*, based on lesion development in excised stem segments.

## MATERIALS AND METHODS

The strain of *S. sclerotiorum* used in this study was isolated from sclerotinia rot lesions on infected stems of sweetpotato grown at the Pukekohe Research Centre (lat. 36° 57'S). The strain (ICMP 13158) is held in the International Collection of Microorganisms from Plants (ICMP), Landcare Research, Auckland, New Zealand. The same strain was used by Broadhurst et al. (1997) for field inoculation and evaluation of sweetpotato cultivars.

Four sweetpotato cultivars were tested: 'Owairaka Red', 'Beauregard', 'Toka Toka Gold', and 'Northland Rose'. The screening experiments were repeated on three dates: 13 April, 26 July, and 23 October 2000.

Sweetpotato sprouts were produced in an unheated greenhouse on storage roots placed in polystyrene planter boxes (595 × 420 × 190 mm high) containing 20 litres of commercial bedding mix (1 part peat : 1 part pumice, with slow release fertilisers added). Fresh, actively growing sprouts were harvested above ground level when they had reached at least 200 mm in length. Fifty stems were trimmed to 100 mm lengths for each of the four cultivars, by cutting 10–20 mm below the apical tip of each stem and then trimming the distal end to the required length. The leaves and petioles were removed from each stem piece. Stem pieces were inoculated as soon as practical after excising the plant material.



**Fig. 1** Boxplots showing length (mm) and variability of *Sclerotinia sclerotiorum* lesions in four sweetpotato (*Ipomoea batatas*) cultivars following moist 48 h incubation at 20°C. Data grouped by cultivar across three experiment dates.

Ten moist chambers were prepared by lining the bottom of tight-sealing plastic boxes (300 × 210 × 90 mm high) with corrugated cardboard that had been moistened with distilled water. Each chamber was marked into quarters, and five stem pieces of each cultivar were placed in each quarter of the chamber, with individual stems spaced 20 mm apart. Each box of 20 stems constituted a block and the experiment comprised of 10 high humidity chambers (blocks).

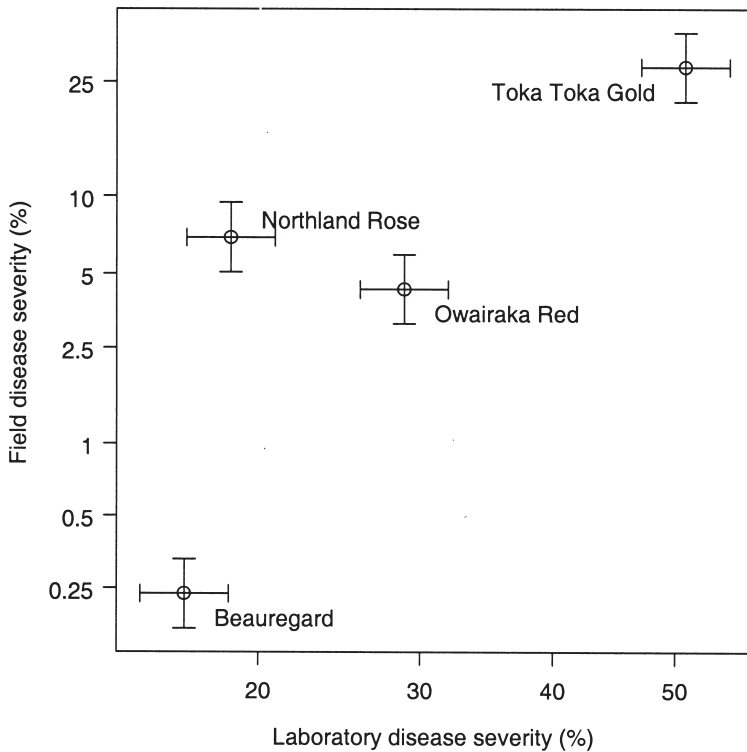
Two holes, 3 mm apart, were made into the centre of each stem using a flame-sterilised 0.45-mm-diameter hypodermic needle. The wounds were made near the middle of the stem, midway between points of leaf petiole insertion. A sterile cork borer was used to take 4-mm-diameter plugs of *S. sclerotiorum* from the actively growing margins of 48 h cultures growing on potato dextrose agar (PDA), incubated at 20°C under 12-h light/dark conditions. One plug was placed mycelium downwards over the puncture wounds in each stem. Control treatments included wounded stems

inoculated with sterile agar plugs. The inoculated stems were lightly misted with sterile water before the chambers were sealed shut. Inoculated stems were incubated inside the moist chambers at 20°C for 48 h, after which time sclerotinia rot lesion length was recorded. Data were subject to analysis of variance using the Genstat® statistical package (2000).

## RESULTS

Infected tissue of inoculated stems had a water soaked appearance and was discoloured. Lesion colour varied from pale pink to dark brown, depending on the cultivar. White, cottony mycelium was present on some infected stem tissue.

After a preliminary analysis that showed not only large differences in susceptibility between cultivars within each of the three experiments, but also several data values at each date with large residuals, the data were transformed to the log-scale and reanalysed.



**Fig. 2** Mean severity of *Sclerotinia sclerotiorum* infection in four sweetpotato (*Ipomoea batatas*) cultivars after field screening (% diseased vine tissue) and laboratory screening (% diseased stem tissue) tests. Percentages on horizontal and vertical scales plotted on logit axes. Barred lines parallel to each axis represent  $LSD_{0.05}$  values for each disease incidence. If horizontal or vertical lines for two cultivars overlap then fitted laboratory or field disease incidences respectively do not differ significantly.

**Table 1** Mean length (mm) of *Sclerotinia sclerotiorum* lesions in four sweetpotato (*Ipomoea batatas*) cultivars following moist 48 h incubation at 20°C. Data combined across three independent assessment dates. (LSD based on 81 degrees of freedom.)

Cultivar	Mean lesion length (mm)
Toka Toka Gold	51
Owairaka Red	32
Northland Rose	20
Beauregard	16
$LSD_{0.05}$	5.4
<i>P</i>	<0.001

occasion, ‘Toka Toka Gold’ (TG) was most susceptible to *S. sclerotiorum*, followed in order by ‘Owairaka Red’ (OR), ‘Northland Rose’ (NR), and ‘Beauregard’ (Be), which was relatively resistant. None of the non-inoculated (control) stems developed sclerotinia rot lesions.

The length of the sclerotinia rot lesions was consistent across the three assessment dates for each of the four cultivars, except for ‘Northland Rose’ on 15 April which had several stems with relatively long lesions (Fig. 1).

When analysis was carried out on the combined data for the three experiments, there was no evidence of an assessment date by cultivar interaction ( $P = 0.33$ ), despite the slightly anomalous behaviour of ‘Northland Rose’ on 15 April. Furthermore, there was no overall difference between the experiments ( $P = 0.84$ ). The data from the three experiments were, therefore, combined to demonstrate statistically significant differences ( $P < 0.001$ ) in cultivar susceptibility to *S. sclerotiorum* (Table 1).

The relationship between disease severity (% diseased vine tissue) of plants under field (Broadhurst et al. 1997) and laboratory evaluation

However, the distribution of this transformed data was not approximately normal, with some stems with small values skewing the data strongly towards low values. Because of this, the data were left untransformed for all subsequent analyses.

The rank order of the four sweetpotato cultivars for mean length of sclerotinia rot lesions was the same for all assessment dates (Fig. 1). On each

techniques was explored (Fig. 2). The four cultivars gave similar results under both systems. However, the relative positions of 'Owairaka Red' and 'Northland Rose' changed. Although both cultivars were of average resistance, the field technique ranked 'Northland Rose' as being slightly more susceptible than 'Owairaka Red' (11% versus 8% disease,  $P = 0.12$ ), while in the laboratory 'Northland Rose' showed much lower disease incidence (19% versus 29%,  $P < 0.001$ ).

## DISCUSSION

The laboratory assessment technique was reproducible across the three experiments for the four sweetpotato cultivars tested. This indicates that the technique is a useful screening method for evaluating the susceptibility of sweetpotato to infection by *S. sclerotiorum*.

In the field, incidence and severity of *S. sclerotiorum* infection is the product of tissue susceptibility and plant growth habit. Chun et al. (1987) and Nelson et al. (1991) reported that lesion lengths on stems of soybean cultivars inoculated in the laboratory with *S. sclerotiorum* had either varying or no correlation with incidence of sclerotinia stem rot in field experiments using the same cultivars. In our experiments, we found that resistance levels to *S. sclerotiorum* using the laboratory inoculation method were similar to those seen in the field (Broadhurst et al. 1997), using the same cultivars. In both instances, 'Toka Toka Gold' was the most susceptible cultivar to *S. sclerotiorum*, and 'Beauregard' the least susceptible. However, in the two cultivars that showed less extreme responses to *S. sclerotiorum* infection the order was reversed, the laboratory tests ranked 'Owairaka Red' as more susceptible than 'Northland Rose', whereas the field evaluations ranked 'Northland Rose' as slightly more susceptible than 'Owairaka Red'.

These results suggest that the increased disease severity observed in 'Northland Rose' in the field may be partly because of the growth habit of this cultivar. Although relatively bushy, 'Northland Rose' produces a larger number of primary vines than 'Owairaka Red' so that inter-vine and vine-soil contact around the immediate crown of the plant is increased. Several researchers, including Steadman (1983), Campbell et al. (1985), and Huang & Kemp (1989) have reported that bean plants with upright and bushy growth often have a lower incidence of *Sclerotinia* infection than dense, viny plants which

have a greater proportion of plant tissue on and near the soil. Saindon et al. (1993) reported that a bean cultivar (LRS92-1) did not carry genes for resistance to infection by *S. sclerotiorum* disease, but rather avoided sclerotinia disease because of its strong upright growth habit.

*Sclerotinia sclerotiorum* is not considered an important pathogen of sweetpotato in the United States (Walker 1952; Clark & Moyer 1988), suggesting that sweetpotato cultivars grown there may be relatively resistant to *S. sclerotiorum*. The laboratory experiments described in this paper and the field experiments carried out by Broadhurst et al. (1997) confirm that the main North American cultivar 'Beauregard' is considerably more resistant to sclerotinia rot than the dominant New Zealand cultivar 'Owairaka Red' and particularly, 'Toka Toka Gold'.

Successful control of sclerotinia rot in sweetpotato will primarily be achieved by growing resistant cultivars and by lowering the risk of infection. This would involve not planting sweetpotato in fields with an immediate history of severe *Sclerotinia* problems and not using alternate hosts to *S. sclerotiorum* in crop rotations. Losses caused by *S. sclerotiorum* in New Zealand could be significantly reduced if local growers moved away from traditionally grown sweetpotato cultivars that are susceptible to sclerotinia rot to cultivars that are more resistant to the disease. However, development and assessment of resistant sweetpotato cultivars would require considerable investment of time and resources then subsequent commercial acceptance.

The advantages of the laboratory method for evaluating sweetpotato cultivar resistance to sclerotinia rot include economies in material inputs, experimental space, and response time. The direct inoculation and controlled incubation of the laboratory technique produced more infected sweetpotato tissue than the field screening test and consequently delivered more precise (sensitive) results.

The method will be further used to determine the response of diverse sweetpotato germplasm to *S. sclerotiorum* infection and will aid in the selection of cultivars for commercial release in New Zealand. Because disease incidence and severity are related to pathogen exposure as well as host resistance, further studies will investigate the relationship between tissue resistance to *S. sclerotiorum* and plant growth habit. In addition, resistance identified by the laboratory inoculation method will be confirmed with field tests during cultivar evaluation.

## ACKNOWLEDGMENTS

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