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Somaclonal selection in potato for resistance to common scab provides concurrent resistance to powdery scab

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Variant somaclones of potato cultivar Russet Burbank, selected for resistance to common scab using *in vitro* cell selection techniques, were tested for resistance to powdery scab, another important disease of potato caused by *Spongospora subterranea*. This pathogen also invades roots, producing root galls. Most variants consistently showed increased resistance to powdery scab, both in field and glasshouse challenge, when compared to the parental cultivar, several significantly so. On average, the best variant reduced powdery scab incidence by 51% and severity (tuber surface coverage) by 64%. In contrast, no improvement in the extent of root infection and root galling was seen. These results suggest host interactions during root and tuber infection are distinct. Correlation analyses of disease indices amongst the selected variants showed no association between *Sp. subterranea* root infection and gall scores, nor between root infection and tuber disease severity. However, a weak positive association was found between root gall score and tuber disease, and a strong correlation between tuber disease incidence and severity scores. Interestingly, positive correlations were also found between the extent of powdery and common scab resistance expressed and both incidence and severity of these diseases within the variants, suggesting a common defence mechanism. The role of thaxtomin A in selecting for concurrent resistance to both diseases is discussed.

Keywords: root disease, Russet Burbank, *Spongospora subterranea*, tuber disease

Introduction

There are two distinct diseases of potato that result in scab-like symptoms on harvested tubers; common scab caused by infection with actinomycete plant pathogens of the genus *Streptomyces* and powdery scab caused by infection with the plasmodiophorid protozoan *Spongospora subterranea* f. sp. *subterranea*. Both are important diseases of potato (*Solanum tuberosum*) across most potato production regions of the world (Harrison *et al.*, 1997; Loria *et al.*, 1997; Merz & Falloon, 2009). They are both primarily considered quality-limiting, where presence of lesions on tubers results in a reduced value of fresh potatoes for sale to consumers; likewise, severely diseased tubers used in processing may be downgraded or rejected and may require additional processing to remove defects. Seed tubers that are heavily infested with common scab or powdery scab (>4% or >2% incidence, respectively, in Australia) will not be certified as seed quality and will be devalued as they are deemed potential inoculum sources for contamination of potato fields and subsequent crops. There is also increasing evidence that root infection with *Sp. subterranea* can have a significant impact on potato productivity (Falloon *et al.*, 2005).

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Whilst these two diseases often produce similar symptoms, the aetiology and epidemiology of each is distinct. Pathogenic *Streptomyces* spp. that induce common scab are excellent saprophytes and survive in the soil indefinitely, associated with organic matter. They are believed to have wide host ranges and exhibit significant disease in several root crops such as potato, carrot and swede (Loria *et al.*, 1997). They are disseminated by the movement of contaminated soil and seed potato tubers. Tuber infection occurs during early tuber development. Essential to induction of disease is the ability of the pathogen to produce the phytotoxin thaxtomin A, which inhibits cellulose biosynthesis in expanding plant tissues (Loria *et al.*, 2003). Disease is encouraged by warm, dry, neutral pH soil conditions with high soil organic matter (Loria *et al.*, 1997; Lacey & Wilson, 2001).

In contrast, *Sp. subterranea* is an obligate plant parasite. It infects root hairs, producing zoosporangia and infectious zoospores in a wide range of plant species from at least 20 plant families (Jones & Harrison, 1969, 1972; Anderson *et al.*, 2002). However, it only completes its full life cycle, including production of resting spores or sporosori, on a few species, mostly from the Solanaceae, although Qu & Christ (2006) also report presence of sporosori in infected yellow mustard (*Brassica campestris*) and oat (*Avena sativa*). The sporosori can persist for over 20 years in soil in the absence of a host and are therefore important for pathogen survival (de Boer, 1991; Harrison *et al.*, 1997; Merz, 2008), and thus hosts not supporting their production do not neces-

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sarily aid pathogen persistence. Plant infection occurs following release of motile zoospores from the sporosori that then encyst on the surface of host roots or stolons and penetrate the epidermal cells of those tissues. Within infected cells, plasmodia develop zoosporangia, each containing many secondary zoospores that are released and can infect further host cells. The secondary cycle repeats several times during host growth and development, leading to heavily infected plants if conditions are favourable. Infected roots and stolons eventually develop small galls that mature to become filled with sporosori (Harrison *et al.*, 1997; Merz, 2008). Developing tubers succumb to infection and produce blister-like lesions, which at tuber maturity may release additional sporosori into the soil, or carry inoculum with seed tubers to infect subsequent planted crops. Disease is encouraged by cool soil conditions with sufficient free water to enable zoospore movement (de Boer, 1991; Merz, 2008).

Management options for both common scab and powdery scab are limited, with no single effective strategy available for either disease. Longevity of both pathogens in the soil and diverse host range reduces the efficacy of crop rotation. Manipulation of soil moisture and temperature through irrigation scheduling are used, but ensuring abundant soil moisture and lower soil temperatures to reduce common scab may encourage powdery scab and vice versa (Lapwood *et al.*, 1973; Wilson *et al.*, 2001). Soil and seed tuber pesticides, manipulation of soil pH and nutrients, biological treatments and green manures have been variously trialled as part of integrated disease management practices, but success is variable (Keinath & Loria, 1989; Harrison *et al.*, 1997; Wilson *et al.*, 1999; Lacey & Wilson, 2001; Lazarovits *et al.*, 2001; Falloon, 2008; Merz & Falloon, 2009).

Host resistance is a valuable disease management tool that can be central to an effective integrated management solution. In particular, resistance is likely to be the most efficient long-term strategy for preventing build-up of field inoculum and spread of powdery scab (Merz *et al.*, 2004; Merz & Falloon, 2009). Potato cultivars vary in their susceptibility to both common scab and powdery scab. However, despite the efforts of potato breeding programmes around the world, there are no commercial cultivars with extreme resistance to either disease (McKee, 1958; Falloon *et al.*, 2003; Merz *et al.*, 2012). Selecting disease resistance traits whilst maintaining other essential agronomic and quality features is extremely difficult, particularly for the French fry sector of the potato industry where there are additional demands on tuber processing characteristics. An alternate approach uses tissue culture and directed mutagenesis to select somaclonal variants with specific traits of interest. This offers rapid production of clonal variants without the genetic reassortment associated with breeding crosses. Such approaches have been used to improve resistance to several important potato pathogens including *Phytophthora infestans*, *Verticillium dahliae*, *Alternaria solani*, *Fusarium oxysporum* and *Streptomyces scabiei* (Matern *et al.*, 1978; Behnke, 1980; Shepard *et al.*, 1980; Sebastiani *et al.*, 1994; Wilson *et al.*, 2009).

A novel somaclonal cell selection technique was used here, with thaxtomin A as a positive selection agent to efficiently select potato somaclonal variants likely to possess tolerance to the toxin and hence resistance to common scab (Wilson *et al.*, 2009). Many variants of cultivar Russet Burbank were generated that showed substantially enhanced resistance to common scab (Wilson *et al.*, 2010b). Not all of these possessed enhanced tolerance to thaxtomin A, suggesting additional resistance mechanisms were selected. Powdery scab disease was recorded in field performance assessments of a selection of common scab resistant somaclonal variants. In addition to reduced common scab, most of the variants also appeared to show less powdery scab. The present study tested the hypothesis that the common scab resistant somaclonal variants possess concurrent enhanced resistance to powdery scab. Tests were then carried out to see whether this protection extends to reduced *Sp. subterranea* root infection. Finally, a possible physiological mechanism of enhanced resistance is suggested.

Materials and methods

Somatic cell selection and regeneration of common scab resistant Russet Burbank variants

The cell selection process used to obtain somaclonal variants was as previously reported (Wilson *et al.*, 2009, 2010b). In brief, 7–14-day-old callus cultures of cv. Russet Burbank (Vancouver clone) initiated on callus induction (CI) medium (MS salts and vitamins, plus sucrose, 30 g L^{-1}; ascorbic acid, 40 mg L^{-1}; casein hydrolysate, 500 mg L^{-1}; BAP, 2 mg L^{-1}; NAA, 0·2 mg L^{-1}; GA_3, 5 mg L^{-1}; and 0·8% agar (Sigma-Aldrich), with pH adjusted to 5·8) were transferred to CI liquid medium containing thaxtomin A at either 2 mg L^{-1} (4·57 μM) or 3 mg L^{-1} (6·86 μM) and agitated gently for 1–8 days. Toxin-treated cells were plated onto a sterile Whatman no. 1 filter paper that had been placed on top of a nurse culture of *Nicotiana plumbaginifolia* grown on CI medium. After 3–4 weeks, rescued potato cell colonies were transferred to callus regeneration (CR) medium (MS salts and vitamins, plus sucrose, 5 g L^{-1}; ascorbic acid, 40 mg L^{-1}; casein hydrolysate, 500 mg L^{-1}; zeatin, 1 mg L^{-1}; NAA, 0·2 mg L^{-1}; and GA_3, 5 mg L^{-1}, with pH adjusted to 5·8) and incubated under reduced light intensity (7 μmol m^{-2} s^{-1}) for 2–3 months. Regenerated shoots and the parent cv. Russet Burbank were micropropagated on potato multiplication (PM) medium (MS salts and vitamins, plus sucrose, 30 g L^{-1}; ascorbic acid, 40 mg L^{-1}; casein hydrolysate, 500 mg L^{-1}; and 0·8% agar, with pH adjusted to 5·8). Plants were routinely subcultured as two-node segments every 3–4 weeks and incubated at 22°C with a 16 h photoperiod under cool white fluorescent lamps (65 μmol m^{-2} s^{-1}). A total of 253 regenerated plants were recovered from 212 individual cell colonies. Of these, 51 plants consistently showed enhanced resistance to common scab disease incidence and severity in glasshouse and field challenges (Wilson *et al.*, 2010b).

Spongospora subterranea resistance screening

A selection of 29 Russet Burbank variants with demonstrated improved resistance to common scab (when compared to the parent cultivar) were tested for resistance to *Sp. subterranea*

induced disease in four field and three glasshouse pot trials. These included variants both with and without enhanced tolerance to thaxtomin A (Tables 1 & 2). The parent cultivar (Russet Burbank) was included in each trial as a control and comparator.

Field trials

The four field trials were established at commercial farms in northwest Tasmania between 2008 and 2010 on potato growing soils typical of the region; field trials 1 (F1), 3 (F3) and 4 (F4) on red ferrosol soils and field trial 2 (F2) on a brown dermosol soil. Locations and dates of planting were: F1 (West Pine) and F2 (Bishopsbourne) both planted in October 2008; F3 (Stowport) planted in November 2009 and F4 (Stowport) planted in November 2010. At all field sites powdery scab had been previously recorded in prior potato crops. The four trials assessed 27, 29, six and six variants, respectively, with the parent cv. Russet Burbank included in each trial. Planting material for all field trials was disease-free mini-tubers produced by glasshouse culture of tissue-cultured plantlets.

Table 1 Powdery scab incidence (percentage of infected tubers) evaluation of selected potato cv. Russet Burbank somaclones in field (F1, West Pine – 2008/09; F2, Bishopsbourne – 2008/09; F3, Stowport – 2009/10; F4, Stowport – 2010/11) and glasshouse (GH1 and GH2 – 2009; GH3 – 2010) trials

Variants	Thaxtomin A tolerance[a]	Relative common scab incidence[b]	F1 Infected tubers (%)[c]	F2 Infected tubers (%)	F3 Infected tubers (%)	F4 Infected tubers (%)	GH1 Infected tubers (%)	GH2 Infected tubers (%)	GH3 Infected tubers (%)	Relative powdery scab incidence[b]
A380	No	0·27	**47·5***[d]	**12·5***	93·3	86·2	47·2	0	**0***	0·53
A168a	No	0·32	–	20·0	83·3	–	45·5	0	**0***	0·49
A289	No	0·40	61·3	18·8	–	–	–	–	–	0·75
A358	No	0·42	**56·3***	16·3	–	–	–	–	–	0·67
A184a	Yes	0·45	75·0	16·3	–	–	–	–	–	0·77
A386	No	0·48	**57·5***	16·3	–	–	–	–	–	0·67
TC10-C1	Yes	0·48	–	17·5	86·7	91·2	55·0	0	**31·5***	0·66
TC9-M4	No	0·50	61·3	**11·3***	93·3	86·2	50·0	0	**18·0***	0·58
A146a	No	0·52	73·8	17·5	–	–	–	–	–	0·79
A179	n.t.	0·53	66·3	**32·5****	–	–	–	–	–	1·08
TC9-S4	Yes	0·57	76·3	22·5	–	–	–	–	–	0·91
A227	n.t.	0·58	75·0	**12·5***	–	–	–	–	–	0·68
A255	No	0·62	76·3	20·0	–	–	–	–	–	0·86
TC9-M4-7	Yes	0·63	70·0	18·8	95·0	71·2	52·5	0	**27·5***	0·65
A174	No	0·65	78·8	16·3	–	–	–	–	–	0·79
A231	n.t.	0·65	77·5	20·0	–	–	–	–	–	0·86
TC-RB8	No	0·66	70·0	32·2	95·0	80·0	63·5	15·0	85·6	0·99
A154	No	0·66	80·0	17·5	–	–	–	–	–	0·82
TC9-M1	No	0·67	**58·8***	18·8	–	–	–	–	–	0·74
TC9-B3	No	0·67	72·5	21·3	–	–				0·87
TC9-M3	Yes	0·69	**60·0***	12·5*	–	–	–	–	–	0·60
A321	Yes	0·71	67·5	16·3	–	–	–	–	–	0·73
A405	n.t.	0·72	76·3	20·0	–	–	–	–	–	0·86
A397	No	0·72	72·5	23·8	–	–	–	–	–	0·92
A377	n.t.	0·75	72·5	22·5	–	–	–	–	–	0·89
A362	n.t.	0·78	71·3	18·8	–	–	–	–	–	0·80
A118	No	0·80	81·3	20·0	–	–	–	–	–	0·88
A392	Yes	0·81	68·8	26·3	–	–	–	–	–	0·96
A410	n.t.	0·81	73·8	25·0	–	–	–	–	–	0·95
Russet Burbank (parent)		1·00	92·5	22·5	98·3	90	62·7	17·5	82·3	1·00
P			0·04	0·01	0·22	0·31	0·12	0·47	0·02	
LSD (0·05)			32·4	9·73	ns	ns	ns	ns	35·6	

–, lines not tested within the specified trial; ns, not significant.

[a]Enhanced tolerance of variants to thaxtomin A compared to the Russet Burbank parent. Data from Wilson *et al.* (2010b) and unpublished (n.t. = not tested).

[b]Mean relative disease incidence for common and powdery scab diseases were generated for each variant by dividing the mean disease score of each variant by the mean disease score of the parent cultivar in each trial in which they appeared and averaging the result. Common scab data from Wilson *et al.* (2010b) and unpublished data.

[c]Percentage of tubers with any powdery scab lesions.

[d]Values in bold and marked * are significantly less, and ** significantly more ($P < 0·05$) than the Russet Burbank parent.

Table 2 Powdery scab severity (percentage tuber surface lesion coverage) evaluation of selected potato cv. Russet Burbank somaclones in field (F1, West Pine - 2008/09; F2, Bishopsbourne – 2008/09; F3, Stowport - 2009/10; F4, Stowport - 2010/11) and glasshouse (GH1 and GH2 – 2009; GH3 – 2010) trials

Variants	Thaxtomin A tolerance[a]	Relative common scab severity[b]	F1 Surface cover score 0–6 (%)[c]	F2 Surface cover score 0–6 (%)	F3 Surface cover score 0–6 (%)	F4 Surface cover score 0–6 (%)	GH1 Surface cover score 0–6 (%)	GH2 Surface cover score 0–6 (%)	GH3 Surface cover score 0–6 (%)	Relative powdery scab severity[b]
A380	No	0·19	**0·32*[d]** (0·64)	0·13 (0·32)	**0·94*** (2·93)	**0·89*** (2·68)	**0·80*** (3·30)	0 (0)	**0*** (0)	0·44
A168a	No	0·21	–	0·19 (0·54)	**0·64*** (1·54)	–	**0·65*** (2·25)	0 (0)	**0*** (0)	0·36
A289	No	0·29	0·56 (1·51)	0·19 (0·56)	–	–	–	–	–	0·80
A358	No	0·41	**0·43*** (1·01)	0·18 (0·54)	–	–	–	–	–	0·70
A184a	Yes	0·50	0·59 (1·41)	0·16 (0·49)	–	–	–	–	–	0·75
A386	No	0·39	**0·39*** (0·77)	0·18 (0·54)	–	–	–	–	–	0·67
TC10-C1	Yes	0·46	–	0·18 (0·53)	**0·81*** (2·24)	1·18 (3·89)	0·99 (3·50)	0 (0)	**0·40*** (1·62)	0·58
TC9-M4	No	0·29	0·51 (1·29)	**0·11*** (0·31)	**0·93*** (2·73)	0·95 (3·03)	**0·73*** (2·53)	0 (0)	**0·25*** (0·80)	0·49
A146a	No	0·36	0·57 (1·45)	0·17 (0·49)	–	–	–	–	–	0·76
A179	n.t.	0·46	0·54 (1·46)	0·34 (1·05)	–	–	–	–	–	1·15
TC9-S4	Yes	0·63	0·56 (1·28)	0·23 (0·67)	–	–	–	–	–	0·90
A227	n.t.	0·46	0·64 (1·69)	0·13 (0·37)	–	–	–	–	–	0·71
A255	No	0·34	0·53 (1·13)	0·19 (0·54)	–	–	–	–	–	0·78
TC9-M4-7	Yes	0·52	0·51 (1·21)	0·19 (0·59)	1·00 (3·27)	**0·68*** (1·92)	0·89 (3·45)	0 (0)	**0·41*** (1·54)	0·54
A174	No	0·49	0·68 (1·79)	0·16 (0·46)	–	–	–	–	–	0·81
A231	n.t.	0·50	0·61 (1·47)	0·20 (0·60)	–	–	–	–	–	0·86
TC-RB8	No	0·57	0·59 (1·61)	0·36 (1·17)	1·07 (3·44)	**0·82*** (2·44)	1·35 (9·80)	0·20 (0·70)	1·56 (10·41)	0·98
A154	No	0·58	0·83 (2·64)	0·16 (0·43)	–	–				0·90
TC9-M1	No	0·58	**0·43*** (0·94)	0·18 (0·53)	–	–	–	–	–	0·70
TC9-B3	No	0·72	0·53 (1·16)	0·23 (0·72)	–	–	–	–	–	0·88
TC9-M3	Yes	0·51	**0·43*** (0·90)	**0·11*** (0·28)	–	–	–	–	–	0·53
A321	Yes	0·55	0·56 (1·51)	0·18 (0·57)	–	–	–	–	–	0·78
A405	n.t.	0·57	0·60 (1·45)	0·20 (0·60)	–	–	–	–	–	0·85
A397	No	0·58	0·58 (1·41)	0·24 (0·71)	–	–	–	–	–	0·93
A377	n.t.	0·64	0·52 (1·14)	0·22 (0·64)	–	–	–	–	–	0·85
A362	n.t.	0·66	0·59 (1·52)	0·18 (0·50)	–	–	–	–	–	0·80
A118	No	0·60	0·77 (2·36)	0·20 (0·59)	–	–	–	–	–	0·96
A392	Yes	0·63	**0·47*** (0·97)	0·33 (1·06)	–	–	–	–	–	1·08
A410	n.t.	0·68	0·56 (1·33)	0·25 (0·74)	–	–	–	–	–	0·95
Russet Burbank (parent)		1·00	0·88 (2·49)	0·24 (0·76)	1·15 (4·32)	1·09 (3·64)	1·79 (13·53)	0·22 (0·75)	1·50 (10·21)	1·00
P			0·04	0·02	<0·001	<0·001	0·04	0·47	0·03	
LSD (0·05)			0·40	0·12	0·21	0·19	0·95	ns	0·85	

–, lines not tested within the specified trial.

[a]Enhanced tolerance of variants to thaxtomin A compared to the Russet Burbank parent. Data from Wilson *et al.* (2010b) and unpublished (n.t. = not tested).

[b]Mean relative disease severity for common and powdery scab diseases were generated for each variant by dividing the mean disease score of each variant by the mean disease score of the parent cultivar in each trial in which they appeared and averaging the result. Common scab data from Wilson *et al.* (2010b) and unpublished data.

[c]Tuber disease surface cover score, 0 = no disease, 0·5 $\leq$ 1%, 1 = 2–5%, 2 = 6–10%, 3 = 11–30%, 4 = 31–50%, 5 = 51–70%, 6 > 70%. In brackets: estimated tuber surface coverage is calculated from disease cover score using median percentile scores within the allocated range.

[d]Values in bold and marked * are significantly less ($P < 0·05$) than the Russet Burbank parent.

Plots of each variant or the control cultivar contained 8–20 plants. They were arranged in randomized block designs and replicated four (F1 and F2) or three (F3 and F4) times in each trial. No seed or soil pesticide treatments were applied. Fertilizer, foliar fungicide application (for late and early blight control) and irrigation scheduling followed standard commercial practice. In F4, 60 days after plant emergence, two plants per replicate were harvested with roots for assessment of zoosporangium infection and gall development. All other plants were grown until senescence. All tubers were harvested and a random sample of 20 tubers per plot selected for disease assessment.

Glasshouse trials

Three glasshouse pot trials (GH1–GH3) compared six of the somaclonal variants (A168a, A380, TC-RB8, TC9-M4, TC9-M4-7 and TC10-C1) to the parent cultivar for resistance to *Sp. subterranea* infection and disease. For GH1 and GH3, two harvest treatments were included: (i) 70 days after transplanting (root disease assessment), and (ii) after senescence (tuber disease assessment); while for GH2 a single harvest at senescence (tuber disease assessment) was carried out. Each clone was replicated in three pots for each harvest treatment. Plastic pots (20 cm diameter) were filled with pasteurized potting mix (1 part course sand, 1 part peat, 8 parts composted pine bark; pH 6·0). Planting material was disease-free tissue-cultured plantlets transplanted into pots (one per pot). *Spongospora subterranea* inoculum was obtained from heavily diseased potato tubers harvested from Riana, northwest Tasmania, that had been stored (for a maximum of 3 months) in ambient cool conditions (*c.* 10°C) until use. Tubers were scraped and peeled with a sharp knife near and around the disease lesions, collecting infected skin and underlying tissues up to 1 cm depth. The peeled tissue from approximately six (large) to 10 (medium sized) tubers was placed in a 3 L plastic beaker and homogenized in a small volume of sterile water using a handheld blender. The inoculum solution was made up to 2–2·5 L total volume with sterile water. A standard aliquot (100 mL) of the constantly agitated inoculum solution was then added to the surface of each pot at time of transplanting, with the inoculum thoroughly watered in. Pots were subsequently watered by overhead sprinklers at 2-day intervals, with additional hand irrigation when required to maintain constant wet soil conditions (Falloon *et al.*, 2003). Root infection, root galling (GH1 and GH3) and powdery scab severity (all three trials) were assessed.

Disease assessment

Powdery scab

Harvested tubers were stored at 4°C for up to 4 weeks, prior to disease assessment. Tubers were washed and each tuber scored according to a visual tuber surface cover score ranging from 0 to 6 (0 = no visible disease on tuber surface, 0·5 $\leq$ 1%, 1 = 2–5%, 2 = 6–10%, 3 = 11–30%, 4 = 31–50%, 5 = 51–70%, 6 > 70% tuber surface affected). The percentage of tuber surface covered by lesions was then estimated by taking the mid-values of these score ranges. The proportion of healthy tubers with no visible lesions was also recorded (Wilson *et al.*, 1999).

Root infection and galling

Root infection was then assessed by direct microscopic examination using a method modified from Falloon *et al.* (2003). From each plant, five samples of root (2–5 cm long) were cut at 30–50 mm from the crown region and thoroughly washed. Each sample was mounted on a glass slide, stained with lactogycerol blue and observed with a compound light microscope (Leica Microsystems) at × 100 magnification. Sections of root were examined at random, counting the number of zoosporangia clusters within a total of 10 mm of root per sample. Mean root width per sample was estimated and data were converted to zoosporangia number per mm^2 of root.

Root galling was assessed at 60 days after planting (F4) or 70 days after transplanting (GH1 and GH3). Plants were carefully harvested and roots washed and a root galling score per plant was estimated according to a 0–4 visual rating scale modified from that of van de Graaf *et al.* (2007); 0 = no galls; 1 = 1–2 galls; 2 = 3–10 galls, most <2 mm in diameter, 3 = >10 galls, some >2 mm in diameter; 4 = most major roots with galls, some or all >4 mm in diameter.

Data analysis

Disease data for all tubers per replicate both in field and glasshouse trials were averaged prior to analysis. Data were analysed by one-way analysis of variance (ANOVA) using GENSTAT v. 9.1 (VSN International Ltd) after ensuring an approximate normal distribution for each variable. The estimated percentage tuber surface cover was to illustrate disease severity and not subject to further statistical comparisons.

Mean disease scores (tuber incidence and severity; root infection and galling) relative to the control cultivar were generated for each variant by dividing the mean score of each variant by the mean score of the control in each trial in which they were tested, and averaging the result. Root infection, mean gall scores and mean tuber disease severities were compared for six variants using correlation analyses with GENSTAT v. 9.1. Similarly, the relative tuber powdery scab severity and incidence data were compared for all 29 variants. Finally, the relative powdery scab tuber disease incidence and severity scores of all 29 variants were compared to relative common scab disease incidence and severity scores generated in a prior study (Wilson *et al.*, 2010b; C.R. Wilson *et al.*, unpublished data) using correlation analysis.

Results

Powdery scab tuber assessment

Incidence of powdery scab across the field and glasshouse trials was moderate to heavy, ranging from 17·5–98·3% of tubers with lesions in the parent cultivar. However, disease severity was low in the parent cultivar, with 0·8–4·3% average tuber surface coverage. In two field trials and one glasshouse trial, significant differences in disease incidence (percentage of infected tubers) were found while, in all four field, and in two of three glasshouse trials, significant differences in disease severity were shown. Most variants had less disease than the parent cultivar (Fig. 1). Ten variants had significantly less disease incidence and ten had significantly less disease severity than the parent in at least one trial (Tables 1 & 2).

Spongospora subterranea root infection and galling

Examination of root infection and gall scores in one field (F4) and two glasshouse (GH1, GH3) trials showed no significant differences between the six variants and the

control parent, and showed no apparent trends (Table 3). Root infection scores were variable across all clones. Greater numbers of zoosporangia per mm^2 root were found in the two glasshouse trials (ranging from 7·1 to 32·4) than the field trial (3·5 to 16·5). In contrast, expression of galling symptoms was greater in the field trial (ranging from 3·0 to 4·0), than in the glasshouse trials (2·0 to 3·3).

Relationships between *Spongospora subterranea* root and tuber symptoms

There was no obvious association between relative *Sp. subterranea* root infection and gall scores for the six variants assessed ($r = 0{\cdot}49$; Fig. 2a). In contrast, there was a strong positive correlation between relative tuber powdery scab severity and incidence scores for the 29 variants assessed ($r = 0{\cdot}96$; Fig. 2b). Comparisons of relative root and tuber severity indices for six variants showed no association between root infection and tuber severity ($r = 0{\cdot}27$) but a positive association between root galling and tuber severity score ($r = 0{\cdot}74$; Fig. 2c).

Relationship between resistance to common scab and powdery scab

Comparison of relative powdery scab incidence and severity scores (mean of 2–7 trials) and relative common scab incidence and severity scores (mean of 3–8 trials) for each tested variant revealed weak positive correlations ($r = 0{\cdot}61$, incidence; $r = 0{\cdot}64$, severity; Fig. 3).

Discussion

This study confirms that selection for resistance to common scab within somaclonal variants, generated through *in vitro* cell selection techniques, concurrently produces resistance to powdery scab, but that this does not extend to protection of roots from infection with *Sp. subterranea* and root gall formation. Consistent variation in the extent of resistance observed amongst the different variants indicates the resistance obtained is incomplete and can be additive. The value of resistance enhancement obtained is perhaps best demonstrated in glasshouse trials 2 and 3, where the best lines would meet Australian seed certification standards (maximum of 2% disease incidence), whilst the unselected parent would not.

The positive correlations between common scab and powdery scab resistance in both disease incidence and severity indices within the somaclonal variants of near genetic identity, are indicative of a common resistance mechanism. This is intriguing, as common scab and powdery scab vary considerably in their aetiology and epidemiology. The causal agents are very different, one an actinomycete bacterium, the other a protozoan, as are the environmental conditions favouring the two diseases.

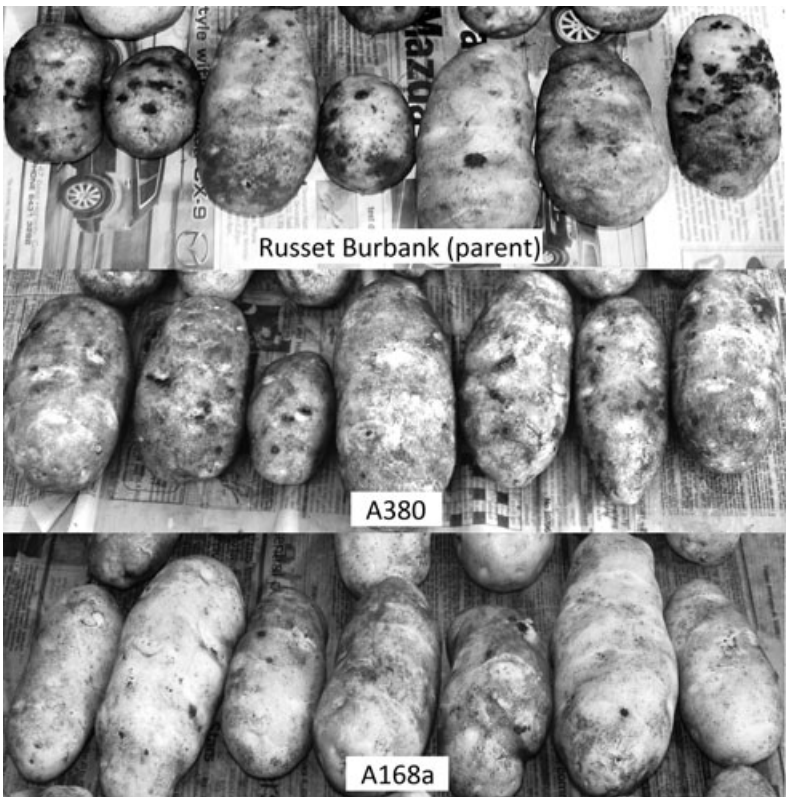

Figure 1 Typical incidence and severity of powdery scab on potato somaclonal variants A380 and 168a and the parent cv. Russet Burbank (tubers from trial F3).

Table 3 *Spongospora subterranea* root infection and gall formation in selected potato somaclones in field (F4, Stowport – 2010/11) and glasshouse (GH1 – 2009; GH3 – 2010) trials

	F4		GH1		GH3	
Variants	Root infection[a]	Mean root gall score (0–4)[b]	Root infection	Mean root gall score (0–4)	Root infection	Mean root gall score (0–4)
A380	4·7	3·5	29·3	2·0	23·5	2·7
A168a	16·5	3·5	7·1	2·0	19·4	3·0
TC9-M4	15·9	3·5	17·6	3·0	32·4	2·0
TC10-C1	3·5	4·0	21·2	2·0	15·9	2·5
TC9-M4-7	10·6	4·0	14·1	2·5	21·8	3·0
TC-RB8	16·5	3·0	12·9	3·0	27·6	3·3
Russet Burbank (parent)	10·6	4·0	17·6	2·0	20·6	2·7
P	0·38	0·42	0·30	0·43	0·50	0·54
LSD (0·05)	ns	ns	ns	ns	ns	ns

ns, not significant (α = 0·05).

[a]Mean number of zoosporangia per mm^2 root.

[b]0 = no root galls; 1 = 1–2 galls; 2 = 3–10 galls, most < 2 mm in diameter; 3 > 10 galls, some >2 mm in diameter; 4 = most major roots with galls, some or all >4 mm in diameter.

The relative resistance of potato cultivars to the two diseases is also quite distinct, although Russet Burbank is moderately resistant to both (Wilson, 2001; Falloon *et al.*, 2003). Both diseases have been suggested to share a common point of entry into developing tubers. In early tuber development, stomata on the underground stolons develop into lenticels which mature with tuber development, becoming increasingly suberized and filled with packing cells. Both common scab and powdery scab pathogens are suggested to use immature lenticels (but not stomata) as a means for initiating infection in developing tubers (Adams & Lapwood, 1978; de Boer, 1991; Diriwachter & Parberry, 1991; Khatri *et al.*, 2011). Once mature and fully suberized, lenticels appear no longer susceptible to pathogen entry (Adams, 1975; de Boer, 1991). However, zoospores of *Sp. subterranea* also actively penetrate the epidermal cells of roots and root hairs following encystment, and possibly invade stolons and developing tubers in a similar manner (Merz, 1997). Similarly, pathogenic *Streptomyces* spp. may use wounds or growth cracks as means of entry, and there is also some evidence for direct penetration of tuber periderm cells (Loria *et al.*, 2003).

The initial study targeting common scab resistance used the pathogen's key pathogenicity determinant, thaxtomin A, as a positive cell selection agent (Wilson *et al.*, 2009). The aim was to obtain toxin-tolerant plants that express disease resistance. Strong and robust disease resistance was obtained, but not always in variants expressing toxin tolerance (Wilson *et al.*, 2010b). Thaxtomin A has the capacity to induce scab-like symptoms when applied to developing tubers in the absence of the *Streptomyces* pathogen (Lawrence *et al.*, 1990). This suggests it stimulates the defence response of the host, resulting in the formation of suberized cork-like tissues that form the scab. It is therefore tempting to postulate that the use of thaxtomin A as a screening agent in the cell selection experiments might have selected for variants with a rapid suberization response within lenticels and periderm which led to enhanced disease resistance. There is preliminary evidence that variants with enhanced resistance to both common and powdery scab have greater suberin content in the tuber lenticular and periderm tissues and that they possess a greater number of tuber periderm cell layers, providing a further physical barrier to tuber infection (Khatri *et al.*, 2011). Such a mechanism may help to explain the broad-spectrum resistance to these distinct diseases and perhaps why *Sp. subterranea* root infection was not protected against. Further examinations of tuber suberization responses within these variant somaclones are currently underway.

Despite the evidence of a significant impediment on root function and yield (Falloon *et al.*, 2005), management of root infection by *Sp. subterranea* has generally received less emphasis than the tuber disease. In a large comparative study of different cultivars, Falloon *et al.* (2003) showed most, but not all, cultivars that are resistant to powdery scab on tubers also possess resistance to root infection and gall formation. They showed Russet Burbank had moderate resistance to tuber infection, but produced moderately high numbers of galls and moderately large numbers of zoosporangia (*c.* 50 mm^2) in infected roots, similar results to those found in the present study. However, Merz *et al.* (2012) found no relationship between tuber and root susceptibility in the cultivars they examined. In the present study of somaclonal variants of Russet Burbank, there was also no association found between zoosporangial infection and tuber severity scores, but there was a weak positive association between gall and tuber disease indices. Some reports suggest that zoosporangial root infection does not necessarily correlate with expression of root galling (Merz *et al.*, 2004). The results here, albeit from a limited data set, support this, with no clear association between zoosporangial root infection and galling observed. This collation of data, and the results showing enhancement of resistance to tuber but not root infection in the somaclonal variants, suggest different resistance mechanisms may operate in tubers and roots and that root disease expression is further influenced by the host genetics.

Russet Burbank is a cultivar more than 130 years old and is the most cropped potato cultivar in the world, with tuber characteristics and cooking qualities that make it ideal for French fry processing (Wilson *et al.*, 2010a). Whilst it has moderate resistance to both common scab and powdery scab, substantial epidemics of both diseases occur frequently (Wilson *et al.*, 1999, 2010a; Wilson, 2001; Merz *et al.*, 2012). This can have substantial impact on profitability of potato cropping for seed and for consumption. Traditional breeding approaches to improving disease resistance are problem-

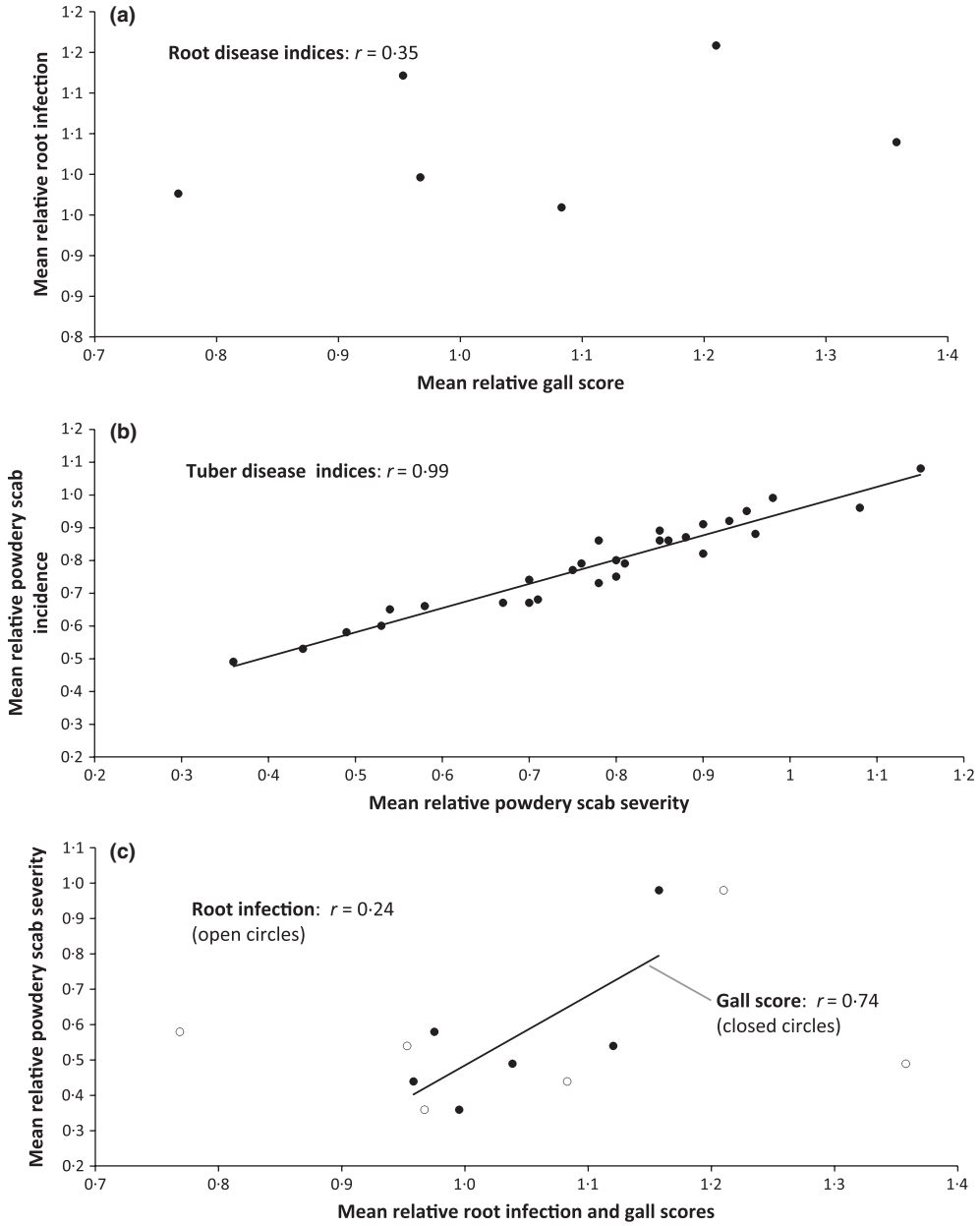

Figure 2 Correlation between *Spongospora subterranea* disease scores for six or 29 somaclonal variants. Compared disease data were root infection (zoosporangia per root length), gall score (severity of root galling) and powdery scab incidence (proportion of the tuber covered with lesions). All data were mean relative disease incidence compared to the parent cultivar. Comparisons were (a) root infection and gall score, (b) tuber severity and tuber incidence score, (c) root infection and gall score compared to tuber severity score.

atic because they involve genetic reassortment and concurrent change in important cultivar characteristics, and the polygenic nature of resistance to powdery scab and common scab (Haynes *et al.*, 1997; Falloon *et al.*, 2003) make selection difficult. This present study highlights the benefits of using cell selection approaches to generate novel resistance traits. Enhanced resistance was successfully obtained to two distinct diseases, while retaining the agronomic and processing characteristics of the target cultivar Russet Burbank (Wilson *et al.*, 2010a). In future work, it is anticipated that cell selection procedures that use thaxtomin A could be very valuable for generation of dual common and powdery scab resistance in a range of other cultivars without the loss of key cultivar characteristics.

Different approaches may be required to obtain enhanced resistance to *Sp. subterranea* root infection and galling. As natural variation exists in susceptibility of potato cultivars to *Sp. subterranea* root infection, for example, cv. Gladiator seldom shows galling and infected roots show few zoosporangia per mm^2 root whilst cv. Agria produces abundant galls and 10-fold

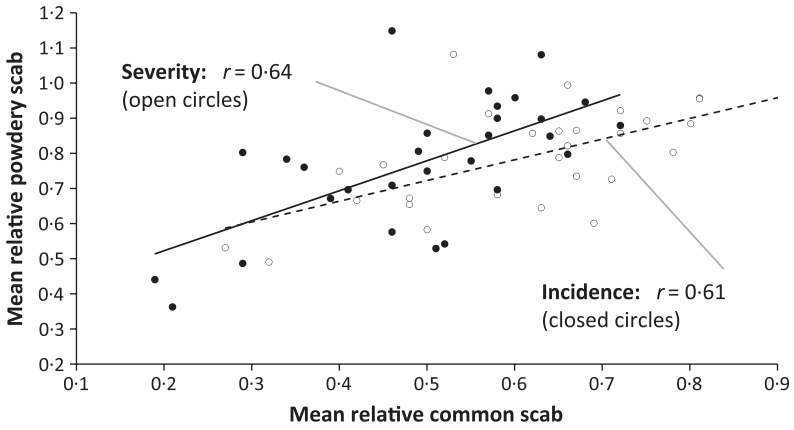

Figure 3 Correlation between mean common scab and powdery scab resistance scores for 29 somaclonal variants. Compared disease data were incidence (proportion of tubers with disease) and severity (proportion of tubers covered with lesions). All data were relative to that shown by the Russet Burbank parent.

more zoosporangia within infected roots (Falloon *et al.*, 2003), it is felt that there is opportunity for using cell selection approaches to select for enhanced resistance to root infection by this pathogen. Efficient selection techniques to screen for variation in root infection are required.

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References

Adams MJ, 1975. Potato tuber lenticels: development and structure. *Annals of Applied Biology* **79**, 265–73.

Adams MJ, Lapwood DH, 1978. Studies on the lenticel development, surface microflora and infection by common scab (*Streptomyces scabies*) of potato tubers growing in wet and dry soils. *Annals of Applied Biology* **90**, 335–43.

Anderson BAB, Nicolaisen M, Nielsen SL, 2002. Alternate hosts for potato mop-top virus, genus *Pomovirus* and its vector *Spongospora subterranea* f. sp. *subterranea*. *Potato Research* **45**, 37–43.

Behnke M, 1980. Selections of dihaploid potato callus for resistance to the culture filtrates of *Fusarium oxysporum*. *Zeitschrift fur Pflanzenzuchtung* **85**, 254–8.

de Boer RF, 1991. Evaluation of potato cultivars in the greenhouse and field for resistance to powdery scab. *Australian Journal of Experimental Agriculture* **31**, 699–703.

Diriwachter G, Parberry DG, 1991. Infection of potato by *Spongospora subterranea*. *Mycological Research* **95**, 762–4.

Falloon RE, 2008. Control of powdery scab of potato: toward integrated disease management. *American Journal of Potato Research* **85**, 53–60.

Falloon RE, Genet RA, Wallace AR, Butler RC, 2003. Susceptibility of potato (*Solanum tuberosum*) cultivars to powdery scab (caused by *Spongospora subterranea* f. sp. *subterranea*), and relationships between tuber and root infection. *Australasian Plant Pathology* **32**, 377–85.

Falloon RE, Curtin D, Lister RA, Butler RC, 2005. Root function and growth of potato plants reduced by *Spongospora subterranea* infection. *American Journal of Potato Research* **82**, 68.

van de Graaf P, Wale SJ, Lees AK, 2007. Factors affecting the incidence and severity of *Spongospora subterranea* infection and galling in potato roots. *Plant Pathology* **56**, 1005–13.

Harrison JG, Searle RJ, Williams NA, 1997. Powdery scab disease of potato – a review. *Plant Pathology* **46**, 1–25.

Haynes KG, Goth RW, Young RJ, 1997. Genotype × environment interactions for resistance to common scab in tetraploid potato. *Crop Science* **37**, 1163–7.

Jones RAC, Harrison BD, 1969. The behaviour of potato mop-top virus in soil, and evidence for its transmission by *Spongospora subterranea* (Wallr.) Lagerh. *Annals of Applied Biology* **63**, 1–17.

Jones RAC, Harrison BD, 1972. Ecological studies on potato mop-top virus in soil in Scotland. *Annals of Applied Biology* **71**, 47–57.

Keinath AP, Loria R, 1989. Management of common scab of potato with plant nutrients. In: Enelhard AW, ed. *Soilborne Plant Pathogens: Management of Diseases with Macro- and Microelements*. St Paul, MN, USA: APS, 152–66.

Khatri BB, Tegg RS, Brown PH, Wilson CR, 2011. Temporal association of tuber development with susceptibility to common scab infection and pathogen induced responses in the potato periderm to *Streptomyces scabiei*. *Plant Pathology* **60**, 776–86.

Lacey MJ, Wilson CR, 2001. Relationship of common scab incidence of potatoes grown in Tasmanian ferrosol soils with pH, exchangeable cations and other chemical properties of those soils. *Journal of Phytopathology* **149**, 679–83.

Lapwood DH, Wellings LW, Hawkins JH, 1973. Irrigation as a practical means to control common scab (*Streptomyces scabies*), final experiments and conclusion. *Plant Pathology* **22**, 35–41.

Lawrence CH, Clark MC, King RR, 1990. Induction of common scab symptoms in aseptically cultured potato tubers by the vivotoxin, thaxtomin. *Phytopathology* **80**, 606–8.

Lazarovits G, Tenuta M, Conn KL, 2001. Organic amendments as a disease control strategy for soilborne diseases of high-value agricultural crops. *Australasian Plant Pathology* **30**, 111–7.

Loria R, Bukhalid RA, Fry BA, King RR, 1997. Plant pathogenicity in the genus *Streptomyces*. *Plant Disease* **81**, 836–46.

Loria R, Coombs J, Yoshida M, Kers J, Bukhalid R, 2003. A paucity of bacterial root diseases: *Streptomyces* succeeds where others fail. *Physiological and Molecular Plant Pathology* **62**, 65–72.

Matern U, Styrobel G, Shepard J, 1978. Reaction to phytotoxins in a potato population derived from mesophyll protoplasts. *Proceedings of the National Academy of Sciences, USA* **75**, 4935–9.

McKee RK, 1958. Assessment of the resistance of potato varieties to common scab. *European Potato Journal* **1**, 65–80.

Merz U, 1997. Microscopical observations of the primary zoospores of *Spongospora subterranea* f. sp. *subterranea*. *Plant Pathology* **46**, 670–4.

Merz U, 2008. Powdery scab of potato – occurrence, life cycle and epidemiology. *American Journal of Potato Research* **85**, 241–6.

Merz U, Falloon R, 2009. Review: powdery scab of potato – increased knowledge of pathogen biology and disease epidemiology for effective disease management. *Potato Research* **52**, 17–37.

Merz U, Martinez V, Schwärzel R, 2004. The potential for the rapid screening of potato cultivars (*Solanum tuberosum*) for resistance to powdery scab (*Spongospora subterranea*) using a laboratory bioassay. *European Journal of Plant Pathology* **110**, 71–7.

Merz U, Lees AK, Sullivan L *et al.*, 2012. Powdery scab resistance in *Solanum tuberosum*: an assessment of cultivar × environment effect. *Plant Pathology* **61**, 29–36.

Qu X, Christ B, 2006. The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. *American Journal of Potato Research* **83**, 343–7.

Sebastiani L, Lenzi A, Pugliesi C, Fambrini M, 1994. Somaclonal variation for resistance to *Verticillium dahliae* in potato (*Solanum tuberosum* L.) plants regenerated from callus. *Euphytica* **80**, 5–11.

Shepard JF, Bidney D, Shahin E, 1980. Potato protoplasts in crop improvement. *Science* **208**, 17–24.

Wilson CR, 2001. Variability within clones of potato cv. Russet Burbank to infection and severity of common scab disease of potato. *Journal of Phytopathology* **149**, 625–8.

Wilson CR, Ransom LM, Pemberton BM, 1999. The relative importance of seed-borne inoculum to common scab disease of potato and the efficacy of seed tuber and soil treatments for disease control. *Journal of Phytopathology* **147**, 13–8.

Wilson CR, Pemberton BM, Ransom LM, 2001. The effect of irrigation strategies during tuber initiation on marketable yield and development of common scab disease of potato in Russet Burbank in Tasmania. *Potato Research* **44**, 243–51.

Wilson CR, Luckman GA, Tegg RS *et al.*, 2009. Enhanced resistance to common scab through somatic cell selection in cv. Iwa with the phytotoxin thaxtomin A. *Plant Pathology* **58**, 137–44.

Wilson CR, Tegg RS, Hingston LH, 2010a. Yield and cooking qualities of somaclonal variants of cv. Russet Burbank selected for resistance to common scab disease of potato. *Annals of Applied Biology* **157**, 283–97.

Wilson CR, Tegg RS, Wilson AJ *et al.*, 2010b. Stable and extreme resistance to common scab of potato obtained through somatic cell selection. *Phytopathology* **100**, 460–7.