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Relationship of resistance to common scab disease and tolerance to thaxtomin A toxicity within potato cultivars

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Abstract Thaxtomin A has a central and implicit role in common scab disease expression in potato. Thaxtomin A tolerance has been suggested as a possible rapid means for screening potato germplasm for disease resistance, during breeding selections. We have tested a range of genetically diverse cultivars with varying resistances to common scab disease in both pot and field based studies and measured their mean necrosis response to thaxtomin A. We found no association between resistance to common scab disease and tolerance to thaxtomin A toxicity. For example, disease resistant cultivars 'Russet Burbank' and 'Atlantic' were sensitive and tolerant to thaxtomin A toxicity respectively. Similarly; disease susceptible cultivars 'Bismark' and 'Tasman' showed susceptibility and tolerance to thaxtomin A. This demonstrates that whilst thaxtomin A is critical to disease expression, reaction to this toxin is only one component influencing resistance to common scab disease and many other anatomical, physiological or biochemical factors are critical to defence against this disease.

Keywords Potato · *Streptomyces scabiei* · *Solanum tuberosum* L.

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Common scab disease caused by infection with pathogenic *Streptomyces* spp. is one of the most important diseases of the potato (*Solanum tuberosum* L.) worldwide (Loria et al. 2006). Annual losses in Tasmania, Australia alone are estimated at approximately 4% of the industry value (Wilson et al. 2009). All pathogenic *Streptomyces* spp. produce the phytotoxin thaxtomin A, whilst non-pathogenic strains do not (Babcock et al. 1993; King et al. 1991), and its essential role in disease induction appears implicit (Goyer et al. 1998; Kers et al. 2005).

Cultivar selection is one of the key management practices used to negate or reduce the impact of this disease. There exists wide variability in resistance to common scab disease (Darling 1937; Goth et al. 1995; Park et al. 2002) but specific factors associated with disease resistance are not well defined. Physical tuber features such as thickened skin, skin russetting, stomata/lenticel density, aperture and structure are likely to play some role in disease resistance expression although further evidence is required (Adams and Lapwood 1978; Darling 1937; Loria et al. 2006; Tegg et al. 2008). Other physiological parameters, such as glucose content in peel or the ability to detoxify thaxtomin A have been postulated as associated with enhanced resistance (Acuña et al. 2001; Goto 1981).

The necessity for thaxtomin A for expression of common scab disease has led our group to look at selection of potato somaclonal mutants with tolerance to this toxin (Wilson et al. 2009, 2010) and

others to use thaxtomin A as a screening tool during conventional breeding to identify lines with enhanced toxin tolerance, with the expectation that these will have greater resistance to disease (Acuña et al. 1998; Delserone et al. 1991; Hiltunen et al. 2006).

There has however, been no attempt to examine in detail more diverse (non-clonal or non-sibling) potato germplasm for thaxtomin A tolerance to see if toxin reaction could be used as a broad predictor of common scab disease resistance potential.

In this study we screened a selection of nine commercial potato cultivars representing a range of known sensitivities to common scab disease (that we confirm in field and glasshouse pathogenicity trials) for reaction to thaxtomin A to address whether toxin tolerance can predict disease resistance.

Potato cultivars were obtained as certified seed (Department of Primary Industries, Devonport, Tasmania). They represented a spread of cultivars with a broad range of resistance to common scab: 'Atlantic' and 'Russet Burbank'—medium to high; 'Bismark', 'Coliban' and 'Shepody'—low to medium; 'Desiree', 'Maris Piper', 'Pontiac' and Tasman—low to very low (Science and Advice for Scotish Agriculture 2009). Production and purification of the toxin thaxtomin A was as previously described (Wilson et al. 2009).

In a preliminary trial, 'Russet Burbank' and 'Desiree' were used to compare the response of different potato tissue types to thaxtomin A and validate the toxin assay. Firstly, leaf tissue of similar physiological age (sourced from tissue culture grown plants) was placed on potato multiplication medium containing 4.6 μM thaxtomin A (Wilson et al. 2009). Three leaflets per plate with three plates per cultivar were used. The toxicity response was measured after 10 days using a 0–5 rating scale (0=no necrosis, 1= 1–5 %; 2=6–15 %, 3=16–50 %, 4=51–80 %, 5 = >80 % of leaf area showing necrosis). Secondly, intact tubers (2–4 weeks after initiation) growing in pots in an open vermiculite mix were uncovered and thaxtomin A containing paper discs (14 μM thaxtomin A, 0.2% agarose solution) placed on each tuber. Ten tubers per cultivar were treated. The toxicity response was measured after 5 days using a 0–4 scale (0=no necrosis, 0.5=very sparse flecks, 1=few light brown flecks, 1.5= few dark brown flecks, 2=light brown flecks in determined necrotic area, 2.5=dark brown flecks in determined necrotic area, 3=light brown necrosis, 3.5= dark brown necrosis and 4=black necrosis).

Lastly, a tuber slice bioassay refined from Tegg et al. (2008) was conducted. In brief, tubers were cut into 0.5 cm slices and placed in Petri dishes with moist filter paper. Three tuber slices were used for each tested cultivar per Petri dish (replicate of three). Filter paper disks of 6 mm diameter were immersed in a 1% acetone solution containing 28 μM thaxtomin A for 1 h and air-dried. Three disks were placed on each potato slice. Disks immersed in 1% acetone solution were used as controls. Tuber slices were incubated at 24°C in the dark. Necrosis assessments were made after 7 days following the same rating system used for intact tubers.

The nine selected potato cultivars were then screened for common scab disease resistance and thaxtomin A sensitivity in two glasshouse pot trials and one field trial. For pot trials, inoculum was prepared from pathogenic *Streptomyces scabiei* isolate #20 obtained from diseased potato tubers from NW Tasmania and maintained on ISP2 slopes (Tegg et al. 2008). Spores were harvested from a two-week-old culture, suspended in 5 ml sterile water, added to a sterilized mixture of 100 g vermiculite and 500 ml SAY solution (20 g sucrose, 1.2 g l-asparagine, 0.6 g K_2HP0_4, 10 g yeast extract, in 1 l water: adjusted to pH 7.2) and incubated for 2 weeks at 24°C. Inoculum (10 g) was mixed into the top one third of the soil (1:1:8 mix of peat, course sand and composted pine bark; premixed with 6 kg/m^3 fertilizer (16:3.5:10 NPK); pH 6.0) of each pot (20 cm in diameter). Five tubers of each cultivar were directly planted into individual pots (2008 trial: 14th March; 2009 trial: 30th January). Overhead irrigation, providing even distribution of water to all pots was supplied every second day taking care to maintain low soil moisture content and allow the soil to periodically dry. Glasshouse temperatures were maintained between 18 and 24°C and no pesticides were applied during the trial period. In each experiment, treatment replicates were arranged in randomized blocks and all care taken to ensure equivalent environmental conditions across all treatments. Plants were grown to senescence (2008 trial: 28th August; 2009 trial: 23rd June). All tubers were harvested, washed, counted and weighed. Those with a mass greater than 4 g were assessed for common scab disease incidence and severity using the rating scales previously described (Wilson et al. 2009).

The field trial was planted on 21st October 2008 on a commercial farm at Bishopsbourne in NW Tasmania, Australia, having brown dermosol soils with a known cropping history of common scab disease. No additional inoculum was introduced but lime was applied (1.0 kg/ha) at the time of planting to increase disease severity. Plots were arranged in a randomized block design comprising four replicated plots, each containing three plants for each cultivar. Fungicide application and irrigation were applied following standard commercial practice. Plants were grown until senescence. Tubers were harvested on 2 April 2009 and a representative sample of 10 tubers per plot selected for disease assessment using the same scales and ratings as for the glasshouse trials.

Tubers harvested from all cultivars from both glasshouse and field trials were tested for thaxtomin A (14 μM) tolerance using the tuber slice bioassay described above. A single bioassay trial was conducted for 2008 pot trial, while duplicate bioassays on separate tuber samples were conducted for both the field trial and the 2009 pot trial.

Disease data for all tubers per replicate in both glasshouse and field trials were averaged prior to analysis. Pathology and toxin assay data for the preliminary and subsequent trials were analysed by one way ANOVA using GENSTAT 9.1 software (VSN International Ltd, Hemel Hempstead, UK) ensuring distribution approximated normal.

The preliminary trial showed that 'Russet Burbank' was significantly ($P<0.01$) more sensitive to thaxtomin A than 'Desiree' and that toxicity response was consistent across a variety of treated tissue types including detached leaf tissue, internal tuber slices and intact tubers (Table 1).

The subsequent study identified significant differences between cultivars in disease expression (tuber surface coverage, mean lesion depth, and proportion of infected tubers) and thaxtomin A tolerance in all three trials (Table 2). There was greater disease incidence within the field trial than the two glasshouse trials, however, the relative resistance to disease amongst the tested cultivars remained relatively consistent across all disease measures. Cultivars performed as expected (Darling 1937; Goth et al. 1995; Park et al. 2002; Science and Advice for Scotish Agriculture 2009). 'Atlantic' and 'Russet Burbank' were consistently the most disease resistant, 'Shepody' and 'Bismark' showed modest resistance. 'Coliban' showed modest resistance in the pot trials but was susceptible in the field. 'Desiree', 'Maris Piper' (except in pot trial 1), and 'Pontiac' were relatively susceptible and 'Tasman' was consistently the most susceptible cultivar to common scab (Table 2).

The pattern of cultivar reaction to thaxtomin A was quite different. 'Russet Burbank' and 'Bismark' were consistently highly sensitive to the toxin, 'Desiree', 'Pontiac', 'Maris Piper', and 'Shepody' showed

Table 1 Thaxtomin A sensitivity of various tissue types from 'Russet Burbank' and 'Desiree' grown in tissue culture and pot conditions. Thaxtomin A sensitivity was measured as a mean necrosis rating with leaf tissue, tuber slice and intact tuber surface exposed to 4.6 , 28 and 14 μM thaxtomin A for 10, 7 and 5 days respectively

Cultivar	Thaxtomin A sensitivity		
	Leaf tissue[a]	Tuber slice[b]	Intact tuber surface[b]
Desiree	0.87 b	3.01 b	0.50 b
Russet Burbank	2.59 a	3.90 a	1.20 a
P	<0.001	<0.01	<0.01
LSD (0.05)	0.770	0.106	0.485

[a] Leaflet necrosis assay 0–5 rating system: 0=no necrosis, 1=1–5%; 2=6–15%, 3=16–50%, 4=51–80%, 5≥80% of leaf area showing necrosis (Wilson et al. 2009)

[b] Tuber necrosis ratings: 0=no necrosis, 0.5=very sparse flecks, 1=few light brown flecks, 1.5=few dark brown flecks, 2=light brown flecks in determined necrotic area, 2.5=dark brown flecks in determined necrotic area, 3=light brown necrosis, 3.5=dark brown necrosis and 4=black necrosis

Means followed by same letter within the same column are not significantly different at $P=0.05$ using Fisher's LSD test

Table 2 Common scab disease development and thaxtomin A sensitivity of nine potato cultivars grown in pot and field conditions. Thaxtomin A sensitivity was measured as a mean necrosis rating from tuber slices exposed to 14 μM thaxtomin A

Cultivar	Common scab disease rating			Thaxtomin A sensitivity	
	Tuber surface cover (%)[a]	Lesion depth score (1–4)[b]	Infected tubers (%)[c]	Mean necrosis rating (0–4)[d]	
				Assay 1	Assay 2
2008 pot trial					
Atlantic	0.68 b	1.65 bc	29.5	1.08 c	–
Bismark	2.40 b	2.20 ab	46.3	1.67 b	–
Coliban	1.45 b	1.96 bc	41.3	1.15 c	–
Desiree	3.49 ab	2.17 ab	37.2	1.64 b	–
Maris Piper	0.67 b	1.69 bc	21.1	1.46 b	–
Pontiac	2.24 b	1.67 bc	44.2	1.16 c	–
Russet Burbank	0.45 b	1.24 c	27.3	2.27 a	–
Shepody	1.32 b	1.89 bc	35.0	1.18 c	–
Tasman	6.32 a	2.71 a	71.2	1.13 c	–
P	0.021	0.021	0.117	<0.001	–
LSD (0.05)	3.285	0.731	ns	0.252	–
2009 pot trial					
Atlantic	0.18 b	1.00 c	6.1 d	1.98 e	2.59 c
Bismark	0.76 b	2.20 ab	25.3 bcd	3.20 a	2.85 b
Coliban	0.61 b	1.90 b	25.0 bcd	2.19 de	2.65 bc
Desiree	2.06 ab	2.72 a	35.0 abc	2.65 bc	2.87 b
Maris Piper	3.36 a	2.80 a	53.6 a	2.39 cd	2.65 bc
Pontiac	3.15 a	2.33 ab	56.5 a	2.80 b	2.70 bc
Russet Burbank	0.45 b	1.00 c	17.8 cd	2.91 ab	3.19 a
Shepody	1.90 a	2.40 ab	40.3 abc	2.30 d	2.74 bc
Tasman	3.51 a	2.83 a	47.8 ab	2.20 de	2.74 bc
P	0.002	<0.001	0.004	<0.001	<0.001
LSD (0.05)	1.970	0.668	26.35	0.294	0.236
2008–09 field trial					
Atlantic	11.3 d	2.44 b	100	2.69 d	2.65 d
Bismark	16.6 cd	3.13 a	100	2.95 b	3.15 a
Coliban	23.3 abc	3.12 a	100	2.80 bcd	2.67 cd
Desiree	24.2 abc	3.47 a	100	2.72 cd	2.72 cd
Maris Piper	27.5 ab	3.39 a	100	2.67 d	2.65 d
Pontiac	23.1 bc	3.31 a	100	2.82 bcd	2.94 b
Russet Burbank	13.6 d	2.43 b	100	3.17 a	3.22 a
Shepody	15.4 cd	3.05 a	100	2.93 bc	2.85 bc
Tasman	32.2 a	3.48 a	100	2.70 d	2.67 cd
P	<0.001	<0.001		<0.001	<0.001
LSD (0.05)	9.00	0.491	ns	0.212	0.195

[a] Estimated tuber surface coverage is calculated from disease cover score (0=no disease, 0·5=0–1%, 1=1–5%, 2=5–10%, 3=10–30%, 4=30–50%, 5=50–70%, 6≥70%) using median percentile scores within the allocated range

[b] Depth of deepest lesion per tuber (excluding disease free tubers); 1≤1 mm deep, 2=1–2 mm deep, 3=2–3 mm deep, 4≥3 mm deep

[c] Proportion of tubers with at least one common scab lesion

[d] Necrosis ratings: 0=no necrosis, 0.5=very sparse flecks, 1=few light brown flecks, 1.5=few dark brown flecks, 2=light brown flecks in determined necrotic area, 2.5=dark brown flecks in determined necrotic area, 3=light brown necrosis, 3.5=dark brown necrosis and 4=black necrosis

Means followed by same letter within the same column are not significantly different at $P = 0.05$ using Fisher's LSD test; ns=non significant

– indicates assay not undertaken within the specified trial

moderate toxin tolerance whilst 'Tasman', 'Coliban' and 'Atlantic' were generally the most toxin tolerant of the cultivars tested (Table 2, Fig. 1).

Few studies have reported on potato varietal or cultivar susceptibilities to thaxtomin A (Delserone et al. 1991; Acuña et al. 1998; Hiltunen et al. 2006). They reported positive associations between common scab disease resistance and thaxtomin A toxicity in potato shoots (Acuña *et al.* 1998; Hiltunen et al. 2006) and tuber tissues (Delserone et al. 1991). In contradiction to these reports we have shown that extent of resistance to common scab disease measured across nine cultivars (in three separate trials) was not associated nor showed any consistent pattern of association with the levels of tolerance to thaxtomin A toxicity.

This is most clearly demonstrated by the important processing cultivar 'Russet Burbank', regarded as having moderate common scab disease resistance (Darling 1937; Goth et al. 1995; Park et al. 2002), yet we show it is highly sensitive to thaxtomin A. Also, the fresh market variety 'Tasman' which was shown to be highly susceptible to common scab disease showed high tolerance to thaxtomin A. Following the anticipated trend, 'Atlantic' a moderately disease resistant cultivar (Science and Advice for Scotish Agriculture 2009) was tolerant to thaxtomin A, and the relatively disease susceptible 'Bismark' showed thaxtomin A sensitivity. These findings suggest thaxtomin A tolerance cannot be used reliably as a rapid screen for common scab resistance expression. They may however provide guidance for germplasm enhancement in future potato breeding programmes aiming to combat common scab disease. As an example, 'Russet Burbank' has high sensitivity to thaxtomin A and improving toxin tolerance is likely to be highly beneficial. This is something we have recently successfully achieved (Wilson et al. 2010). On the contrary, for cultivar 'Tasman' with considerable thaxtomin A tolerance already yet poor physical resistance to the pathogen, a breeding programme aimed at improving physical resistance properties (skin russeting etc.) may be of greater benefit for overall common scab resistance. This study demonstrates that resistance parameters, other than tolerance to thaxtomin A toxicity, are very important in common scab disease resistance expression.

The cultivar responses to common scab in this study reflect the general consensus of previous published reports (Darling 1937; Goth et al. 1995; Park et al. 2002; Science and Advice for Scotish Agriculture 2009). However to the best of the authors' knowledge this represents the first published report of relative common scab disease resistance for cultivars 'Bismark', 'Coliban' and 'Tasman' confirming field observations by industry.

In summary, we showed that necrosis response to the common scab phytotoxin, thaxtomin A, was not associated with relative disease resistance when comparing different distinct cultivars.

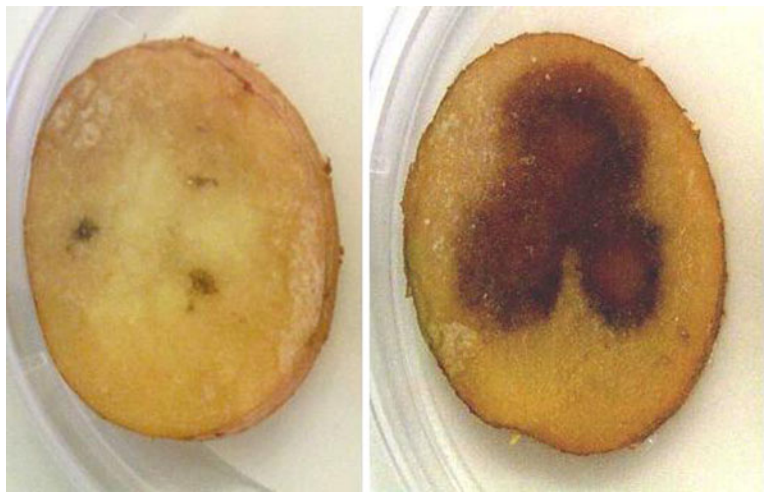

Fig. 1 Typical necrosis expressed in tuber slices of 'Tasman' plant (*left panel*; necrosis rating of 1.5) and 'Russet Burbank' (*right panel*; necrosis rating of 3.5) after 7 days exposure to 14 μM thaxtomin A

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