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Development of extreme resistance (immunity) to common scab disease within current commercial cultivars

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Tasmanian Institute of Agricultural Research

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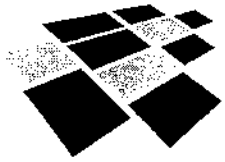

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DEVELOPMENT OF EXTREME RESISTANCE (IMMUNITY) TO COMMON SCAB DISEASE WITHIN CURRENT COMMERCIAL CULTIVARS

FINAL REPORT
(30TH NOVEMBER 2001)

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DEPARTMENT of PRIMARY INDUSTRIES, WATER and ENVIRONMENT

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"Development of extreme resistance (immunity) to common scab disease within current commercial cultivars" HAL PT98015

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MEDIA SUMMARY

Common scab disease is the greatest economic constraint facing the Australian French potato processing industry and an important disease worldwide wherever potatoes are grown. Although not directly affecting tuber yields, disease lesions markedly reduce tuber value and require extra processing steps during French fry production. As a result, crops with severe disease are often rejected by processing companies and seed crops with moderate levels of disease will be downgraded to ware quality resulting in substantial losses to the producers. The frequency and severity of common scab in Australia (primarily Tasmania and parts of Victoria) is increasing. Current conservative estimates of losses due to this disease in Tasmania alone are in excess of A$3.5 million per annum with greater losses forecast without appropriate control. This disease seriously threatens the sustainability of certain cropping areas for potato production.

Management of common scab disease has proven extremely difficult both in Australia and overseas. Past practices and current studies have utilised chemical and cultural control strategies and investigations into biological control have been attempted. Chemical control can be effective under conditions of low soil inoculum but is generally less useful where a high soil inoculum exists. Furthermore the costs associated with chemical applications add an additional burden to producers. Whilst offering a good short term management strategy, reliance on chemicals for disease control is seen as undesirable by industry and may limit future market opportunities. Similarly, cultural management strategies have had limited success in control of this disease. Most are difficult to implement within current cropping practices and may in time exacerbate other problems (e.g. increased irrigation at tuber initiation can increase powdery scab and black leg incidence; soil pH depression which limits the success of notation crops). Biological control has some potential but clear demonstration of cost effective control under commercial conditions is yet to be demonstrated.

Incorporation of durable resistance to the pathogen is an obvious long-term goal to improve sustainability of potato production. However, effective resistance is not present within current commercial cultivars. Also traditional breeding programs have significant weaknesses, requiring long time periods (in excess of ten years) to develop resistant cultivars with commercially suitable agronomic characteristics.

Use of plant tissue culture technologies, in the form of cell selection techniques offers a rapid method to obtain resistant clones of existing cultivars without the genetic re-assortment associated with breeding crosses. This makes it possible to retain the desirable agronomic characters and market acceptability of the original cultivar. This is now possible for this disease as in recent years a phytotoxin (thaxtomin) produced by the pathogen has been found which is fundamental to the development of common scab disease in potatoes. This project has utilised this toxin as a selection agent to select for resistant cell lines of current commercial cultivars.

The project has successfully developed a routine system for production of moderate quantities of highly pure thaxtomin required for cell selection studies. Following cell selection protocols a series of 20 toxin tolerant cell lines have been obtained which tolerate greatly elevated levels of the toxin. Regeneration of these cell lines has required significant level of experimentation of a range of media and culture conditions to ensure successful transfer from callus media to regeneration media. This has now

been achieved but full regeneration will take a further few months culture. Additional studies have demonstrated that all pathogenic strains of the disease agent in Australia produce thaxtomin (which is essential for the robust application of the resistance generated). Furthermore, within existing clones of the pathogen, significant variability of common scab resistance exists.
This is the first successful selection of potato cultures with extreme resistance to the common scab toxin. Subsequent studies should prove these lines to show extreme resistance to common scab disease.

Future work will focus on assessment of regenerated toxin tolerant plants for expression of resistance to common scab disease and agronomic (and processing) performance with the aim of commercial release on scab resistant potato clones of existing commercial varieties. Understanding the mechanisms of resistance will also allow specific markers for cultivar identification to be developed, and may assist in further refinement of resistance selection and strategies.

TECHNICAL SUMMARY

Common scab disease is the greatest economic constraint facing the Australian French potato processing industry and an important disease worldwide wherever potatoes are grown. Although not directly affecting tuber yields, disease lesions markedly reduce tuber value and require extra processing steps during French fry production. As a result, crops with severe disease are often rejected by processing companies and seed crops with moderate levels of disease will be downgraded to ware quality resulting in substantial losses to the producers. The frequency and severity of common scab in Australia (primarily Tasmania and parts of Victoria) is increasing. Current conservative estimates of losses due to this disease in Tasmania alone are in excess of A$3.5 million per annum with greater losses forecast without appropriate control. This disease seriously threatens the sustainability of certain cropping areas for potato production.

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Incorporation of durable resistance to the pathogen is an obvious long-term goal to improve sustainability of potato production. However, effective resistance is not present within current commercial cultivars. Also traditional breeding programs have significant weaknesses, requiring long time periods (in excess of ten years) to develop resistant cultivars with commercially suitable agronomic characteristics.

Use of plant tissue culture technologies, in the form of cell selection techniques offers a rapid method to obtain resistant clones of existing cultivars without the genetic re-assortment associated with breeding crosses. This makes it possible to retain the desirable agronomic characters and market acceptability of the original cultivar. This is now possible for this disease as in recent years a phytotoxin (thaxtomin) produced by the pathogen has been found which is fundamental to the development of common scab disease in potatoes. This project has utilised this toxin as a selection agent to select for resistant cell lines of current commercial cultivars.

The project has successfully developed a routine system for production of moderate quantities of highly pure thaxtomin required for cell selection studies. The techniques developed have delivered milligrams of bright orange crystals of thaxtomin. The identity of the extracts has been confirmed using mass spectroscopy and liquid chromatography have confirmed the identity of extract as being Thaxtomin A. A novel recrystalisation step in the extraction technique has been a key to recovery of highly

pure (98%) thaxtomin in useable quantities exceeding that of other researchers working with this toxin. This is significant as low purity samples could have had a significant effect on plant growth as the target molecule. Experiments examining possible elicitors of thaxtomin production did not clarify the reasons behind occasional variable yields of the toxin.

Following cell selection protocols a series of 20 toxin tolerant cell lines have been obtained. Three distinct phenotypes were observed within the selected callus lines.

1. Several cell lines (5 lines) showed a softer more friable callus than would be expected in unselected callus. These calli grew quickly on callus media which had facilitated rapid production of sufficient quantities callus for regeneration (and other) studies. However, some of these cell lines have proven difficult to handle during attempts at regeneration, due to sensitivity to abrupt changes in media resulting in tissue death. In some cell lines we believe that this is due to changes in osmotic potential of the media but in other cell lines it appears that there is sensitivity to changes in hormone concentration, particularly cytokinin. Sensitivity to changes in cytokinin levels presents a particular challenge, as this is central to the induction of shoots from callus. Differences in plant hormone sensitivity are yet to be quantified in all cell lines but may point to a physiological basis for the Thaxtomin tolerance observed in some, but not all, cell lines.
2. One callus line was harder than normal but was much slower in growth. Osmotic sensitivity does not appear to be as prominent in this line.
3. The remaining 14 lines showed no phenotypic difference to unselected callus.

Despite initial sensitivity to changes in media, all cell lines have been successfully transferred to a thaxtomin free callus medium and are also maintained on a thaxtomin-containing medium.
In assays for maximal thaxtomin tolerance of selected lines, all tested lines tolerated (and grew) on media with ten times the selection concentration of thaxtomin (i.e. 22.5 mg/L). The selection level (2.25 mg/L) exceeds that likely to be found in naturally infected potatoes, so this greatly enhanced tolerance is pleasing and suggests that extreme resistance to thaxtomin has been selected within these lines. The control callus died at the normal selection concentration.

Regeneration of these cell lines has required significant level of experimentation of a range of media and culture conditions to ensure successful transfer from callus media to regeneration media. This has now been achieved but full regeneration will take a further few months culture.
Successful regeneration has not yet been achieved using standard media for thaxtomin tolerant cell lines. However significant progress toward this aim has been made. Cell lines have now all been successfully transferred onto regeneration media without adverse growth effects through careful manipulation of the transfer process, media and timing of transfers. Specific media combinations for each of the cell lines that result in the death of cultures have been identified. Notably, there are significant differences between different cell lines in media requirements.
The calli of all lines are all showing promising signs of differentiation prior to regeneration (including forming semi-structured lumps on callus, greening of callus etc).

New selections are continually being made (as younger callus has a greater regeneration efficiency) and it may be that now that likely specific media conditions are known that these younger calli can be habituated onto regeneration media faster.
That in recent experiments control (unselected) callus has also failed to regenerate has led us to believe that the current limitation may not be a direct consequence of the mutation and selection process but rather some fundamental media or growth room environmental factor.
To overcome this, duplicates of all cultures have been shipped to Prof. Conner (project partner chief Investigator) in New Zealand. Given that his laboratory routinely regenerates potato callus, one can assume that if it is a growth room effect inhibiting regeneration, that this should be overcome in Prof Conner's laboratory. These cultures will require a further 3-4 months before success of regeneration can be ascertained.
Regeneration is a time dependant process, and each experiment requires several months to determine outcomes. The requirement for extensive media studies has slowed progress toward the desired outcome of fully regenerated plants, however, we feel confident that the progress made and state of current cultures (growth dynamics and structures including "greening" and semi-differentiation) and the multi-site trials should result in successful regeneration of toxin resistant plants within 3-4 months.

Additional studies have shown that exogenous and exogenous auxin ameliorates thaxtomin toxicity and conversely thaxtomin interferes with auxin activity. This has implications in the cell selection work, as alternative media had to be developed suitable for callus induction to avoid false tolerance reactions. Furthermore, this finding may link with glasshouse and field studies from the UK where foliar applied auxins reduced common scab disease. This provides a possible mechanism of thaxtomin toxicity and may assist in development of additional disease control strategies as has been suggested and trialed by researchers from the UK in the 1980's.

We also demonstrated that at least four distinct strain groups of pathogens exist in Tasmanian soil, at least two of which are novel species. Notably all pathogenic strains of the disease agent in Australia produce thaxtomin (which is essential for the successful application of the resistance generated in this project).

Lastly a brief study has shown that even within current commercial clones of Russet Burbank, significant difference in resistance to common scab disease exist. We are currently attempting to correlate this differences with tolerance to thaxtomin.

This project is the first successful selection of potato cultures with extreme resistance to the common scab toxin. Subsequent studies underway now should prove these lines to show extreme resistance to common scab disease and if agronomically sound should lead to commercial release of scab resistant clones of commercially important varieties.

INTRODUCTION

Common scab - the disease

Common scab disease is present in all potato growing areas of the world. Yield losses may occur from severe infections but usually damage is restricted to tuber blemishes reducing tuber quality (Hooker, 1981). Despite the relatively mild effect on tuber productivity and plant growth common scab infection may cause major economic losses to the potato industry by down grading of seed stocks to ware quality, consumer resistance to blemished ware tubers, restrictions to export markets, and with deep pitted scab, losses to processors through the necessity for double peeling or tuber rejection.

In Tasmania, losses to the French fry processing and seed potato industries have been estimated at approximately $3 M and $0.5 M per annum respectively (McCain Foods (Aust) & Simplot Aust., pers. comm.) with forecasted increases in the future. Prevalence of common scab disease in seed crops has forced industry to use late generation uncertified potatoes for seed, increasing the chance of losses due to other seed borne diseases.

Fig 1. Deep pitted lesions of common scab disease

Symptoms of the disease

Common scab lesions are highly variable (McKee, 1958). Typical symptoms may be divided loosely into surface or pitted lesions (Goth *et al.*, 1995) and are usually roundish or star shaped with cracked and torn edges. Surface lesions may range from a superficial cork-like layer (russet scab; Harrison, 1962) to an erumpent raised corky lesion (raised scab) caused by a proliferation of suberised material. Pitted scab lesions may be only slightly sunken or deeply pitted with cavities up to 10 mm deep developing within infected tubers. Pitted lesions tend to be darker in colour surrounded by straw coloured translucent tissues (Archuleta & Easton, 1981). Lesions may be discrete or can

coalesce to form irregular shaped lesions and may completely cover infected tubers (Emilsson & Gustafssen, 1953).

Beside tuber symptoms, brown necrotic spots have been noted on roots and stolons of affected plants (Hooker *et al.*, 1950; Dutt, 1979). No disease symptoms are found on aerial parts of the plant (Smith, 1968). In Australia, deep-pitted lesions are a common symptom and a major reason for the economic losses associated with this disease (Wilson *et al.*, 1999, Wilson, 2001).

Taxonomy of the causal agent

Common scab of potato is caused by infection of developing tubers by actinomycetes of the family Streptomycetaceae. The specific causal organism is generally regarded as *Streptomyces scabies* although several other species of *Streptomyces* have been implicated. *Streptomyces* spp. are aerobic, gram positive filamentous, soil-borne bacteria. They differ from most prokaryotes by production of hyphae and terminal spores (Chater & Merrick, 1979). It has been suggested that they occupy an evolutionary opposition between the eubacteria and the lower fungi (Stonesifer & Baltz, 1985).

Thaxter (1891) first isolated the pathogen and named it *Oospora scabies* based on melanin production, and grey spores borne in spiral chains. The organism was renamed to *Actinomyces scabies* (Gussow, 1914) and again to *Streptomyces scabies* (Waksman & Henrici, 1948). Waksman (1961) redescribed the species and mistakenly used strain IMRU 3018 as the neotype strain. This strain was used in the International *Streptomyces* Project (ISP) and in all the keys derived from the ISP (Kurster, 1972, Szabo *et al.*, 1975). However, this strain lacks spiral spore chains and melanin production (Shirling & Gottlieb, 1968) and does not represent the common strain types found associated with common scab disease.

Besides *S. scabies*, more than 30 *Streptomyces* species have been described which induce common scab disease in potato. These include *S. acidiscabies* (Lambert & Loria, 1989; Faucher *et al.*, 1992), *S. caviscabies* (Goyer *et al.*, 1996), *S. europaeiscabiei, S. stelliscabiei, S. reticuliscabiei* (Bouchek-Mechiche *et al.*, 2000), *S. griseus* (Hoffman, 1958; Corbaz, 1964; Hutter, 1967; Gordon & Horan, 1968; Doering-Saad *et al.*, 1992), *S. olivaceous* (Hutter, 1967), *S. aureofaciens* (Corbaz, 1964), *S. flaveolus* (Millard & Burr, 1926), *S. globisporus* (Siderevich *et al.*, 1981), S. violaceus (Doering-Saad *et al.*, 1992) and others (Millard & Burr, 1926; Harrison; 1962; Krassilnikov *et al.*, 1970; Archuleta *et al.*, 1981). The situation has been clarified somewhat with the revival of the name *S. scabies* to describe the common strains from USA, Canada and Hungary (Lambert & Loria, 1989). These *S. scabies* strains were described on the basis of 42 phenotypic characters and differentiate these from atypical pathogenic and saprophytic strains.

Typical strains have smooth grey aerial arthrospores (0.6-0.7 um x 1.0-2.0 um) borne in spiral chains, are melanin producers, and utilise all ISP sugars (Elesaway & Szabo, 1979). Most do not degrade xanthine and are susceptible to 25 ug/ml oleandomycin, 10IU/ml pennicillin G, 20 ug/ml streptomycin, 10 ug/ml thallium acetate and 0.5 ug/ml crystal violet. The type strain is ATCC 49173 (Schottel, 1995).

The organism grows well in the temperature range of 25-30°C with an optimal growth at 29°C (Sharpavalov, 1915). It shows practically no growth below 5°C or above 40°C but can survive 90°C for 10 minutes. *S. scabies* may also withstand desiccation for extended periods (Millard, 1923).

Disease cycle

Infection occurs through young lenticels during tuber elongation or through wounds in the tuber surface. Some evidence suggests stomata might be infected also (Fellows, 1926). It is suggested that internodes that form successively at the tuber apex each pass through a period of susceptibility to common scab infection (Lapwood & Adams, 1973). Each internode reaches its susceptibility phase about 10-14 days after initiation during which time the stomata present become lenticels and the guard cells protecting inner tissues are lost (Lapwood & Adams, 1973). Lenticels remain susceptible for approximately 6 days. On lenticel maturity it becomes resistant to infection which coincides and may be related to suberisation of the tissues (Fellows, 1926; Jones, 1931; Lapwood & Adams, 1973).

On infection, meristematic tissues are stimulated to form wound tissue layers. The pathogen may penetrate the wound periderm layer after which a second or third layer is formed (Lapwood, 1973). This results in lesion formation. Severity of lesions may be determined by host genotype. Those cultivars with greater resistance may restrict infection through formation of a single periderm layer whilst others require successive layers as the infection progresses (Hooker, 1981). In addition to initiating wound sites, larval feeding can also aid disease development through damage to wound periderm layers allowing the pathogen to penetrate to greater depths in to the tuber (Afanasiev, 1937; Agrios, 1988).

In addition to potato common scab affects a number of other root crops such as radish, red and sugar beet, carrot, rutabaga, parsnip, and turnip (Locci, 1994). A distinct *Streptomyces* sp. causes scab of sweet potato.

Pathogenic *Streptomyces* survive between potato crops either in the soil as an efficient saprophyte on decaying plant parts, on alternate hosts (perhaps including roots of hosts as yet unidentified) or on infected potato seed tubers. There is great debate as to the importance of seed and soil borne inoculum on subsequent disease levels (Lapwood, 1973; Adams & Hide, 1981; Singh *et al.*, 1987) and it is likely that the relative role of each varies with ecosystem. The pathogen is disseminated on infected seed tubers and in infested soil transported by vehicles or stock, or carried by wind or water (Agrios, 1988; Sharma & Sharma, 1989). The arthrospores remain viable after long periods of freezing and desiccation in soil and may persist in passage through the digestive tract of animals and spread through manure (Morse, 1912).

Several soil environmental factors such as pH, moisture, temperature, and microbial activity as well as host cultivar are known to alter predisposition of crops to infection, although no single factor is solely responsible (Sharma & Sharma, 1989). The mechanisms of disease suppression by such factors are poorly understood.

Thaxtomin - the key to unlocking this disease

Thaxtomins

It has been demonstrated (King *et al*, 1989, Babcock *et al.*, 1993) that all plant pathogenic strains of *Streptomyces* sp. produce a group of (up to 11) related phytotoxins (thaxtomins) whilst non-pathogenic strains do not. Of this group, one (thaxtomin A) is the predominant compound produced by potato pathogenic strains (King *et al.*, 1992; King *et al.*, 1994; King & Lawrence, 1996) and many of the other isolated compounds are likely precursors to, or breakdown products of thaxtomin A. These toxins are capable of inducing complete common scab disease symptoms when applied to

developing tubers in absence of the pathogen itself (Lawrence *et al.*, 1990). These findings have provided a key insight into the nature of the pathogenesis reaction and suggest a probable role of the toxin(s) in killing potato cells to sequester nutrition on which the pathogen may then subsequently grow saprophytically.

Thaxtomins are modified dipeptide molecules which may arise biosynthetically from tryptophan and phenylalanine (King, 1997) by as yet uncharacterised pathways. Essential to the phytoxic activity is the presence of the 4-nitroindol-3-yl and phenylalanine groups linked in an L,L configured cyclodipeptide (King *et al.*, 1992). Production of organic compounds containing nitro (NO_2) groups is relatively unusual in nature (Loria *et al.*, 1997) adding interest to the study of these bioactive compounds.

Thaxtomin toxicity

Thaxtomins have been shown to affect all higher plant species to which they have been applied (Leiner *et al.*, 1996). Symptoms associated with application of thaxtomins include cell hypertrophy and growth reduction (at sublethal concentrations), and cell death with associated necrosis, probably preceded by chlorosis (at concentrations similar to those found in diseased potatoes (Lawrence *et al*, 1990; Loria *et al.*, 1997; B Fry pers. comm). Isolated protoplasts from thaxtomin sensitive plants are insensitive until replication and reformation of cell wall structures indicating a probable target for toxicity (B Fry, Cornell University, manuscript in progress). The mechanism(s) of toxicity are not clear but there is histological evidence that thaxtomin inhibits laying down of cellulose in the cell wall and affects normal cell plate formation probably also through disruption of cellulose deposition (B Fry, Cornell University, manuscript in progress).

Research on common scab disease control undertaken to date in Australia

Studies on various aspects of common scab disease control have been a major undertaking of various Tasmanian researchers over the past decade, reflecting the prevalence and importance of this disease to that State. In recent time common scab has become a rapidly increasing problem in Victoria leading to increased interest in disease management studies in that State. We will not expand on the findings of this work in this report but rather refer the reader to a series of summary reports available from Horticulture Australia Ltd, or the University of Tasmania.

Previous studies initially focussed on development of detection systems for the pathogen based on immunoasay approach, which were generally unsuccessful (Gillian, 1991, Wilson, 1995). In this aspect, research from the USA and UK have revealed a genetic marker associated with common scab pathogenicity which may prove a useful detection tool. In this report we discuss the diversity of pathogenic strains in Tasmania and we are currently trialing the UK derived genetic marker detection system in conjunction with the UK researchers against a range of pathogenic and non-pathogenic isolates to determine likely usefulness as a detection tool.

Further studies examined disease management through cultural, biological and chemical control (Wilson, 1995, Pung 2000, Lacey, 2000). While significant disease control is possible through careful irrigation management and use of chemical seed dressings, and good progress has been made toward the use of biological suppression of the disease, no single management approach led to robust and reliable control, emphasising the importance of integrated solutions to minimise risk. However, under

heavy soil inoculum conditions, these control strategies generally have limited efficacy and significant losses can occur.

Given the difficulty in prediction of likely disease risk (and hence the need for disease control investment), the limited control offered by these management strategies, and the financial and environmental limitations placed on commercial potato cropping which can limit best disease practice (e.g. availability of water for appropriate irrigation strategies during tuber set, the capacity for soil pH amendment during crop rotations etc), the disease remains a significant problem for potato growers.

To address this, the current project took a novel approach to enhance resistance to the disease using the recently described thaxtomin as a pathogen specific target.

Resistance to common scab disease

Host resistance is generally the most effective tool in the management of plant disease. By definition, the interaction between host and pathogen (or substances of origin from the pathogen) is of prime importance in disease development. This interaction is quite specific and influenced by the genomes of both pathogen and host. The complexity of the common scab pathosystem and the difficulty experienced in maintaining disease control through chemical and cultural means emphasises the need for effective host resistance to this disease. However, selection of common scab resistant germplasm is made more difficult by the wide variation in pathogen types.

No commercial potato cultivars are immune to infection by *S. scabies* but there is considerable variation in cultivar susceptibility (McKee, 1958). Common scab disease does not systemically rot plant tissues seriously affecting productivity of the host plant. This is due to effective resistance responses by the potato through production of suberised cork layers at the sites of attack, limiting further penetration of the pathogen (Agrios, 1988; Fischl, 1990; Mishra & Sivastava, 1991). It is however, these defensive cork layers which disfigure the tubers and reduce fresh market value and processability. The defence response is induced through the production of the thaxtomin phytotoxin.

Potatoes with the greatest resistance to common scab disease have an ability to localise infection sites more rapidly with production of a single wound barrier. More susceptible varieties require several layers of wound periderm to form (Mishra & Sivastava, 1991). However, the ultimate severity of disease will be influenced by pathogen strain and environmental conditions (Keinath & Loria, 1991. There are no currently available commercial genotypes that exhibit complete resistance to common scab disease. Even the most resistant of varieties currently described can suffer heavily from disease under highly conducive conditions. Of particular concern is the association with other soil micro-organisms and insects which may feed on damage caused by the common scab pathogen, penetrating the defensive layers allowing the common scab pathogen to advance further into the tuber and cause deep pitting (Agrios, 1988).

One major difficulty facing breeding programs seeking enhanced scab resistance is the difficulty of rapidly screening numerous clones and breeding lines. Traditionally most tests were done in field exposure trials (e.g. Langton, 1972; Freve, 1987; Goth *et al.*, 1993) but these may be somewhat compromised by variations in environmental conditions and infection pressure (Loria & Kempster, 1986). More reliable and less costly assays can be done in the glasshouse (Bjor & Roer, 1980; Calgarie & Wastie, 1985; Murphy, 1988; Marais & Vorster, 1988) but these may still be time consuming and difficult to interpret. More recently cultivars are being screened using reaction to

purified preparations of the major phytotoxin produced by pathogenic *S.scabies* (R. King, pers. comm.).

Despite the development and selection of several cultivars with superior resistance to common scab (e.g. Holm *et al.,* 1992), this has been of limited value to the Australian french fry producers. The clients of the processors demand that the potato cultivars processed must be Russet Burbank or cultivars with processing qualities similar or better. Russet Burbank is recognised as moderately resistant in the US (Goth *et al.,* 1995) but may still be severely affected by this disease (Archuleta & Easton, 1981; Wilson *et al.,* 1999).

Enhancing resistance through mutation and cell selection

Controlled mutation using natural somaclonal variation during tissue culture or mutagenic agents may be used to generate useful traits in commercial crop plants. In experiments from the former Soviet Union, potatoes were treated with ionising radiations or chemical mutagens and resulting mutants screened for enhanced resistance to common scab disease. One particular mutant produced a thick corky tuber skin, not previously recognised in commercial potato genotypes, which was genetically stable and conferred full resistance to *S. scabies* (Saulite, 1986; 1987). Furthermore, selections from somaclonal mutants have also shown enhanced resistance to common scab to parental material (Yakauleva & Gancharova, 1990).

With the recent finding of the importance of the phytotoxin thaxtomin in the pathogencity of the common scab pathogen, this has provided a specific target for resistance studies. Indeed, purified phytotoxin is currently used in breeding programs to rapidly screen new lines for probable common scab resistance (King, pers. comm.). Recent publications have demonstrated that resistance to thaxtomin in potato seedlings is well correlated with decreased incidence of scab lesions in tubers. (Dongman & Lim, 2000; Ivette *et al.,* 2000) However, traditional breeding programs have significant weaknesses, requiring long time periods (in excess of ten years) to develop resistant cultivars with commercially suitable agronomic characteristics. On the other hand, tissue culture technologies utilizing directed mutation offer more rapid production of new clones without the genetic re-assortment associated with breeding crosses, and can retain the desirable agronomic characters and market acceptability of the original cultivar.

Cultured plant cells offer a convenient system for the selection of mutants in plants with resistance to phytotoxic stress. A selection pressure such as the incorporation of the common scab phytotoxin into the culture, can be easily imposed on a large population of plant cells in a single Petrie dish. Rare variants, with resistance to the stress imposed can then regenerated into complete plants (e.g. Conner, 1986). To increase the frequency of recovering mutants, plant cells may be exposed to mutagenic agents prior to selection.

Cell selection techniques also have significant advantages over genetic engineering approaches which have also been used to generate phytotoxin resistance (e.g. resistance to *Pseudomonas syringae* pv. phaseolicola, Fuente-Martinez *et al.,* 1993; glyphosate resistance, Shah *et al.,* 1986). The technology avoids much of the legislative, social and economic barriers associated with the adoption of genetic engineering.

Such a cell selection system are well suited to potatoes as this plant is readily cultured and has been regenerated from single cells and callus. Techniques and conditions for regeneration from callus have been published from several studies and a wide range of varieties (Shepard 1977, de Garcia and Martinez, 1994 Curry and Cassells,1999). This procedure has been effectively used to develop resistance to

several plant pathogens in a range of crop plants (Reviewed by Gengenbach & Rines, 1986) and herbicide-resistant mutants in potato and other crop plants (Reviewed by the partner chief investigator, Conner & Field. 1995). Examples include resistance to the *bacterium Erwinia caratovora* in potato (Taylor &, Secor, 1990), the fungi *Alternaria solani* in potato (Cho *et al.,* 1993), *Phoma lingum* in rape (Sacristan, 1984). *Helminthosporium oryzae* in rice (Ling *et al.,* 1985) and resistance to the herbicidal auxin analogues in tobacco (Chaleff and Parsens 1 978), atrazine in soybean (Wrather & Fretag, 1991) and chlorosulfuron in rape (Conner *et al.,* 1994).

We believe this approach to common scab disease control through induction of resistance is the first real possibility for significant, substantial and sustainable disease management without compromising production practices. As the resistance targets the toxin that is central to disease induction, both extreme and durable resistance is likely. It is novel, achievable and perhaps the only sustainable disease management option available for cropping of current commercial cultivars.

PROJECT AIMS

1) To enable sustainable management of common scab disease of potato based on extreme and durable resistance within commercial cultivars to the pathogen to complement short term solutions under development

2) To develop valuable export potato lines of current commercial cultivars exhibiting extreme disease resistance in demand worldwide wherever common scab disease limits potato production.

3) To increase our knowledge of this common scab:potato pathosystem with the aim of identifying additional targets for development of effective control strategies.

4) To assist in facilitation of the export of seed tubers by reducing or eliminating tuber contamination with this persistent soil and tuber-borne pathogen.

EXPERIMENTAL SECTION

THAXTOMIN PRODUCTION AND PURIFICATION

Background

The phytotoxins produced by *Streptomyces* scabies, named thaxtomins, were first identified in 1989. All are derived from the ester linkage of 4 nitrotryptophan and phenylalanine molecules to form a dioxopiperazine. Thaxtomin A is the predominant form found in cultures. Most of the other forms appear to be either precursors or breakdown products in the synthesis of thaxtomin A.

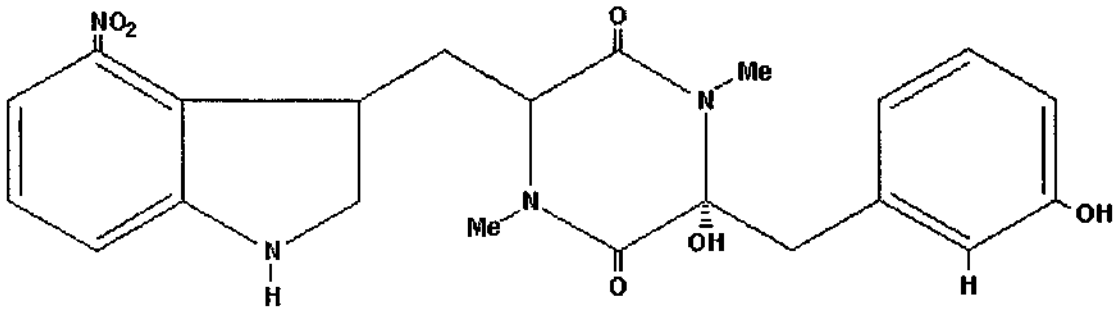


Fig 2: Chemical structure of thaxtomin A

Accumulation of thaxtomin in growth media occurs after the major growth phase of the culture. This is assumed to indicate that it is produced by *Streptomyces* as a secondary metabolite.
The first step in this project was a requirement to isolate highly pure thaxtomin in sufficient quantities for selection of toxin tolerant cell lines.
Although *Streptomyces scabies* is able to grow on a wide range of media, it has been found that the conditions in which thaxtomin production occurs at detectable levels are very much restricted. In liquid media, thaxtomin production has only been measured in culture media containing plant-derived components such as oatmeal or oat bran. Growth of *S. scabies* on fully defined media inhibits production of thaxtomin, as does the presence of glucose in liquid media, although the presence of glucose does not inhibit thaxtomin production on solid media. Optimal production of thaxtomin occurs in oatmeal broth at a temperature of 28°C. Such conditions are well outside the range under which *Streptomyces* infection occurs in field conditions.
Thaxtomin production techniques have been described previously (King *et al.*, 1992). In this study we adapted these techniques to produce greater quantities of very pure product (without the need for reverse-phase HPLC etc) which we felt was necessary in order to ensure optimal cell selection conditions.

Materials and Methods

Australian *S. scabies* isolate G32 was chosen from our culture collection as the source culture for thaxtomin production. As part of a prior international collaboration, G32 had previously been demonstrated to produce "good" quantities of thaxtomin by Dr

Russell King (Agriculture Canada, pers. comm.). G32 was maintained on slopes of ISP2 medium at 4°C and spores stored at –20°C in glycerol.

***Streptomyces* culture**
Freshly grown slopes of isolate G32 were used to inoculate 500 mls of oatmeal broth in 1 litre Erlenmeyer flasks. The spores were suspended in 5 ml sterile water using vigorous scraping which was then transferred the broth. Flasks were loosely closed with cotton wool plugs, capped with aluminium foil. Cultures are placed in an incubator and grown at 25°C on an orbital shaker at 120 rpm for 5-10 days.

Extraction
Thaxtomin was extracted from culture broths by three successive washes of chloroform. 250ml of chloroform was added to each 500ml of broth culture and shaken for 2 hours on an orbital shaker. The chloroform and broth mixture was allowed to settle in a separating funnel and the chloroform decanted. The chloroform collected from all three washes was combined and redistilled under vacuum at 40°C leaving an oily residue containing thaxtomin and other unidentified products. This crude extract has been stored at -80°C prior to further purification using Thin Layer Chromatography (TLC).

Thaxtomin Purification
The crude extract was purified using a two stage thin layer chromatograph technique, modified to maximise yield of purified thaxtomin A.
Glass TLC plates were coated with Sigma type GF silica gel to a thickness of 1.1mm using a Shandon Plate spreader. After drying, the plates were activated by heating to 110°C for three hours then cooled in a desiccator. The plates were then heavily loaded with a band of crude extract and developed in 9:1 chloroform: methanol for about 70 minutes. The resulting Thaxtomin A band (Fig 3), found at an Rf value of approximately 0.3, was scraped from the plate and extracted from the silica gel with acetone. After drying down the thaxtomin from several plates was combined and re-applied to a fresh TLC plate and redeveloped under the same conditions. The resultant band is collected and re-extracted in acetone, dried down and redissolved in chloroform.

The final stage of purification was by re-crystallisation from chloroform. The final extract was dissolved in minimal volumes of chloroform and stored for several days at -20°C. At this temperature, thaxtomin crystallises from chloroform in a highly pure form. The resulting crystals were filtered from the chloroform at low temperature, washed with cold chloroform and dried.

Induction of thaxtomin production
While sufficient quantities of highly pure thaxtomin have been generated by this procedure, yields of thaxtomin were variable between extraction batches despite careful control over production systems. It was not clear as to what factors were responsible for this variability. It appeared that the brand and batch of oatmeal used in preparation of the oatmeal broth significantly altered thaxtomin yields, but variation between replicates of the same broth batches were also occasionally apparent.

Preliminary experiments were undertaken in an attempt to identify potential elicitors of thaxtomin production, in the expectation that use of such components would result in higher yields of thaxtomin. An understanding of elicitors of thaxtomin production would also provide useful information on how *Streptomyces* infections are initiated.

Incorporation of potato extracts has been found not to be beneficial to thaxtomin production in liquid media. (Beausejour *et al.,* 1999)

Extraction of fats from oatmeal

Suggestions that extracellular esterases may be involved in release of inducers from lipids (Schottel 1995) led to consideration whether the high proportion of lipids in oatmeal that might play a role in thaxtomin production. To test this hypothesis soluble fats were extracted from oatmeal with chloroform. Experimental broths were 1) standard oatmeal broth, 2) standard oatmeal broth + added fats, 3) oatmeal broth with fats extracted. Inoculum was added and thaxtomin cultured, extracted and purified as described previously. The relative thaxtomin content was assayed by TLC.

Auxin as an elicitor

Addition of the auxin IAA to growth media was assayed to test the hypothesis that the presence of auxin (structurally related to thaxtomin) could form part of the mechanism by which thaxtomin production is induced by the organism. Since auxin is one of the products that is produced as a wound response and is found in low concentrations on the developing epidermis at the time of initial *Streptomyces* infection which is believed to be during lenticel formation

Standard broth cultures were supplemented with either 1mg/L or 0.1 mg/L IAA and thaxtomin cultured, extracted, purified and assayed as before.

Results and Discussion

The techniques outlined have delivered milligrams of bright orange crystals of thaxtomin. The identity of the extracts has been confirmed using mass spectroscopy (Fig 4) and liquid chromatography have confirmed the identity of extract as being Thaxtomin A.

The recrystalisation step is a novel adaptation of extraction techniques used by other researchers working with this toxin. With this simple technique relative large quantities of very purity thaxtomin was obtained, exceeding that achieved by other researchers. Samples of up to 98% purity have been obtained which makes subsequent experiments using the high purity samples more significant. If low purity samples are used in experiments, there is always a possibility that the impurities may have as significant effect on plant growth as the target molecule.

The experiments assessing fat extracts and addition of IAA as possible elicitors of thaxtomin production in culture failed to show any difference (positive or negative) in thaxtomin yield compared to control treatments. The reasons behind variable yields between production batches remains unknown and may be an inherent factor of thaxtomin production, but sufficient product was obtained from successive extractions to more than meet our requirements.

During the course of this study, Agriculture Canada have begun to sell thaxtomin A extracts (primarily targeting potato breeding programs who may choose to use thaxtomin sprays as a common scab resistance selection tool amongst seedlings), but these are in a substantially less pure form than can be produced by the techniques in use

here. There may be a possibility that pure thaxtomin production in Australia could be marketed.

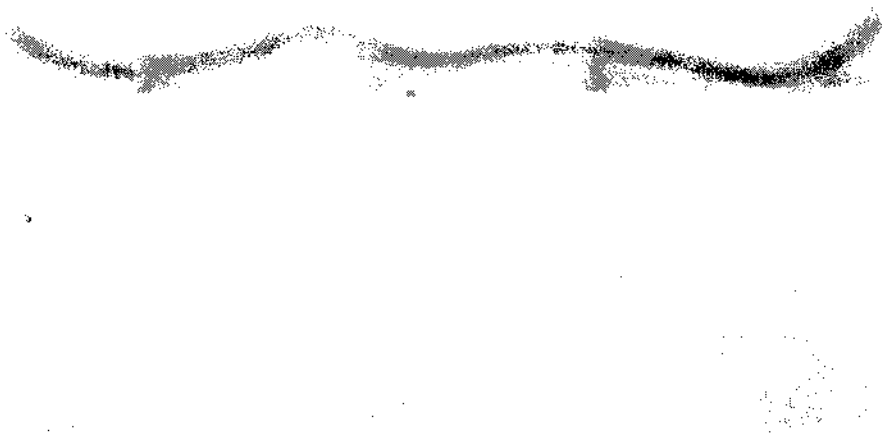


Fig 3: Thaxtomin purification using TLC.

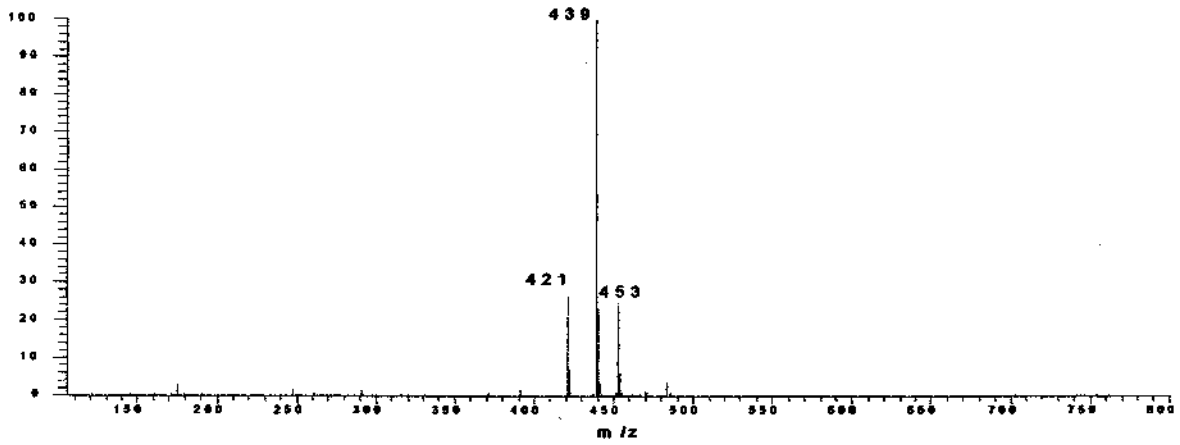


Fig 4: Mass spectrum of thaxtomin A, obtained by LC/MS
(Acknowledgment: N Davies, University of Tasmania)

GENERATION OF THAXTOMIN RESISTANT CELL LINES

Background

Following successful development of a production system for highly pure thaxtomin we were able to commence cell selection trials. Callus or protoplasts could have been used for this study. Callus cells were chosen as a suitable isolated cell type for selection as they are readily produced from potato tissues, and easier to handle.

Materials & Methods

Tissue cultured plantlets of commercial clones of Russet Burbank (Vancouver clone no. 5), and Shepody were obtained from Ms Linda Wilson, Department of Primary Industry, Water & Environment, Tasmania. The cultures were maintained *in vitro* on standard potato propagation media.

Initiation of potato callus and identification of callus culture techniques,
Callus cultures of both Russet Burbank and Shepody were initiated by placing 6 to 8 mm stem sections cut from 4 week old *in vitro* grown plants onto a 2,4D-free callus induction media and incubation in dark for five weeks.
The callus induction media used was developed to give good growth rates of callus, without the use of 2,4D, which is commonly used to initiate potato callus cultures. The use of 2,4D was avoided because of research that has indicated that 2,4 D, at low concentrations, can provide some protection from thaxtomin toxicity. Although a mechanism for this protective effect has not been identified, there is a possibility that 2,4 D might interfere with thaxtomin resistance if incorporated into the medium. The use of 2,4D in culture media has also been associated with changes in ploidy levels in callus culture, which could result in a higher percentage of "off type' regenerants.

Once a suitable callus induction medium was identified, small scale regeneration trials were conducted to test published shoot induction media. These tests resulted in small numbers of shoots being successfully regenerated from test callus. Further experimentation to test and refine callus culture media continued, using media from published reports and experimental observation. The resulting media (PCM5) has been used for all subsequent callus and cell selection experiments. The medium was found to be very suitable for cell selection studies as it gave rise to a soft callus that was readily dispersed, a trait required for efficient cell selection.

Mutagenesis Treatment
Pre-treatment of cell cultures with the mutagen ethyl methane sulphonate (EMS) was used to increase the probability of obtaining variant cell lines. EMS was selected as the most suitable mutagen for treatment of the cell cultures as it is believed to provide an high proportion of point mutations and a lower incidence of chromosome abnormalities (van Harten 1998). Callus cultures were exposed to concentrations of EMS up to 0.8% for three hours. Concentrations of EMS in excess of 1% were found to result in the death of cultures. After treatment with mutagen cultures were returned to callus medium for 24 to 48 hours to allow for recovery prior to exposure to thaxtomin

Toxin resistance selection
Following mutagenesis, callus cultures were dispersed by shaking in liquid media and transferred to a medium containing 2.25 mg/L thaxtomin. This thaxtomin concentration was determined from preliminary assays of unselected callus and potato leaf tissues, 2.25 mg/L thaxtomin was sufficient to demonstrate severe symptoms in organised tissues and is lethal to unselected callus cultures. Cell cultures took between 8 and 12 weeks for cell growth to become apparent in the presence of thaxtomin in the culture medium.

Thaxtomin tolerance studies
A series of selected lines were screened at elevated thaxtomin concentrations to determine strength of tolerance selected. Varying levels of the toxin, up to ten times the selection concentration, were incorporated into the callus growth medium. Control unselected lines were included in the study.

Results and Discussion

Mutagenesis and toxin resistance selection
The growth of callus that was not exposed to thaxtomin showed no obvious effect from the mutagen treatment suggesting limited evidence for undesirable gross phenotypic change (Fig. 5).
Initial growth of callus during the selection process was invariably very poor and very slow. This is most likely due to the large quantities of dead and dying callus cells which has a very negative effect on nearby live cells (through production and release of stress metabolites and decay of cells). At the initial screening stage any callus showing signs of life were picked off with forceps and transferred to fresh thaxtomin containing media. Further sub-culture on thaxtomin containing media was required to establish rapid growth and confirm thaxtomin tolerance. Approximately half of the initial selections were found not to grow at all. Others were subsequently found to have inadequate growth rates for further experimentation and were discarded. Thaxtomin tolerant cultures were isolated from nine separate selection experiments. A total of twenty cell lines are currently being maintained for regeneration.

Phenotypic description of selected lines and cell line bulking
Three distinct phenotypes were observed within the selected callus lines

1. Several cell lines (5 lines) showed a softer more friable callus than would be expected in unselected callus. These calli grew quickly on callus media which had facilitated rapid production of sufficient quantities callus for regeneration (and other) studies. However, some of these cell lines have proven difficult to handle during attempts at regeneration, due to sensitivity to abrupt changes in media resulting in tissue death. In some cell lines we believe that this is due to changes in osmotic potential of the media but in other cell lines it appears that there is sensitivity to changes in hormone concentration, particularly cytokinin. Sensitivity to changes in cytokinin levels presents a particular challenge, as this is central to the induction of shoots from callus. Differences in plant hormone sensitivity are yet to be quantified in all cell lines but may point to a physiological basis for the thaxtomin tolerance observed in some, but not all, cell lines.

2. One callus line was harder than normal and much slower in growth. Osmotic sensitivity did not appear to be prominent in this line.

3. The remaining 14 lines did not differ phenotypically from unselected callus

All cell lines have been successfully transferred to a thaxtomin free callus medium and are also maintained on a thaxtomin-containing medium.

Thaxtomin tolerance studies

All selected lines tolerated (and grew) on media with ten times the selection concentration of thaxtomin (i.e. 22.5 mg/L). The selection level (2.25 mg/L) exceeds that likely to be found in naturally infected potatoes, so this greatly enhanced tolerance is pleasing and suggests that extreme resistance to thaxtomin has been selected within these lines. The control callus died at the normal selection concentration.

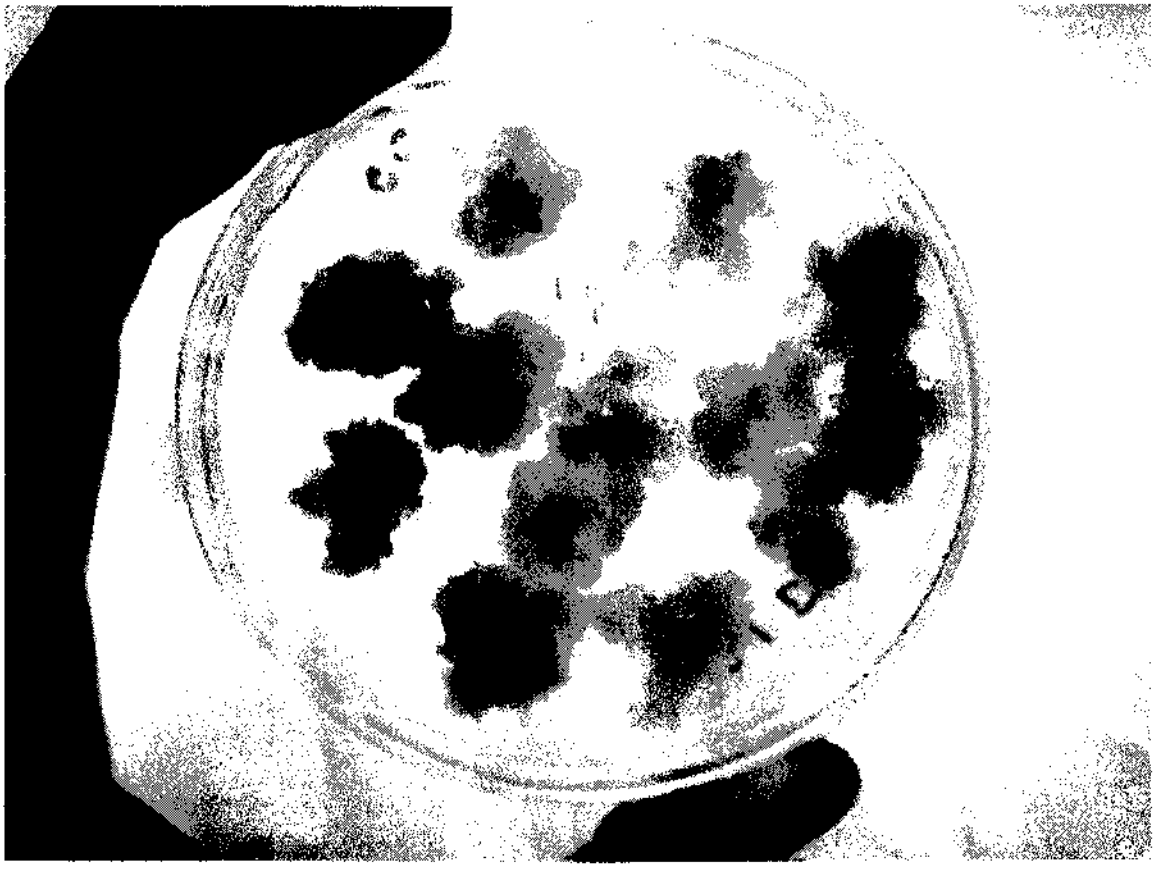


Fig 5: Callus selections on thaxtomin containing media

REGENERATION OF RESISTANT PLANTS

Background

Plant tissue culture techniques for potatoes are well established. Techniques for the regeneration of shoots from callus and suspension cultures have been published extensively using a variety of media and involving direct regeneration from callus and via somatic embryogenesis. (Shepard, 1977; de Garcia & Martinez, 1994; Curry & Cassells, 1999).

Materials and Methods

Regeneration - initial observations
Prior to the commencement of cell selection experiments, an initial test of a regeneration protocol yielded a small but satisfactory number of shoots from unselected callus. Although there have been subsequent changes to the callus media used it was not expected that these would materially affect the regeneration potential of the callus.

Throughout the regeneration experiments four distinct published media for potato callus regeneration have been used as a basis for the multiple experiments including numerous variations on these media.

Difficulties were encountered with some cell lines being highly intolerant of changes in media components, particularly plant hormones were following transfer to regeneration media the calli often died. This has significantly impeded progress in identifying the conditions required to regenerate whole plants.

Initial experiments were aimed at improvement of the survival of callus during transition of cultures from proliferation media to root initiation media.

Successful transfer to regeneration media
Some regeneration protocols (Shepard, 1977) use a short-term transitional medium before transfer of callus to a shoot induction medium. The use of this media was assessed to assist in transfer of healthy callus to regeneration media.
Other work focussed on careful handling and frequent transfers to fresh media.

Media supplement trials
During the assessment of the four different published shoot regeneration media, it became apparent that the media requirements vary substantially between individual cell selections. It appears necessary to use quite different media to achieve regeneration for groups of different cell lines, even though those cell lines have similar levels of thaxtomin tolerance.

A series of experiments were then undertaken to assess the effect of altering various media constituents and growth conditions for each of the cell lines. Experimental studies have concentrated on adapting known successful techniques for shoot

regeneration in potato callus. By their very nature, the experiments undertaken have generally been qualitative rather than quantitative.
Experimentation in this area has concentrated on four issues:

Optimal plant hormone regimes

1. **Cytokinin:** Zeatin is the most commonly used cytokinin for regeneration. Experiments looked at varying concentration of Zeatin in the media.

2. **BAP:** Kinetin is predominantly used in callus medium. Increased concentrations were trialed for effect on regeneration.

3. **Auxin:** NAA has been the predominant auxin used in regeneration studies. We have examined the effect of NAA at a range of concentrations.

4. **Gibberellin:** Addition of Gibberellic Acid (GA3) to media where it was not already contained has shown some benefits. Cell cultures have a greater tendency to become substantially greener and growth rates may improve marginally. Although there is variation in the concentration of GA3 used by other researchers

Organic media amendments.

1. **Adenine:** Addition of adenine to media is common and been trialed.

2. **Casein hydrolysate:** Casein hydrolysate has been included in many of the media.

3. **Vitamins:** The more complex vitamin mixture proposed by Shepard have been trialed.

Other culture conditions.

1. **Light:** Light intensity has been investigated as a possible influence on root initiation. Literature evidence is contradictory, some researchers have claimed that shoot initiation only occurs at low light intensity, others claim that high light intensity is required for shoot formation to occur.

2. **Agar:** A variety of types and brands of agar and agar substitutes have been used.

3. **Culture vessel seals:** Closure/sealing of Petrie dishes with Parafilm or polyethylene film (cling wrap) has also been investigated for the possible effects on shoot regeneration. No obvious differences have been detected between cultures maintained in sealed Petrie dishes, or unsealed screw topped flasks.

Growth room environment
A subsequent failure of regeneration of unselected callus in later experiments despite previously success in initiation of shoots from unselected callus was perplexing resulting in considerable attention being devoted to growth conditions that might have changed since those initial experiments were undertaken.
The possibility of ethylene build up in the culture room (especially with the increased numbers of callus cultures and associated cell death during selection and transfer) or

some other inhibitory gas (e.g. refrigerant leakage) has been suspected. To investigate this duplicates of all cultures have been moved to alternative growth rooms in Tasmania and New Zealand. Also, ventilation within the existing growth room has been improved.

Results and discussion

Transfer of callus to shoot proliferation media

The use of transitional media as described by Shepard (1977) was not successful with the selected cell lines. Many of the cell lines were severely affected by transfer to this medium. Other cell lines have also been severely affected by transfer to other callus media such as that of Connor (appendix 2) and Curry & Cassells (1999).

These effects have to some extent been alleviated by ensuring that transfers occur more frequently and by using larger explants to transfer between media types, however there remain some cell lines that do survive or have very low survival during transfer between particular media combinations.

All cultures have no been successfully transferred to at least one of the four regeneration media.

Media supplement trials

1. **Cytokinin:** Higher concentrations of zeatin (above 2 mg/L) often resulted in death of cultures. Lower concentrations of zeatin (0.05 to 0.2 mg/L) did not damage cell cultures but did not result in any signs of regeneration. Most recent experiments again using moderately high concentrations of zeatin (2 mg/L) have showed much more promise, particularly with some of those cell cultures that have "greened up" indicating acclimatisation to regeneration media.

2. **BAP:** Increased concentrations of Kinetin have not been found to be beneficial in regeneration.

3. **Auxin:** The selected callus cultures do not appear to be as sensitive to variations in NAA as unselected callus

4. **Gibberellin:** Addition of Gibberellic Acid (GA3) to media where it was not already contained has shown some benefits. Cell cultures have a greater tendency to become substantially greener and growth rates may improve marginally. Although there is variation in the concentration of GA3 used by other researchers

Organic media amendments.

1. **Adenine:** Addition of adenine to media is common and has been adopted.

2. **Casein hydrolysate:** Casein hydrolysate is now included in many of the media but no specific benefits have been noted.

3. **Vitamins:** the more complex vitamin mixture as proposed by Shepard have been used, although no specific benefits have been observed.

Other culture conditions.

1. **Light:** To date no differences have been determined using the selected cultures, both high light intensity and low light intensity continue to be used for different media combinations

2. **Agar:** The agar brand nor agar substitutes did not appear to effect success of regeneration. Experiments looking at root initiation in liquid media (in absence of agar) were attempted, without success to date.

3. **Culture vessel seals:** Sealing of Petrie dishes with Parafilm or polyethylene film (cling wrap) has also not shown any differences in growth or regeneration capacity.

Summary of regeneration trials

Successful regeneration has not yet been achieved using standard media for thaxtomin tolerant cell lines. However significant progress toward this aim has been made. Cell lines have now all been successfully transferred onto regeneration media without adverse growth effects through careful manipulation of the transfer process, media and timing of transfers. Specific media combinations for each of the cell lines that result in the death of cultures have been identified. Notably, there were significant differences between different cell lines in media requirements.
The calli of all lines are all showing promising signs of differentiation prior to regeneration (including forming semi-structured lumps on callus, greening of callus etc).
Notably regeneration of unselected lines (that previously were successfully regenerated) has also failed in recent experiments over the last 6-12 months. This points to a fundamental problem in the regeneration media or growth room conditions being used.
New selections are continually being made (as younger callus has a greater regeneration efficiency) and it may be that now that likely specific media conditions are known that these younger calli can be habituated onto regeneration media faster.
That in recent experiments control (unselected) callus has also failed to regenerate has led us to believe that the current limitation may not be a direct consequence of the mutation and selection process but rather some fundamental media or growth room environmental factor.
To overcome this, duplicates of all cultures have been shipped to Prof. Conner (project partner chief Investigator) in New Zealand. Given that his laboratory routinely regenerates potato callus, one can assume that if it is a growth room effect inhibiting regeneration, this should be overcome in Prof Conner's laboratory. These cultures will require a further 3-4 months before success of regeneration can be ascertained.

Regeneration is a time dependant process, and each experiment requires several months to determine outcomes. The requirement for extensive media studies has slowed progress toward the desired outcome of fully regenerated plants, however, we feel confident that the progress made and state of current cultures (growth dynamics and structures including "greening" and semi-differentiation) and the multi-site trials should result in successful regeneration of toxin resistant plants within 3-4 months.

Fig 6: Greening callus on regeneration media with semi-differentiated lumps

EFFECT OF AUXINS ON COMMON SCAB DISEASE AND TOXICITY OF THAXTOMIN

Background

During the callus selection experiments it was noted that the toxic effect of thaxtomin was ameliorated significantly if auxin was incorporated into the media. This was an interesting an potentially important finding prompting further work to confirm this linkage. Of particular note is that during the 1980's British workers began a series of experiments using foliar applied auxins and auxin-analogues for common scab control (McIntosh *et al.*, 1981, 1985, 1988). Di-substituted phenoxyacetic acids, such as 2,4 dichloro-phenoxyacetic acid (2,4 D) and some di substituted benzoic acids which are structurally similar produce significant decreases in damage both in pot trials and in field trials. The effects were measured using concentrations of 2,4 D below those that cause any damage for the auxin activity of 2,4 D (which is a potent auxin).

The mode of action of this effect has not been determined. Our postulate is that perhaps resistance to the disease through foliar application may be linked to an increased tolerance to thaxtomin during the critical infection period. Thaxtomin is believed to cause disruption of the cytoskeleton of the dividing potato cells but precise details of its mode of action are as yet undetermined. It is possible that the effect of thaxtomin A on cell cytoskeleton is inhibited by the auxin-like activity of the dichloro-phenoxyacetic acids and structural analogues. A number of small-scale experiments were initiated to investigate this.

Materials and Methods

Demonstration and confirmation of toxicity amelioration with auxin.

A series of experiment were conducted with model plant systems to analyse the interactive effects of auxins and thaxtomin.

Experiment 1:
Arabidopsis seed (cv. Colombia) were surfaced sterilised and plated onto the surface of a series of plates (Arabidopsis basal media) containing a varying levels of thaxtomin (0, 0.01, 0.1, 0.25 and 1.0 μM) and auxin (both IAA and NAA assessed at 0, 0.01, 0.1, and 1.0 μM). Plates were incubated at 25°C under light. Seedlings were observed for growth rate, expression of symptoms of thaxtomin toxicity (death, stunting, chlorosis, and hypertrophy).

Experiment 2:
Arabidopsis seedlings grown for 1 week on thaxtomin-free media were transferred to media containing 0, 0.01, 0.1, 0.25 and 1.0 μM thaxtomin. Plates were incubated at 25°C under light and subsequent growth observed.

Experiment 3:
The second experiment was repeated but this time using decapitated seedlings to reduce endogenous auxin levels

Experiment 4:
Tobacco protoplasts were cultured in liquid media containing thaxtomin (0.1 μM) either with or without auxin (IAA at 1.0 μM). Samples of protoplast were taken at 24, 36 and 72 hours and observed for hypertrophy and necrosis symptoms associated with thaxtomin toxicity.

Glasshouse treatment of plants with substituted auxin analogues

Tubers of cv. Shepody were planted in 20 cm pots in a sand/loam potting mix amended with 20 g inoculum of pathogenic strain G32. Inoculum was produced following the method of (Labruyère, 1971). Single foliar applications of di-substituted benzoic acid (DBA; at 1.6mM rate) or 2,4D (0.9mM rate) was applied to 10 pots of potatoes at tuber initiation (as determined by examination of developing stolons for hooking stage). 10 pots were left untreated as controls. Potato were grown to senescence whereupon all tubers were harvested and assessed for common scab disease following the visual keys of Bjor & Roer, (1980).

Interactive effect of thaxtomin and di-substituted benzoic acid (DBA) - a weak auxin-analogue

Cultures of thaxtomin resistant callus and control unselected callus were grown on media containing DBA at a range on concentrations, in the presence or absence of 2.25 mg/L thaxtomin.
Growth rate of callus (measured by successive weighing of callus cultures) was determined. Comparisons of the curves generated were used for determination of any interactions.

Results and Discussion

Demonstration and confirmation of toxicity amelioration with auxin.

Experiment 1:
The Arabidopsis seeds germinated on all media but seedlings were quickly killed in presence of 1uM thaxtomin only. Inclusion of 1 μM auxin in this media protected seedlings allowing some limited growth.
At 0.1 μM concentration thaxtomin induced extensive hypertrophy and stunting when present alone, but with equimolar auxin seedlings showed reduced hypertrophy, and less chlorosis.
At 0.01 μM thaxtomin symptom expression was minimal but a similar ameliorating effect with auxin was suggested.

Experiment 2:
When introduced to thaxtomin containing media, the Arabidopsis seedlings slowed growth. One interesting observation is that when the seedlings were transferred to a media with a moderately high level of thaxtomin (0.25 μM), subsequent root development slowed with a proliferation of terminal root hairs, symptoms very similar to what one would expect if an auxin transport inhibiting material (e.g. NPA; Schiavone *et. al.* 1987) was applied.

Experiment 3:
Thaxtomin induced hypertrophy and chlorosis was more evident in decapitated seedlings than those in experiment 2 presumably due to lack of endogenous auxin. A clear effect of added auxin on expression of these symptoms was found (Table 1).

Experiment 4:
As with the Arabidopsis experiment, auxin reduced expression of thaxtomin induced symptoms in tobacco protoplasts.

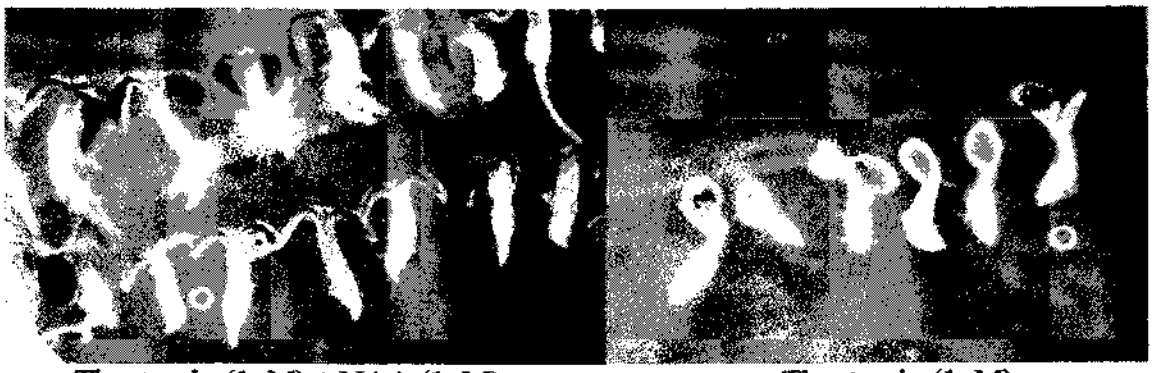


Fig 7. Amelioration of thaxtomin toxicity with auxin

Table 1: Effect of auxin on induced chlorosis and hypertrophy of sublethal level of thaxtomin (0.1 μM) on decapitated Arabidopsis seedlings (Colombia).

	Chlorosis (%)	Cell hypertrophy (%)
Control	0	0
Thax. only	94	94
Thax. + IAA (0.01μM)	88	94
Thax + IAA (0.1μM)	50	69
Thax. + IAA (1.0μM)	20	53
IAA only (all conc.s)	0	0

Table 2: Effect of auxin (0.1 μM) on thaxtomin (1.0 μM) toxicity in tobacco cell cultures after 72 hours growth.
(Measures estimated from microscope counts).

	Cell necrosis (%)	Cell hypertrophy (%)
Thax. only	50-70	50-70
Thax + IAA	< 20	< 10
IAA only	0	0

Fig 8. Effect of thaxtomin on mature Arabidopsis root tip growth
(shares similarities with symptoms of auxin transport inhibitor treatment)

Summary of these experiments

These studies have clearly shown that the presence of exogenous auxin inhibits the toxicity of thaxtomin in an apparent dosage dependant manner (e.g. Tables 1 & 2). This was shown with cultured tobacco cells and Arabidopsis seedlings where chlorosis and cell death rates at elevated thaxtomin concentrations, and cell hypertrophy and growth retardation at sublethal concentrations were significantly diminished (e.g. Tables 1 & 2).

There was also an indication that endogenous source of auxin also alleviate thaxtomin toxicity in comparisons of results from experiments using whole and decapitated seedlings. Thaxtomin induced toxicity was much greater in decapitated seedlings where endogenous sources of auxin were diminished.

The mode of auxin induced amelioration of thaxtomin toxicity is not clear and may be the result of indirect activities.

Glasshouse treatment of plants with substituted auxin analogues

In the pot trial we were unable to show the same level of disease control with 2,4D that McIntosh had observed previously (using same rate etc). DBA (the weaker auxin) also failed to show an obvious effect on disease incidence. It is unclear as to why there may be a discrepancy between our results and those of McIntosh, but perhaps timing of application of the material may have varied, as determination of critical infection period can be difficult.

Interactive effect of thaxtomin and di-substituted benzoic acid (DBA) - a weak auxin-analogue

This *in vitro* trial gave interesting further indications of the interactions between auxins and auxin-like compounds and thaxtomin.
The results of the trial are presented graphically in Fig 9.

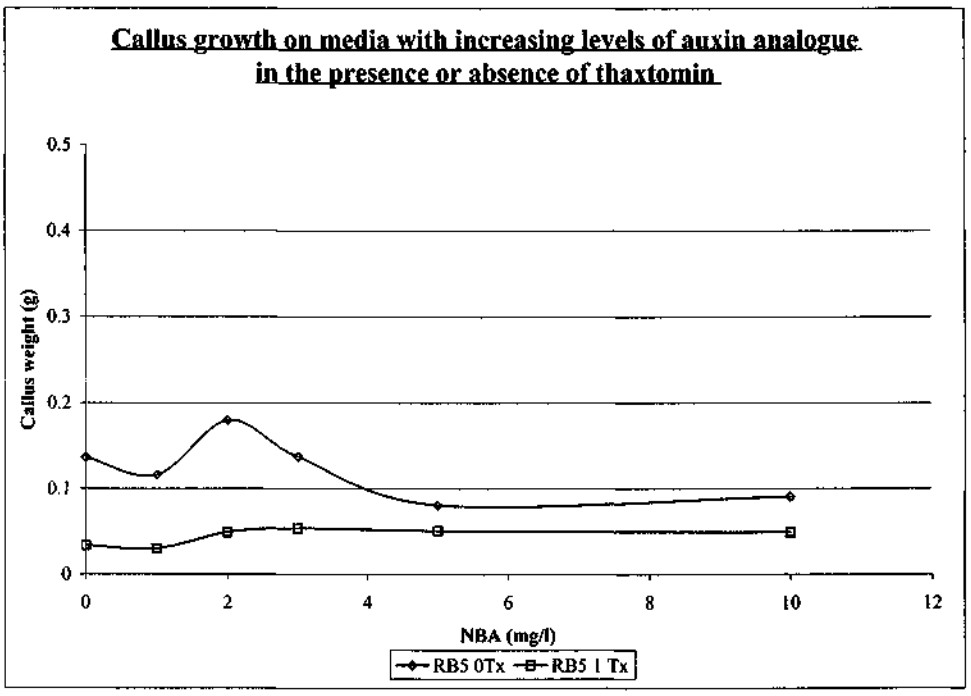
In the toxin sensitive (unselected) line (a):

- DBA was shown to provide weak auxinic activity (as expected from this weak auxin analogue) as demonstrated by the small peak in growth at 2 mg/L NBA in absence of thaxtomin. Greater concentration slightly reduced growth but had less detrimental effect than one would expect from a more potent auxin (like IAA or 2,4D).
- This auxinic activity was not evident when thaxtomin was present in the media. This is the first evidence to suggest that thaxtomin has the capacity to inhibit auxin activity (as well as auxin ameliorating thaxtomin toxicity as previously shown).
- Presence of thaxtomin in the media affected growth of the unselected lines (as would have been expected). Increasing DBA concentration did amelerioate this reduction in growth (as seen by comparing difference in weight at 0 mg/L DBA and above 5 mg/L DBA).

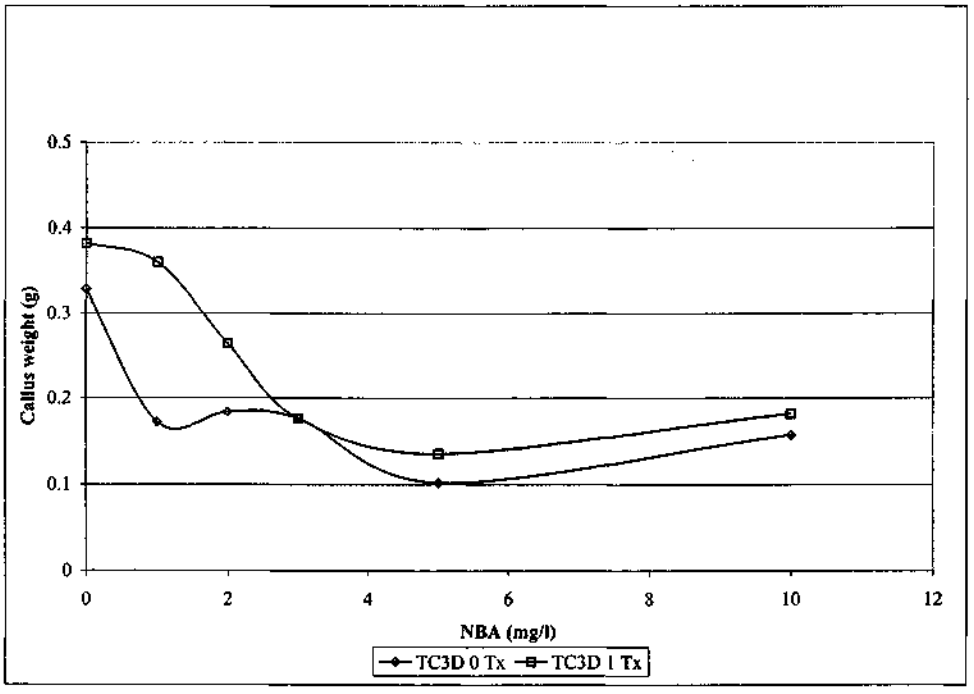
In the toxin tolerant line (b):

- DBA again showed a weak auxinic activity as demonstrated by the small peak in growth at 3 mg/L NBA in absence of thaxtomin.
- Incorporation of thaxtomin in the media again removed this small auxinic activity.
- There was no real evidence for toxicity of thaxtomin in this line (as expected), and thus the effect of thaxtomin on auxinic peaks is interesting suggesting the interaction may occur independently of the mechanism for thaxtomin tolerance

Fig. 9. Callus growth on media with increasing levels of auxin analogue DBA in the presence or absence of thaxtomin (2.25 mg/L)



a) Unselected callus



b) Thaxtomin tolerant callus

VARIABILITY OF PATHOGENIC STRAINS OF *STREPTOMYCES* IN TASMANIA

Background

We undertook a brief study of the variability of pathogenic strains of *Streptomyces* present in Tasmanian soils. This is important for several reasons:

a) We needed to ascertain whether all pathogenic strains produced thaxtomin (and thus whether the resistance offered by the toxin tolerant plants was likely to be robust).
b) We wanted to determine whether there was presence of novel Australian strains that may assist in explaining the alarming level of disease we find in Tasmania (and more recently Victoria).
c) We want to examine the possibility of using specific markers for detection of pathogenic strains of *Streptomyces*.

Materials and Methods

We have a moderate collection of Tasmanian *Streptomyces* isolates from diseased potatoes during the past decade. In this study these isolates were characterised phenotypically and genetically.

1) Phenotypic characterisation of strains

Methods for characterisation of the strains generally followed that of Lambert and Loria (1989) and included assessment of morphology, growth on standard sole carbon and nitrogen sources and in presence of inhibitory substances.

2) Genetic analysis

DNA was extracted from a selection of strains from representative *Streptomyces* sp. groups as determined by phenotypic characterisation (Healy & Lambert, 1991), and amplification of a portion of the 16s rRNA gene done by PCR (Takeuchi *et al.*, 1996). Amplicons were sequenced and compared with other *Streptomyces* sp. derived from the GenBank database using CLUSTAL analysis. Genetic relatedness was visualised in a taxonomic tree (Fig 10).

Pathogenicity tests

Pathogenicity of selected strains was confirmed by *in vitro* and *in vivo* pathogenicity assays. Initially all strains were screened using a potato disk assay (Loria *et al.*, 1995). A selection of strains was re-examined using radish seedling (Leiner *et al.*, 1996), and minituber assays (Fry pers. comm.). These assays all rely on detection of toxicity induced by thaxtomin production and may give false positive results if other phytotoxic metabolites are produced by test strains. To overcome this *in vivo* pathogenicity of smaller selection of strains was also assessed on potato plants in a glasshouse trial (Labruyère, 1971).

Thaxtomin assays

Thaxtomin production was determined from representatives of pathogenic species by following the thaxtomin production and purification protocol and assaying by TLC.

Results and Discussion

Strain Characteristics

Most of 115 strains characterised could be sorted into 7 major groups based on morphological, physiological and 16s data characteristics (Fig 10). The majority were identified as *Streptomyces* species, with the exception of three strains producing globular sporangia, identified as *Streptosporangium* sp.

There were four distinct groups of pathogenic strains described including novel species.

Group 1 Strains in this group (14 strains) are closest to *S. scabies*. Most were pathogenic in both *in vitro* and *in vivo* assays (this group includes strain G32).

Group 2 This group (25 strains including pathogenic isolates) showed morphological characteristics similar to *S. scabies* (Lambert and Loria, 1989a) but were genetically quite distinct and represent a novel pathogenic species. Some showed pathogenic characteristics in both *in vitro* and *in vivo* assays and produced thaxtomin.

Group 3 There was one pathogenic strain (as determined from *in vivo* assay and thaxtomin production) that clustered with the pathogen *S turgidiscabies,* but is morphologically distinct from this species and represents a second novel pathogenic group.

Group 4 There were two putative pathogenic strains (*in vivo* results pended retest) which clustered with the putative pathogen *S. caviscabies* isolated from deep-pitted lesions in Canada. The thaxtomin assay was positive.

Group 5 Characteristics of members of this group (29 strains) most closely fitted the probabilistic description of *S. halstedii* Williams *et al.* (1983a) and were non-pathogenic in all assays.

Group 6 This group (11 strains) fitted the description of *S. violaceusniger* (Williams *et al.* 1983a). All *S. violaceusniger* strains gave strong pathogenic reactions in *in vitro* assays but failed to show pathogenicity in *in vivo* assay. They did not produce thaxtomin (the phytotoxic compounds responsible for the *in vitro* reactions were subsequently identified as nigricin and geldanamycin by GCMA analysis). Thus this group are not common scab pathogens.

Group 7 consisted of three *Streptosporangium* strains. They were non-pathogenic in all assays.

Other strains were non-pathogenic and did not clearly fit into any of these other groups.

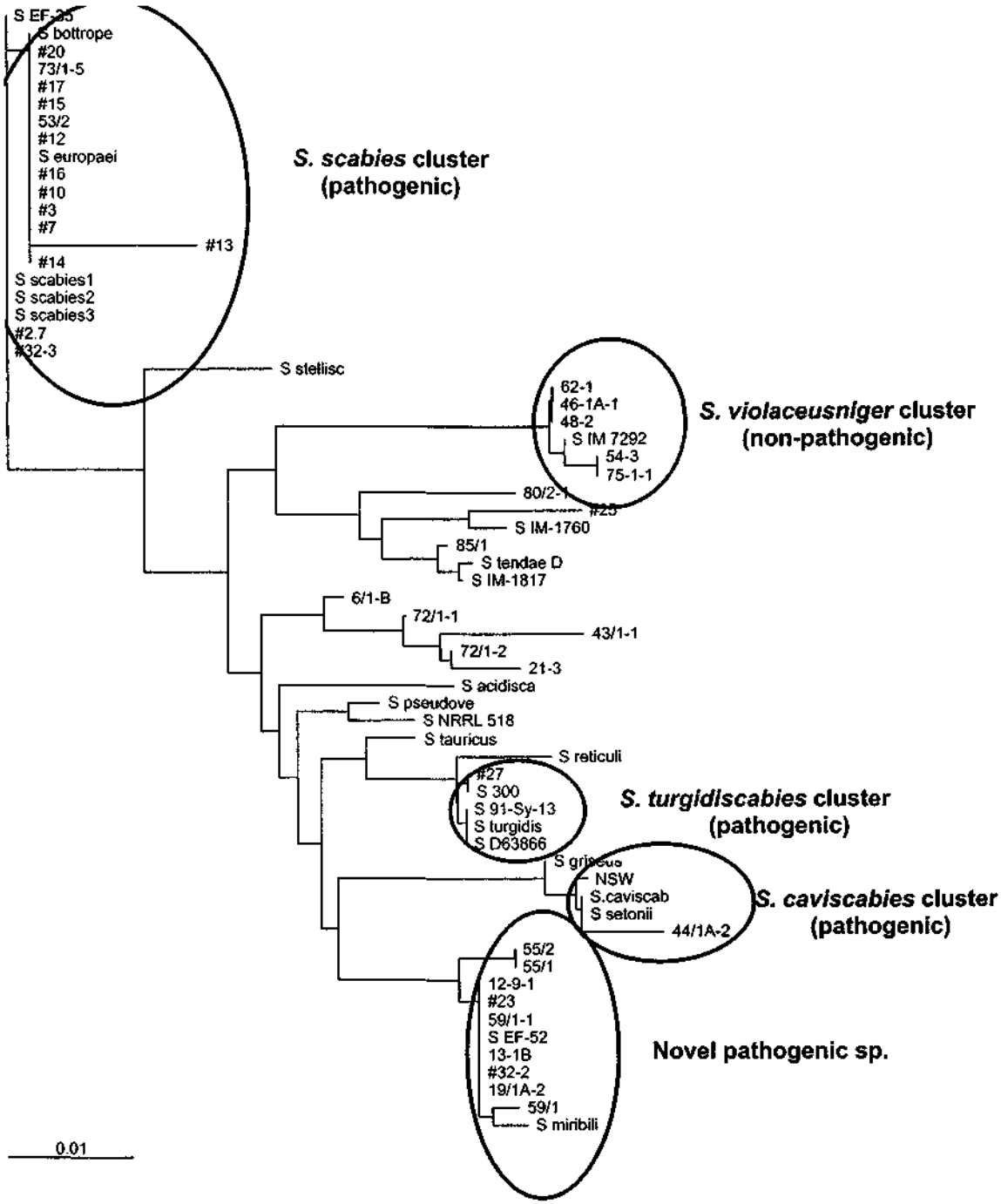


Fig. 10 Phylogenic tree of Tasmanian *Streptomyces* sp. isolated from potatoes derived from analysis of ribosomal 16s sequences
(representative sequences from characterised species have been included)

NATURAL VARIABILITY WITHIN RUSSET BURBANK CLONES

Background

In a field trial assessing a variety of processing cultivars and clones apparent differences in susceptibility of common scab disease within clones of Russet Burbank was suggested (L.R. Ransom, AQIS, Canberra, pers. comm). Commercial production in Tasmania utilises five clonal units of Russet Burbank originally derived from the clone Vancouver. These Vancouver units are used interchangeably with apparently little difference in agronomic performance, although some physiological differences have been noted (B. Beattie, TIAR, pers. comm.). However, the relative susceptibility of these units and other Russet Burbank clones to important diseases like common scab has not been determined. Indeed susceptibility of locally used clonal lines may represent one of the factors increasing local risk.

Materials and Methods

A selection of Russet Burbank clonal variants were assessed for disease tolerance under controlled glasshouse conditions. In the first trial 13 Russet Burbank clones and three cultivars (Russet Norkotah, Russet Nugget, Shepody) were assessed. These clones included the five used commercially in Tasmania. Planting material was minitubers sourced from glasshouse grown tissue cultured plants to guarantee freedom from tuber contamination with the pathogen. Five or six tubers of each cultivar were planted into sterilised potting mix (mix of peat moss, sand and composted pine bark with a slow release fertiliser (Osmocote, The Scotts Company, USA)) amended with (*c.* 10% v/v) inoculum of pathogenic *Streptomyces* sp. (isolate G32) produced on vermiculite following the method of Loria & Davis (1988). A further two pots of each cultivar were planted into non-pathogen amended soil as control treatments. Irrigation was supplied daily from overhead sprinklers and no pesticides were used. Plants were grown to senescence whereupon all tubers within each pot were harvested and assessed using modified disease rating scales of Bjor & Roer (1980) for disease incidence (0 = no visible disease, 1 = <5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50-70%, 6 = >70% tuber surface affected) and severity, (0 = no disease, 1 = superficial lesions < 1mm deep, 2 = typical star cracked lesions of 1-3 mm deep, 3 = deep pitted lesions > 3 mm deep). Treatment data were compared by REML variance components analysis (using GENSTAT) to allow for the unbalanced data set following confirmation of adherence to the assumptions inherent with this analysis and a least significant difference between means determined.

The same cultivars/clones were used in trial 2, with the omission of Shepody. Tubers were sourced from the disease-free control treatments of the previous trial with six replicates of each treatment used. Disease assessments were as before. Data then compared by analysis of variance (using GENSTAT) again following confirmation of adherence to the appropriate assumptions and least significant difference between means calculated.

Results and Discussion

Significant variability between cultivars and within the Russet Burbank clones in both disease incidence (Fig.1) and severity (Fig. 2) were found in both glasshouse trials. Differences in disease indexes were generally greater between the different cultivars than within the Russet Burbank clonal lines. In neither trial was infection noted within control treatments.

In trial 1, three overlapping cluster groups were shown for disease incidence and five for disease severity (Figs. 1 & 2). Cv. Russet Norkotah (4.41) had significantly higher disease incidence than Russet Burbank clones Vancouver unit 5 (2.74), Manitoba (2.64), unit 2 (2.32), and cvs. Shepody (2.18) and Russet Nugget (1.47). Furthermore Russet Nugget had a significantly lower disease incidence index than clones unit 1 (3.71), unit 4 (3.68), Ruen (3.50), and Victoria (3.27; Fig. 1). Lesion severity of Russet Norkotah (2.59) was significantly greater than clones Regular (1.83), unit 1 (1.81), Ruen (1.81), Idaho (1.78), unit 5 (1.73), British Colombia (1.69), Manitoba (1.44), unit 2 (1.25), and cv. Russet Nugget (1.36). Cv. Shepody (2.41) showed significantly greater disease severity than British Colombia, Manitoba, unit 2, and cv. Russet Nugget. Unit 4 (2.15) disease severity was significantly greater than Manitoba, unit 2, and cv. Russet Nugget, and clones Victoria (2.01), and Starks (2.00) showed significantly greater disease severity than unit 2 (Fig. 2).

In trial 2, four overlapping cluster groups were shown for disease incidence and four for disease severity (Figs. 1 & 2). Clone unit 4 (3.70) had significantly greater disease incidence than clones unit 1 (2.70), British Colombia (2.49), and cv. Russet Nugget (1.1). Furthermore British Colombia had significantly lower disease incidence index than cv. Russet Norkotah (3.59) and clone unit 5 (3.55), while cv. Russet Nugget had significantly lower disease incidence than all tested clones and cvs (Fig. 1). Disease severity of clones Starks (2.16) and Manitoba (2.04) was significantly greater than clones unit 4 (1.60), Victoria (1.59), British Colombia (1.39), and cv. Russet Nugget (1.29). Cv. Russet Nugget and clone British Colombia had significantly lower disease severity index than clones unit 1 (1.98), Luther (1.89), unit 2 (1.88), Ruen (1.88) and unit 5 (1.87). Furthermore cv. Russet Nugget showed lower disease severity than clones Regular (1.81), unit 3 (1.80), Idaho (1.80), and cv. Russet Norkotah (1.78; Fig. 2).

Comparing results across the two trials for the disease incidence index, cv. Russet Norkotah and clone unit 4 consistently grouped within the cluster showing greatest disease incidence (and outside the cluster with least disease incidence), and cvs. Shepody (one trial only) and Russet Nugget consistently grouped within the cluster of least disease incidence. Clonal units 1, 2, and 5 showed inconsistent results across the trials. For the severity index cvs. Russet Norkotah, Shepody (one trial only) and clone Starks were consistently found within the cluster showing the greatest disease severity while cv. Russet Nugget and clone British Columbia consistently grouped within the cluster of least disease severity. Variable results were showed for clones Victoria, Manitoba, and units 2 and 4.

Strain selection for desirable characters within potato varieties have been successfully used for traits such as skin colour, and vigour and yield (Miller *et al.*, 1999). There exist several clonal selections of Russet Burbank that perform differently under varying growth conditions. Cultivars are commonly ranked for disease resistance as a guide to

producers (eg Anon 1990) but presence of clonal variants within cultivars, may require additional comparisons to confirm results. The clone Vancouver was shown to have suitable characteristics for production and French fry processing under Tasmanian conditions (B.M. Beattie, Personal Communication). From this clone five subclones (units) were derived and maintained as nuclear stock to support the certification scheme. Over time occasionally small physiological changes have been observed within these units (e.g. increased tendency for hairy root production in storage) which if perceived as detrimental have meant that the unit was culled from the nuclear stock and a replacement unit created from one of the remaining four. Notably for the Tasmanian industry, there were some differences shown between the five Vancouver units; unit 4 always rating in the most susceptible cluster group for disease incidence and showing significantly greater disease severity than unit 2 (trial 1 only) and significantly greater incidence than unit 1 (trial 2 only). However, the extent of increased disease never exceeded 1.75 times the best performing of the other four clonal units. Whether this has had a significant impact on the severity of common scab disease in Tasmania under field production is unclear, but with no agronomic benefits over other commercial clones, it may pay for Vancouver unit 4 to be omitted from Tasmanian production. Given that unit 2 averaged the lowest mean disease score (both indexes) across both trials perhaps the use of this clone in preference to others should be encouraged.

While it has been generally shown that surface area affected and lesion severity are correlated, and thus a combined disease index often used (eg Leach *et al.,* 1938; Bjor & Roer, 1980; Wilson *et al.,* 1999), other work has shown that separate analysis of these factors may be more reliable for cultivar resistance assessment (Goth *et al.,* 1993). Results from the current study also highlighted a few variant outcomes where relative resistance to disease incidence and severity were not necessarily similar (eg. cv. Shepody). Furthermore, for the French fry processing market, the incidence of common scab disease is generally not as critical as the severity, as superficially lesions are removed during the peeling process, but deep lesions remain causing defects in fries. In contrast both incidence and severity are important for the fresh market, where appearance of tubers is the key factor compromised by common scab infection. Therefore separate analysis of incidence and severity scores as used here may be useful when considering specific product use.

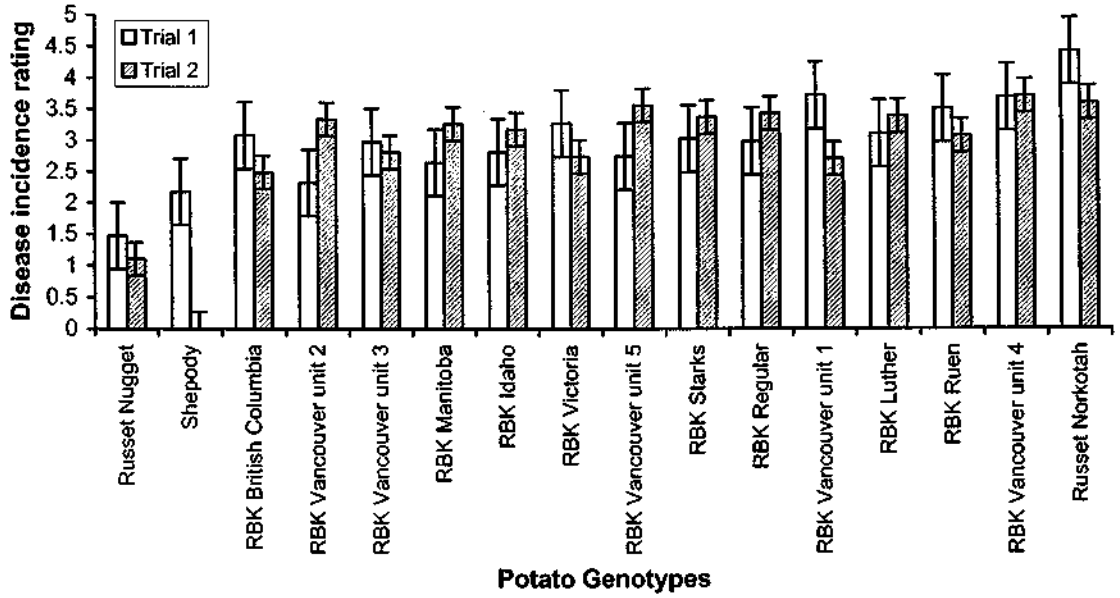


Fig. 11. Glasshouse comparison of common scab disease incidence of selected French fry processing potato cultivars and clones of Russet Burbank.
Those lines prefixed with RBK are clones of cv. Russet Burbank. Bars indicate L.S.D. values (P=0.05). Cv. Shepody was not assessed in trial 2.

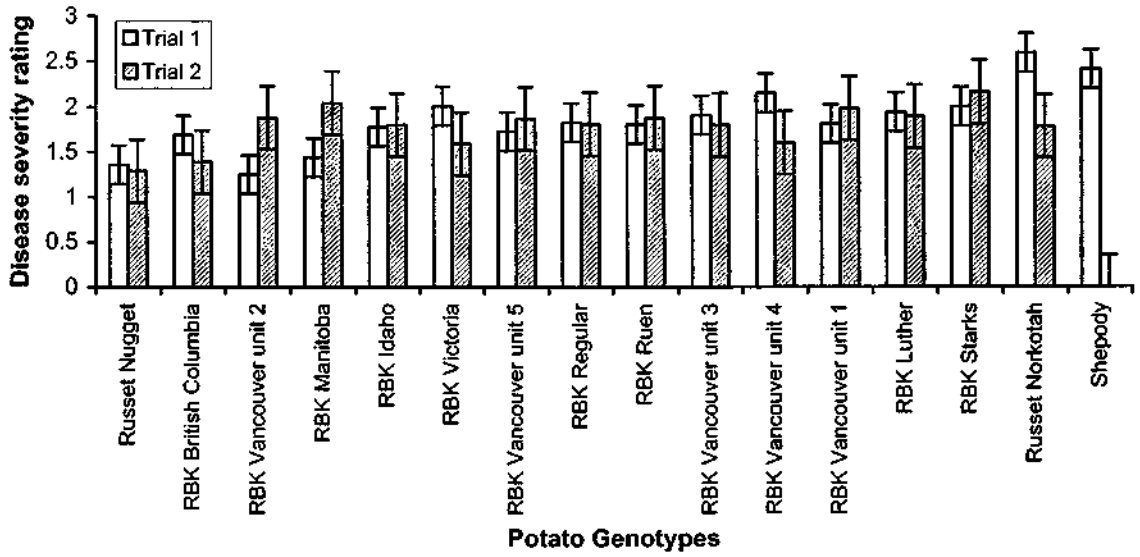


Fig. 12. Glasshouse comparison of common scab disease severity of selected French fry processing potato cultivars and clones of Russet Burbank.

TECHNOLOGY TRANSFER ACTIVITY

The project by its nature was laboratory based leading to preliminary outcomes of significance to the potato industry.

During the course of the project we have regularly conveyed the progress and outcomes of the project and related studies on common scab disease research in Tasmania through industry publications, workshops and radio broadcasts.

We have also been in regular contact on a one-to-one basis with local and Interstate potato growers and other industry personnel who have showed a keen interest in the progress and outcomes of the project.

A summary of this activity is given below.

Industry Meetings & Workshops

Presentation at the TIAR annual research presentation day, Launceston, 1998

"Development of extreme resistance (immunity) to common scab disease within commercial potato cultivars". Paper presented at the Tasmanian Potato Industry Research (ARAC) Presentation Day, 27th July, 1999, Ulverstone.

Presentation at the TIAR annual; research presentation day, Launceston, 1999

"Resistance to common scab of potato - progress toward a long term solution". Paper (poster & full abstract) presented at the Australian Potato Research, Development and Technology Transfer Conference, 31 July – 3 August, 2000, Adelaide, Australia.

"Development of extreme resistance (immunity) to common scab disease within commercial potato cultivars". Paper presented at the Tasmanian Potato Industry Research (ARAC) Presentation Day, 10th August, 2000, Ulverstone.

Presentation at the TIAR annual; research presentation day, Launceston, 2000

"Development of extreme resistance (immunity) to common scab disease within current commercial potato cultivars". Paper presented at the Tasmanian Potato Industry Research (ARAC) Presentation Day, 15th August, 2001, Devonport.

"Common scab disease of potato - current and future options for control". Paper presented at the Seed Potatoes Victoria Industry workshop, Warrugul Victoria, 21 August 2001.

"Common scab disease of potato". Paper presented at the Central Highlands Integrated Production Systems, Industry seminars, Ballarat Victoria, 22 August 2001.

Industry Publications & Media releases

"Protecting the humble spud". New article presented in University news, 1999.

"Breakthrough in potato Research". Media release May 11 2000 announcing successful completion of generation of toxin resistant callus. Media release covered by major Tasmanian newpapers, and television.

"Common scab resistance in processing potatoes is possible". Article published in Potato Australia, Sept. 2000, vol 11: 20.

"Development of extreme resistance to common scab". Article published in Potato Australia, Sept 2001, vol 12: 28.

We have also conducted three presentations on the "Country hour" on ABC radio outlining project progress.

Scientific Publications

Refereed journal articles:

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: 13-18.

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: (in press).

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: 625-628.

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: (in press).

Conference presentations:

Wilson (1998). "Common scab disease of potato: causal agent and disease management" *Microbiology Australia* 19: A47
(Oral paper presented at the Actinomycete workshop of the Australian Society for Microbiology, Annual Scientific Meeting and Exhibition. 27 September - 2 October, 1998, Hobart, Australia.)

Lacey MJ & Wilson CR (2000). "Actinomycetes associated with common scab lesions of potatoes in Tasmania." *Phytopathology* 90: S44.
(Poster presented at American Phytopathology Society Annual Meeting, 12th-16th August, New Orleans, USA)

Luckman GA & Wilson CR (2000). "Induction of resistance to common scab in potato by selection for tolerance to thaxtomin A." *Phytopathology* 90: S48. (Poster presented at American Phytopathology Society Annual Meeting, 12th-16th August, New Orleans, USA)

RECOMMENDATIONS FOR FUTURE RESEARCH

This project has successfully determined the feasibility of selection of toxin resistant cell lines of potato. This in turn now opens the door for development of commercially important common scab resistant potato clones.
Obviously resistance to the disease is not sufficient in its own right to warrant commercial acceptance, therefore further studies are required to evaluate the resistant clones produced for disease resistance, agronomic performance and product quality (processing and ware).

Therefore a new project has been developed and approved by Horticulture Australia Ltd to progress commercial development of these lines. This new project will focus on:

- Assessment of regenerated toxin tolerant plants for expression of resistance to common scab disease under controlled and field conditions
- Assessment of regenerated toxin tolerant plants for agronomic performance under field conditions
- Assessment of regenerated toxin tolerant plants for potato product quality (processing performance etc)
- Commercially valuable lines developed will protected through plant breeder's rights (PBR) and commercially developed for release to industry.
- Commercially valuable lines will be analysed physiologically and genetically to generate suitable markers for identification of clones and protection of PBR.
- Further research will also be undertaken on important but poorly understood facets of the disease, making use of the development of toxin resistant cell lines attempting to further understand the mechanism of the disease resistance and possible development of alternative methods of control for those varieties where resistance has not yet been developed.

In addition, work examining detection methodologies for pathogenic strains using defined molecular markers would be a useful adjunct, allowing evaluation of soil pathogen populations and giving estimates on likely disease risk. We will follow this work, in conjunction with researchers from the Scottish Crop Research Institute to evaluate potential in Australia.

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APPENDIX 1. CULTURE MEDIA

ISP2 medium for cultivation of *Streptomyces*

Malt extract	10 g
Yeast extract	4 g
Glucose	4 g
Agar	15 g
Water: make up to	1 L

Adjust pH to 7.4 and autoclave

Oatmeal Broth

Broth prepared by boiling 60 g of oatmeal in 2 litre of water for 12 minutes, strain the mixture through muslin, cool to room temperature add 3ml trace salts solution and make up to 3 litres with water. Adjust pH to 6.8 and autoclave.

Trace Salt solution

Fe SO4.7H2O	100 mg
MnCl2.4H2O	100 mg
ZnSO4.7H2O	100 mg
Water: make up to	100 ml

Arabidopsis basal media

Arabidopsis growth media	20ml
Phytoagar	0.8g
Water: make up to	100 ml

Table 3. *Media used for tissue culture*

	Potato Propagation	PCM5	PRM2	M2	M3	Connor Callus	Connor Regeneration
MS salts and vitamins	4.43	4.43 g	4.43 g	4.43 g	4.43 g	4.43 g	4.43 g
Thiamine		0.40 mg	0.40 mg				
Nicotinic acid		0.45 mg	0.45 mg				
Folic acid		0.50 mg	0.50 mg				
Biotin		0.05 mg	0.05 mg				
Myo-Inositol					100 mg		
Glutamine		200 mg	200 mg				
MES		500 mg	500 mg		1000 mg		
PVP		500 mg	500 mg				
Adenine		40 mg	40 mg				
Casein hydrolysate	500 m	100 mg	100 mg		1000 mg	500 mg	500 mg
Sucrose	30			10 g	3 g	5 g	5 g
Mannitol		20 g	20 g	40 g	36 g		
Glucose		20 g	20 g	10 g	10 g		
Ascorbic Acid	40 m					40 mg	40 mg
Zeatin					0.5 mg		1.0 mg
Kinetin		0.1 mg	0.2 mg		0.5 mg		
BAP			1 mg	0.1 mg		2.0 mg	
IAA					0.1 mg		
NAA		3 mg		0.2 mg		0.2 mg	0.2 mg
GA3		0.1 mg	0.1 mg	0.2 mg	0.2 mg	5.0 mg	5.0 mg

pH adjusted to 5.8 prior to autoclaving. GA3 and Zeatin were filter sterilised and added after autoclaving.