

Know-how for Horticulture™

### Cleaning and disinfection practices for potato farms

Dolf de Boer VIC Department of Primary Industries

Project Number: PT98018

#### PT98018

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the potato industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the potato industry.

All expressions of opinion are not to be regarded as expressing the opinion of Horticulture Australia Ltd or any authority of the Australian Government.

The Company and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this report and readers should rely upon their own enquiries in making decisions concerning their own interests.

ISBN 0 7341 06629 9

Published and distributed by: Horticultural Australia Ltd Level 1 50 Carrington Street Sydney NSW 2000 Telephone: (02) 8295 2300 Fax: (02) 8295 2399 E-Mail: horticulture@horticulture.com.au

© Copyright 2003



Horticulture Australia

# Cleaning and disinfestation practices for potato farms

Final Report Horticulture Australia Project PT98018

Rudolf de Boer *et al.* Department of Primary Industries Institute for Horticultural Development, Knoxfield, Victoria





Horticulture Australia

Horticulture Australia Project PT98018 – Cleaning and disinfestation practices for potato farms

Rudolf F de Boer Department of Primary Industries Institute for Horticultural Development, Knoxfield Private Bag 15 Ferntree Gully Delivery Centre, VIC 3156 Phone: 03 9210 9222 Fax: 03 9800 3561 Email: dolf.deboer@nre.vic.gov.au Website: www.nre.vic.gov.au/ihd

Project Team

Rudolf de Boer (Department of Primary Industries, Victoria) Jacky Edwards (Department of Primary Industries, Victoria) Ross Mann (Department of Primary Industries, Victoria) Nigel Crump (Department of Primary Industries, Victoria) Rajendra Gounder (Department of Primary Industries, Victoria) Prabhpreet Inder (Department of Primary Industries, Victoria)





#### **Purpose of project**

Potato growers, particularly seed potato growers, are under increasing pressure to improve potato tuber quality. The potato shed has been implicated as a source of disease in seed stocks and this has raised the issue of hygiene in the shed. The purpose of this project was to examine the risks of contaminating seed stocks with common potato pathogens in the potato shed, to evaluate disinfectant treatments and to develop hygiene protocols, incorporating cleaning and disinfection procedures for the potato shed. These protocols will be an important component of any integrated disease management strategy used on the potato farm.

#### Acknowledgments

The potato growers of Australia, Horticulture Australia and the Victorian Department of Primary Industries funded this project.

#### March 2003

Any recommendations contained in this publication do not necessarily represent current Horticulture Australia Policy. No person should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other comment, without first obtaining specific, independent professional advice in respect of the matters set out in the publication.

#### Contents

1	MEDIA SUMMARY	2
2	TECHNICAL SUMMARY	3
	TECHNICAL REPORT – CLEANING AND DISINFESTATION PRACTICES AUSTRALIAN POTATO FARMS	4
3.1	Background	4
3.2	<ul> <li>3.2.1.1 Evaluation of the shed dust as a potential source of inoculum</li> <li>3.2.1.2 Comparing disease risk in shed dust and field soil</li> <li>2.2 Conclusions</li> </ul>	6 6 10 11
	<ul> <li>B.2 Disinfectants for the potato farm</li> <li>Disinfectant database</li> <li>Testing disinfectants against potato pathogens</li> <li>Testing disinfectants against common fungal and bacterial potato pathogens in vitro</li> <li>Testing disinfectants against powdery scab spore balls in vitro</li> <li>Testing disinfectants against common potato pathogens on different surface materials</li> <li>Testing disinfectants against pathogens on 'dirty' metal and wooden surfaces</li> <li>Testing disinfectants by treating diseased seed tubers</li> <li>Jisinfectants on the potato farm – a summary</li> <li>When to use disinfectants</li> </ul>	14 14 15 17 17 22 25 28 32 37 37 37
3.4	Conclusions	38
<b>3.5</b> 3.5	Hygiene protocols for the potato farm 5.1 HACCP	<b>40</b> 45
3.6	Technology Transfer	46
3.7	Recommendations	49
3.8	Acknowledgments	50
3.9	References	51
	Appendices0.1Appendix 10.2Appendix 2	<b>53</b> 54 55

#### 1 Media Summary

The potato shed is a significant source of contamination and disease in seed potato stocks. This research showed that the dust in potato sheds is heavily contaminated with common potato pathogens that reduce potato quality. In many cases, the dust sampled from potato sheds contained higher levels of pathogen propagules than soil sampled from potato paddocks. Even the air in the sheds and cool stores was laden with pathogen spores. Other sources of contamination within the shed are potato boxes and grading equipment that are smeared with diseased tubers, as well as stocks of stored potatoes which have skin blemish diseases that produce thousands of airborne spores. Without a hygiene program, growers can quickly erode the benefits of their investments in disease management such as crop rotation, the use of high health seed potatoes and purchase or lease of new land.

Hygiene protocols have been developed to minimise the risk of contaminating healthy seed stocks. These include the installation of dust extraction fans, mechanical cleaning, such as vacuuming of floors and pressure washing of bins, equipment and floors and walls of sheds and stores, and disinfection. Other recommendations are to concrete (or asphalt) floor and traffic areas both inside and outside the shed, to keep grading areas apart from storage areas and to store high value seed stocks separately, away from work areas and other potatoes.

We tested the ability of some commercially available sanitisers to disinfect potato pathogens from the types of surfaces that are commonplace in the potato shed. All were effective when used on clean, non-porous surfaces such as metal and plastic, but wood and dirty surfaces were much more difficult to disinfect. The tough-walled spores of the silver scurf fungus also proved very challenging. Two chemicals tested, a phenolic detergent/sanitiser and a peroxygen sanitiser, were the most effective against all pathogen/surface combinations tested at label rates.

Implementation of a shed hygiene program can lead to tangible improvements in the health of seed and ware potatoes. A good hygiene program also means that the potato grower and his staff enjoy cleaner, safer working conditions and the image of 'clean' farm is better for business.

### 2 Technical Summary

The market demand for washed, blemish-free fresh potatoes together with the trend in cropping potatoes in 'new' or 'clean' ground (no previous history of potato cropping) to avoid disease has put pressure on seed growers to significantly improve the quality of their produce. The potato shed was implicated as a source of infection for high value seed stocks. This project assessed the disease risk in Australian potato sheds, evaluated the effectiveness of different classes of disinfectants against common potato pathogens and developed hygiene protocols incorporating cleaning and disinfection practices.

This research confirmed that potato sheds are sources of inoculum of common potato pathogens. Dust was swept from the floors of twelve potato sheds across two districts and baited with potato plants. The development of silver scurf, black dot, black scurf, powdery scab and common scab on progeny tubers revealed the presence of inoculum of *Helminthosporium solani*, *Colletotrichum coccodes*, *Rhizoctonia solani*, *Spongospora subterranea* and *Streptomyces scabies* in the dust from both districts. Comparative disease incidences reflected the disease incidence on seed potato stocks grown in each district. There was no apparent difference in disease risk between dust from concrete or rammed-earth shed floors. However, higher levels of silver scurf developed on tubers grown in shed dust than on tubers grown in 'new' ground soil, indicating that shed dust poses a serious contamination risk. The pathogens *C. coccodes*, *R. solani*, *S. subterranea*, *Fusarium* spp and *H. solani* were also detected in the air in sheds and cool stores, with *H. solani* the most common.

Disinfectant chemicals representing the halogen, aldehyde, synthetic phenol, peroxygen and QAC chemical classes were evaluated against potato pathogens in vitro and on various surface materials. This research indicated that in a clean environment, most commercially available disinfectants used at recommended label rates for hard surface disinfection are suitable for disinfecting clean non-porous surfaces (eg metal and plastic) that may be contaminated with the common potato pathogens (bacteria and fungi). The exception was the melanised spores of Helminthosporium solani (silver scurf) against which only Biogram (synthetic phenol) and Peratec 5 Sanitiser (peroxygen) proved to be effective. Wooden surfaces were more difficult to disinfect than non-porous surfaces such as metal, and Peratec 5 Sanitiser and Perfoam 2 (peroxygens) were the most effective at disinfecting a wooden surface contaminated with *Erwinia* or *H. solani*. Higher rates (x5) and longer exposure times of other chemicals (eg Biogram, Virkon S and Phytoclean) required to achieve a similar effect. Our research also showed that to be certain of killing cystosori of the powdery scab pathogen, Spongospora subterranea, relatively high rates (5x label rates for hard surface disinfection) of disinfectants from the phenol, peroxygen and QAC groups were required. For best results, disinfectant treatments should only be used after mechanical cleaning.

Hygiene protocols, which include mechanical cleaning, such as vacuuming of floors and pressure washing of bins, equipment and floors and walls of sheds and stores, followed with a disinfectant treatment, have been developed as a guide to growers and store managers. Other hygiene strategies outlined include concreting or asphalting working areas inside and outside the shed, separation of grading areas from the storage areas and separate storage for high value seed stocks. Overseas research shows that improvements in shed hygiene can lead to tangible improvements in the health of seed and ware potatoes.

#### 3 Technical Report – Cleaning and disinfestation practices for Australian potato farms

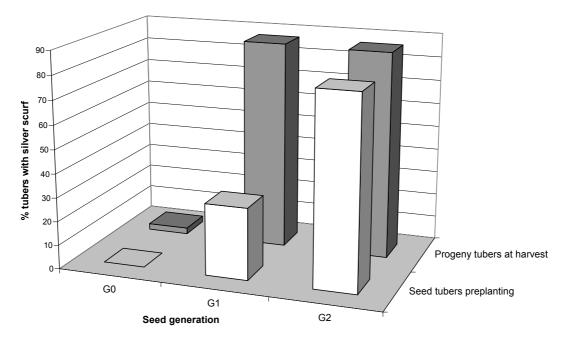
#### 3.1 Background

The market demand for washed, blemish-free fresh potatoes together with the trend in cropping potatoes in 'new' or 'clean' ground (no previous history of potato cropping) to avoid disease, have put the spotlight firmly on seed-borne diseases. These trends are putting pressure on seed growers to produce seed potatoes which exceed the quality required by seed certification schemes, particularly for the common skin blemishing diseases silver scurf (*Helminthosporium solani*), black dot (*Colletotrichum coccodes*), black scurf (*Rhizoctonia solani*), powdery scab (*Spongospora subterranea*) and common scab (*Streptomyces scabies*).

To meet this challenge, a strategy commonly used by seed growers is to grow all generations of seed potatoes (generations G0-G5) in new ground, starting with disease-free minitubers (G0) produced on tissue-cultured potato plantlets in glasshouses. Another is to offer early generation seed such as G3, instead of the usual G5, for commercial use. The underlying assumption is that there will be less disease in the G3 generation, particularly if it is grown in new ground.

In reality, early generations (field grown generations G1-G3) of seed potatoes grown in new ground have been found to have a relatively high incidence and severity of some of the blemishing diseases (de Boer 1997, de Boer and Curtis 1999). For example, a very high incidence of silver scurf was found in both the seed and progeny of tubers of three generations grown only in new ground know to be free of the pathogen *H. solani* (Figure 1). A Scottish report (Carnegie *et al.* 1996) had identified the potato store as a source of infection for a number of potato diseases, indicating the need for growers to adopt hygiene practices on their farms. In light of this, we suspected that the potato shed may have been the source of the seen on potatoes grown in new ground.

This project aimed to provide growers with practical hygiene protocols to help improve potato quality and minimise the risk of the inadvertent spread of the major seed and soilborne potato pathogens. It assessed the risk posed by shed and field dust as sources of inoculum and evaluated the effectiveness of different classes of commercially available disinfectants against the major potato pathogens on a range of surfaces found on potato farms. The outcomes are cleaning and disinfection practices specifically for use by potato growers.



Disease incidence in three generations of seed and progeny tubers planted in new ground

Figure 1 The incidence of silver scurf in three generations of seed and progeny potato tubers only ever grown in 'new' ground each successive season (no previous history of potato production (G0 = minitubers, G1 and G2 = first and second field-grown generations)

#### 3.2 Assessing the hygiene risk in potato sheds

#### 3.2.1 The potato shed as a hygiene risk

Potato sheds are inherently dirty and dusty places and earthen floors are not uncommon. A simple experiment conducted in the UK demonstrated that cleaning stores could lead to significant improvements in the health of potato crops (Hall 1996). Thirty percent of the progeny of minitubers that had been exposed for several weeks in a commercial store had silver scurf at harvest, compared with 5% of the progeny of minitubers that had not been length of time in a cleaned experimental store. The progeny of minitubers that had not been exposed had no silver scurf.

Individual growers can produce up to five generations of seed potatoes, starting from diseasefree mini-tubers produced on tissue-cultured plantlets. Generally, all generations are sorted in the one facility and stored together in the same cool-store over winter. Dust, comprising soil organic debris and airborne spores, is recognised as a source of inoculum of common potato pathogens in sheds and cool-stores [Carnegie *et al.* 1996]. Healthy seed stocks are at risk of contamination with potato pathogens during sorting and storage.

The aims of this study were:

- 1. to define the significance of the potato shed as a source of inoculum in Australian potato production,
- 2. to determine whether there is a greater risk of contamination of seed stocks within sheds with 'dirt' floors compared with concrete floors in two major seed producing regions of Victoria, and
- 3. to compare the inoculum load of shed dust with the inoculum load of field soil.

#### 3.2.1.1 Evaluation of the shed dust as a potential source of inoculum

#### **Materials and Methods**

#### Dust Bioassay

Samples of shed floor dust were collected in October 1999 from potato sheds in two major production areas, namely, south of Colac (Colac/Otway) and around Ballarat (Central Highlands) in Victoria. A total of 12 sheds were sampled (6 per region), including dirt-floored and concrete-floored sheds. Within each shed, samples of dust were collected from a general thoroughfare area, from around the main potato bin handling areas and from under the grading equipment.

Plastic pots (15 cm diameter) were half-filled with pasteurised sand-based potting media, which was then overlain with a blended mixture of 100 g of shed dust and potting media. The pots were planted with potato plantlets cv. Sebago (Figure 2). Pots without shed dust were included as control treatments. The pots were arranged in a randomised block design in a glasshouse maintained at 15-28°C. Progeny tubers were harvested after three months and examined for the incidence and severity of disease.

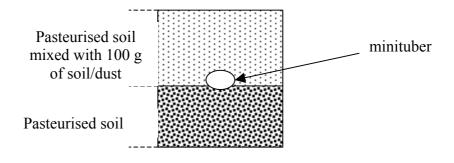


Figure 2 Schematic diagram of a pot bioassay for shed dust

#### Detection of air borne inoculum in potato sheds

The air in the 12 sheds was sampled for 5 minutes using a Rotorod<sup>TM</sup> air sampler, a hand-held device consisting of spinning U-shaped arms. Airborne dust and spores are collected on double-sided adhesive tape attached to the arms. The air in cool-stores was also sampled. Spores and fungal hyphae were counted using 400X magnification to determine the spore load in each shed.

#### **Results and Discussion**

#### Dust Bioassay

The skin blemishing diseases silver scurf, black dot, black scurf, powdery scab and common scab occurred on the progeny of the potato bait plants indicating the presence of the pathogens *H. solani*, *C. coccodes*, *R. solani*, *S. subterranea*, and *S. scabies*, respectively, in the dust samples. These results highlight the risk of contaminating seed stocks with the common potato pathogens through the movement of dust in the potato shed. It should be noted that this type of bioassay does not favour the detection of pathogens associated with tuber damage such as *Fusarium* spp., *Phoma exigua* and *Erwinia carotovora*.

The incidence of each disease in the progeny tubers varied from sample to sample and district to district, reflecting differences in inoculum levels for individual pathogens per unit volume of dust. There was no apparent correlation between the incidence of each disease and floor type (dirt or concrete) or location within a shed.

Generally, silver scurf and black dot were the most common diseases on progeny bait-plant tubers (Figure 3 and Figure 4) reflecting relative disease incidence in seed potatoes (de Boer and Wicks 1994). Silver scurf was the most common disease in dust from the Colac/Otway region (Figure 3), whereas black dot was more common than silver scurf in dust from the Central-Highlands (Figure 4). This is consistent with observed trends in the relative incidence of diseases on field-grown potatoes in these two regions (RF de Boer unpublished data).

Spongospora subterranea was detected in some dust samples from sheds in an area southwest of Colac considered to be powdery scab "free". Consequently, the dust samples were further tested using a DNA based technique specific for the powdery scab fungus *S. subterranea*. Although still in a development stage, this test confirmed the positive bioassay results but also detected *S. subterranea* in dust samples from the Colac district that had tested "negative" with the bioassay (Faggian 2002). This indicates the potential for seed potatoes to be contaminated with *S. subterranea* in a production area where symptoms of powdery scab on tubers occur infrequently. There is a risk that planting these tubers will result in the inadvertent contamination of new areas that are conducive to the development of powdery scab.

#### Air-borne inoculum in potato sheds

Spores of *S. subterranea, H. solani, C. coccodes* and *Fusarium* spp., along with fragments of *R. solani* hyphae, were found on the tapes of air samples taken in sheds and cool-stores (Figure 5). Spores of *H. solani* and fragments of *R. solani* hyphae were relatively common in some sheds and cool-stores. Overall, *H. solani* was the most common pathogen recorded. The relative abundance of spores and hyphal fragments varied from shed to shed.

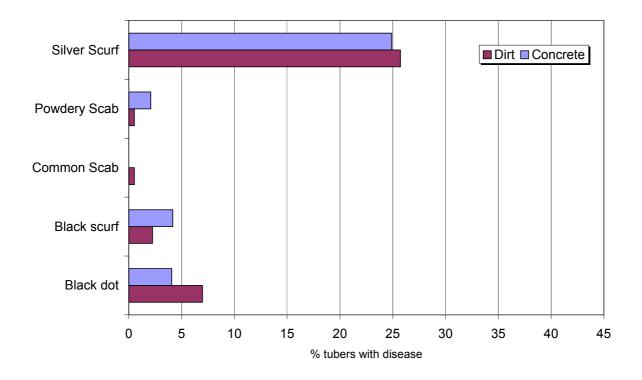


Figure 3 Incidence (% tubers affected) of five diseases in a potato-plant bioassay of dust sampled from dirt and concrete-floored potato sheds in the Colac/Otway region.

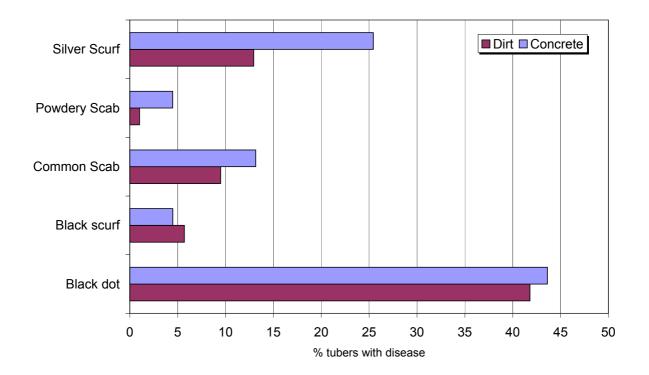
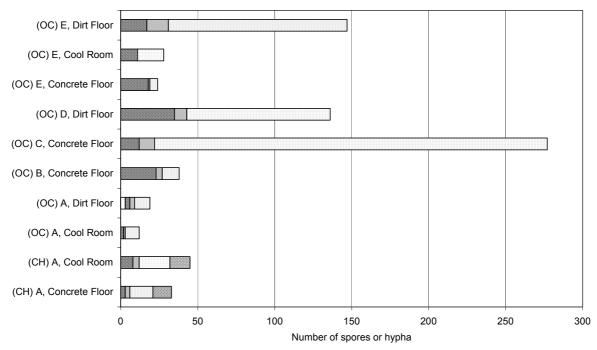


Figure 4 Incidence (% tubers affected) of five diseases in a potato-plant bioassay of dust sampled from dirt and concrete-floored potato sheds in the Central Highlands region.



🗆 Colletotrichum coccodes 🖩 Rhizoctonia solani 🖬 Fusarium spp. 🗆 Helminthosporium solani 🖾 Spongospora subterranea

Figure 5 The frequency of detection of five potato pathogens (as either spores or hyphal fragments) in air sampled from dirt and concrete-floored sheds and cool-stores in the Colac/Otway (OC) and Central Highland (CH) regions.

#### 3.2.1.2 Comparing disease risk in shed dust and field soil

#### **Materials and Methods**

Soil samples were taken from a potato field near Ballarat in the Central Highlands of Victoria ('old' ground with a history of potato production) and from a field near Colac in the Colac/Otway region of Victoria that had not been planted to potato for eight years. Soil was sampled using a 10 cm diameter auger to a depth 15 cm every 10 paces in a 'W' pattern across each field. Samples from within a field were combined and mixed to form a composite sample. Dust was swept from the floor at a number of points within the potato sheds that serviced each of the two potato farms.

The soil and dust samples were air-dried and 100 g sub samples were tested using the sandwich method described for the shed dust bioassay in the previous experiment (Figure 2). Twelve replicate sub samples were baited with minitubers of cv. Sebago.

100 g sub samples of shed dust and field soil was also tested for the presence of *S*. *subterranea* using the tomato seedling bioassay described in Section 3.3.4.2.

#### **Results and Discussion**

In Ballarat, traces of silver scurf (7% of tubers) were detected in the shed dust but not in the field soil (Figure 6). Few tubers grown in the shed dust and field soil developed powdery scab (4% and 7% tubers affected, respectively) and the presence of *S. subterranea* in these samples was confirmed by the tomato seedling bioassay (Figure 7). The farm from which these samples were taken has a high risk of powdery scab. Black scurf was not detected in the Ballarat shed dust or field soil (Figure 6), although the disease is common in this district.

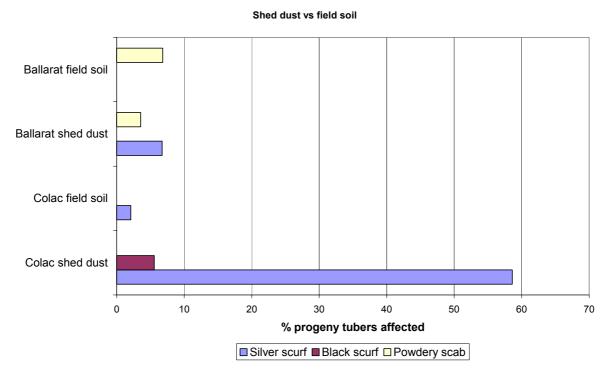


Figure 6 Incidence of silver scurf, black scurf and powdery scab in progeny tubers (% tubers affected) in a potato plant bioasssay of shed dust and field soil from farms at two different locations

In Colac, 59% of the tubers grown in shed developed silver scurf compared with 2% from the field soil (Figure 6). Only 6% of tubers in the shed dust developed black scurf, although the disease was not detected in the field soil. The powdery scab pathogen *S. subterranea* was not detected shed dust or field soil using either the potato plant or the tomato seedling bioassay (Figure 6 and Figure 7).

Black dot occurred on tubers grown in dust and soil samples from both districts. However, the data was not reliable because the disease was also detected in the controls.

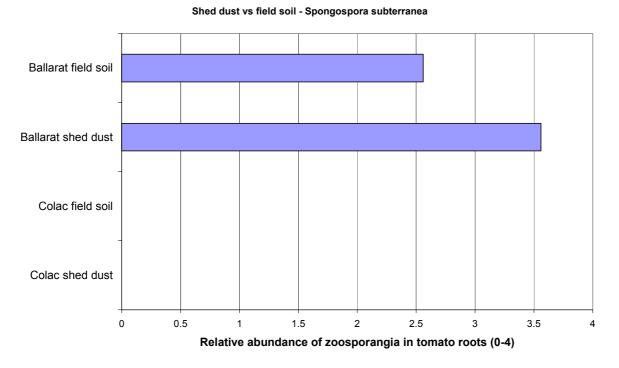


Figure 7 Detection of *Spongospora subterranea* (powdery scab) in shed dust and field soil from two different locations using a tomato seedling bioassay

This study confirms that shed dust is a source of disease inoculum. The disproportionate levels of silver scurf in the Colac shed dust compared with field soil illustrates that inoculum of some pathogens can be highly concentrated in the shed environment. Since seed potatoes on this farm are planted in new ground each year where the risk of silver scurf is relatively low, the potato shed is probably a major source of inoculum of *H. solani* for disease in the produce from this farm. This helps explain the relatively high incidence of silver scurf found in early generations of seed potatoes in these production areas (Figure 1).

#### 3.2.2 Conclusions

This study demonstrates that the potato shed is a source disease in seed potatoes. The dust on the floor of the shed or cool store is contaminated with the common potato pathogens. Spores and other infective units of pathogens, particularly *H. solani*, are found in air currents around the shed. This is consistent with reports from the United Kingdom and the USA (Carnegie *et al.* 1996; Hall 1996; Rodriguez *et al.* 1996).

The relative incidence of the different diseases in dust bioassay generally reflected the relative incidence of those diseases in the different districts overall. Generally, shed dust was as infective or, in some instances more infective, than field soil. For instance, shed dust from a farm in the Colac district was considerably more infective with silver scurf than the field soil, suggesting that the shed may be a major source of infection for potatoes grown on the farm.

The diseases and pathogens that have been detected in dust and air currents in the shed are summarised in Table 1. The sources of infection in the shed environment include:

- Dust contaminated with inoculum of various potato pathogens which can spread throughout the shed and cool store coating seed stocks, equipment and boxes etc..
- Tubers with diseases such as Phoma (Gangrene), Fusarium dry rot, bacterial soft rot and brown rot. Healthy tubers, grading table parts and boxes are smeared with infected material during sorting and handling operations.
- Tubers with 'dry' diseases such as powdery scab. The dry powdery spore balls are redistributed throughout a seed batch during grading (Stuart Wale, SAC, personal communication).
- Spores of pathogens such as *H. solani, Fusarium* spp. and *Phoma* are carried in aircurrents around the shed and contaminate seed stocks and various surfaces in the shed (Carnegie *et al.* 1996, Rodriguez *et al.* 1996).

### Table 1 Potato pathogens detected in dust or in air in potato sheds and their potential for spread through contamination of seed stocks

Potato disease	Organism	Sources of infection	Spread
Silver scurf	Helminthosporium solani	Dust, diseased tubers, surfaces, air	++++
Gangrene	Phoma exigua	Dust, diseased tubers, surfaces, air	++
Fusarium dry rot	Fusarium spp.	Dust, diseased tubers, surfaces, air	++
Powdery scab	Spongospora subterranea	Dust, diseased tubers, surfaces, air	++
Black scurf	Rhizoctonia solani	Dust, surfaces, air	+
Black dot	Colletotrichum coccodes	Dust, surfaces	+
Common scab	Streptomyces scabies	Dust, diseased tubers	+
Soft rot/black leg	Erwinia spp.	Diseased tubers, surfaces	++
Brown rot	Ralstonia solanacearum	Diseased tubers, surfaces	++

The silver scurf pathogen has the highest propensity for rapid multiplication and spread within the shed and cool store environment. The fungus sporulates after condensation on the tuber skin (eg during cooling cycles in cool stores) and spores spread rapidly in the air currents. Enormous quantities of *H. solani* spores can be produced on silver scurf lesions and peaks of up to 12 000 and 24 000 spores per day have been measured in seed (4°C) and processing (10°C) cool stores, respectively, in a US study (Rodriguez *et al.* 1996). Generally, the incidence and severity of silver scurf after storage is higher than after harvest.

Evidence of disease spread in the shed includes:

• In the US, disease-free minitubers developed silver scurf after only one week of exposure in a cool store (Rodriguez *et al.* 1996).

- In the UK, healthy tubers developed gangrene and Fusarium dry rot after being passed over a grading table that had previously been used for contaminated stock (shed and contaminate seed stocks and various surfaces in the shed (Carnegie *et al.* 1996).
- In a study in the UK, grading a seed stock with powdery scab resulted in a higher disease incidence in the progeny compared with ungraded stocks because the grading process redistributed the sporeballs throughout the consignment (Stuart Wale, SAC, personal communication). In an Australian study, the incidence of progeny tubers with powdery scab was as high from seed tubers contaminated with *S. subterranea* (no visible scab) as from seed tubers with visible symptoms of powdery scab (RF de Boer, unpublished data).

It is common practice for post-harvest handling, grading and storage of potatoes to be conducted under the same roof and for all generations of seed potatoes to be stored together in the same cool store after grading. In this situation, healthy seed stocks are at high risk of contamination. It is clear that a hygiene program, which includes cleaning and disinfection, is essential to minimise the risk of contaminating high value seed stocks.

#### 3.3 Cleaning and disinfection

#### 3.3.1 Cleaning – the first and most important step in a disinfection program

Contaminated soil, dust and diseased tubers in grading, storage and packing sheds have been identified as the source of inoculum for many potato diseases. Scientists at the Scottish Agricultural College (SAC), Aberdeen tested several methods of cleaning naturally contaminated and artificially inoculated surfaces floors, rollers and other equipment in order to establish practical guidelines for cleaning and disinfection (Clayton *et al* 1999, Wale 2002 and Clayton *et al*. 2001). Cleaning routines included removal of dust by various means (sweeping, vacuuming), the mechanical cleaning of roller tables and grading lines (wiping, hosing, high pressure washing), followed by a disinfectant treatment. In most cases, the progeny of minitubers or healthy seed stocks exposed left exposed in the store after cleaning were significantly healthier than the controls (Clayton *et al*. 1999). Cleaning routines were found to significantly reduce inoculum (spores and other infective units) levels of pathogens.

The SAC research provides the basis of the cleaning protocols in the hygiene protocols developed as part of this project (Section 3.5). These studies show that the single most important step in a disinfection program is to remove the contaminating material. This may involve:

- 1. Vacuuming dust from around the sheds and stores (how often depends on risk);
- 2. Washing surfaces, floors, boxes and equipment (eg grading lines, seed cutter, planter and harvesters);
- 3. Wash down with a disinfectant

The SAC research shows that, with effective cleaning procedures, the last step may be redundant. This depends, of course on the surfaces being cleaned, the biology of the pathogens involved and the relative risk of spread of those pathogens. The porous surfaces of wooden boxes are more difficult to clean than metal surfaces. For example, bacteria (eg *Erwinia*) remaining on cleaned surfaces are killed when the surface is dried, whereas some fungi can survive this process. Nevertheless, disinfectants should be used when a high level of disinfection is required.

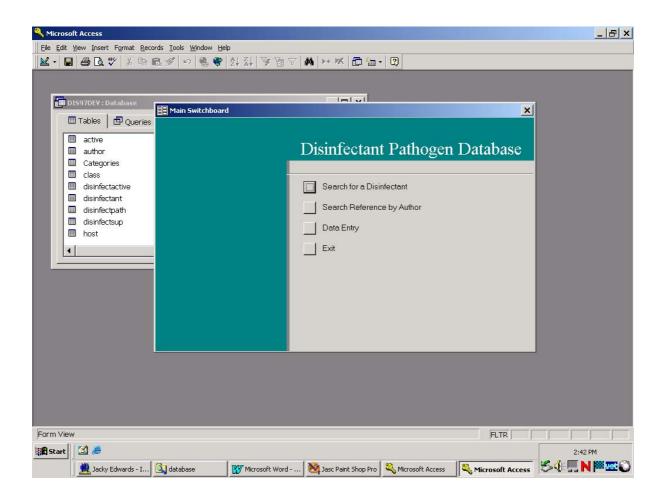
#### 3.3.2 Disinfectants for the potato farm

There are many disinfectants available commercially, and the range covers several different classes of chemicals (Appendix 1, Table 2). Most have been developed for general-purpose applications, such as in the home, the dairy, animal houses, hospitals and farms and, therefore, there are no specific claims made on the labels regarding potato pathogens.

Various studies have shown that the recommended dilution on a label may be effective against bacteria but not against fungi, or it may disinfect glass surfaces but not wood or concrete. Their effectiveness can vary with water supplies, chemical make-up of surfaces and temperature. Many lose effectiveness when applied to surfaces contaminated with organic matter, such as would be the case in a dirty potato shed. It become apparent, therefore, that some evaluation of disinfectants was required before recommendations could be made to the potato producers on the efficacy of disinfectants against potato pathogens.

#### 3.3.3 Disinfectant database

A disinfectant database was developed in order to help compile the comprehensive research information from around the world on the effectiveness of disinfectants against a wide range of plant pathogens. The Microsoft Access 97 database contains information from research papers on the efficacy of disinfection treatments on many vegetable pathogens including those affecting potatoes. Information sourced from brochures regarding trade products, their active ingredients, rates tested, application rates, safe handling and disposal, suppliers, costs, their registration status, and so forth has also been entered into the database. The database is shared with our collaborators, Dr. Robert Holmes and Mr. Martin Mebalds, who have conducted similar research into the use of disinfectants in the fruit, vegetable and nursery industries. The database can be easily queried to provide information on such questions as "What disinfectants have been tested against the potato cyst nematode and how effective were they?" or "List the disinfectants that have been effective on concrete surfaces".



- 🖬 🎒 💽 🖤		<u>T</u> ools <u>W</u> indow <u>H</u>	elo.				 
				📈 🔂 ⁄a - 📿			
name	rate_tested	🗠 👼 😴 💈		time	dirty	rating long	cnama
Sodium Hypoch	-	min	surface.long	5		rating.long	mesophilic bacteria
	10%(0.1g peat		water	10		poor excellent	Fusarium culmorun
Sodium Hypoch Sodium Hypoch		min	lettuce	30		poor	mesophilic bacteria
	100mg/1PAC 10%(0.1g peat		water	1		excellent	Fusarium culmorun
	10%(0.1g peat/L		water	100		excellent	Fusarium culmorun
	10%(1g peat/L		water	100		excellent	Fusarium culmorun
	1%(0.1g peat/L		water	10		ineffective	Fusarium oxysporu
	1%(0.1g peat/L		water	10		ineffective	Fusarium culmorun
	2% dip / 3 sec		rollerpress (smc	5		excellent	Potato Spindle Tub
Sodium Hypoch Sodium Hypoch		1360	PDA (potato de:	5		average	Rhizopus stolonifer
Sodium Hypoch Sodium Hypoch		sec	metal	5		excellent	Potato Spindle Tub
Sodium Hypoch Sodium Hypoch		min	plastic pots	15		excellent	Botrytis cinerea
Sodium Hypoch Sodium Hypoch		min	plastic pots	15		excellent	Rhizoctonia solani
Sodium Hypoch Sodium Hypoch			metal	20			Erwinia carotovora
	10% 10%(1g peat/L	min	water	20		good	
	2% dip / 3 sec			5		poor excellent	Fusarium oxysporu
2.1			rollerpress (knu				Potato Spindle Tub Geotrichum candid
	80 ppm FAC -		water	1		excellent excellent	
21	120 ppm FAC -		water	1			Geotrichum candid
21	120 ppm FAC -		water	5		good	Rhizopus stolonifer
	2% dip / 10 sec >1,000ug/l-<10		rollerpress (smc	5		excellent	Potato Spindle Tub
21			water	20		average	Clavibacter michiga
Sodium Hypoch Sodium Hypoch		min	wood	20		poor	Erwinia carotovora
21	10% 2% dip / 10 sec	min	plastic rollerpress (knu	20		average	Enwinia carotovora Potato Spindle Tub
• •	2% aip / 10 sec 20 ppm FAC -		water	5		excellent	
soulum hypocr	20 ppm r AC - j		water			good	Geotrichum candid
itart 🔟 🥭				r			2:46 PM
licrosoft Access	Iools <u>Wi</u> ndow <u>H</u> elp				Microsoft Access	Microsoft Acces	
iicrosoft Access Edit View Insert : Edit View Insert : Provide the second se	Image: Cools     Window     Help       Image: Cools     Image: Cools     Image: Cools       <	vo   ₩ + bū g_key t	Microsoft Word Microsoft Word pathogen pathogen pathogen pathogen pathogen pathogen pathogen pathogen pathogen pathogen pathogen		1 nt_kee		. Sat <b>in m</b> e
IIICrosoft Access Edit View Inset IIICrosoft Access Edit View Inset IIICrosoft Access IIICrosoft Acces	Image: Cools     Window     Help       Image: Cools     Image: Cools     Image: Cools       <	vo   ₩ + bū g_key t	Pathogen       *       pathype_key       *	Image: second	t t t v	<ul> <li>disinfectactive</li> <li>disinfectactive_key</li> <li>disinfectant_key</li> <li>active_key</li> </ul>	

2:51 PM

Ready

📲 Start 🛛 🙆

🚊 Jacky Edwards - I... 🔍 database

#### 3.3.4 Testing disinfectants against potato pathogens

A series of experiments were conducted to gather data on the effectiveness of several commercially available disinfectant chemicals against the common potato pathogens. Representatives of the main classes of disinfectants were tested. Experiments included an evaluation of disinfectant treatments for their efficacy against

- common fungal and bacterial pathogens *in vitro*;
- powdery scab cystosori in vitro;
- bacterial and fungal pathogens on 'clean' and 'dirty' hard surfaces; and
- sclerotial pathogens, *R. solani* and *C. coccodes* on the potato surface.

The aim of these tests was to provide guidelines for growers on the relative effectiveness of disinfectant chemicals under different circumstances, to allow them to make informed decision about which treatments would be of the most appropriate for use in a cleaning/disinfection program on their potato farm.

### 3.3.4.1 Testing disinfectants against common fungal and bacterial potato pathogens in vitro

#### Materials and methods

Twelve treatments, including eight proprietary compounds used at the recommended label rates, three chemicals and heat, were evaluated for their effectiveness at killing the infective units of seven potato pathogens. The treatments were: the quaternary ammonium compounds (QACs) Phytoclean<sup>®</sup>, Sporekill<sup>®</sup> and Hi-Dab<sup>®</sup>; the phenolic Kendocide<sup>®</sup> and Biogram<sup>®</sup>; Peratec 5 Sanitiser<sup>®</sup> (hydrogen peroxide + peroxyacetic acid); Oxine<sup>®</sup> (chlorine dioxide @ 200 ppm free Cl), sodium hypochlorite + acetic acid (@ 250 ppm free Cl) and sodium hypochlorite (@ 1000 ppm free Cl); a plant extract Citrox 14W<sup>TM</sup>; 70% ethanol and 45°C heat. The organisms tested were the bacteria Erwinia carotovora var. atroseptica (Eca), E. c. var. carotovora (Ecc), Ralstonia solanacearum (Rs), Streptomyces scabies (Ss), and the fungi Fusarium trichothecioides (Ft), Helminthosporium solani (Hs) and Rhizoctonia solani (Rhs). The British Standard quantitative suspension test (Gardner and Peel 1998) was used to test infective unit suspensions of the potato pathogens. The procedure was as follows. Infective unit suspensions were adjusted to the required concentration  $(10^7-10^9)$  for bacteria,  $10^4-10^6$  for fungi) with sterile distilled water and 1 mL of the suspension was added to 9 mL of disinfectant at the test concentration. For the temperature treatments, the 1 mL of suspension was added to 9 mL sterile distilled water that had been preheated in a tube by immersion in a water bath set at 45°C. Following a 2.5, 5, 10 or 20 min exposure time, a 0.5 mL aliquot was mixed with 4.5 mL inactivator solution to halt the disinfection process. Sodium thiosulphate (0.05%) plus 10% Tween<sup>®</sup> 80 was used as the inactivator to neutralise the disinfectants before plating for most treatments, but dilution was considered adequate for neutralising the phenolics and 70% ethanol. Three 0.1 mL samples of each inactivated treatment were plated onto appropriate media (NA or PDA) and incubated at room temperature. A water treatment and an inactivator treatment were used as controls in each test

In order to test the effectiveness of the treatments in the presence of organic matter, an organic load mixture was substituted for sterile water as the diluent for the infective unit suspensions in a second series of tests. The mixture was composed of 5% yeast extract

solution for bacteria or 5% peat solution for fungi. Peat was substituted for yeast extract as the latter proved to be toxic to the fungal organisms. The experimental procedure was the same as previously described.

The number of colony-forming units (cfu) was counted after 3-7 days incubation and means of the three replicates were calculated. The cfu in the control plates varied between experimental runs. The results were standardised by conversion of the number of cfus to percentages of the control. Each treatment was tested at least twice.

#### **Results and Discussion**

The effectiveness of the treatments varied depending on the test organism (Table 3 and Table 4). Biogram (synthetic phenol) and Peratec 5 Sanitiser (peroxygen) were the only treatments that consistently killed all organisms within 2.5 minutes, regardless of the presence of organic matter. Organic matter reduced the effectiveness of sodium hypochlorite and Oxine. Heat at 45°C was the least effective treatment, although some effect was noted on the fungi at the longer exposure times of 10 and 20 minutes.

*Hs*, which causes silver scurf, was resistant to most treatments. The QACs, Kendocide, Citrox 14W, 70% ethanol and 45°C heat had relatively little effect. The sodium hydroxide treatments were relatively effective, consistent with the current recommendations for controlling this pathogen in the USA (Prof. D. Preston, personal communication 1999), but Biogram<sup>TM</sup> and Peratec 5<sup>TM</sup> were the only two which consistently killed *Hs* under all conditions tested. The thick melanised walls of *H. solani* conidia obviously provide good protection. Melanised cells are known to have a relatively high degree of resistance to chemical treatment (Butler and Day 1998). The dry rot fungus, *Ft*, was the easiest organism to kill. All treatments except 45°C heat killed the spores within 2.5 minutes.

It is important to note differences in the type of fungal inoculum used in this laboratory study in comparion with the type of inoculum that may occur in the farm shed. The black scurf fungus *Rs* will occur as thick-walled melanised sclerotia and hyphae in debris in the potato shed, rather than as the less robust hyphae grown in culture.

Product	Active ingredient(s)	Disinfectant class/chemical group	Cost \$/L	Label rates	Comments
Formalin <sup>®</sup>	36% formaldehyde	Reducing agents/aldehydes	\$1.75	-	Probable human carcinogen
Sodium hypochlorite	12.5 % sodium hypochlorite	Halogens and halogen based	\$4.00	1000 ppm	
('bleach')		compounds			
Biogram <sup>®</sup>	2.5% w/v clorofene (Na salt), 16.5% w/v ortho- phenylphenol (Na salt)	Synthetic phenols. Hospital grade Detergent/disinfectant	\$10.00	0.6-5%	Hospital disinfectant
Kendocide <sup>®</sup>	423 g/L dichlorophen Na	Synthetic phenols.	\$36.00	0.1-2.5%	Registered as an algicide
Oxine <sup>®</sup>	2% available chlorine dioxide	Oxidising agent/ peroxygen compounds	\$10.00	5-200 ppm	
Peratec 5 Sanitiser <sup>®</sup>	250 g/L $H_20_2$ , 50 g/L peroxyacetic acid	Oxidising agent/ peroxygen compounds	\$3.50	0.2-1%	
Perfoam 2	5% peroxyacetic acid, 14% hydrogen peroxide	Oxidising agent/ peroxygen compounds Detergent/disinfectant	\$3.50	1%	
Virkon <sup>®</sup> S	Potassium peroxymonosulphate (sulphamic acid, malic acid, sodium hexametaphosphate, dodecyl benzene sulphonate)	Oxidising agent/ peroxygen compounds. Detergent/disinfectant	\$76.00	0.5-1%	Recommended by WHO for foot & mouth eradication programs. Contains oxidising agents, organic acid catalysts, a buffering agent & an anionic surfactant
Phytoclean <sup>®</sup>	100 g/kg benzalkonium chloride	Cationic surfactants – QAC <sup>A</sup>	\$10.00	2-10%	Detergent/disinfectant. NRA registration – <i>Phytophthora</i> <i>cinnamomi</i> (wash down, surface sanitation)
Sporekill <sup>®</sup> Hi Dab <sup>®</sup>	120 g/L didecyldimethylammonium chloride 150 g/L alkyldimethylbenzylammonium chloride, 20 g/L chlorhexidine complex, 150 g/L ethylene oxide surfactant	Cationic surfactants – QAC <sup>A</sup> Cationic surfactants – QAC <sup>A</sup>	\$26.00	0.1-1% 0.3-1.25%	
Castrol <sup>®</sup> Farmcleanse	Not available	Cationic surfactants – QAC <sup>A</sup> . Detergent/disinfectant	\$5.00	2.5-10%	High foaming, detergent degreaser with antifungal properties ( <i>Fusarium</i> spp.)
Citrox 14W <sup>™</sup>	Orange extract (5%), glycerine (5%), Yucca schidegra extract (5%), propylene glycol (5%)	Food grade biocide/sanitiser & potable water treatment	\$25.00	2%	Added to fresh and wash water for washed food

#### Table 2 Details of disinfectant/sanitiser compounds evaluated for their efficacy against potato pathogens

<sup>A</sup> Quaternary ammonium compounds

Treatment	Fusarium trichothecioides		Helminthosporium solani		Phoma exigu	a var. <i>foveata</i>	Rhizoctonia solani	
	-OM <sup>A</sup>	+OM	-OM	+OM	-OM	+OM	-OM	+OM
Phytoclean <sup>®</sup> (2%)	<2.5	<2.5	>20	>20	<2.5	<2.5	<2.5	<2.5
Sporekill <sup>®</sup> (0.2%)	<2.5	<2.5	>20	>20	<2.5	10	<2.5	<2.5
Hi Dab <sup>®</sup> (1.25%)	<2.5	<2.5	>20	>20	20	>20	<2.5	<2.5
Kendocide <sup>®</sup> (1%)	<2.5	<2.5	>20	>20	>20	10	<2.5	<2.5
Biogram <sup>®</sup> (1.5%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Peratec 5 Sanitiser <sup>®</sup> (1%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Oxine <sup>®</sup> (200 ppm Cl)	<2.5	<2.5	>20	>20	<2.5	<2.5	<2.5	<2.5
Sodium hypochlorite + acetic acid	<2.5	<2.5	20	10	<2.5	<2.5	<2.5	<2.5
(250 ppm Cl)								
Sodium hypochlorite (1000 ppm Cl)	<2.5	<2.5	<2.5	>20	5	<2.5	<2.5	<2.5
Citrox 14W <sup>TM</sup> (2%)	<2.5	<2.5	>20	>20	>20	>20	>20	<2.5
70% ethanol	<2.5	<2.5	>20	>20	<2.5	<2.5	<2.5	<2.5
45°C (water bath)	>20	>20	>20	>20	10	10	10	>20

Table 3 Time (minutes) taken to achieve 100% kill of potato pathogenic fungi in quantitative in vitro suspension tests

<sup>A</sup> OM = organic matter

Treatment	Erwinia carotovora var. atroseptica		Erwinia carotovora var. carotovora		Ralstonia solanacearum		Streptomyces scabies	
	-OM	+OM	-OM	+OM	-OM	+OM	-OM	+OM
Phytoclean <sup>®</sup> (2%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Sporekill <sup>®</sup> (0.2%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Hi Dab <sup>®</sup> (1.25%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Kendocide <sup>®</sup> (1%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Biogram <sup>®</sup> (1.5%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Peratec 5 Sanitiser <sup>®</sup> (1%)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Oxine <sup>®</sup> (200 ppm Cl)	<2.5	10	<2.5	<2.5	<2.5	>20	<2.5	>20
Sodium hypochlorite + acetic acid (250 ppm Cl)	<2.5	10	<2.5	5	<2.5	10	<2.5	10
Sodium hypochlorite (1000 ppm Cl)	<2.5	5	<2.5	5	<2.5	5	<2.5	<2.5
Citrox 14W <sup>TM</sup> (2%)	<2.5	<2.5	<2.5	<2.5	>20	>20	<2.5	<2.5
70% ethanol	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
45°C (water bath)	>20	>20	>20	>20	>20	>20	>20	>20

Table 4 Time (minutes) taken to achieve 100% kill of potato pathogenic bacteria in quantitative in vitro suspension tests

<sup>A</sup> OM = organic matter

#### 3.3.4.2 Testing disinfectants against powdery scab spore balls in vitro

The powdery scab pathogen (*Spongospora subterranea*) can be a contaminant in potato sheds (de Boer *et al.* 1982; Section 3.2) and on the surface of apparently healthy tubers (de Boer 1983). The pathogen produces spore masses (cystosori or 'sporeballs') in the scab pustules on tubers and in galls on roots. These spores are robust and can survive in a dormant state for several years. The organism is an obligate parasite, requiring a living host to complete its life cycle and, therefore, cannot be grown in culture like other potato pathogens. However, the viability of cystosori can be tested using a tomato seedling bioassay or 'bait'. The results of a series of experiments to determine the effects of disinfectant treatments on the viability of cystosori are described.

#### Materials and Methods

Preparations of powdered cystosori (sporeballs) of *S. subterranea*, obtained by wet-sieving macerated powdery scab pustules from diseased tubers (smallest sieve size was  $38 \,\mu$ m), were immersed in solutions of different disinfectant chemicals for different periods of time. The reaction was inactivated by adding sodium thiosulphate and Tween<sup>®</sup> 20 to the spore suspension and by further dilution.

The viability of cystosori in the suspensions was determined by tomato seedling bioassays adapted from methods described by Flett (1983), Merz (1989) and Fornier (1997). Preliminary tests had shown that cv. Oxheart was the most susceptible. The relative abundance of zoosporangia in the tomato root hairs and cortical cells was expressed as a disease index described by Merz (1989) where ratings of 0, 1, 2 3, and 4 were assigned to root systems with

- no zoosporangia in root hairs and cortical cells;
- occasional zoosporangia;
- several roots with zoosporangia;
- zoosporangia regularly present, moderate infection;
- zoosporangia regularly present, heavy infection.

Results were analysed by Analysis of Variance (Genstat for Windows 5<sup>th</sup> Edition<sup>TM</sup>, Lawes Agricultural Trust, Rothamsted Experimental Station).

In preliminary experiments, twelve disinfectant treatments were selected from several chemical groups and tested at the recommended label rates for surface disinfection. The treatments were 2% Phytoclean, 0.2% Sporekill and 1.25% Hi-Dab (quaternary ammonium compounds), 1% Kendocide and 1.5% Biogram (phenols), 1% Peratec 5 Sanitiser (peroxygen), Oxine (200 ppm Cl), sodium hypochlorite plus vinegar (256 ppm Cl) and sodium hypochlorite alone (1000 ppm Cl) (halogens), 2% Citrox 14W (plant extract), 70% ethanol (alcohol) and moist heat (water bath at 45°C). Water and water plus inactivator were used as controls. Cystosori were treated for 10 minutes.

Treated cystosori suspensions (20 mg) were added to plastic cups, each with one tomato seedling (1<sup>st</sup> true leaf stage), which were than incubated in the dark at 15°C for 3 days. The pots were then placed on glasshouse benches and grown on for 21 days before assessing roots for infection. All treatments were replicated six times and the experiment was repeated once.

Further experiments were conducted to compare disinfectant chemicals at one and five time recommended label rates and at 1% and 5% product. In these experiments, cystosori suspensions were treated for 2 minutes. The viability of treated cystosori was tested using a tomato seedling bioassay method adapted from the methods of Merz (1989). In this bioassay, tomato seedlings were grown in tomato hydroponic nutrient solutions. The procedure involved a 9 d incubation period (15°C, dark), a 24 hr infection period and a 7 d cultivation period (15°C, 12 hrs light/12 hrs dark). The experimental unit was 6 tomato seedlings per treatment. Each experiment was repeated twice.

#### **Results and Discussion**

Overall, the results show that all classes of chemicals tested could potentially reduce the viability of powdery scab cystosori ((Figure 8, Figure 9 and Figure 10). However, there were no treatments that killed all spores at the recommended rates for surface disinfection. Five times the recommended label rate of some disinfectants was necessary (Figure 9 and Figure 10) to achieve reductions in viability of up to 90-100%. There was considerable variation between experiments and we are unable to explain the reasons for this. However, the phenols (Biogram, Kendocide), peroxygens (Peratec 5 Sanitiser, Perfoam 2, Virkon S) and the QACs (Phytoclean, Sporekill) were generally the most consistent in efficacy.

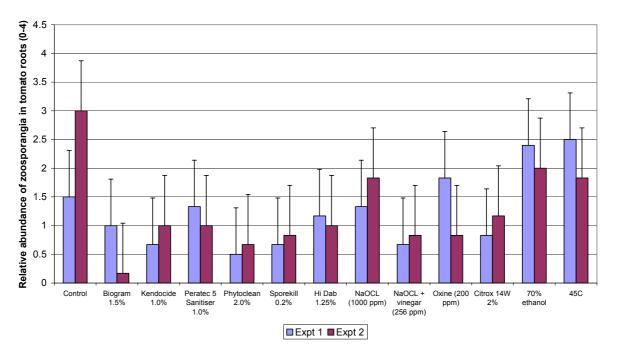
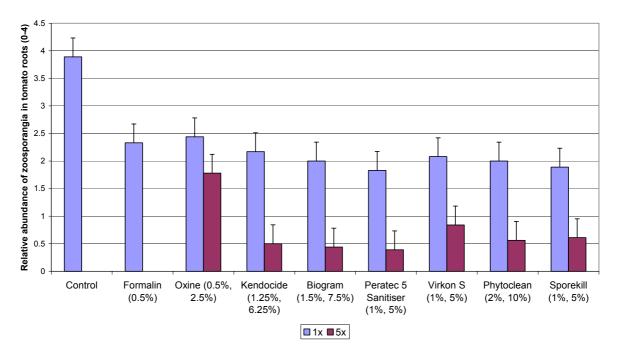


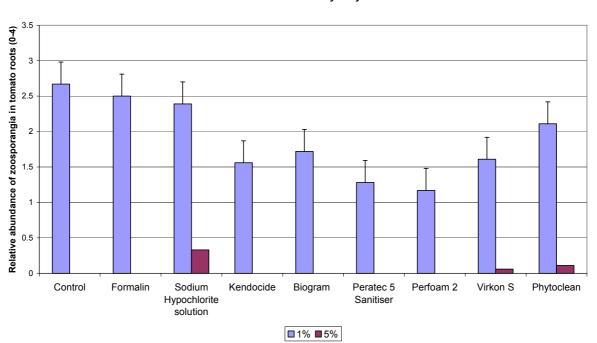


Figure 8 Effects of disinfectant treatments at recommended rates (10 minute immersion) on the viability of cystosori of *S. subterranea* as determined by a tomato seedling bioassay (Bars above the histograms represent the lsd at P=0.05)



Effect of disinfectant treatments on the viability of cystosori of S. subterranea

Figure 9 Effect of disinfectant treatments on the viability of cystosori of *S. subterranea* as determined by a tomato seedling bioassay (Cystosori exposed to disinfectant chemicals for 2 minutes at 1 and 5 times recommended labels rates for surface disinfection) (Bars above the histograms represent the lsd at P=0.05)



Effect of disinfectant treatments on the viability of cystosori of S. subterranea

Figure 10 Effect of disinfectant treatments on the viability of cystosori of *S. subterranea* as determined by a tomato seedling bioassay (Cystosori exposed to disinfectant chemicals for 2 minutes at 1% and 5% of product) (Bars above the histograms represent the lsd at P=0.05)

### 3.3.4.3 Testing disinfectants against common potato pathogens on different surface materials

Protocols for cleaning and disinfection include mechanical cleaning (eg vacuuming) and high pressure washing before using a disinfectant. This ensures that most soil, organic matter and pathogens are removed. Also, organic matter reduces the efficacy of some groups of disinfectant chemicals (see Section 3.3.4.1 and Table 6). Experiments were conducted to determine the effect of several disinfectant treatments on the survival of some common potato pathogens on various surface materials likely to be encountered in the potato shed.

#### Material and methods

Five disinfectant treatments were evaluated for their ability to disinfect four surface materials artificially contaminated with inoculum of five potato pathogens. The pathogens tested were *Erwinia carotovora* var *atroseptica, Erwinia carotovora* var. *carotovora, Ralstonia solanacearum, Fusarium trichothecioides* and *Helminthosporium solani*, responsible for the diseases blackleg, bacterial soft rot, bacterial wilt, dry rot and silver scurf. The surfaces tested were concrete, zincalum metal (used for shed walls and roofs), plastic (pot labels) and wood (matchsticks). Disinfectant choice was based on the results of previous *in vitro* suspension tests (see Section 3.3.4.1). The best representative from each chemical group was chosen and tested at the recommended label rate for dirty surfaces *ie*. Phytoclean<sup>™</sup> (quaternary ammonium compound), Biogram<sup>™</sup> (phenol), Peratec 5 Sanitiser<sup>™</sup> (peroxygen), Sodium hydroxide @ 1000 ppm Cl (halogen) and 70% ethanol (alcohol). Water was used as a control. The disinfection treatments were applied in both the presence and absence of organic matter.

Thirty-six 2 cm<sup>2</sup> samples of each surface were autoclaved prior to use, with the exception of plastic that was soaked in 70% ethanol for 30 mins and then rinsed with sterile distilled water. To simulate dirty conditions, 18 samples of each surface were 'dirtied' by dipping in organic matter (yeast extract for bacteria and peat extract for fungi) and left to air-dry in a laminar flow cabinet.

For each disinfectant treatment, three samples of the four surfaces were placed in a metal tray with a lid (*ie.* 12 pieces per tray). This was duplicated for both clean and dirty surfaces. Fungal spore/bacterial cell suspensions were made up and 0.5 mL added to each surface, then left to dry overnight (12 hours) in the laminar flow cabinet. The number of viable colony-forming units (cfus) was estimated by plating 3 x 0.5 mL aliquots of suspension onto media and counting the resultant colonies after several days incubation at room temperature (20-22°C). The mean of the three replicate 0.5 mL aliquots was taken to represent the original number of cfus placed onto the surfaces prior to disinfection.

Each of the disinfectants was tested at the recommended label rate for ten minutes. They were applied to the surfaces in the metal trays until run-off using an atomiser. The trays were immediately covered with lids to prevent the disinfectant drying out or being inactivated by light. After ten minutes, the treated surface pieces were removed using forceps and placed into test tubes containing two mL inactivator solution (4 g sodium thiosulphate plus 30 mL Tween 80 made up to 300 mL with sterile distilled water) to stop the disinfection process. The tubes were allowed to stand for 1 hour and shaken vigorously. The inactivator solution was removed and plated onto 90 mm petri dishes containing nutrient agar or potato dextrose agar. The plates were incubated for several days at room temperature (20-22°C) and then

examined for cfus. The mean cfus of the three replicate samples were calculated for each surface-disinfectant-pathogen combination.

#### **Results and Discussion**

The recovery of viable potato pathogens after 12 hours was dependent on the type of organism and the type of surface, even when no disinfectant treatment was applied (Table 5). On clean surfaces, very few viable bacteria were recovered in comparison to dirty surfaces. Viable fungi could be recovered from all surfaces, particularly from concrete and wood.

Viable bacterial cells were not readily recovered from untreated surfaces and, therefore, it is difficult to make any real comparisons about the relative effectiveness of each disinfectant treatment. However, it is evident from this study that 70% ethanol was the best general-purpose disinfectant. This contrasts with the results of *in vitro* suspension tests in which Biogram and Peratec 5 Sanitiser were the most effective disinfectant treatments [Section 3.3.4.1], suggesting that different properties of each surface material, such as pH, interact with and/or alter the properties of the disinfectants.

Overall, higher numbers of viable pathogens were recovered from dirty surfaces, suggesting that the efficacy of the disinfectants were reduced in the presence of organic matter. The organic matter may also have provided some protection to the pathogens, improving their survival, or may have reduced the adhesion of the pathogens to the surface allowing a greater recovery of viable pathogens.

Of the surfaces tested, both clean and dirty wood had the highest levels of recoverable cfus for all pathogens and disinfectant treatments. It is noteworthy that wood is itself an organic material, unlike concrete, plastic and metal. This indicates that wooden surfaces may be an important source of contamination in the shed.

Table 5 Recovery of potato pathogen inoculum after disinfectant treatment of clean and dirty surfaces of concrete, metal, plastic and wood. Pathogens tested were *Helminthosporium solani* (*Hs*), *Fusarium trichothecioides* (*Ft*), *Ralstonia solanacearum* (*Rs*), *Erwinia carotovora* var. *atroseptica* (*Eca*) and *E.c.* var. *carotovora* (*Ecc*).

			Cle	ean		Dirty				
Disinfectant	Pathogen	Concrete	Metal	Plastic	Wood	Concrete	Metal	Plastic	Wood	
Control (water)	Hs	+++	+	+	+++	+++	++	++	+++	
. ,	Ft	++++	++++	++++	++++	++++	+++	+++	++++	
	Rs	-	-	-	-	-	++	++	++	
	Eca	-	-	-	+	++	+++	++++	++++	
	Ecc	-	-	-	-	-	-	+	++	
Phytoclean	Hs	+	-	-	++	++	++	++	+++	
-	Ft	+	-	-	+++	++	+	++	++++	
	Rs	-	-	-	-	-	-	-	++	
	Eca	-	-	-	-	-	-	++	+++	
	Ecc	-	-	-	+	-	-	-	+++	
Biogram	Hs	+	-	-	++	+++	+	-	++	
-	Ft	++	-	-	+++	++	-	-	++++	
	Rs	-	-	-	-	-	-	-	++	
	Eca	-	-	-	-	-	+	+++	+++	
	Ecc	-	-	-	-	-	-	-	++	
Peratec 5	Hs	+++	-	-	-	+++	-	-	-	
Sanitiser	Ft	+++	-	-	+++	+++	+	-	+++	
	Rs	-	-	-	-	-	-	-	+	
	Eca	-	-	-	++	++	+	+++	++	
	Ecc	-	-	-	-	-	-	-	+	
Sodium	Hs	+++	-	++	-	++	++	++	+++	
Hypochlorite	Ft	-	-	-	++++	-	-	-	++++	
(1% Cl)	Rs	-	-	-	-	-	+	+	+	
	Eca	-	-	+	+	+	-	+++	++	
	Ecc	-	-	-	-	-	-	+	++	
70% ethanol	Hs	-	-	-	-	+	-	-	-	
	Ft	-	-	-	+++	++++	-	-	+++	
	Rs	-	-	-	-	-	-	+	+++	
	Eca	-	-	-	-	-	-	+	+++	
	Ecc	-	-	-	-	-	-	+	+++	

-=0-0.01% cfus recovered

+ = 0.01 - 0.1% cfus recovered

++=0.1-1% cfus recovered

+++=1-10% cfus recovered

++++ = 10 - 100% cfus recovered

## 3.3.4.4 Testing disinfectants against pathogens on 'dirty' metal and wooden surfaces

The previous study described the testing of disinfectants against potato pathogens on clean surfaces and 'dirty' surfaces coated with yeast or peat extracts for bacteria and fungi, respectively. The results of the tests were inconclusive because the fungal and bacterial pathogens were not readily recovered from the clean, inert surfaces, particularly the bacteria (*Eca, Ecc* and *Ralstonia solanacearum*). Either the pathogens died under these conditions or were removed during the experimental procedure.

Studies on the testing of disinfectants against gram negative bacteria (eg *Eca*, *Ecc* and *Rs*) on hard surfaces have shown that these bacteria are vulnerable to drying, particularly when suspended in water without any proteinaceous material (Van Klingern *et al.* 1998). To test the survival of bacteria on a surface, cells of *R. solanacearum* were suspended in solutions of sterile distilled water, phosphate buffer or two different protein solutions (0.1% tryptone and 20% potato decoction). Droplets of each suspension were allowed to dry on the surface of zinc-alum metal coupons for one hour. Viable bacteria were recovered from the tryptone and potato decoction treated surfaces but not from the water or buffer treated surfaces. This confirms that a pathogenic gram negative bacteria such as *R. solanacearum* will not survive as a pure culture on a clean, dry surface, but could survive in dried potato juice smeared on various surfaces in the potato shed as would occur during sorting and handling of diseased potatoes.

In the light of this, further experiments were carried out to test the efficacy of disinfectant treatments against a bacteria (*E. carotovora* var *carotovora*) and a fungal pathogen with melanised spores (*H. solani*) suspended in potato dextrose broth as a protein source on a wooden and metal surface. The treatment of these 'dirty' surfaces ensured survival of the pathogens, providing a more robust test for the disinfectant chemicals.

#### Materials and Methods

Eight disinfectant products were tested on a wooden surface (smooth-planed 'radiata' pine) and a metal surface (aluminium) smeared with potato dextrose broth contaminated with either *Erwinia carotovora* var. *carotovora* (*Ecc*) or *Helminthosporium solani* (*Hs*), the causal organisms of bacterial soft rot and silver scurf of tubers, respectively. The products tested were Formalin (aldehyde), sodium hypochlorite (halogen), Biogram (phenol), Peratec 5 Sanitiser, Perfoam 2, Virkon S (peroxygens), Phytoclean and Farmcleanse (quaternary ammonium compounds). Water was used as a control.

The wooden and metal blocks had a surface area of 25 cm<sup>2</sup>. Prior to treatment, the metal blocks were sterilised by soaking in 70% ethanol for 30 minutes and rinsed in sterile distilled water. Wooden blocks were sterilised by autoclaving (121°C, 20 minutes).

The surfaces of the metal and wooden blocks were evenly coated with 100  $\mu$ L and 200  $\mu$ L respectively of *Ecc* bacteria or *Hs* spores (10<sup>4</sup> cfus/mL) suspended in potato dextrose broth (Amyl) and allowed to dry in a laminar flow cabinet for 20 minutes. There were three replicate blocks for each surface-pathogen-disinfectant treatment combination. Prior to inoculation, the surfaces of the wooden blocks were wetted with 100  $\mu$ L sterile distilled water to prevent absorption of the test suspension. Disinfectant solutions were sprayed onto the blocks using an atomiser (1 mL/block) at concentrations of 1% or 5% of product. The

controls were sprayed with sterile distilled water instead of disinfectant. The treated surfaces were placed face down into 90 mm diameter petri plates of nutrient agar (for *Ecc*) or Rose Bengal media (for *Hs*) flooded with 1 mL of autoclaved inactivator solution at 1, 5 or 20 minutes after treatment. The metal surfaces were gently rotated across the agar surface (360°) and the wooden surfaces gently tapped onto the surface five times in different directions to dislodge the pathogen cfus. After 60 hrs incubation at 21°C, the resultant colonies of *Ecc* and *Hs* were counted. The mean cfus of the three replicate samples were calculated for each surface–disinfectant–pathogen combination. Each experiment was repeated twice.

Differences between treatments were determined using analysis of variance (Genstat for Windows 5<sup>th</sup> Edition <sup>TM</sup>, Lawes Agricultural Trust, Rothamsted Experimental Station). The raw data was first transformed using the square-root transformation in order to satisfy the assumptions of normality required ANOVA of this type of data.

#### **Results and Discussion**

The numbers of cfus recovered from the control blocks were 2200-2800 *Ecc* (Figure 11 and Figure 12) and 640-680 *Hs* (Figure 13 and Figure 14).

All disinfectant treatments resulted in significant (P<0.001) reductions in the proportion of *Ecc* and *Hs* cfus recovered from both wooden and metal surfaces (Figure 11, Figure 12, Figure 13 and Figure 14). Overall trends showed that treatments were more effective on the metal surface than on the porous wooden surface. It should be noted, however, that wooden surfaces were inoculated with twice the amount of inoculum than the metal surfaces in order to recover measurable numbers of cfus from the latter.

Increasing the time of exposure to a disinfectant resulted in proportionate reductions in the recovery of both *Ecc* and *Hs* from treated surfaces. The relative effects of increasing the concentration of a disinfectant compared with increasing exposure times varied with the chemical. In general, increasing the concentration from 1% to 5% was more effective than increasing the exposure time with a concentration of 1% product.

*Ecc.* The peroxygen compounds, Peratec 5 Sanitiser and Perfoam 2, and the aldehyde Formalin were very effective against *Ecc* at the lower concentration (1%) and the shortest exposure time (1 min) on both wood (Figure 11) and metal (Figure 12) The QACs, Phytoclean and Farmcleanse, were the least effective.

*Hs.* Once again, the peroxygen compounds, Peratec 5 Sanitiser and Perfoam 2, and the aldehyde, Formalin, were the most effective against Hs on both wood (Figure 13) and metal (Figure 14), requiring only the low dose at 1 minute for maximum effect. Virkon S was equally effective when used at the higher rate (5%). Sodium hypochlorite, Biogram, Phytoclean and Farmcleanse were the least effective, particularly on wooden surfaces (NB the recommended concentration for Farmcleanse as an antifungal agent is 10% product).

In conclusion, the results of these experiments demonstrated that all the disinfectant chemicals tested have the potential to disinfect surfaces contaminated with *Ecc* and *Hs*. However, the peroxygen compounds, Peratec 5 Sanitiser and Perfoam 2, were effective against both pathogens at the recommended label rates for one minute, while the others required higher doses or long exposure times to achieve similar efficacies. Formalin was also effective, but is not recommended for general use as a sanitiser because of its potential to cause harm to humans (Anon 1999).



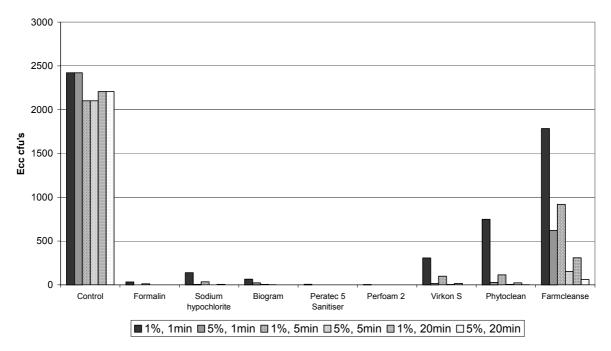


Figure 11 Recovery of *Erwinia carotovora* var. *carotovora* (cfus) from a wooden surface (dressed pine) after treatment with disinfectant chemicals at 1% and 5% product for 1, 5 or 20 minutes (Data back-transformed)

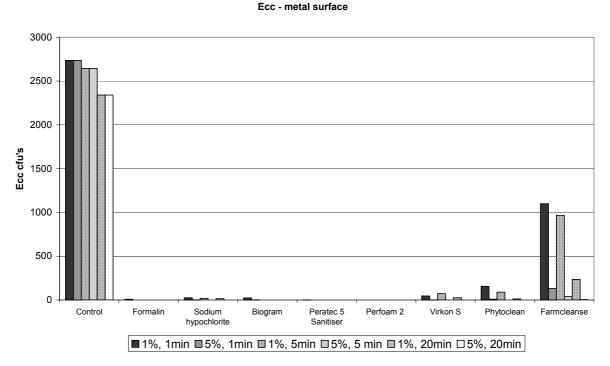


Figure 12 Recovery of *Erwinia carotovora* var. *carotovora* (cfus) from a metal surface (aluminium) after treatment with disinfectant chemicals at 1% and 5% product for 1, 5 or 20 minutes (Data back-transformed)

H. solani - wooden surface

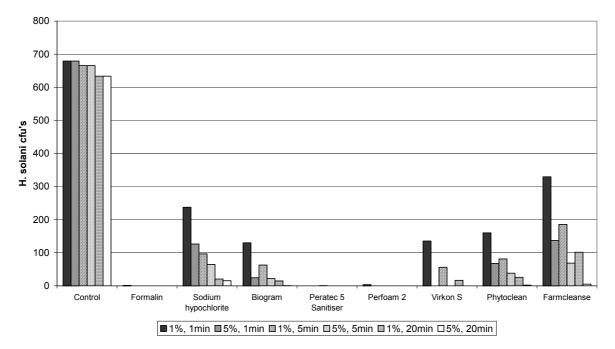


Figure 13 Recovery of *Helminthosporium solani* (cfus) from a wooden surface (dressed pine) after treatment with disinfectant chemicals at 1% and 5% product for 1, 5 or 20 minutes (Data back-transformed)

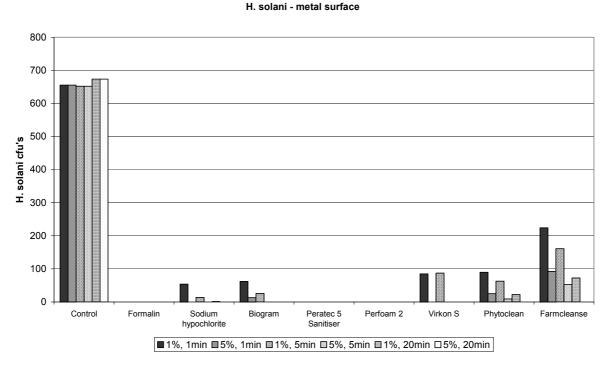


Figure 14 Recovery of *Helminthosporium solani* (cfus) from a metal surface (aluminium) after treatment with disinfectant chemicals at 1% and 5% product for 1, 5 or 20 minutes (Data back-transformed)

Horticulture Australia Project PT97015

#### 3.3.4.5 Testing disinfectants by treating diseased seed tubers

#### Using seed potato tubers as a model for testing disinfectants

Evaluating the effects of disinfectant treatments on biological organisms is often done *in vitro* where the organism is not in its natural environment. Two potato pathogens, *Rhizoctonia solani* (*Rs*) and *Colletotrichum coccodes* (*Cc*), causing black scurf and black dot respectively, occur as sclerotia on the skin of potato tubers and can be excised and cultured on agar with relative ease. A series of experiments was conducted in which diseased tubers were immersed in disinfectant solutions, segments of skin with sclerotia excised from the tuber and plated onto media, and the subsequent fungal growth was measured. In this way, the diseased tubers provided a 'model' with which to obtain data on the efficacy of disinfectants under more natural conditions, as well as providing data on the efficacy of the treatments against the thick walled, melanised survival structures produced by some potato pathogens.

Potato farmers also use disinfectant treatments to treat seed potatoes. Potato eyes and sprouts are at risk from damage by the disinfectant chemicals, especially if potato tubers have broken dormancy. Experiments were also conducted to determine whether any of the disinfectants would cause damage to the emerging potato sprouts.

#### **Materials and Methods**

#### Efficacy

Experiment 1

Six commercially available disinfectants were tested for their efficacy against sclerotia of *R*. *solani in situ* at the recommended label rate for hard surface disinfection. Whole, commercial seed potato tubers (cvs. 91-106-1, Coliban) with symptoms of black scurf (*Rs*) were immersed in the disinfectant solutions or distilled water (control) for 2 minutes, removed and allowed to dry for one hour. Four tubers were used per treatment. Four 3.5 mm diameter skin cores, each with a 3-4 mm diameter sclerotia of *Rs*, were excised from each tuber, rinsed in sterile-distilled-water, cut in half and plated onto potato dextrose agar amended with 25  $\mu$ g/mL tetracycline hydrochloride. The plates were incubated at 21°C and the colony diameters of any subsequent growth was measured 48 and 72 hours.

Experiment 2

The same methodology was used as described above, but with the additional disinfectant products (Figure 16). Each disinfectant was tested at x1 and x5 the recommended rate.

#### Experiment 3

A third experiment was conducted to test whether one of the disinfectants that showed promise in the previous experiments, Peratec 5 Sanitiser, would be more effective if used with a surfactant,. The same methodology described above was used again, but this time to compare Peratec 5 Sanitiser used at x1 and x5 label rates, with and without the surfactant, Triton X-100. In this experiment, colony growth was measured after 45 and 69 hours incubation.

#### Experiment 4

Th same six disinfectants were tested for their efficacy at x1 and x5 the recommended label rate in experiment 2 was tested agaisnt black dot (Cc). Tubers of cv. Russet Burbank covered

with *Cc* micro-sclerotia were subjected to the same experimental procedure as described above, and colony growth was measured after 69 and 93 hours incubation.

#### Phytotoxicity

This experiment was designed to test whether of the six disinfectants tested above would cause damage to emerging potato sprouts when the seed had been treated at both x1 and x5 the label rates. Sebago minitubers which had just broken dormancy and desprouted Coliban commercial seed (physiological age 12 months) were treated with disinfectants as described above and then planted into pasteurised sand/peat in 7.5 and 15 cm diameter plastic pots respectively. The plants were grown in a glasshouse for 41 days, then assessed for the proportion of plants that emerged, the number of stems/plant and plant height.

Differences between treatments were determined by analysis of variance (Genstat for Windows 5<sup>th</sup> Edition <sup>TM</sup>, Lawes Agricultural Trust, Rothamsted Experimental Station).

#### **Results and Discussion**

When applied at the rates recommended for surface disinfection, Formalin and Biogram were very effective against *Rs* sclerotia, although some growth was still apparent after 72 hrs (Figure 15). Peratec 5 Sanitiser caused an initial fungistasis, but by 72 hours the fungal growth was no different from the control. When the disinfectants were applied at x5 the label rates, Peratec 5 Sanitiser proved as effective as Formalin and Biogram, and Phytoclean and sodium hypochlorite also significantly reduced colony growth (Figure 16). In experiment 3, the addition of the surfactant enhanced the efficacy of Peratec 5 Sanitiser at the label rate, but still not to the level of x5 label rate without surfactant (Figure 17). Interestingly, the surfactant by itself was also quite effective.

Only Biogram applied at x5 label rate significantly (P $\leq 0.05$ ) reduced the growth of *Cc* sclerotia, which was apparent at 69 hrs, but not at 93 hrs when the colony diameter did not differ significantly (P>0.05) from the control (Figure 18). The *Cc* micro-sclerotia are partially embedded in the potato periderm giving them some protection from disinfectant chemicals. By comparison, the *Rs* sclerotia sit on the skin surface and are, therefore, more exposed to the chemicals. Treatment with sodium hypochlorite, Peratec 5 Sanitiser and Phytoclean at the high rate enhanced the growth of *Cc* sclerotia compared with the untreated control (Figure 18). This is possibly because disinfectants killed antagonists to the fungus or because the treatments affected the integrity of the sclerotial surface without affecting viability, thereby enhancing, rather than inhibiting germination.

All the disinfectants except Oxine at the label rate were phytotoxic to the commercial Coliban seed tubers (Figure 19). The least damaging were sodium hypochlorite and Phytoclean when applied at the label rate. The Sebago minitubers were less severely affected, although the x5 rates of Biogram and Phytoclean killed all sprouts on these tubers (Figure 19). The treatment of tubers that have broken dormancy or tubers that have been desprouted is a severe test of phytotoxicity. Nevertheless, this demonstrates the risks of treating tubers with disinfectant chemicals. Tubers should only be treated with these chemicals prior before they have broken dormancy.

#### Conclusions

We expected that the sclerotia of *Rs* and *Cc* would be difficult to kill, particularly with the added complication of the organic nature of potato skin. The sclerotia are protected by thick,

melanised walls whose resistance to chemical degradation is well documented (Butler 1998). Although a number of products from the aldehyde, phenol, peroxygen and QAC groups reduced the viability of the sclerotia, Formalin, Biogram and the x5 label rate of Peratec 5 Sanitiser were particularly effective. This is consistent with the results of the *in vitro* study in Section 3.3.4.1 that found Biogram and Peratec 5 Sanitiser to be the most efficacious against another fungus with melanised spores, *Helminthosporium solani* (silver scurf) (Formalin was not tested in this study). This study has shown that the sclerotia of *Cc* are relatively resistant to chemical disinfectants *in situ*.

The Oxine treatments proved to be relatively ineffective when used as a tuber 'dip'. In our experience and that of our colleagues (Robert Holmes, personal communication), Oxine is generally not effective when surfaces requiring treatment, such the potato skin and garden stakes for instance, are dipped into the solution. This may be because agitation of tubers or stakes in the solution causes the chlorine dioxide to vaporise. Sodium hypochlorite also proved to be relatively ineffective, even at the relatively the high rates used here, perhaps because the efficacy is reduced significantly when organic matter is present (see Section 3.3.4.1).

Some of the most effective disinfectant chemicals proved to also by very phytotoxic to potato sprouts. Although farmers usually avoid treating tubers that have broken dormancy, our results highlight the risk of treating tubers with disinfectant chemicals to control pathogens. Tubers should only be treated when potato tubers are dormant.

Treating potato tubers (ie organic material with a large surface area) with disinfectant chemical represents an extreme test of efficacy. It should be noted that when treating hard surfaces on the farm, the cardinal rule is to clean the surface before applying the chemical. Our results showed that the addition of a surfactant to the disinfectant chemical could enhance their efficacy. Surfactants are included in formulations of some sanitisers (see Table 2).

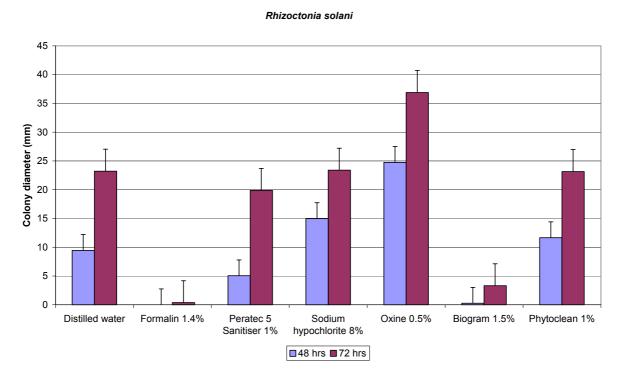


Figure 15 Effects of disinfectant treatments of whole seed tubers affected with black scurf (2 min immersion) on the viability of *Rhizoctonia solani* sclerotia, as measured by the radial growth of the fungi on PDA (Bars above the histograms represent the lsd at P=0.05)

Rhizoctonia solani

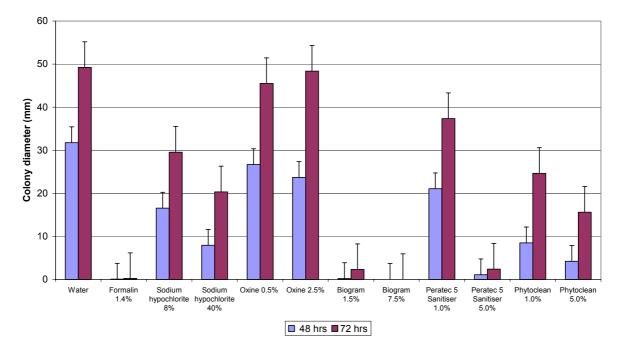


Figure 16 Effects of disinfectant treatments of whole seed tubers with black scurf (2 min immersion) on the viability of sclerotia of as measured by the radial growth of *Rhizoctonia* solani on PDA (48 hr & 72 hrs after plating) (Bars above the histograms represent the lsd at P=0.05)

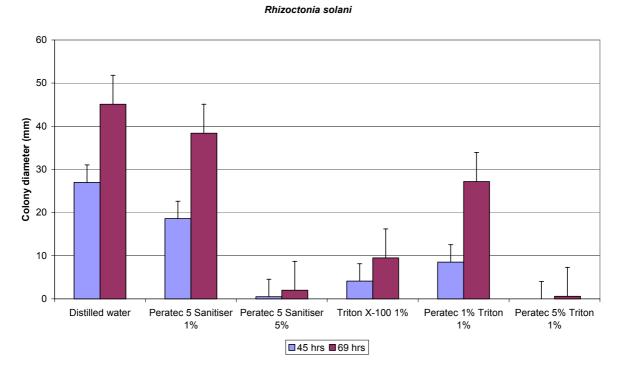


Figure 17 Effects of disinfectant treatments, with and without a surfactant, of whole seed tubers (cv. Coliban) with black scurf (2 min immersion) on the viability of sclerotia as measured by radial growth of *Rhizoctonia solani* on PDA (Seed tubers immersed for 2 minutes) (Bars above the histograms represent the lsd at P=0.05)

Colletotrichum coccodes

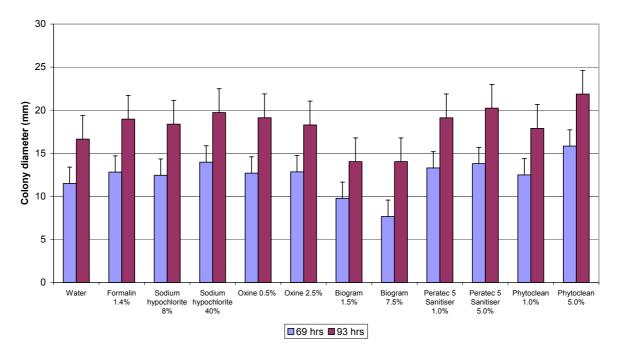


Figure 18 Effects of disinfectant treatments of whole seed tubers with black dot (2 min immersion) on the viability micro-sclerotia as measured by the radial growth of *Colletotrichum coccodes* on PDA (69 hrs & 93 hrs after plating) (Bars above the histograms represent the lsd at P=0.05)

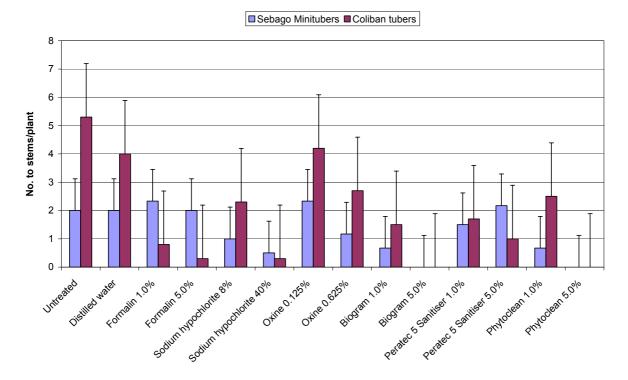


Figure 19 Effects of disinfectant treatments of seed tubers (2 min immersion) on the number of stems/plant (Sprouts were removed from Coliban tubers but were left intact on Sebago minitubers prior to treatment) (Bars above the histograms represent the lsd at P=0.05)

# 3.3.4.6 Disinfectants on the potato farm – a summary

Under laboratory conditions, several disinfectant treatments, representing the main classes of disinfectant chemical groups were effective against cultures of the common potato pathogenic bacteria and fungi at the recommended label rates for hard surface disinfection. However, only two treatments, Biogram (phenol) and Peratec 5 Sanitiser (peroxygen) were effective against the melanised spores of *H. solani*. In these tests, the efficacy of the chlorine-based chemicals (chlorine dioxide and sodium hypochlorite) was reduced in the presence of organic matter. In contrast, the same treatments were relatively ineffective against the cystosori of the powdery scab pathogen *S. subterranea*. Five times the label rates were required to achieve a 95-100% reduction in viability of this pathogen.

The disinfectant treatments Formalin (1.5% product), Biogram (1.5% product) and Peratec 5 Sanitiser (5% product) had the greatest impact on the viability of the melanised sclerotia of R. *solani in situ* (ie on the potato tuber). No treatments effectively reduced the viability of sclerotia of *C. coccodes* on tuber surface.

Most treatments reduced the viability of *Ecc* and *H. solani* on 'dirty' (coated in potato dextrose broth) wooden and metal surfaces. Generally, treatments were more effective in disinfecting a metal surface that a wooden surface. The most effective treatments were Formalin (aldehyde) and two peroxygen products (Peratec 5 Sanitiser and Perfoam 2) at the recommended label rates and the shortest exposure times (1 min).

# 3.3.5 When to use disinfectants

A disinfectant treatment is the last step in a cleaning and disinfection program. A stated earlier, the most important step in a disinfection program is mechanical cleaning which involves the removal of dust, soil and organic debris (eg dried potato juices) through vacuuming and washing. The cleaning process can remove most, if not all, of the contaminants. The wash-down and drying process can render a disinfectant redundant in some cases, particularly with bacterial contamination.

Disinfectants are best used after wash-down:

- When cleaning walls and floors of sheds and stores annually for an extra high standard of hygiene;
- On boxes that have had carried particularly badly diseased stocks;
- On a grading line after sorting diseased stocks when high quality stocks need to be graded next;
- On seed cutting equipment between different seed stocks.
- On planting and harvesting equipment that has been used in a 'diseased' paddock and must be used in 'new' ground or in ground with a lower disease risk.

# 3.3.6 The registration of disinfectants

There is a bewildering array of cleaning/sanitiser/disinfectant products available for the rural producer. The majority of these products are not registered under legislative codes for agricultural chemicals regulated by the National Registration Authority (NRA). If a product

label claims to control a specific plant pathogen it must be registered with the NRA (Anon 2002) and any claims must be supported with the necessary efficacy and toxicological data. The product Phytoclean, for example, carries NRA approval for the claim that it contains active ingredients that control *Phytophthora cinnamomi* when used as a wash-down and hard surface disinfectant. This means that most commercial disinfectants cannot be recommended for specific purposes. However, data from this project can be used to guide growers in choosing appropriate chemicals for use on the potato farm.

# 3.4 Conclusions

#### The shed as a source of infection

The production of early generation seed potatoes, the leasing or buying of new ground to grow potatoes, and the procurement of high quality seed stocks for commercial production represent a significant investment for potato growers. This project has shown that potato sheds are a source of contamination and infection of seed stocks with the common potato pathogens. The process of handing and storing high health seed stocks in the potato shed and cool store can potentially negate the investments made in the process of producing or purchasing high health seed stocks or in avoiding, preventing and controlling diseases in other parts of the farm operation.

#### Disinfection – the importance of cleaning

A good hygiene program is essential in protecting this investment and minimising the risk of contaminating seed stocks in the shed and cool store. A major component of any hygiene program is the disinfection procedure. Disinfection is a two stage process involving mechanical cleaning, ie the removal of dust, soil, debris and other contaminants from floors, walls, ceilings, grading and seed cutting equipment and boxes etc, with an optional follow-up with disinfectant chemicals. Cleaning can involve vacuuming and high-pressure washing. Researchers in Scotland evaluated various cleaning and disinfection strategies for potato stores and grading equipment and demonstrated tangible benefits in disease control after cleaning (Clayton *et al.* 1999, Clayton *et al.* 2000, Wale 2002).

Other strategies in a hygiene program include the separation of working areas from storage areas, concreting or asphalting all working areas within and outside the shed and separating the storage of early generation from older generation seed stocks.

In the study of shed dust, there was no apparent difference in disease risk from dust swept from concrete compared with earthen floors. However, the important distinction between the two is that concrete floors can be vacuumed and washed-down. The risk from the earthen floors cannot be removed.

#### Using disinfectant chemicals

Ideally disinfectants should only be used in a clean environment. The efficacy of disinfectant chemicals is reduced in the presence of soil, organic debris and potato juices, particularly so the chemical groups containing chlorine (eg sodium hydroxide and chlorine dioxide) and some QACs. Also, organic material, namely proteins, aids the survival of pathogens, especially bacteria.

Our research indicates that, in a clean environment, most commercially available disinfectants when used at recommended label rates for hard surface disinfection will be suitable for disinfecting clean non porous surfaces (eg metal and plastic) that may be contaminated with the common potato pathogens (bacteria and fungi). The exception is the melanised spores of *Helminthosporium solani* (silver scurf) against which only Biogram (synthetic phenols) and Peratec 5 Sanitiser (peroxygen) proved to effective.

Wooden surfaces are more difficult to disinfect that non porous surfaces like metal. Peratec 5 Sanitiser and Perfoam 2 (peroxygens) proved to be the most effective in disinfecting a wooden surface contaminated with *Erwinia* or *H. solani* at the label rates. Other treatments (eg Biogram, Virkon S and Phytoclean) required higher rates (x5) and longer exposure times to achieve a similar effect.

Relatively high rates (5x label rates for hard surface disinfection) of disinfectants were required for a high level of surety of killing cystosori of the powdery scab pathogen *Spongospora subterranea*. The most effective treatments were from the synthetic phenol, peroxygen and QAC groups (Kendocide, Biogram, Peratec 5 Sanitiser, Perfoam 2, Virkon S and Phytoclean).

Biogram (label rate) and Peratec 5 Sanitiser (5x label rate) were the most effective treatments against melanised sclerotia of *Rhizoctonia solani* (black scurf) on the surface of tubers. However, two treatments that had some efficacy against *R. solani* proved to be phytotoxic to potato sprouts (Biogram and Phytoclean at 5x label rates). There were no treatments that were effective in killing the sclerotia of the black dot fungus and *Colletotrichum coccodes*.

Generally, Formalin proved to be an effective disinfectant against potato pathogens under the different scenarios tested but is not recommended as a general-purpose sanitiser in the potato shed.

The effectiveness of a disinfectant chemical is a function of the concentration and time of exposure the higher the concentration or the longer the exposure time the more effective the treatment. Unlike fungicides, these chemicals do not have residual activity.

#### The benefits of a hygiene program

The adoption of a hygiene program on the potato farm can have a number of benefits. These include:

- Protection of high value seed stocks passing through the shed and cool store;
- The protection land, the most valuable asset on a farm, from contamination (new land) or reinfection (old land);
- Minimised disease risk and improvements in the quality of produce from the farm;
- Management and staff that are 'hygiene aware' and ready to adopt Quality Assurance programs if necessary;
- A better working environment for staff; and
- A 'clean' image which is good for business.

# 3.5 Hygiene protocols for the potato farm

# HYGIENE PROTOCOLS FOR THE POTATO FARM

# MENU

### Shed hygiene

Why bother with shed hygiene?

#### What can be done?

- Implement a hygiene policy for the farm
- Review the operations in the shed
- Reduce the amount of dirt and dust in the shed.
- When to use a disinfectant?
  - Use of disinfectants

#### Hygiene protocols for key areas in the shed

- Boxes
- Floors
- Walls and roof
- Grader (between seasons)
- Grader (during operations)
- Seed cutting

#### General potato farm hygiene

# SHED HYGIENE

### Why bother with shed hygiene?

• The potato shed is a source of disease



- The dust in the shed contains propagules of disease-causing organisms (pathogens) and is distributed around the shed resulting in the contamination of high value seed stocks, boxes, graders, walls and floors
- Diseased tubers contaminate other tubers, boxes, grading equipment and seed cutters
- Infective propagules of several pathogens can spread around the shed in air and contaminate high value seed stocks, boxes and equipment
- Infective propagules of pathogens are often more concentrated in the shed dust than in the fields in which seed stocks are planted
- Contamination of high value seed stocks can negate labour and financial investments in early generation seed production, leasing of new land and grading seed stocks for certification
- Contamination of seed stocks in the shed can increase the risk of contaminating new production areas and adds to disease pressure in traditional production areas

- Hygiene is an integral component of disease management programs. Poor hygiene can negate the benefits of other control options such as fungicides and crop rotation
- Shed dust may contain fungicide-resistant strains of pathogens that can be introduced into new areas
- A high level of dust in shed is an OH& S issue. Breathing dust for long periods is detrimental for human health. Keeping dust levels to a minium results in a better working environment
- Shed hygiene is an important disease management strategy and should be adopted as part of a quality assurance program

# What can be done?

- Implement a hygiene policy for the farm
  - A hygiene policy is an important component of any quality assurance program
  - It provides guidelines for you and your workers
  - Increases awareness of the need to be clean
  - Creates a better working environment
  - Is good for business: 'clean farm, clean image'



Return to menu

#### • Review the operations in the shed

- Can equipment be relocated to reduce the amount of dust produced or disturbed?
- Can the grading area be separated from the storage area to prevent contamination during storage?
- Can you store early and late seed generations separately, or store seed separately according to disease status?
- Is the floor sealed (concrete or asphalt) to allow cleaning?
- Are the working areas at the entrance to the shed sealed (concrete, asphalt)?
- Do you have a wash down area for bins and equipment?
- Do you own a vacuum cleaner and high pressure washing equipment?

#### Return to menu

#### • Reduce the amount of dirt and dust in the shed.

- Vacuum the traffic areas to keep dust to a minimum. Do not sweep
   this just redistributes the dust
- Wash down equipment to remove dirt
- Install extraction fans above grading area to expel dust from the shed

- Install scrapers on rollers and conveyer belts to prevent the build up of dirt and make cleaning easier and quicker
- Adopt a cleaning regime and USE it regularly

#### Return to menu

# When to use a disinfectant?

- When cleaning the shed (walls and floors) at the end of the season for an extra high standard of hygiene
- On boxes that have contained particularly badly diseased tubers or soil
- On the grading line during the season, especially if a high value seed stock needs to be graded after grading a diseased batch of tubers.
- On the seed cutter during the season between different batches of seed

#### Use of disinfectants

- Always READ THE LABEL first before use and disposal (get advice from the supplier if necessary)
- Clean first, THEN disinfect
- Always wear the recommended personal protection equipment when using disinfectants
- Disinfectants are a "one shot" treatment. They do not have a residual action which protects the treated surface from re-contamination. Keep the shed clean!

#### Return to menu

### Hygiene protocols for key areas in the shed

#### **Boxes**

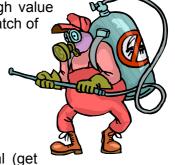
- It may be impractical to clean all boxes.
   However, boxes that are heavily soiled and those that contained diseased tubers or rotted tubers should be cleaned
- High-pressure wash boxes with detergent to remove debris and soil. Rinse and spray with a disinfectant
- Early generation seed growers should consider managing box use earmark boxes for early generation stocks only. Also consider cleaning all boxes as described above

#### Return to menu

#### Floors

- Vacuum dirt and dust from floors. Do not sweep sweeping redistributes the dust and increases potential for contamination
- High-pressure wash floors between seasons





 Vacuum floors at least once a day during the season, particularly in heavy fork-lift travel areas

#### Return to menu

#### Walls and roof

- Vacuum where dust collects (eg ledges and beams)
- Vacuum or wash walls taking care around electrical equipment

#### Return to menu

#### Grader (between seasons)

- Remove dirt from all surfaces using scrapers, brushes etc.
- Vacuum dirt from around the grader
- Vacuum the grading line
- High-pressure wash the grader and associated equipment

#### Return to menu

#### Grader (during operations)

- When and how often the grader needs cleaning will depend on the condition of the potatoes being graded
- The grader should be cleaned before grading a healthy stock, especially if a particularly diseased or rotted seed stock was graded previously
- Grade out diseased or rotted tubers early on the grading line to minimise the spread of spores over the grading line
- Clean soil from around and on the grader, particularly after grading heavily soiled stocks
- To clean the grading line, remove as much dirt and encrusted soil as possible along the grading line by vacuuming, scrapping and using a brush followed by a high pressure wash. Lightly spray with a disinfectant and leave to dry

#### Return to menu

#### Seed cutting

 High-pressure wash the seed cutter with detergent, rinse and follow up with a disinfectant between different seed stocks

#### Return to menu

Shed/equipment	Boxes	Floors	Grader	Shed/Store	Seed Cutter
Daily		~			
Between seed batches	~		~		~
Annual	~	~	~	~	~

### General cleaning schedule

# **GENERAL POTATO FARM HYGIENE**

A hygiene program in the potato shed will be of limited value without an overall farm hygiene program, especially if you use new ground to produce your crop. Your hygiene policy should cover your farm, the machinery and equipment that is taken onto new paddocks, and any contractors and visitors who visit your farm.

- Dirty machinery carries disease. Scrape off encrusted soil with scrapers or brushes. Consider installing a concrete washpad for washing down tractors, cultivators, planters, harvesters, etc, between paddocks and between seasons. High-pressure wash with detergent to remove as much soil as possible and follow with a disinfectant treatment if the machinery has been on soils with serious disease problems (eg bacterial wilt).
- Train staff in the importance of hygiene
- Develop an appropriate policy for visitors and contractors entering the farm or bringing machinery and bins onto the farm. Use footbaths with disinfectants

Return to menu

3.5.1 HACCP

Potato growers will be under increasing pressure to improve hygiene practices on their farms, not only to improve potato health but to comply with Quality Assurance and Quality Management schemes such as ISO 9000 and SQF 2000<sup>TM</sup>. HACCP (hazard analysis and critical control points) protocols for the seed potato production cycle were done. Flow charts, risk analysis tables and HACCP audit tables were done for many parts of the process and are presented in Appendix 2. These tools are useful in ascertaining the critical control points on the farm in developing and implementing hygiene and Quality Assurance programs.

# 3.6 Technology Transfer

The results of this project were presented to growers across Southern Australia through workshops, seminars and field days. Details of many of the presentations are listed below.

- Seed Potato Industry Workshop, Colac, 16-17 September 1998
- National Potato Field Day, Institute for Horticultural Development Toolangi, 18 February 1999
- Series of Grower Meetings, Tasmania, 18-19 June 1999
- Visit to IHD by growers from Dorrigo, NSW, 25 March 1999
- Agriculture Victoria, Knoxfield/VicSPA Consultative Committee Meeting, 15 June 1999
- Series of Grower Information Sessions, Perth, Bunbury, Manjimup, Albany, Western Australia, October 1999
- Series of half-day workshops held for Victorian Seed Growers, Thorpdale, Ballarat, Gellibrand, Portland, 9, 10, 16 and 17 November 1999
- Potatoes 2000, Linking Research to Practice. Australian Potato Research, Development and Technology Transfer, Adelaide, 31 July–3 August 2000
- Gippsland Seed Potato Growers Discussion Group, Trafalgar, Victoria, 9 August 2000,
- Potato Growers Seminar CHIPS Demonstration Farm, Bullarook, 10 August 2000
- Potato Growers Seminar CHIPS Demonstration Farm, Bullarook, 22 August 2001
- Gippsland growers meeting, Mirboo North, 10 October 2001
- Koo-Wee-Rup growers meeting, Cora Lynn, 30 October 2001
- Post-harvest handling course for Costa and Co personnel, 5 August 2002
- Potato Growers Seminar CHIPS Demonstration Farm, Bullarook, 27 August 2002
- Grower workshops, Colac, Portland and Ballarat, Victoria, 2-3 September 2002
- Grower workshops Devonport and Scottsdale, Tasmania, 7-8 October 2002
- ViCSPA Certification Workshop Toolangi, 17 January 2003

Publications from this project include conference papers, abstracts and posters, as well as articles in industry journals and the popular press.

de Boer RF (1998) Dirty potato sheds are for suckers. *Eyes on Potatoes* (Australian Potato Industry Council Newsletter). **5**, December 1998, p 4.

de Boer RF, Edwards J (1999) Managing seed potato health - the role of hygiene. *In*, Field notes for the National Potato Field Day (Potatoes for quality and profit). Institute for Horticultural Development, Toolangi, 18 February 1999.

Crump NS, de Boer RF (2000) Hygiene in the potato shed. Notes for Bullarook Growers Seminar, 10 August 2000, Bullarook, Victoria.

Crump NS, Gounder RK, Edwards J, Mann RC, de Boer RF (2001) The potato shed as a potential source of soil-borne pathogens on seed potatoes. *In* Proceedings of the Second Australasian Soilborne Diseases Symposium, The Cumberland Resort, Lorne, Victoria, 5-8 March 2001'. (Eds IJ Porter *et al.*), pp 133-134. (Second Australasian Soilborne Diseases Symposium, Victoria, Australia).

de Boer RF(2001) Summary of session on recognising the components of an integrated control approach to powdery scab and the potato mop top virus. *In* 'Proceedings of the First

European Powdery Scab Workshop, Scottish Agricultural College, Craibstone Estate, Aberdeen, Scotland, July 20-22, 2000. (Eds Ueli Merz and Alison K. Lees). pp 101-104.

de Boer R F (2001) Is a 'foot and mouth' type scenario likely for Aussie potatoes? *Eyes on Potatoes*. Australian Potato Council Newsletter, Volume 13, June 2001. Page 4.

de Boer, R.F. (2001). Research into the biology and control of powdery scab of potatoes in Australia. *In* 'Proceedings of the First European Powdery Scab Workshop, Scottish Agricultural College, Craibstone Estate, Aberdeen, Scotland, July 20-22, 2000. (Eds Ueli Merz and Alison K. Lees). pp 79-83.

de Boer RF, Curtis LW (2000) The significance of old and new ground as sources of disease in potatoes. *In* 'Potatoes 2000, Linking Research to Practice. Conference Proceedings of the Australian Potato Research, Development and Technology Transfer Conference, 31 July – 3 August 2000, Adelaide, South Australia'. (Eds C.M. Williams and L.J. Walters). pp 259-260. (South Australian Research and Development Institute, South Australia).

de Boer RF, Crump NS, Curtis LW (2001) Disinfecting potato tubers – an option for seedborne disease management? *In* 'Conference Handbook of the 13<sup>th</sup> Biennial Plant Pathology Conference of the Australasian Plant Pathology Society, Cairns, Queensland, 24-27 September 2001'. (Eds Veronica Oliver, Peter Trevorrow and Richard Davis). p 196.

de Boer RF, Inder P, Crump NS (2003) Effects of disinfectant treatments on the viability of *Spongospora subterranea* cystosori. *In* '8<sup>th</sup> International Congress of Plant Pathology, Volume 2 – Abstracts of Offered Papers, Christchurch, New Zealand, 27 February-2 March 2003'. p 128. (International Society for Plant Pathology).

Edwards J, de Boer RF (1999) "Hygiene affects seed-potato health" *Good Fruit and Vegetables* June 1999, p 77.

Edwards J, de Boer RF (1999) Hygiene in the potato shed – a role for disinfectants? *Potato Australia*, **10**, 56-57.

Edwards J, de Boer RF (2000) Hygiene and disinfection in the potato shed. *In* 'Conference Proceedings of the Potatoes 2000 Australian Potato Research, Development and Technology Transfer Conference, Adelaide, South Australia, 31 July-3 August 2000'. (Eds CM Williams and LJ Walters). pp 59-62. (South Australian Research and Development Institute, Adelaide, South Australia).

Edwards J, Mann RC, de Boer RF (1999) Evaluation of disinfectants against some common potato pathogens. *In*' Conference Handbook, Australasian Plant Pathology Society 12<sup>th</sup> Biennial Conference. Canberra, 27-30 September 1999'. (Ed Louise Morin), Australasian Plant Pathology Society.

Edwards JE, Mann RC, Crump NS, de Boer RF (2001) Evaluation of disinfectants against common potato pathogens on different surface materials in the potato shed. *In* 'Proceedings of the Second Australasian Soilborne Diseases Symposium, The Cumberland Resort, Lorne, Victoria, 5-8 March 2001'. (Eds IJ Porter *et al.*), pp 131-132. (Second Australasian Soilborne Diseases Symposium, Victoria, Australia).

Pitt T, de Boer RF (1998) Farm hygiene – potato sheds as a source of disease on seed potatoes. *Potato Australia* **9**, 56-57.

Pitt AJ, de Boer RF (2000) The European Powdery Scab Workshop, Aberdeen, Scotland, 20-22 July, 2000.A report for the Horticultural Research and Development Corporation and Seed Potatoes Victoria. September 2000. (Department of Natural Resources and Environment, Victoria).

"Potato hygiene essential" In Western District Farmer April 1999 p 8.

Wicks T, de Boer R (2000) Diseases – issues and control practices. *In* 'Potatoes 2000, Linking Research to Practice. Conference Proceedings of the Australian Potato Research, Development and Technology Transfer Conference, 31 July – 3 August 2000, Adelaide, South Australia'. (Eds CM Williams and LJ Walters). pp 33-34. (South Australian Research and Development Institute, South Australia).

# 3.7 Recommendations

This project has highlighted the importance the need for a hygiene program on the potato farm and that good hygiene practices could lead to tangible benefits in terms of minimising disease risk and improving the quality of seed, ware and processing potatoes. The hygiene protocols presented here form the basis for a hygiene program on any farm. In order to make farmers aware of importance of hygiene it is recommended that:

- 1. Hygiene protocols be further developed for distribution to farmers. This could take the form of material on WWW sites, posters and brochures outlining the importance of hygiene, cleaning and disinfection procedures and guidelines on the use of disinfectants;
- 2. Develop an extension/workshop program that demonstrates the importance of hygiene and outlines the essence of a hygiene program using the hygiene protocols developed here.

# 3.8 Acknowledgments

There are many people and organisations that provided assistance in one way or another to make this research possible. They include:

- The project team, Jacky Edwards, Ross Mann, Nigel Crump, Rajendra Gounder, Prabhpreet Inder for their invaluable contribution in the conduct of this project
- The potato farmers who graciously allowed us to sample their sheds, cool stores and fields
- Biometricians Graham Hepworth, Nam Ky Nguyen and Fiona Thomson for their assistance in the design and analysis of trials
- Bruce Fry for assisting in the shed dust survey
- Chemical companies who provided samples of their products
- Robert Holmes, Paul Harrup and Martin Mebalds for sharing their knowledge of disinfectants

The potato growers of Australia, Horticulture Australia and the Victorian Department of Primary Industries funded this project.

### 3.9 References

Anon (1976) Vegetable Pest and Disease Control Guide. (Department of Agriculture Victoria and Agriculture and Veterinary Chemical Association).

Anon (1999) Formaldehyde. National Cancer Institute Cancer Facts (Cancer Information Service) (http://cis.nci.nih.gov/fact/3 8.htm)

Anon (2002) Registration of post harvest treatments. A guide to the product types which must be registered by the NRA. Agricultural and Veterinary Chemicals Code Act 1994. *Commonwealth of Australia Gazette, No. NRA* 8, 6 August 2002.

Butler MJ, Day AW (1998) Fungal melanins: a review. Canadian Journal of Microbiology 44, 1115-1136.

Carnegie SF, Cameron AM, Haddon P. (1996) The potato store – a source of infection. In 'Abstracts of Conference Papers, Posters and Demonstrations of the 13<sup>th</sup> Triennial Conference of the European Association for Potato Research, Veldhoven, The Netherlands, 14-19 July 1996', eds P.C. Struik, J. Hoogendoorn, J.K. Kouwenhoven, I. Mastenbroek, L.J. Turkensteen, A. Veerman, and J. Vos, pp.257-8. (European Association for Potato Research, Wageningen, the Netherlands).

Clayton R (1999) Potato store hygiene and disinfection to improve seed health and ware quality. *British Potato Council Project Report No.1999/22*.

Clayton R, Wale S, Blackwood J (1999) Improving tuber health by mechanical and chemical store cleaning and disinfection. In 'Abstracts of Conference Papers, Poster and Demonstrations, 14<sup>th</sup> Triennial Conference of the European Association for Potato Research, Sorrento, Italy, 2-7 May 1999', eds A. Leone, S. Foti, P. Ranalli, A. Sonnino, V. Vecchio, A Zoina, L. Monti, L. Fruscinante, pp.257-8. (Assessorato Agricoltura, Regione Campania).

Clayton R, Wale SJ, Blackwood JM, Black S (2001). Potato store hygiene and disinfection to improve seed health and ware quality. *British Potato Council Project Report No. 2001/5*.

de Boer RF (1983) Dust in potato growers sheds - a source of inoculum of *Spongospora subterranea* - cause of powdery scab of potatoes. *In* Proceedings of the 4<sup>th</sup> International Congress of Plant Pathology, Melbourne, August 1983. Abstract No. 628. p.158.

de Boer, RF and Curtis, LW (1997) The incidence of blemishing diseases on tubers of G1 generation seed potatoes in the Victorian Seed Potato Certification Scheme. Results of a survey, July 1997. *ExpHORT 2000 Publication No. 28.* (Department of Natural Resources and Environment, Knoxfield, Victoria, Australia).

de Boer RF, Flett SP, Taylor PA (1982) A survey of Foundation Seed Potatoes for spore balls of *Spongospora* subterranea. Australasian Plant Pathology **11**, 6-7.

de Boer R, Wicks T (1994) Survey of black dot and other diseases of potato tubers. Potato Australia 5, 40-41.

Faggian R (2002) The application of molecular biology techniques for the detection and genetic differentiation of the plasmodiophorids. PhD Thesis. Department of Biotechnology and Environmental Biology, RMIT University.

Flett SP (1983) A technique for detection of *Spongospora subterranea* in soil. *Transaction of the British mycological Society* **81**, 424-425.

Fornier N (1997) Epidemiology of *Spongospora subterranea*, the cause of powdery scab of potatoes. PhD Thesis, University of Aberdeen, Scotland, UK.

Gardner JF, Peel MM (1998) *Sterilization, disinfection and infection control (3<sup>rd</sup> Edn)*. Churchill Livingstone, Melbourne; pp. 182-3.

Hall S (1996) Sources of *Helminthosporium solani* inoculum during the potato crop cycle and its importance in contaminating healthy tubers. In 'Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial Conference of the European Association for Potato Research'. Velhoven, The Netherlands. (Eds P Struik, J Hoogendoorn, J Kouwenhoven, I Mastenbroek, L Turkensteen, A Veerman, and J Vos) (The Scientific Committee of the 13th Triennial Conference of the European Association for Potato Research: Wageningen, The Netherlands)

Te Giffel MC, Beumer RR, Van Dam WF, Slaghuis BA, Rombouts FM (1995) Sporidical effect of disinfectants on *Bacillus cereus* isolated from the milk processing environment. *International Biodeterioration and Biodegradation* **36**, 421-430.

van Klingeren B Koller W, Bloomfield SF, Böhm R, Cremieux A, Holah J, Reybrouck G, Rödger HJ (1998) Assessment of the efficacy of disinfectants on surfaces. *International Biodeterioration and Biodegradation* **41**, 289-296.

Wale SJ (2002) Potato store hygiene and disinfection to improve seed health and ware quality. *British Potato Council Final Project Report, Reference No.* 807/171.

# 3.10 Appendices

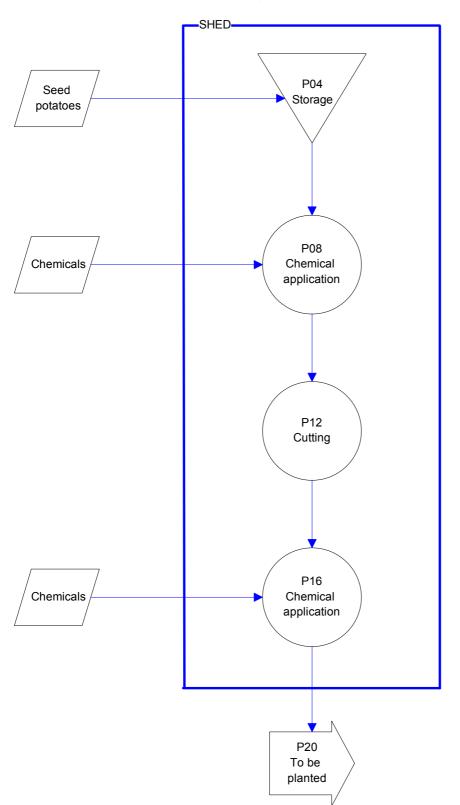
# 3.10.1 Appendix 1

### Table 6 Common disinfectant groups: their pros and cons

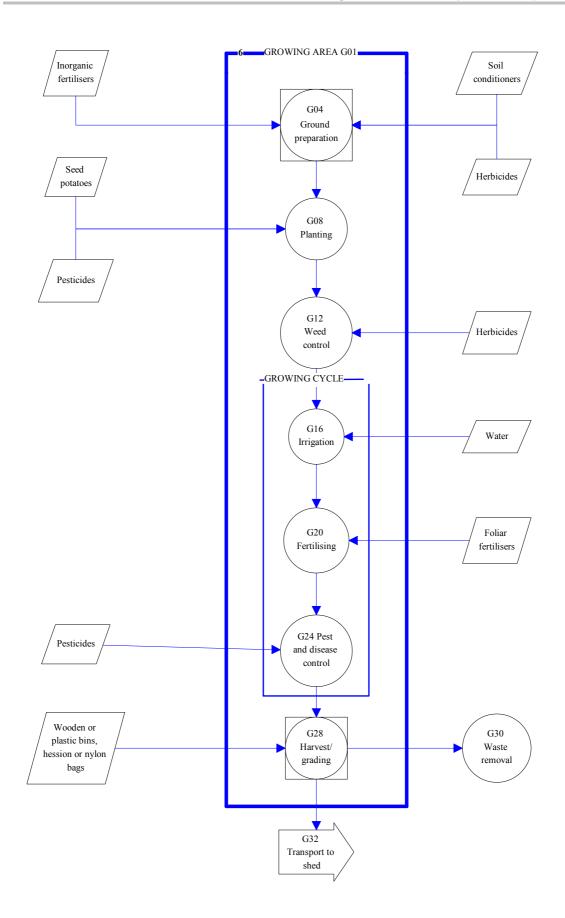
Disinfectant group	Inactivated by organic matter	Corrosive to metal	Activity dependent on pH (most effective pH range)	Safety	Comments
Alcohols	No	No	No	Relatively safe (see comments)	Flammable; can irritate skin; cracks rubber and plastics.
eg methylated spirits					
Aldehydes eg. Formalin	No	No	No	Poisonous	No longer recommended; toxic and carcinogenic, causing rashes, nausea and asthma attacks.
Chlorine dioxide	No	No	No	Irritant vapour	Comes as two components (base and activator) which are mixed together when required for use.
Hypochlorites eg. bleach	Yes	Yes	Yes (Acid)	Irritant vapour	Quick-acting and inexpensive, but activity is lost rapidly.
Iodine compounds	Some	Yes	Yes (Acid)		Similar properties to hypochlorites, but more stable.
Peroxygen compounds eg. peroxyacetic acids	No	Some	Some	Irritants in the concentrated form	This group varies in toxicity and corrosiveness, so generalisations cannot be made.
Phenolic compounds – phenols and inorganic phenols	No	No	No	Poisonous	Suspected carcinogen. Activity lost rapidly if diluted below recommended concentration.
Quaternary ammonium compounds	Yes	Slight	Yes (Alkaline)	Use caution (see comments)	Many different types of QACs, often formulated as mixtures. Diluted disinfectant relatively safe, concentrated form poisonous.

# 3.10.2 Appendix 2

HACCP flow charts, risk analysis tables and audit tables for hygiene on the potato farm



Pre-planting storage and seed treatments



HACCP audi	IACCP audit table Pre-planting storage and treatments								
Step	Hazard	Control measure	CCP, CP	Critical limit	Monitoring procedure	Corrective action	Records		
P04 Storage	Contamination of shed environment and future crop by pathogens	• Use clean, healthy seed			<ul> <li>Sort seed potatoes and remove dirty or diseased tubers</li> <li>Clean areas and bins where dirty/diseased tubers were kept</li> </ul>	• Source and condition of seed potatoes			
P04 Storage	Contamination of seed potatoes by dirt in the shed, by dirt from other potatoes or by spores from waste potatoes	<ul> <li>Good hygiene practices</li> <li>Clean shed</li> </ul>	СР	Good hygiene practices are followed	What: Shed environment before and after packing How: Visual Where: On-site When: Ongoing Who: Manager	<ul> <li>Clean the shed</li> <li>Reinforce hygiene practices</li> <li>Train staff</li> </ul>	• Cleanin g records		
P08 Chemical application	Contamination of grading equipment with rot-causing pathogens, particularly the roller table, leading to contamination of seed potatoes	<ul> <li>Sort out rotted tubers</li> <li>Clean rollers regularly</li> </ul>	СР	Sorting all tubers and cleaning rollers between runs	What: Tubers and rollers How: Visual Where: At grading table When: Ongoing Who: Operators	<ul> <li>Thoroughly clean equipment</li> <li>Train staff</li> </ul>	Log book		
P12 Cutting	Contamination of grading equipment with rot-causing pathogens, particularly the roller table, leading to contamination of seed potatoes	<ul> <li>Sort out rotted tubers</li> <li>Clean rollers and knives regularly</li> </ul>	ССР	Sorting all tubers and cleaning rollers and knives between runs	What: Tubers, rollers and knives How: Visual Where: At cutting table When: when seed is cut Who: Operators	<ul><li>Thoroughly clean equipment</li><li>Train staff</li></ul>	Log book		

HACCP audi	ACCP audit table Growing area								
Step	Hazard	Control measure	CCP, CP	Critical limit	Monitoring procedure	Corrective action	Records		
G01 Growing area	Infection of the potato crop with pathogens	<ul> <li>do not use ground previously used for potatoes</li> <li>good crop hygiene</li> </ul>	ССР	New ground used Weed hosts removed	What: Ground How: Past history Where: On-site When: Time of choosing ground Who: Manager	• Lease new ground	<ul> <li>Log book of paddock history</li> </ul>		
G04 Ground preparation	Contamination of ground with soil containing pathogens	• monitor all equipment and bins coming onto the paddock	ССР	No dirt on machinery, bins, etc.	What: Equipment, bins, etc. How: Visual Where: At or before the gate When: Ongoing Who: Operators	<ul> <li>Wash equipment</li> <li>Reinforce hygiene practices</li> <li>Train staff</li> </ul>	• Log book		
G08 Planting	Contamination of ground with pathogens, contamination of progeny potatoes	• Use clean disease-free seed	ССР	Clean healthy seed potatoes used	What: seed How: Visual Where: source When: time of choosing seed Who: Manager	• New source of seed	Source and condition of seed potatoes		
G08 Planting	Contamination of ground with pathogens, contamination of progeny potatoes	Clean planting equipment	ССР	Equipment washed before entering paddock	What: equipment How: Visual Where: prior to entering paddock When: at planting Who: operators	<ul><li>Thoroughly clean equipment</li><li>Train staff</li></ul>	Log book		
G12 Cutting	Contamination of grading equipment with rot-causing pathogens, particularly the roller table, leading to contamination of seed potatoes	<ul> <li>Sort out rotted tubers</li> <li>Clean rollers and knives regularly</li> </ul>	ССР	Sorting all tubers and cleaning rollers and knives between runs	What: Tubers, rollers and knives How: Visual Where: At cutting table When: when seed is cut Who: Operators	<ul><li>Thoroughly clean equipment</li><li>Train staff</li></ul>	Log book		

#### Pre-Planting Storage and Treatments - skin blemish diseases

Step	Process input	Hazards	Cause	Significance (High or Low)		ligh or Low)	Control measure
				Severity	Risk	Significance	
P04	seed potatoes	possible contamination of storage	buying dirty or diseased	Н	М	Н	Use clean, healthy seed
Storage		environment and progeny by pathogens	seed potatoes				
P04	storage	contamination of seed by dirt in the shed,	pathogen inoculum carried in	М	М	М	keep the shed clean and
Storage	environment	by dirt from other potatoes or by spores	the dirt/dust; sporulation on the				free of diseased tubers
		from waste potatoes	surfaces of stored/waste tubers				ie good hygiene practices

#### Pre-Planting Storage and Treatments - rots

Step	Process input	Hazards	Cause	Significa	Significance (High or Low)		cance (High or Low)		ignificance (High or Low) Control n		Control measure
				Severity	Risk	Significance					
P08 chemical application	seed potatoes people chemicals equipment		rotted tuber smeared onto rollers not cleaning between runs	Η	Μ		sort out rotted tubers clean rollers regularly				
P12 cutting	people	particularly the roller table, leading to	knife cuts rotted tubers; rotted tuber smeared onto rollers; not cleaning between runs	Н	Η		sort out rotted tubers clean rollers/knife regularly				

#### Growing Area - new ground

Step	Process input	Hazards	Cause	Significance (Hig		
•				Severity	Risk	
growing area	environment	Infection of crop with common scab,	inoculum in the soil,	Н	L	
G01		rhizoctonia and other diseases.	weed hosts present			
ground preparation	equipment	contamination of ground with	dirty equipment carrying contaminated	Н	Н	
G04		soil containing pathogens	soil from other potato paddocks			
planting	seed potatoes	contamination of ground with pathogens	planting diseased seed	Н	Н	
G08		contamination of progeny potatoes				
planting			Н	Н		
G08		contamination of progeny potatoes	soil from other potato paddocks			
weed control	veed control equipment contamination of ground with pathogens dirty equipment carrying contaminated		dirty equipment carrying contaminated	Н	Н	
G12	herbicides	contamination of progeny potatoes	soil from other potato paddocks;			
			inadequate control of weed hosts			
irrigation	water	severe powdery scab infection	cold, wet conditions at tuber initiation	М	L	
G16		pink rot infection	increases the chance of powdery scab infection;			
			water source may be contaminated			
fertilising	equipment	contamination of ground with pathogens	dirty equipment carrying contaminated	Н	Н	
G20	fertiliser	contamination of progeny potatoes	soil from other potato paddocks			
pest/disease control	equipment	contamination of ground with pathogens	dirty equipment carrying contaminated	Н	Н	
G24	pesticides	contamination of progeny potatoes	soil from other potato paddocks			
			inadequate control of pests/diseases			
harvest/grading	bins, bags, etc.	contamination of ground with pathogens	dirty bins and bags	Н	М	
G28		contamination of progeny potatoes	bins left sitting in field for days			
harvest/grading	equipment	contamination of ground with pathogens	dirty equipment; damage to tubers;	Н	Н	
G28	people	contamination of progeny potatoes	leaving tubers in the ground for long period after			
			vine death increases severity of silver scurf			
waste removal	equipment	contamination of ground with pathogens	inadequate waste removal leads to build-up of	Н	М	
G30	people		pathogen inoculum			

#### Growing Area - old ground

Step	Process input	Hazards	Cause	Significance (High or Low)			
-				Severity	Risk	Significance	
growing area	environment	infection of the potato crop	inoculum built up in soil from previous	Н	Н	Н	
G01		with pathogens	potato crops; self-sown potatoes as pathogen hosts				
ground preparation	equipment	contamination of ground	dirty equipment carrying contaminated	L	Н	L	
G04		with pathogens	soil from other potato paddocks				
planting	seed potatoes	contamination of progeny	using diseased seed	L	М	L	
G08	equipment	potatoes					
planting	equipment	contamination of progeny	dirty equipment carrying contaminated	L	Н	L	
G08		potatoes	soil from other potato paddocks				
weed control	equipment	contamination of progeny	dirty equipment carrying contaminated soil from	L	М	L	
G12	herbicides	potatoes	other paddocks; inadequate control of weed hosts				
irrigation	water	severe powdery scab infection	cold, wet conditions at tuber initiation increases	Н	Н	Н	
G16			chance of powdery scab infection;				
			water source may be contaminated				
fertilising	equipment	contamination of progeny	dirty equipment carrying contaminated	L	Н	L	
G20	fertiliser	potatoes	soil from other potato paddocks				
pest/disease control	equipment	contamination of progeny	dirty equipment;	Н	М	Н	
G12	pesticides	potatoes	inadequate control of pests/diseases				
harvest/grading	bins, bags, etc.	contamination of progeny	dirty bins;	L	М	L	
G28			leaving bins sitting in the field for days				
harvest/grading	equipment	contamination of ground and/or	dirty equipment; damage to tubers;	Н	Н	Н	
G28	people	contamination of progeny	leaving tubers in the ground for long period after				
		with pathogens	vine death increases severity of silver scurf				
waste removal	equipment	contamination of ground	inadequate waste removal leads to build-up of	Н	М	Н	
G30	people	with pathogens	pathogen inoculum				

#### Process input Significance (Hic Step Hazards Cause Severity Risk S01 storage and diseased/dirty potatoes brought potatoes contamination of shed and other tubers Н Н sorting shed with pathogen inoculum into the shed S01 storage and contamination of tubers with pathogens fungal spores in dirt and dust Μ environment Н sorting shed sporulation on diseased tubers in shed potatoes damage to tubers high humidity inducing sporulation of pathogens; Н Μ curing S04 environment contamination of tubers with pathogens inadequate time for curing process temperature too hot or too cold tipping equipment rough handling of potatoes Μ Μ damage to tubers S08 potatoes people sorting and grading damage to tubers rough handling of potatoes equipment Μ Μ rotted tubers contaminating equipment S12 contamination of tubers potatoes people such as rollers with rot-causing pathogens inadequate waste removal leads to build-up of Н waste removal people contamination of shed and other tubers Н S14 with pathogen inoculum equipment pathogen inoculum packing damage to tubers rough handling of potatoes М equipment S16 contamination of tubers with pathogens dirty bags and bins carrying pathogen inoculum potatoes people off-farm storage environment contamination of tubers with pathogens potatoes stored next to diseased/dirty tubers Н М S20 from other growers potatoes ambient storage high temperature causes condensation on environment contamination of tubers with pathogens Н M tubers and subsequent fungal sporulation S28 potatoes

#### Storage and Sorting Shed

#### **HACCP Control Points**

#### <u>In the field</u>

- **CP1:** Is the ground old or new? What is the paddock history? Are self-sown potatoes present in the paddock?
- **CP2:** Do the seed potatoes come from clean or dirty storage? Were they stored separately from ware potatoes?
- **CP3:** How disease free are the seed potatoes?
- **CP4:** Chance of contamination during cutting
- **CP5 CP7:** Contaminated machinery
- **CP8:** Contaminated dam water
- **CP9:** Timing of irrigation is critical eg. if it is cold and wet at tuber initiation, powdery scab will be severe.
- **CP10:** Contaminated machinery
- **CP11:** Contaminated machinery; how effective is the pest and disease control?
- **CP12:** Are the containers clean or dirty? How long are the containers left sitting on soil in the paddock?
- CP13: Contaminated machinery; damage to tubers during handling process
- **CP14:** If waste removal is not thorough, there may be contamination of the ground with pathogens, and self-sown potatoes will become weeds in subsequent crops.

#### In the shed

- **CP15:** How much soil comes on tubers? Are the tubers carrying disease? How long were the tubers left in the ground after vine death?
- **CP16:** Duration and temperature of curing process?
- **CP17:** Source of seed?
- CP18: How clean is the shed? Are there any diseased tubers already in storage?
- **CP19:** Are the containers clean?
- **CP20:** A lot of dust and dirt is generated, contaminating tubers and machinery; tubers can be bruised and damaged during processing; tubers can pick up disease from the rollers if a rotted tuber has been processed.
- **CP21:** Storage with potatoes from other growers contamination?
- **CP22:** How thorough is the waste removal? Where is the disposal site situated?
- **CP23:** Are the bags clean?