Know-how for Horticulture™

Analysising imported dried horticultural products for microbiological and chemical contamination

Bruce Tomkins
VIC Department of Primary Industries

Project Number: VG01085

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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

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Analysing Imported Dried Horticultural Products for Microbiological and Chemical Contamination

Project No. VG01085
Horticulture Australia Limited

Janine Jaeger[1], Julia Behrsing[1], Brendan Rodoni[1], Robert Premier[1], Pei Zhang[2], Gavin Rose[2], Bruce Tomkins[1]

Department of Primary Industries
Research and Development Division
1. Knoxfield
2. State Chemistry Laboratory

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July 2003

Project Leader

Bruce Tomkins

Department of Primary Industries
Research and Development Division, Knoxfield
Private Bag 15
Ferntree Gully Delivery Centre, Vic, 3156
Phone: (03) 9210 9222
Fax: (03) 9800 3521
E-mail: Bruce.Tomkins@dpi.vic.gov.au

Other key personnel were:

Knoxfield
Janine Jaeger
Julia Behrsing
Brendan Rodoni
Robert Premier

State Chemistry Laboratories
Gavin Rose
Pei Zhang

Purpose:
To conduct a preliminary survey, to provide information on the status of some imported dried horticultural products with respect to contamination with pests, diseases, viable seeds and chemical residues.

The project was funded by Horticulture Australia and the Department of Primary Industries, Victoria.

CONTENTS

1 MEDIA SUMMARY

Australia has been fortunate in avoiding many of the pests and diseases that cause great economic losses for horticultural producers around the world. In order to avoid these problems in Australia great care is taken, on an on-going basis, to ensure that plant material being imported into Australia does not pose a risk to our agricultural industries.

Dried horticultural products are being imported from many countries and are readily available in commercial outlets across Australia. Surprisingly, there is little information available regarding the survival of plant pathogens, insects, weeds and seed viability in dried products. There is also considerable consumer concern about chemicals in food, but there is little known about chemical residues on dried horticultural products imported into Australia.

The aim of this study was to conduct a preliminary survey to provide information on the status of some imported dried horticultural products with respect to contamination with pests, diseases, viable seeds and chemical residues.

Over 100 dried horticultural products were collected from commercial outlets across Melbourne. Selected products were subjected to tests for the presence and viability of bacteria, fungi, virus and seeds. The presence of insects, weed seeds and chemical residue levels was also checked.

Many bacteria and fungi were isolated from most products. One bacterium and one fungus were found that can cause disease in some plants. Other fungi were found that were not identified to species, but were from genera that have plant pathogenic species. The identification of a plant virus was not conclusive, but there were indications that viruses may have been present in some products. Insects were found in a few products, but they were all dead. Seed products and products containing seeds showed varying degrees of seed viability. Several products had foreign seeds in them.

It has been suggested that the introduction of exotic pests and diseases is possible through dried horticultural products. The findings from this pilot study indicate that dried horticultural products are a potential pathway for the introduction of exotic pests, diseases and weeds and that consumers may be exposed to chemical residues on these products. The risk of introducing exotic bacterial diseases and insects appears to be quite low. However, fungi viruses and weeds could be of greater concern and the issue of chemical residues needs addressing. More work is needed to properly assess these risks. To achieve this, collaboration is needed between appropriate regulatory authorities to properly plan and evaluate future studies.

2 TECHNICAL SUMMARY

The aim of this study was to conduct a preliminary survey to provide information on the status of some imported dried horticultural products with respect to contamination with pests, diseases, viable seeds, weed seeds and chemicals. There is little known about this issue and the risks associated with importing these products into Australia are poorly understood.

A total of 120 imported dried horticultural products were collected from 19 retail shops located across seven Melbourne suburbs. A selection of 100 samples were tested for bacteria, 100 for fungi and 100 for viruses and 50 samples were screened for chemical residues.

Bacteria and fungi were isolated from many products. Several of these were found to be from genera that have plant pathogenic species. Some fungi were not identified due to lack of spores on the test media. The identification of a plant virus was inconclusive. Symptoms on herbaceous indicators suggested that there may have been virus present in some products, but this could not be confirmed. Seed viability was variable, but most had some degree of germination. Foreign seeds were found in some products of which some could have been weed seeds, whilst others could have been food seeds from the place where the products were packed. Insects were found, but they were all dead. Chemical residues were found in some products, but it was difficult to interpret the significance of the detected levels of residues.

The results of this preliminary study suggest that there is some risk involved with the importation of dried horticultural products into Australia. Bacteria, fungi and possibly viruses survived the drying processes and could therefore be a means by which exotic disease could enter Australia. Seed transmitted diseases are a potential risk given the high levels of seed viability recorded during this pilot study. The generally low numbers of bacteria isolated suggest that the likelihood of exotic bacterial disease being introduced into Australia via this route is fairly low, but the likelihood of introducing fungi and viruses is of greater concern. The presence of weed seeds in some products is also a concern in that exotic weed species could be introduced. The issue of chemical residues in these products also needs addressing. Clarification of the category of food into which these products fall is needed so that there is better understanding by importers and consumers as to what levels of chemicals can be tolerated.

More work is needed to properly assess the risk of introducing pests, diseases and chemical residues in these products. To achieve this, collaboration is needed between appropriate regulatory authorities to properly plan and evaluate future studies.

3 INTRODUCTION

Australia has been fortunate in avoiding many of the pests and diseases that cause significant economic losses for horticultural producers around the world. In order to remain free from these problems in Australia great care is taken with regard to the importation of plant material into this country. There is an increasing and extensive range of dried horticultural products being imported from many countries. These products are readily available in commercial outlets across Australia. There is potential for these products to be a pathway for the introduction of exotic pests and diseases. It has been found that dried foods including herbs and spices have a high degree of contamination with human pathogens such as *Salmonella* (Bockemuhl and Wohiers, 1984). Some plant pathogens are more tolerant of dessication than many human pathogens and it is possible that these will survive transport in dried food products. However, apart from a suggestion that fungi are common in sun-dried plant material (Ekundayo, 1987), there is little information regarding survival of plant pathogens in dried products.

Seed transmission of pests and diseases is a widely recognised quarantine risk. The presence of weed seeds is also a risk, because both the weed itself and seedborne pathogens can be exotic to Australia. The viability of seeds accompanying dried horticultural products is poorly understood. Plant seeds may be part of the product (eg. chillies) or the products itself (eg. legumes and herbs / spices) or a weed seed contaminant.

There is a concern that the dried horticultural products may have chemical residues that are restricted in Australia. There is also considerable consumer concern about chemical residues in food. Tolerance levels for chemical residues vary between countries. Little is known about the presence and levels of chemical residues on dried horticultural produce imported into Australia.

The aim of this study was to conduct a preliminary survey to provide information on the status of some imported dried horticultural products with respect to contamination with pests, diseases, viable seeds and chemicals.

4 MATERIALS AND METHODS

4.1 Collection

A total of 120 dried horticultural products were collected from 19 retail shops located across seven Melbourne suburbs. A selection of 100 of these samples were tested for bacteria, fungi and viruses, but not always the same product and a selection of 50 samples were screened for chemical residues. Twenty four products with seeds were set aside for seed germination tests. All products were checked for characteristics that may indicate disease. Insects and foreign seeds were noted and collected.

4.2 Bacteria

Samples were prepared for bacterial isolation as per Australian Standard AS 17663.3-1991, Method 3.3: Examination of specific products – Dehydrated foods. Approximately 10g of the product was weighed into a sterile stomacher bag and 90 or 190mL of peptone buffer (0.1%) was added. The samples were left to rehydrate at room temperature for 15 minutes then blended for 1 minute using the stomacher. Serial dilutions were made and 0.1mL plated onto Kings B medium and/or Nutrient Agar with yeast media and incubated at 26°C for up to 2 weeks.

Bacterial colony forming units were counted for 50 of the products tested and calculated as colony forming units per gram (cfu/g). Colonies with similar morphology to target genera, including *Agrobacterium, Erwinia, Pseudomonas* and *Xanthomonas,* were isolated for further testing. Possible *Xanthomonas*, *Erwinia* and *Agrobacterium* spp. were transferred to Nutrient Agar and possible fluorescent *Psuedomonas* spp. to Kings B medium. These were incubated at 26°C for at least 48 hours. A gram stain was performed on colonies of interest and if this was consistent with the gram response of known pathogenic bacteria then further confirmation tests were performed.

4.2.1 Confirmatory Tests for the Identification of Plant Pathogenic Bacteria

Xanthomonas-like colonies were line streaked onto Starch, Tween and Milk agar along with a positive control and incubated at 26°C for at least 2 days. Positive reactions typical of *Xanthomonas* spp. included clearing around the streak when starch agar was flooded with iodine, a white deposit around the streak on Tween agar and clearing around the streak on Milk agar.

Erwinia-like colonies were inoculated, along with positive and negative controls, into two tubes of Oxidative - Fermentative (OF) medium, one of which was covered with sterile paraffin oil. These were incubated at 26°C for up to 1 week. Production of acid indicated by a colour change in both tubes indicated fermentative metabolism, which is positive for *Erwinia* spp. Cultures with positive fermentative metabolism were inoculated onto potato slices. Rotting of potato indicated *Erwinia* spp.

Agrobacterium-like colonies, along with a positive control were line streaked onto induction medium and incubated for 2-3 days. When grown they were spotted onto reaction medium and incubated for up to 2 days. Once a colony formed, the plate was flooded with Benedict's reagent and left for 15-30 minutes. A positive result for *Agrobacterium* spp. was indicated by the development of a yellow colouring around the colony.

Pseudomonas-like colonies were checked for fluorescence on King B medium with UV light. If fluorescent, cultures were dilution streaked onto Sucrose Nutrient Agar (SNA), inoculated

onto potato slices, inoculated into Arginine dihydrolase medium, and incubated for at least 48 hours. Positive results included levan type shiny raised colonies on SNA, rotting of potato and a colour change from yellow to purple of the Arginine dihydrolase medium. Colonies were tested for the oxidase reaction using commercially available test sticks (Oxoid) and tobacco plants were inoculated to check for hypersensitivity reaction. The resulting positive or negative reactions give a presumptive identification of the species of *Psuedomonas.*

4.3 Fungi

Moist incubation of plant material was prepared by placing pieces of product on wet paper towel in plastic containers with lids. These were incubated at room temperature until fungal growth appeared or for up to 3 months. Fungal isolation was carried out using four types of media, including malt extract agar, potato dextrose agar, rose-bengal agar and water agar. If necessary, plant material was cut into small pieces, using a sterile scalpel. The material was then steeped in hypochlorite for at least one minute to surface sterilise and then drained. Five or six pieces from each sample were pressed into the media and incubated at room temperature until fungal growth appeared or for up to 3 months. Fungi were identified by a fungal taxonomist.

4.4 Viruses

Several species of plants were grown as indicators for virus determination. These included, *Chenopodium quinoa, Nicotiana tabacum, Nicotiana glutinosa, Nicotiana rustica, Cucumis sativus* and for some products, *Physalis floridana*. Dried plant samples were macerated using a mortar and pestle with 5-10mL of 0.05M Na_2HPO_4-NaH_2PO_4, pH 7.4, buffer containing 0.1% sodium sulphite. Two leaves per indicator plant were coated with carborundum. These leaves were wiped with the prepared sample, left for 1-2 minutes and then rinsed with distilled water. Plants were observed over time for symptoms. Leaves from plants thought to have positive symptoms were sub-inoculated onto a new set of plants by macerating with buffer and applying as described above.

4.5 Chemical Residues

Fifty products were tested by Department of Primary Industries' State Chemistry Laboratories (SCL), for chemical residues including Organochlorine (OC), Organophosphorus (OP), Synthetic Pyrethroid (SP) and selected fungicides and Carbamate pesticides. The methods used were based on SCL 10062 (SCL, Sneydes Rd, Werribee, Victoria) method with the following modifications.

4.5.1 Sample Extraction

Samples were chopped into small pieces and 10g of homogenised sample weighed into a 250mL beaker and 70mL of filtered distilled water added. The samples were soaked overnight and next day, 130mL of acetone was added and blended for 5 minutes. Extract was filtered under suction through Buchner funnel fitted with a glass fibre filter paper. The filtrate was transferred into a 1L separating funnel with 650mL of 4% sodium sulphate solution added and extracted with 1× 100mL and 2 × 50mL dichloromethane (DCM). The dichloromethane extract was passed through a drying column containing 30g anhydrous sodium sulphate into a 500mL flat bottom flask. The extract was concentrated and inverted into hexane on a water bath. The final extract was transferred into a 5mL measuring cylinder and made up to 5mL with hexane.

4.5.2 Column Chromatography Clean-up

4.5.2.1 Organochlorines and Synthetic Pyrethroids

A chromatographic column was prepared with 3g of 2% (w/w) deactivated Florisil® (magnesium-silica gel) topped with 1.5-cm anhydrous sodium sulphate and prewashed with 25mL hexane. A 0.5mL aliquot of the sample extract was loaded on the column and eluted with Eluent 1 consisting of 25mL 10% ether/hexane for organochlorides (OC) and synthetic pyrethroids (SP) except β-Endosulfan and Endosulfan or Eluent 2 consisting of 25mL 0.5% acetonitrile/49.5% hexane/50% dichloromethane. This eluent is used only for the determination of β-Endosulfan and Endosulfan sulphate.

Each eluent was collected in a Kuderna/Danish (KD) flask, concentrated and inverted into hexane to a final volume of 1mL. For OC 200µl was diluted to 1000µl and injected on a gas-liquid chromatograph with electron capture detector (GLC-ECD). SP samples were injected on a GLC-ECD without further dilution.

4.5.2.2 Organophosphorus and selected carbamate pesticides

A chromatographic column was prepared with 3g of 2% (w/w) deactivated Florisil® as above. A 1mL aliquot of the sample extract was loaded on the column and eluted with 25mL 20% acetone/dichloromethane. The eluent was collected in a Kuderna/Danish (KD) flask, concentrated and inverted into hexane to a final volume of 1mL. OP samples were injected on a gas-liquid chromatograph with pulsed flame photometric detector (GLC-PFPD). Carbamate samples were injected on a gas-liquid chromatograph with nitrogen phosphorus detector (GLC-NPD).

4.5.3 Method modification for determination of pirimicarb in bamboo

Using the above sample extraction method for the bamboo samples resulted in low recovery for pirimicarb due to pirimicarb being highly soluble in the acidic aqueous phase and was therefore not extracted into the organic solvent. The pH was adjusted from acidic to neutral to extract pirimicarb out of the bamboo sample into the organic solvent phase.

4.6 Seed germination

Between 100 and 400 seeds, depending on size, were laid evenly on moistened paper (Grade 2H on top and Grade U5 underneath) which was placed on a moistened towel in trays and covered with lids. These were left at room temperature and examined weekly for signs of germination. Germinated seeds were removed and the remainder left for up to 4 weeks.

4.7 Extraneous material - insects and foreign seeds

Products were examined for any other extraneous materials including insects and seeds. Insects were sent to an insect taxonomist for identification. Seeds were separated and kept aside for photographing, to show the variety of foreign seeds found in the products and then set up for germination as described above.

5 RESULTS

5.1 Bacteria

Results of the 100 bacterial isolations are presented in Appendix 1. A variety of types and numbers of bacteria were isolated from all products. The number of bacteria isolated from 50 products varied from as few as one colony forming unit per gram (cfu/g) for cardamon pods, watermelon seeds, marjoram and tomatoes, to over 2000 cfu/g for mung beans and curry leaves. However, most products had less than 500 cfu/g.

Biochemical tests on some bacteria with selected colony morphologies did not confirm the presence of pathogens (Appendix 1). Others exhibited fluorescence under UV light and were therefore considered to be fluorescent *Pseudomonas* spp. Seven products had fluorescent colonies and biochemical tests were performed. Presumptive identification was established from the results of biochemical tests (Table 1).

Table 1: Presumptive identification of bacteria from the target genera that were identified from dried horticultural products.

Code	Product	Family	Genus / Species	Origin	Count cfu/g	Presumptive identification
D4/21	Dill seeds	Apiaceae	*Anethum graveolens*	Turkey	4	Fluorescent *Pseudomonas*, but not identified to species
S3/3	Chinese cabbage	Brassicaceae	*Brassica rapa var pekinensis*	China	NC	Saprophytic *Pseudomonas*
BR1/5	Cumin seeds	Apiaceae	*Cuminum cyminum*	China	55	*P. marginalis* *P. fluorescens*
BR3/2	Liquorice	Leguminosae	*Glycyrrhiza glabra*	Syria	34	*P. marginalis* *P. tolaasii* or a saprophyte*
D4/24	Aniseed star	Illiciaceae	*Illicium anisatum*	China	88	*P. tolaasii* or a saprophyte*
BH3/17	Black beans	Leguminosae-Papilionoideae	*Phaseolus vulgaris*	China	3	*P. marginalis*
BR3/5	Sesame seeds	Pedaliaceae	*Sesamum orientale*	Lebanon	1069	*P. marginalis*

* - *P. tolaasii* gives the same LOPAT results as some saprophytic Pseudomonads.

Results of biochemical tests on fluorescent Pseudomonads indicated that the likely species isolated were *P. fluorescens, P. tolaasii* or a saprophyte and *P. marginalis*. One isolate from dill seeds was identified as a fluorescent pseudomonad, but did not show the typical responses to the biochemical tests that would identify the species.

5.2 Fungi

A wide range of fungi was isolated from most of the 100 products tested (Appendix 1). One pathogenic fungus, *Macrophomina phaesolina* (Tassi) Goid, was identified from mung beans (D1/3) from Pakistan. The most common genera isolated included *Penicillium, Rhizopus, Aspergillus, Cladosporium* and *Chaetomium*. Other genera included *Absidia, Acremonium, Alternaria, Aureobasidium, Botrytis, Chromelosporium, Chrysonilia, Coniella, Epicoccum, Khuskia, Melanospora, Mucor, Neurospora, Nodulisporium, Papulaspora, Periconia, Phoma, Pithomyces, Sporormiella, Stachybotris, Syncephalastrum, Thielavia, Trichoderma* and *Ulocladium.* Several fungi could not be identified because no spores were formed. No fungi was isolated from a few products, including sweet turnip (daikon), lily flower and betel leaves.

5.3 Viruses

From the 100 products tested for viruses (Appendix 1) several induced symptoms on some of the herbaceous indicator plants (Table 2).

A range of symptoms was expressed by the herbaceous indicators in response to inoculation with sap from certain dried products including leaf necrosis and chlorosis (yellowing), deformed leaves (drooping, distorted), stunting and bronzing. It was difficult to determine if these symptoms were induced by other factors, such as water or nutritional deficiencies or high concentrations of salts and other reagents in the dried plant product.

Of particular interest were the bamboo and barwon flower samples. Four bamboo products BH2/7, BH3/10, R1/3 and S2/5 induced symptoms on cucumbers that appeared to indicate the presence of virus. The chlorosis, necrosis and stunting observed on the cucumber indicator plants after inoculation with sap from the bamboo samples, were virus-like (Table 2). Sub-inoculation of the symptomatic cucumber tissue onto young cucumber plants yielded milder symptoms. Examination of the symptomatic cucumber tissue using the electron microscope did not identify any rod shaped virus particles.

Sap from the barwon flower tissue produced chlorotic and bronzing symptoms on several tobacco indicator plants and on *Physalis floridiana*. Stunting was also observed on *N. glutinosa* (Table 2). Sub-inoculation of the symptomatic indicator tissue onto young indicator plants yielded only minor symptoms and no rod shaped virus particles were observed using the electron microscope. It is unclear whether the presence of virus in the dried bamboo and barwon flower tissue was the cause of the symptoms observed.

5.4 Chemical Residues

From the fifty products screened, chemical residues were detected in sixteen products. Chemicals found were from the organophosphate, organochloride, synthetic pyrethroid and carbamate groups.

Table 2: Virus-like symptoms observed on herbaceous indicators inoculated with dried horticultural products.

Code	Product	Family	Genus / Species	Origin	Herbaceous Indicators					
					Chenopodium quinoa	***Nicotiana tabacum***	***Nicotiana glutinosa***	***Nicotiana rustica***	***Cucumis sativus***	***Physalis floridana***
BH1/8	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	b	-	d	d	e	d
BH2/7	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	-	-	-	-	abc	x
BH3/10	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	-	-	-	-	abc	x
R1/3	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	Thailand	-	-	-	-	abc	x
R2/2	Bamboo leaf	Gramineae (Poaceae)	*Bambusa*	Thailand	-	-	-	bd	e	-
S2/5	Bamboo shoot tip	Gramineae (Poaceae)	*Bambusa*	Thailand	-	-	-	-	abc	x
S1/9	Meigancai	Brassicacae (Cruciferae)	*Brassica juncea*	China	-	-	-	-	e	-
S1/3	Chrysanthemum	Asteraceae	*Chrysanthemem coronarium*	China	-	-	-	-	e	f
BR5/3	Watermelon seed	Cucurbitaceae	*Citrullus lantatus*	Iran	-	-	-	-	b	d
BH1/10	Lime skin	Rutaceae	*Citrus aurantifolia*	Thailand	b	-	-	-	b	-
BH3/2	Lime leaves	Rutaceae	*Citrus aurantifolia*	Thailand	b	-	-	d	-	-
BR1/5	Cumin seeds	Apiaceae	*Cuminum cyminum*	China	-	-	-	-	ab	b
R1/2	Lily flower	Liliacae	*Hermerocallis fulva*	China	-	-	-	-	a	ab
S1/5	Barwon flower	Cactaceae	*Hylocereus undatus*	China	b	-	e	d	e	abdf
BR5/2	Mint	Labiatae	*Mentha*	India	b	-	-	bd	-	-
D4/2	Curry leaves	Rutaceae	*Murraya koenigii*	Sri Lanka	-	-	-	d	-	-
BR4/1	Oregano bunches	Labiatae	*Origanum vulgare*	Greece	b	-	d	-	-	f
D1/2	Kidney beans	Leguminosae-Papilionoideae	*Phaseolus vulgaris*	Pakistan	e	-	-	-	-	de
BH2/8	Prunella vulgaris	Lamiacae	*Prunella vulgaris*	China	-	-	-	bf	-	-
BH2/6	Sweet turnip	Brassicacae (Cruciferae)	*Raphanus sativus*	China	-	-	-	d	ab	d
BR3/1	Sage leaves	Labiatae	*Salvia*	Jordan	-	-	-	ed	-	f
D4/26	Ginger roots	Zingiberaceae	*Zingiber officinale*	India	-	e	e	d	-	bd
S1/4	Ginger slice	Zingiberaceae	*Zingiber officinale*	China	-	-	-	-	-	d

a= necrosis b= chlorosis c= drooping leaves d= deformed leaves e= stunted f= bronzing x= not tested

5.5 Seed germination

There were several products that were seeds or contained seeds. Nearly all the seeds tested showed some degree of germination (Table 4). The percentage that germinated varied from 0.5% to 95%. Only four out of the 25 products tested showed no germination.

Table 4: The percentage of seeds germinated from dried horticultural products.

Code	Product	Family	Genus	Origin	% germinated
D4/21	Dill seeds	Apiaceae	*Anethum graveolens*	Turkey	15.5
BH3/6	Chillies	Solanaceae	*Capsicum annuum*	China	75.5
D4/14	Chillies	Solanaceae	*Capsicum annuum*	India	0.5
D4/15	Chillies	Solanaceae	*Capsicum annuum*	India	78.5
S3/4	Chillies	Solanaceae	*Capsicum annuum*	Thailand	9
D4/22	Papita seeds	Caricaceae	*Carica papaya*	China	0
BR5/3	Watermelon seed	Cucurbitaceae	*Citrullus lantatus*	Iran	0
KC/1	Cumin seeds	Apiaceae	*Cuminum cyminum*	India	20
BR1/6	Brown cardamon pods	Zingiberaceae	*Elettaria cardamonum*	India	0.5
BR1/3	Cardamon seeds	Zingiberaceae	*Elettaria cardamonum*	PNG	0.5
BR1/4	Cardamon pods	Zingiberaceae	*Elettaria cardamonum*	Guatemala	0.5
BR3/4	Fennel seeds	Apiaceae	*Foeniculum vulgare*	Lebanon	75.5
D4/23	Fennel seeds	Apiaceae	*Foeniculum vulgare*	India	80.3
D4/24	Aniseed star	Illiciaceae	*Illicium anisatum*	China	1
BR1/7	Star anise	Illiciaceae	*Illicium anisatum*	Iran	0
BR2/1	Tomatoes	Solanaceae	*Lycopersicon esculentum*	Italy	28.5
D4/12	Tomatoes	Solanaceae	*Lycopersicon esculentum*	Turkey	30.5
D2/1	Poppy seeds	Papaveraceae	*Papaver somniferum*	Poland	52.5
BH3/17	Black beans	Leguminosae-Papilionoideae	*Phaseolus vulgaris*	China	48
D1/2	Kidney beans	Leguminosae-Papilionoideae	*Phaseolus vulgaris*	Pakistan	23
BH2/8	Prunella vulgaris (self heal)	Lamiacae	*Prunella vulgaris*	China	0
D4/6	Senna pods	Fabaceae	*Senna*	India	22.5
BR3/5	Sesame seeds	Pedaliaceae	*Sesamum orientale*	Lebanon	0.5
D1/3	Mung beans	Leguminosae-Papilionoideae	*Vigna radiata*	Pakistan	95
D4/19	Horse gram	Leguminosae-Papilionoideae	*Vigna unguiculata*	Sri Lanka	68.3

5.6 Extraneous material - insects and foreign seeds

The remains of several insects were found in several products (Table 5). No live insects were found in any of the products. Several products, especially seed products, contained foreign seeds. Examples of seeds found are shown in Figures 1 - 9. Some of the foreign seeds were tested for germination and some of the seeds were found to be viable (Table 6).

Table 5: Arthropods found in dried horticultural products.

Code	Product	Family	Genus	Origin	Insect
BH1/11	Orange peel	Rutaceae	*Citrus sinensis*	China	Part of pyralid larva, possibly *Plodia* sp.
BH3/3	Tangerine peel	Rutaceae	*Citrus tangerina*	China	Shed skin of a spider Forewing of a beetle, possibly Dermestidae Fragment of a beetle larva, possibly Dermestidae Few incomplete book lice, Psocidae
S3/2	Snake beans	Leguminoseae	*Vigna sesquipedalis*	China	Three larvae of Bean pod borer moth, *Maruca vitrata*

Table 6: Germination of foreign seeds.

Code	Product	Family	Genus	Origin	Observations
BR1/5	Cumin seeds	Apiaceae	*Cuminum cyminum*	China	33 out of 76 seeds germinated. 6/14 seed types had some degree of germination.
KC/1	Cumin seeds	Apiaceae	*Cuminum cyminum*	India	24/76 seeds germinated. 8/17 seed types had some degree of germination.
D4/19	Horse gram	Leguminosae–Papilionoideae	*Vigna unguiculata*	Sri Lanka	30/69 seeds germinated. 5/17 seed types had some degree of germination.
D 4/23	Fennel seeds	Apiaceae	*Foeniculum vulgare*	India	14/82 seeds germinated. 7/17 seed types had some degree of germination.
D4/21	Dill seeds	Apiaceae	*Anethum graveolens*	Turkey	4/16 seeds germinated. 2/10 seed types had some degree of germination.
D2/1	Poppy seeds	Papaveraceae	*Papaver somniferum*	Poland	4/44 seeds germinated. 1/4 seed types had some degree of germination.
BR3/4	Fennel seeds	Apiaceae	*Foeniculum vulgare*	Lebanon	4/12 seeds germinated. 3/6 seed types had some degree of germination.
BR3/5	Sesame seeds	Pedaliaceae	*Sesamum orientale*	Lebanon	No germination from 4 seeds.
D3/1	Camomile	Compositae	*Chamaemelum nobile*	Yugoslavia	No germination from the seeds in the pods.

Figure 1: Foreign seeds (L) found in product BR1/5 cumin seeds (R) from China. The circles indicate the different types of foreign seeds found.

Figure 2: Foreign seeds (L) found in product KC/1 cumin seeds (R) from India. The circles indicate the different types of foreign seeds found.

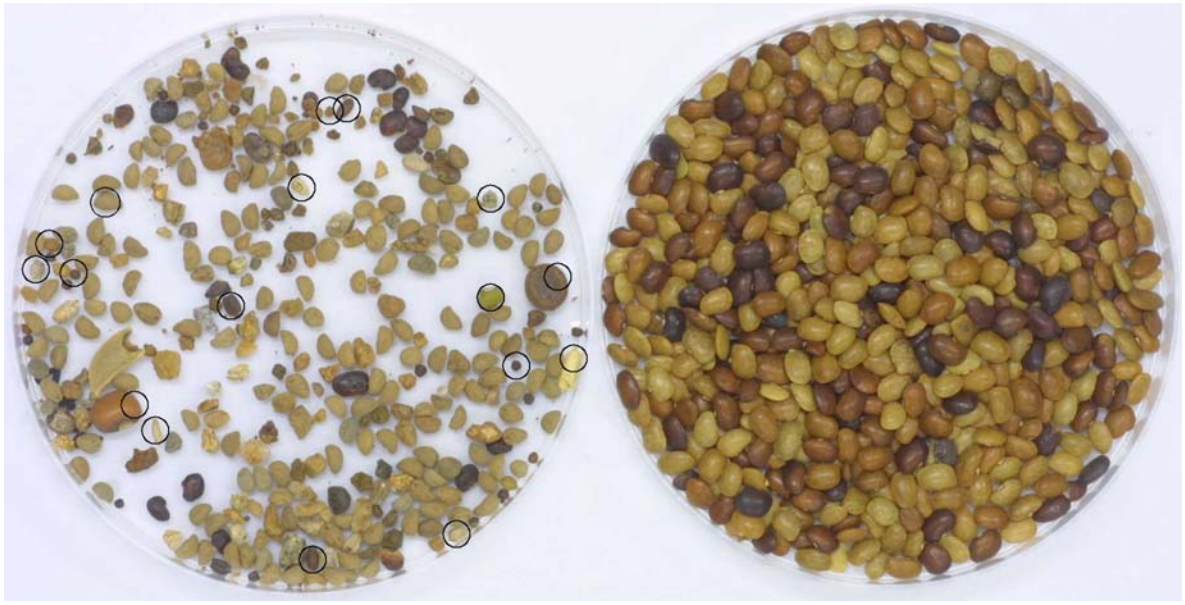

Figure 3: Foreign seeds (L) found in product D4/19 horse gram (R) from Sri Lanka. The circles indicate the different types of foreign seeds found.

Figure 4: Foreign seeds (L) found in product D4/23 fennel seeds (R) from India. The circles indicate the different types of foreign seeds found.

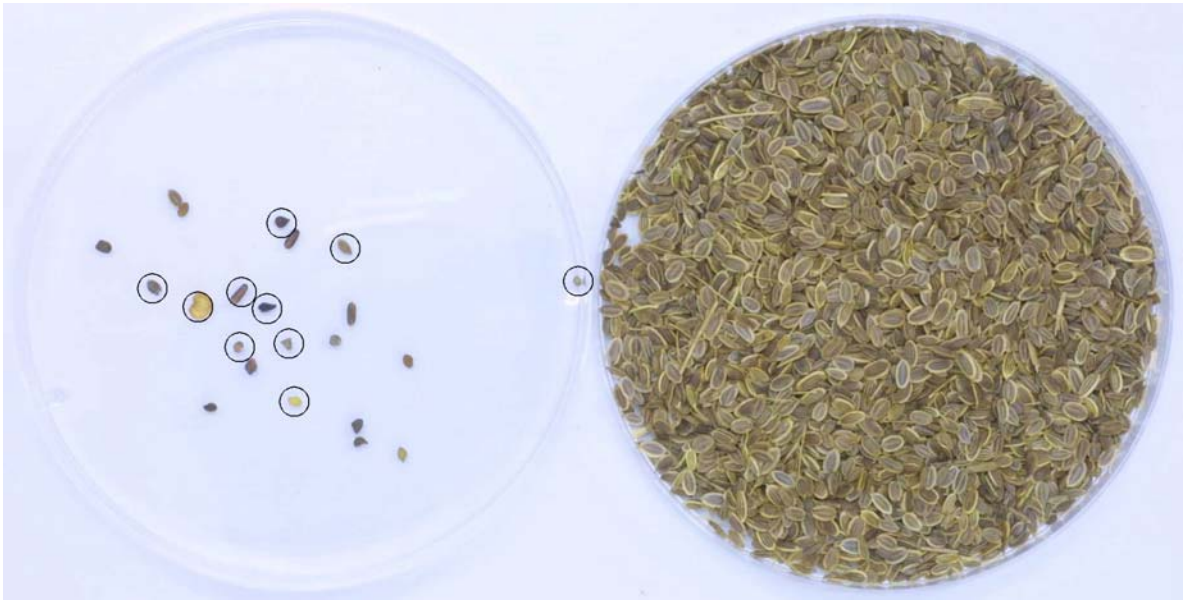

Figure 5: Foreign seeds (L) found in product D4/21 dill seeds (R) from Turkey. The circles indicate the different types of foreign seeds found.

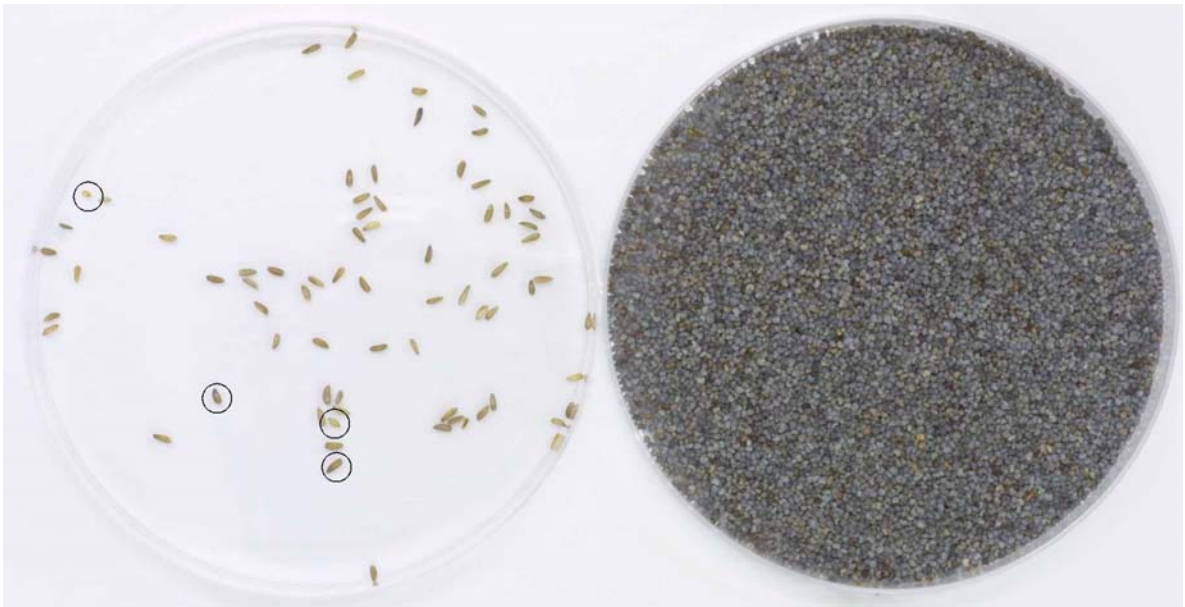

Figure 6: Foreign seeds (L) found in product D2/1 poppy seeds (R) from Poland. The circles indicate the different types of foreign seeds found.

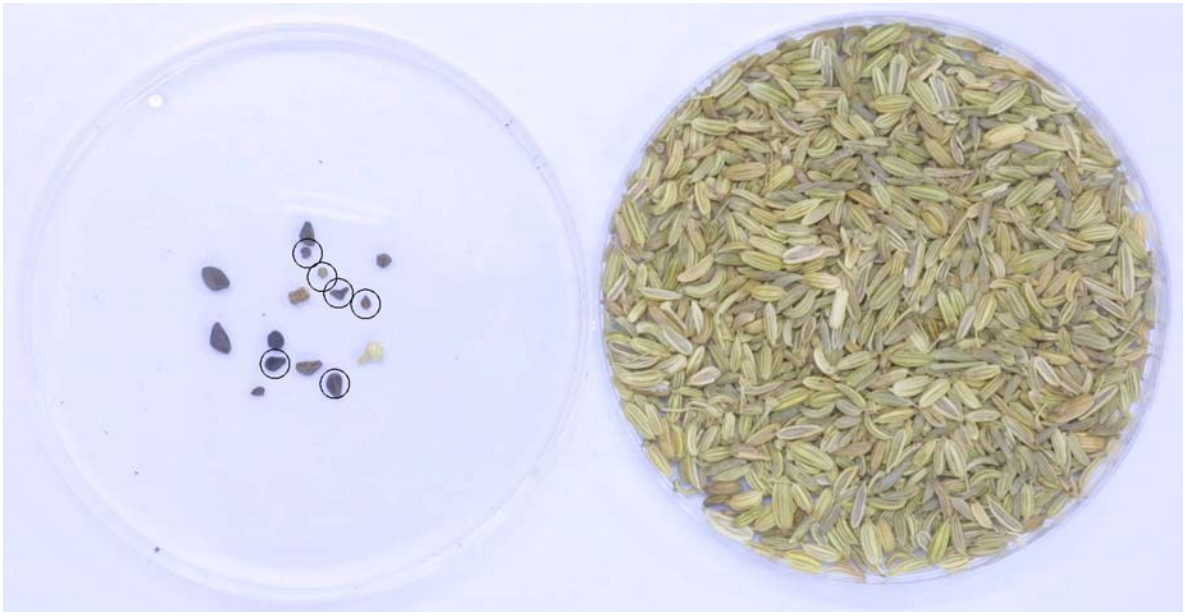

Figure 7: Foreign seeds (L) found in product BR3/4 fennel seeds (R) from Lebanon. The circles indicate the different types of foreign seeds found.

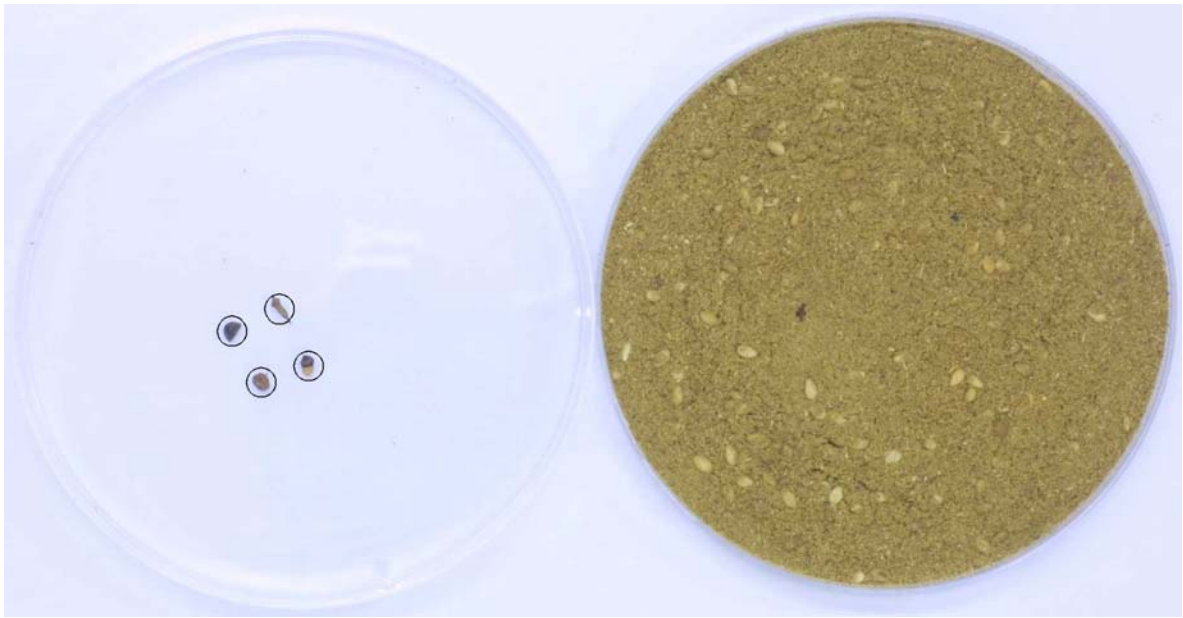

Figure 8: Foreign seeds (L) found in product BR3/5 sesame seeds (R) from Lebanon. The circles indicate the different types of foreign seeds found.

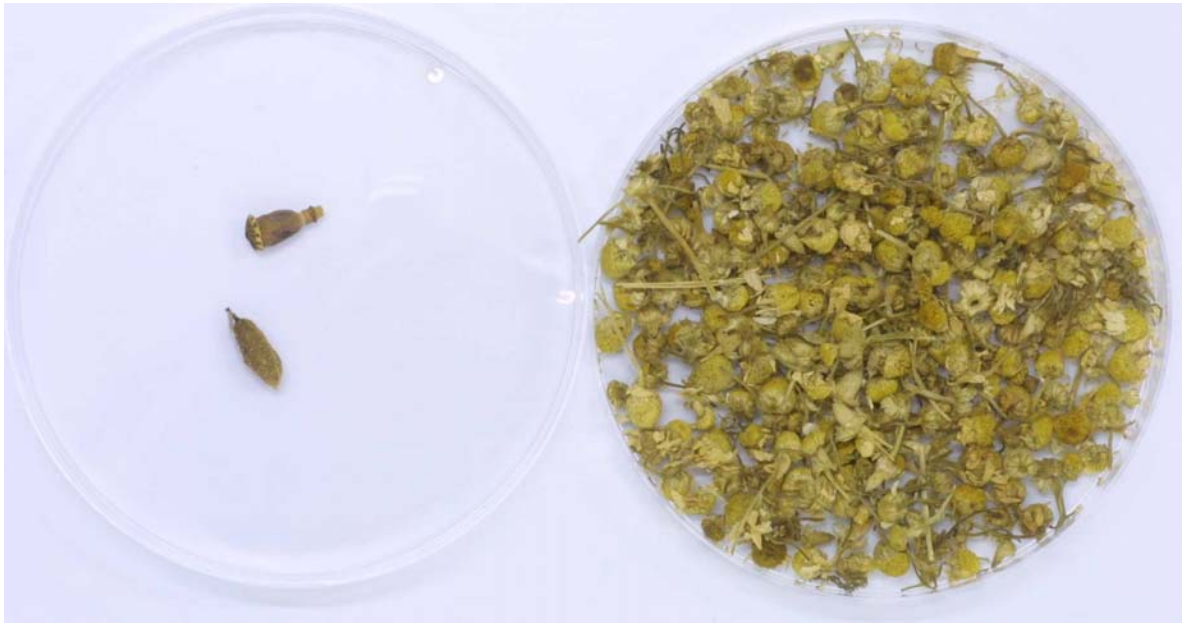

Figure 9: Foreign seed pods (L) found in product D3/1 camomile (R) from Yugoslavia. Each pod contained many tiny seeds.

6 DISCUSSION

6.1 Bacteria

Determining the presence of pathogenic bacteria was difficult, because of limited resources. Therefore, an attempt was made to determine the presence of common genera that have pathogenic species. As a result, some pathogenic isolates may not have been detected and it is these that may be of concern. Many more resources would have been required to identify every colony isolated. Of the bacteria isolated, *P. fluorescens* is saprophytic, but *P. marginalis* and *P. tolaasii* are pathogenic to many agricultural hosts and mushrooms, respectively (Bradbury, 1986). The low numbers of bacteria isolated and the absence of many common pathogenic genera suggests that the risk of introducing exotic bacterial pathogens on dried horticultural products is quite low. However, bacteria were consistently isolated from the dried horticultural products which indicates that the introduction of exotic bacterial pathogens into Australia via dried horticultural products is possible.

6.2 Fungi

There were many fungal species isolated from the dried horticultural products, most of which are saprophytic and found in soil. *Macrophomina phaesolina* causes charcoal rot and ashy stem blight on legumes, jute, potato, cotton, peanut and conifers (Holliday and Punithalingam, 1970). This fungus is usually transmitted via plant debris in the soil and may also be seed-borne in some crops. This is of concern since it was isolated from a seed product that had a high germination rate. Other genera were isolated that have pathogenic species of which some are exotic to Australia. These include *Alternaria, Aspergillus, Cladosporium, Penicillium, Periconia*, and *Phoma*.

Fungi are generally well equipped to survive the drying processes used to produce the products from which they were isolated. The wide variety of fungi isolated, including those

that could not be identified suggest that dried horticultural products could be a means by which exotic fungi or new strains of existing fungi could be introduced into Australia. The right conditions in a backyard compost heap or people planting the seeds could lead to infected seeds like mung beans growing and pathogens like *M. phaesolina* spreading. The fact that the mung beans exhibited a 95% germination rate and this fungus can be seed transmitted, increases the chances of spread. Even though this fungus is found in Australia, it clearly demonstrates that new pathogenic and exotic strains of fungi could be introduced via this route.

6.3 Viruses

The aim of our studies was to screen 100 samples of dried horticultural products for the presence of viable virus particles, as measured by infectivity. Mechanical inoculation of sap from test plant tissue onto sensitive indicator plants is one of the most basic means for detecting the presence of plant viruses and is extremely useful in studies such as this when confronted with a wide range of botanically diverse samples. A major drawback of this approach is that the test result indicates only the presence of plant viruses and does not definitively identify a specific virus. A second drawback is that the symptoms expressed on virus infected indicator plants can sometimes be caused by other factors such as nutritional or chemical imbalances. During the experiments conducted in this study a number of *Physalis floridiana* indicator plants were affected by a chemical spray and therefore any symptoms observed on these plants had to be ignored.

Many viruses will loose their infectivity during the processing of dried plant tissue. However, a number of viruses will survive in dried tissue for many years, particularly those viruses belonging to the Tobamovirus (*Tobacco mosaic virus*) and Potexvirus (*Potato virus X*) groups. Some of these viruses (ie. *Pepino mosaic potexvirus*) do pose a quarantine risk to Australia. With these points in mind, our screening of 100 samples suggests that some of the samples of dried plant tissue may have been infected with a plant virus. Of particular interest were the bamboo and barwon flower samples. Further work is required to verify the presence of virus in these samples.

6.4 Chemical Residues

Although chemical residues were detected in a number of samples, it is not possible to properly interpret the data and make recommendations. The aim of this study was to do a preliminary investigation to see whether chemicals were present. Samples were not collected according to internationally agreed protocols so any interpretation against the Food Standards Code would not be legitimate. The information would need to be examined by the appropriate national and state authorities to determine what further work, if any, needs to be done.

6.5 Seed germination

The number of seeds that germinated varied greatly both between and within plant species. The results show that the growth of seeds that may be harbouring fungal, bacterial or viral pathogens is possible from dried horticultural products whether they are seed products or within products such as dried chillies and tomatoes. *Macrophomina phaesolina* in mung beans is an example of a seed borne pathogen that was found in a seed product. There is also a range of seed borne diseases that could be transmitted by the viable seeds that were present in the dried horticultural products.

6.6 Extraneous material - insects and foreign seeds

It is encouraging that no live insects were found in any of the dried horticultural products assessed and only three products showed evidence that insects had been present in the products. The risk of insect transmission by these products appears to be unlikely.

The presence of so many foreign seeds in some of the products is cause for concern. Some of the foreign seeds appeared to be other food seeds such as chickpeas and sesame seeds and these may have been introduced when the product was packaged, in either Australia or the exporting country. There were other seeds detected that are likely to be weed seeds. The foreign seeds that germinated suggest that given the right conditions exotic weed species could be introduced into Australia via dried seed products. Further work is needed to assess the risk of importing noxious weeds into Australia in dried horticultural products.

From the results of this study of bacteria, fungi, viruses and seeds, we believe that it is possible for exotic diseases to be introduced into Australia via imported dried products. A possible route could be if products were disposed of in the backyard compost. Elimination of pathogens is possible in compost piles set up to achieve optimum temperatures and that are properly turned (Wijnen *et al*., 1983). However, most backyard compost piles do not achieve this. Ryckerboer, Cops and Coosemans (2002), found that backyard composting was only effective in eliminating tobacco mosaic virus and only partly successful in eliminating the nematode, *Heterodera schachtii* and tomato seeds. Composting was also found to be unsuccessful in eliminating the fungus *Plasmodiophora brassicae*. Another route could be if seed products were grown in backyards or on commercial properties.

6.7 Conclusions

This project was conducted in response to the suggestion that exotic pests and diseases could be introduced into Australia on dried horticultural products. A possible route could be if products were disposed of in the backyard compost or if seed products are grown in backyards or on commercial properties. This study has shown the potential for pathogenic bacteria, fungi and viruses and exotic weeds to be introduced in dried products. However, the study was intended to only be preliminary and the small sample size makes results difficult to interpret. More work that targets specific problems is needed to properly assess the risk of introducing exotic diseases. To achieve this, collaboration is needed between appropriate regulatory authorities to properly plan and evaluate future studies.

7 Recommendations

- AQIS, Plant Health Australia and state regulatory bodies need to be notified of the results of this pilot study.

- Future work, particularly the issue of chemical contaminants, needs to be carefully planned and interpreted. The information gathered here needs to be examined by the appropriate national and state authorities to determine what further work, if any, needs to be done.

- Clarification of food standards for dried horticultural products is required.

- More extensive studies including reviews need to be supported that target specific pests / pathogens / weeds. For example, seed borne diseases in pulses, Solanaceous species and noxious weeds.

- This study was a pilot study and should not be used as a basis for conclusion on risks associated with imported dried produce. It should however, be made available to government agencies which have a mandate for regulating imports.

8 ACKNOWLEDGEMENTS

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10 APPENDICES

Appendix 1: Bacteria, fungi and virus isolated from dried horticultural products.

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
BH2/10	Wu hua cha (5 flowers tea)			China	NC	CNT		*Aspergillus, Rhizopus, Syncephalastrum*	-
BH3/4	Mushroom			China	NC	CNT		*Rhizopus, Tricoderma, Penicillium, Chaetomium*	x
D4/1	Onion flakes	Alliaceae	*Allium cepa*	India	367	CNT		*Rhizopus, Chaetomium, Penicillium, Aspergillus,* unidentified fungi	-
S2/6	Galangal	Zingiberaceae	*Alpinia galanga*	Thailand	NC	CNT		*Rhizopus, Penicillium*	-
D4/21	Dill seeds	Apiaceae	*Anethum graveolens*	Turkey	4	*Pseudomonas*	Fluorescent *Pseudomonas*, but not identified to species	*Chaetomium, Cladosporium,* unidentified fungi	-
D4/20	Celery seeds	Apiaceae	*Apium graveolens*	Turkey	98	*Erwinia*	Not *Erwinia*	*Rhizopus, Cladosporium, Chromelosporium,*	-
BH1/12	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	NC	CNT		*Cladosporium, Penicillium,* unidentified fungi	-
BH1/13	Bamboo shoot strip	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	-
BH1/3	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	-
BH1/8	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	NC	CNT		*Penicillium*	?
BH2/7	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	NC	CNT		*Cladosporium, Penicillium*	?
BH3/10	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	?
BH3/11	Bamboo shoot tips	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	-
BH3/12	Bamboo shoot tips	Gramineae (Poaceae)	*Bambusa*	Vietnam	NC	CNT		*Cladosporium,*	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
BH3/13	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	-
BH3/9	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	-
R1/3	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	Thailand	NC	CNT		*Penicillium, Rhizopus, Cladosporium,* unidentified fungi	?
R2/2	Bamboo leaf	Gramineae (Poaceae)	*Bambusa*	Thailand	NC	CNT		*Penicillium*	?
S1/8	Bamboo	Gramineae (Poaceae)	*Bambusa*	China	x			*x*	-
S2/4	Bamboo shoots	Gramineae (Poaceae)	*Bambusa*	Vietnam	NC	CNT		*Cladosporium,* unidentified fungi	-
S2/5	Bamboo shoot tip	Gramineae (Poaceae)	*Bambusa*	Thailand	NC	CNT		*Rhizopus,* unidentified fungi	?
BH2/4	Mustard green	Brassicacae (Cruciferae)	*Brassica juncea*	China	NC	CNT		*Aspergillus, Rhizopus, Penicillium, Aureobasidium*	-
R2/1	Preserved mustard green	Brassicacae (Cruciferae)	*Brassica juncea*	China	NC	*Erwinia*	Not *Erwinia*	*Aspergillus*	-
S1/9	Meigancai	Brassicacae (Cruciferae)	*Brassica juncea*	China	NC	CNT		*Penicillium, Cladosporium,* unidentified fungi	?
S3/3	Chinese cabbage	Brassicaceae	*Brassica rapa var pekinensis*	China	NC	*Pseudomonas*, *Erwinia*, *Agrobacterium*	Saprophytic *Pseudomonas*, not *Erwinia*, not *Agrobacterium*	*Mucor, Penicillium, Rhizopus*	-
BH1/4	Cole (bok choi)	Brassicacae (Cruciferae)	*Brassica rapa var chinesis*	China	NC	CNT		*Rhizopus, Penicillium, Cladosporium,* unidentified fungi	-
BH2/3	Cole (bok choi)	Brassicacae (Cruciferae)	*Brassica rapa var chinesis*	China	NC	CNT		*Penicillium, Aspergillus, Cladosporium, Epicoccum*	-
BH1/1	Chillies	Solanaceae	*Capsicum annuum*	China	NC	Xanthamonas, *Erwinia*, Agrobactium	Not *Xanthomonas*, not *Erwinia*, not *Agrobacterium*		-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
BH3/5	Chillies	Solanaceae	*Capsicum annuum*	China	NC	Xanthamonas, *Erwinia*	Not *Xanthomonas*, not *Erwinia*	*Rhizopus, Chaetomium,* unidentified fungi	-
BH3/6	Chillies	Solanaceae	*Capsicum annuum*	China	NC	CNT		*Rhizopus, Penicillium, Aspergillus, Chaetomium,* unidentified fungi	-
BH3/7	Chillies	Solanaceae	*Capsicum annuum*	China	NC	CNT		*Rhizopus, Penicillium, Chaetomium, Cladosporium*	-
BH3/8	Chillies	Solanaceae	*Capsicum annuum*	Thailand	NC	CNT		*Penicillium, Cladosporium*	-
BR1/1	Chillies	Solanaceae	*Capsicum annuum*	India	178	CNT		*Cladosporium, Rhizopus, Penicillium, Aspergillus,* unidentified fungi	-
D1/1	Chillies	Solanaceae	*Capsicum annuum*	Thailand	2	*Pseudomonas*	Not *Pseudomonas*	*Rhizopus, Cladosporium*	-
D3/2	Chillies	Solanaceae	*Capsicum annuum*	Sri Lanka	526	CNT		*Penicillium, Cladosporium, Chaetomium, Pithomyces,* unidentified fungi	-
D4/13	Chillies - without stems	Solanaceae	*Capsicum annuum*	India	10	*Pseudomonas*	Not *Pseudomonas*	*Rhizopus, Aspergillus, Chaetomium, Cladosporium,* unidentified fungi	-
D4/14	Chillies - round	Solanaceae	*Capsicum annuum*	India	90	CNT		*Penicillium, Cladosporium, Aspergillus*	-
D4/15	Chillies - small	Solanaceae	*Capsicum annuum*	India	2	*Pseudomonas*	Not *Pseudomonas*	*Penicillium, Rhizopus, Aspergillus,* unidentified fungi	-
D4/16	Chillies - kashmiri	Solanaceae	*Capsicum annuum*	India	81	CNT		*Penicillium, Rhizopus, Aspergillus*	-
D4/17	Chillies - crushed	Solanaceae	*Capsicum annuum*	India	18	*Xanthomonas*	Not *Xanthomonas*	*Rhizopus, Cladosporium*	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
S3/4	Chillies	Solanaceae	*Capsicum annuum*	Thailand	NC	CNT		*Penicillium, Rhizopus*	-
D4/22	Papita seeds - papaya	Caricaceae	*Carica papaya*	China	96	CNT		*Rhizopus, Penicillium, Cladosporium*	-
D3/1	Chamomile flowers	Compositae	*Chamaemelum nobile*	Yugoslavia	18	*Erwinia*, *Agrobacterium*	Not *Erwinia*, not *Agrobacterium*	*Rhizopus, Chaetomium, Cladosporium, Periconia,* unidentified fungi	-
S1/3	Chrysanthemum	Asteraceae	*Chrysanthemem coronarium*	China	NC	CNT		*Penicillium, Botrytis, Aspergillus*	?
BH3/14	Cassia bark	Lauraeae	*Cinnamomum aromaticum*	China	NC	CNT		*Penicillium, Aspergillus, Epicoccum, Botrytis, Aureobasidium, Cladosporium,* unidentified fungi	x
D4/25	Cassia bark	Lauraeae	*Cinnamomum aromaticum*	Sri Lanka	11	*Agrobacterium*	Not *Agrobacterium*	*Rhizopus, Aspergillus*	x
BR5/3	Watermelon seed	Cucurbitaceae	*Citrullus lantatus*	Iran	1	CNT		*Penicillium, Rhizopus, Aspergillus, Cladosporium*	?
BH1/10	Lime skin	Rutaceae	*Citrus aurantifolia*	Thailand	NC	CNT		*Penicillium*	?
BH3/2	Lime leaves	Rutaceae	*Citrus aurantifolia*	Thailand	NC	CNT		*Aspergillus, Penicillium, Cladosporium*	?
R1/4	Kaffir (lime) leaf	Rutaceae	*Citrus aurantifolia*	Thailand				*Penicillium,* unidentified fungi	-
R1/5	Lime leaves	Rutaceae	*Citrus aurantifolia*	Thailand	NC	CNT		*Aspergillus, Penicillium, Rhizopus*	x
S2/3	Lime leaves	Rutaceae	*Citrus aurantifolia*	Thailand	NC	CNT		*Penicillium, Aureobasidium*	x
BH1/11	Orange peel	Rutaceae	*Citrus sinesis*	China	NC	CNT		*Chaetomium, Rhizopus, Penicillium, Aspergillus*	-
BH3/3	Tangerine peel	Rutaceae	*Citrus tangerina*	China	NC	CNT		*Aspergillus, Penicillium, Rhizopus, Penicillium, Tricoderma*	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
BH1/9	Codonopsis	Campanulaceae	*Codonopsis*	China	10	*Pseudomonas*, *Erwinia* or *Agrobacterium*	Not *Pseudomonas*, not *Erwinia* or *Agrobacterium*	*Penicillium, Rhizopus, Aspergillus, Mucor, Cladosporium*	x
BR1/2	Taro leaves	Araceae	*Colocasia esculenta*	Philippines	263	*Erwinia*	Not *Erwinia*	*Penicillium, Chaetomium, Aspergillus, Cladosporium, Sporormiella, Ulocladium,* unidentified fungi	-
BR5/1	Molakia (Jew's mallow)	Tiliaceae	*Corchorus olitorius*	Egypt	11	*Pseudomonas*, *Xanthomonas*, *Erwinia*, *Agrobacterium*	Not *Pseudomonas*, not *Xanthomonas*, not *Erwinia*, not *Agrobacterium*	*Alternaria, Penicillium, Rhizopus, Cladosporium, Aspergillus, Coniella, Chaetomium*	-
BR1/5	Cumin seeds	Apiaceae	*Cuminum cyminum*	China	55	*Pseudomonas*	Possibly *P. marginalis* and saprophytic *Pseudomonas*	*Rhizopus, Cladosporium, Aspergillus, Ulocladium,* unidentified fungi	?
KC/1	Cumin seeds	Apiaceae	*Cuminum cyminum*	India				*Penicillium, Rhizopus, Chaetomium, Absidia,* unidentified fungi	-
BH1/5	Lemon grass	Gramineae (Poaceae)	*Cymbopogon citratus*	China	NC	CNT		*Rhizopus,* unidentified fungi	-
D4/8	Lemon grass	Gramineae (Poaceae)	*Cymbopogon citratus*	Turkey	82	CNT		*Rhizopus, Aspergillus, Chaetomium, Sporomiella, Epicoccum, Melanospora,* unidentified fungi	-
D4/9	Lemon grass	Gramineae (Poaceae)	*Cymbopogon citratus*	Sri Lanka	158	CNT		*Penicillium, Rhizopus, Aspergillus, Sporormiella,* unidentified fungi	-
BR1/3	Cardamon seeds	Zingiberaceae	*Elettaria cardamonum*	PNG	446	*Erwinia*, *Agrobacterium*	Not *Erwinia*, not *Agrobacterium*	*Penicillium, Aspergillus, Cladosporium, Epicoccum,* unidentified fungi	-
BR1/4	Cardamon pods	Zingiberaceae	*Elettaria cardamonum*	Guatemala	1	*Erwinia*, *Agrobacterium*	Not *Erwinia*, not *Agrobacterium*	*Aspergillus, Rhizopus, Penicillium, Chaetomium*	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
BR1/6	Cardamon brown pods	Zingiberaceae	*Elettaria cardamonum*	India	3	CNT		*Cladosporium, Penicillium*	x
BR3/4	Fennel seeds	Apiaceae	*Foeniculum vulgare*	Lebanon	4	*Xanthomonas*	Not *Xanthomonas*	*Rhizopus, Stachybotris, Penicillium, Thielavia*	-
D4/23	Fennel seeds	Apiaceae	*Foeniculum vulgare*	India	23	*Xanthomonas*, *Erwinia*, *Agrobacterium*	Not *Xanthomonas*, not *Erwinia*, not *Agrobacterium*	*Rhizopus, Alternaria, Chaetomium, Ulocladium, Cladosporium,* unidentified fungi	-
BR3/2	Liquorice	Leguminosae	*Glycyrrhiza glabra*	Syria	34	CNT		*Penicillium, Rhizopus, Cladosporium, Aspergillus, Chaetomium, Mucor, Ulocladium, Papulaspora, slime mould*	-
BH1/7	Lily flower (golden needles)	Liliacae	*Hermerocallis fulva*	China				*Penicillium, Aspergillus, Cladosporium*	-
R1/2	Lily flower (golden needles)	Liliacae	*Hermerocallis fulva*	China	NC	CNT		*No growth*	?
BH2/2	Barwon flower (nightblooming cactus)	Cactaceae	*Hylocereus undatus*	China	NC	CNT		*Penicillium, Alternaria, Rhizopus*	-
S1/5	Barwon flower (nightblooming cactus)	Cactaceae	*Hylocereus undatus*	China	NC	CNT		*Penicillium, Cladosporium*	?
BR1/7	Star anise	Illiciaceae	*Illicium anisatum*	Iran	55	CNT		*Penicillium, Cladosporium, Aspergillus*	-
D4/24	Aniseed star	Illiciaceae	*Illicium anisatum*	China	88	*Pseudomonas*	Possibly *P. marginalis*	*Penicillium, Rhizopus, Epicoccum*	-
BH3/1	Bay leaves	Lauraceae	*Laurus nobilis*	Indonesia	NC	CNT		*Penicillium, Cladosporium, unidentified*	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
D4/3	Bay leaves	Lauraceae	*Laurus nobilis*	India	1887	*Xanthomonas*	Not *Xanthomonas*	*Rhizopus, Absidia, Alternaria, Epicoccum, slime mould,* unidentified fungi	-
S2/1	Bay leaves	Lauraceae	*Laurus nobilis*	Turkey	NC	CNT		*Penicillium, Cladosporium, Rhizopus,* unidentified fungi	-
S3/5	Bay leaves	Lauraceae	*Laurus nobilis*	Indonesia	NC	CNT		*Penicillium*	-
R2/4	Honey suckle	Caprifoliaceae	*Lonicera sp.*	China	NC	CNT		*Penicillium, Rhizopus, Chaetomium*	-
BR2/1	Tomatoes	Solanaceae	*Lycopersicon esculentum*	Italy	7	CNT		*Penicillium, Rhizopus, Aspergillus*	-
D4/12	Tomatoes	Solanaceae	*Lycopersicon esculentum*	Turkey	1	*Pseudomonas*	Not *Pseudomonas*	*Penicillium, Rhizopus, Aspergillus, Chaetomium, Melanospora*	-
BR5/2	Mint	Labiatae	*Mentha*	India	10	*Pseudomonas*, *Agrobacterium*	Fluorescent *Pseudomonas*, but not identified to species, not *Agrobacterium*	*Stachybotris, Acremonium, Aspergillus, Rhizopus, Chaetomium*	?
S3/1	Bitter gourd	Cucurbitaceae	*Momordica charantia*	China	NC	CNT		*Aspergillus, Penicillium*	-
BH1/6	Curry leaves	Rutaceae	*Murraya koenigii*	Indonesia	NC	CNT			x
D4/2	Curry leaves	Rutaceae	*Murraya koenigii*	Sri Lanka	2002	*Pseudomonas*, *Agrobacterium*	Not *Pseudomonas*, not *Agrobacterium*	*Penicillium, Rhizopus, Aspergillus, Cladosporium, Mucor*	?
S2/2	Curry leaves	Rutaceae	*Murraya koenigii*	Sri Lanka	NC	CNT		*Chaetomium, Penicillium, Aspergillus,* unidentified fungi	x
S1/1	Water cress	Brassicacae (Cruciferae)	*Nasturtium officinale*	China	NC	CNT		*Aureobasidium, Phoma, Penicillium*	-
BH3/15	Lotus leaves	Nelumbonaceae	*Nelumbo nucifera*	Thailand	NC	CNT		*Penicillium, Rhizopus,* unidentified fungi	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
D4/10	Olive leaves	Oleaceae	*Olea europaea*	Turkey	301	*Pseudomonas*	Not *Pseudomonas*	*Penicillium, Aspergillus, Cladosporium*	-
D4/5	Marjoram	Labiatae	*Origanum majorana*	Turkey	1	*Pseudomonas*	Not *Pseudomonas*	*Penicillium, Rhizopus, Aspergillus, Chaetomium*	-
BR4/1	Oregano bunches	Labiatae	*Origanum vulgare*	Greece	19	CNT		*Penicillium, Aspergillus, Chaetomium, Sporormiella,* unidentified fungi	?
D4/11	Rampe leaves (pandan)	Pandanaceae	*Pandanus amaryllifolius*	Sri Lanka	6	*Pseudomonas*	Not *Pseudomonas*	*Chaetomium*	-
D2/1	Poppy seeds	Papaveraceae	*Papaver somniferum*	Poland	7	*Pseudomonas*, *Xanthomonas*, *Agrobacterium*	Not *Pseudomonas*, not *Xanthomonas*, not *Agrobacterium*	*Penicillium, Rhizopus, Aspergillus, Cladosporium,* unidentified fungi	-
BH3/17	Black beans	Leguminosae-Papilionoideae	*Phaseolus vulgaris*	China?	3	*Pseudomonas*, *Xanthomonas*	Possibly *P. marginalis*, not *Xanthomonas*	*Penicillium, Cladosporium, Aspergillus,* unidentified fungi	-
D1/2	Kidney beans	Leguminosae-Papilionoideae	*Phaseolus vulgaris*	Pakistan	131	CNT		*Rhizopus, Penicillium,* unidentified fungi	?
S1/2	Betel leaves	Piperaceae	*Piper betel*	Thailand	NC	CNT		*No growth*	-
BH2/8	Prunella vulgaris (self heal)	Lamiacae	*Prunella vulgaris*	China	NC	*Erwinia*	Not *Erwinia*	*Penicillium,* unidentified fungi	?
BH2/1	Chili prunella	Lamiacae	*Prunus*	China	NC	*Erwinia*	Not *Erwinia*	*Aspergillus, Chaetomium, Penicillium*	-
BH2/6	Sweet turnip (daikon)	Brassicacae (Cruciferae)	*Raphanus sativus*	China	NC	CNT		*No growth*	?
BR3/3	Roses	Rosaceae	*Rosa*	Jordan	4	CNT		*Rhizopus, Aspergillus, Penicillium, Ulocladium,* unidentified fungi	-
D4/7	Rosemary	Labiatae	*Rosmarinus officinalis*	Spain	392	*Pseudomonas*	Not *Pseudomonas*	*Penicillium, Aspergillus, Cladosporium,* unidentified fungi	-

Sample Code	Product	Family	Genus / Species	Origin	Bacteria			Fungi isolated	Virus
					Count cfu/g	Possible Pathogen	Pathogen Confirmation		Possible symptoms
BR3/1	Sage leaves	Labiatae	*Salvia*	Jordan	3	CNT		*Aspergillus, Neurospora, Chaetomium, Alternaria, Papulaspora*	?
D4/6	Senna pods	Fabaceae	*Senna*	India	1629	CNT		*Penicillium, Rhizopus,* unidentified fungi	x
BR3/5	Sesame seeds	Pedaliaceae	*Sesamum orientale*	Lebanon	1069	*Pseudomonas*	Possibly *P. marginalis*	*Rhizopus, Penicillium, Aspergillus*	-
D4/4	Fenugreek leaves	Leguminosae-Papilionoideae	*Trigonella corniculata*	India	269	*Pseudomonas*, *Xanthomonas*, *Erwinia*	Not *Pseudomonas*, not *Xanthomonas*, not *Erwinia*	*Rhizopus, Penicillium, Cladosporium,* unidentified fungi	-
D1/3	Mung beans	Leguminosae-Papilionoideae	*Vigna radiata*	Pakistan	2183	*Pseudomonas*, *Xanthomonas*, *Agrobacterium*	Fluorescent *Pseudomonas*, but not identified to species, not *Xanthomonas*, not *Agrobacterium*	*Rhizopus, Cladosporium, Aspergillus, Penicillium, Macrophomina phaesolina* (Pathogen),* unidentified fungi	-
S3/2	Snake beans	Leguminoseae	*Vigna sesquipedalis*	China	NC	CNT		*Khuskia oryzae, Chrysonilia sitophila, Nodulisporium, Cladosporium*	-
D4/19	Horse gram	Leguminosae-Papilionoideae	*Vigna unguiculata*	Sri Lanka	38	*Erwinia* or *Agrobacterium*	Not *Erwinia* or *Agrobacterium*	*Penicillium, Rhizopus, Aspergillus, Cladosporium,* unidentified fungi	x
D4/26	Ginger roots	Zingiberaceae	*Zingiber officinale*	India	54	*Erwinia*	Not *Erwinia*	*Penicillium, Aspergillus, Rhizopus, Cladosporium, Epicoccum*	?
S1/4	Ginger slice	Zingiberaceae	*Zingiber officinale*	China	NC	CNT		*Cladosporium, Rhizopus, Penicillium*	?

NC = not counted CNT= colonies not typical

x = not tested ? = possible symptoms observed - = no symptoms were observed