Citrus exotic pest and disease survey -2010

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A report for:

PLANT STANDARDS BRANCH, BIOSECURITY VICTORIA

by:

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Contents

Definitions

BV	Biosecurity Victoria
DPI	Department of Primary Industries Victoria
MVCB	Murray Valley Citrus Board
CTV	Citrus Tristeza Virus
Citrus patch	An area of citrus trees of the same variety, rootstock and age
Citrus block or property	A property that includes one or more citrus patches but that may also carry other crop types

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- Citrus growers across the region for their cooperation and willingness to be involved in the survey

Executive summary

Citrus trees on 40 properties in the Murray Valley growing districts of Victoria, from Kerang to the South Australian border, were surveyed between June and August 2010 for a range of exotic diseases and pests as part of an ongoing surveillance program. A high degree of cooperation from the industry and assistance from the Murray Valley Citrus Board ensured the successful operation of the survey. Survey and hygiene procedures followed during the survey were adapted from protocols developed during previous surveillance activities for citrus canker and other exotic pests and diseases of concern.

The field survey and subsequent laboratory testing of leaf samples did not detect any of the target diseases and pests, namely: citrus canker (*Xanthomonas citri* subsp. *citri*), bacterial spot (*Xanthomonas axonopodis* pv citrumelo), mel secco (*Phoma tracheiphila*), citrus scab (*Elsinoe fawcetti*), sweet citrus scab (*Elsinoe australis*), citrus black spot (*Guignardia citricarpa*), citrus stubborn (*Spiroplasma citri*), citrus tristeza closterovirus (CTV), citrus greening/ Huanglongbing (*Candidatus Liberibacter* sp.), powdery mildew (*Oidium tingitaninum*), cercospora spot (*Phaeoramularia angolensis*), Satsuma dwarf virus, citrus snow scale/white louse scale (*Unaspis citri*), citrus red mite (*Panonychys citri*), glassy-winged sharp shooter (*Homalodisca vitripennis*), spiralling whitefly (*Aleurodicus dispersus*) and purple round scale/Florida red scale (*Chrysomphalus aonidum*).

Statistical analysis of accumulative surveillance results yielded estimates of the probability of Victoria being free from these exotic diseases and pests to be 99.7% for citrus canker and 98.2% for the other targeted organisms.

Citrus tristeza closterovirus was detected in samples from 34 of the forty inspected sites, but whether any severe CTV strains were present could not be determined with the available tests.

Introduction

Under a Commonwealth Government biosecurity program, Australian farming industries are periodically surveyed for the presence of exotic pests and diseases. The surveys, performed with industry support, are intended to reassure our trading partners that certain plant pests and diseases are absent from our farms.

Victorian citrus properties were surveyed in 2004 for citrus canker and in 2006 for a broader range of exotic pests and diseases. As part of this ongoing surveillance program, Biosecurity Victoria (BV) commissioned DPI Victoria to undertake a survey for exotic pests and diseases on citrus properties in the Murray Valley region of Victoria. The survey was to include approximately 40 sites and provide a broad geographical cover of the citrus districts within the region.

Methods

Geographical region to be surveyed

Citrus properties to be surveyed were to be located in the Murray Valley, on the Victorian side of the Murray River between Koondrook in the east and the South Australian border in the west.

Selection of properties and citrus patches for inspection

To ensure the survey provided a good cover of the region, Sunrise21 Inc was contracted to produce a map of all citrus property locations in the region, based on 2010 data. The map was used to highlight potential survey sites at a district level. This information was then used by the Survey Officer and Murray Valley Citrus Board (MVCB) to select specific properties to be contacted. The targeted selection of properties ensured that the survey included:

- small and large properties
- family and corporate properties
- organic and conventional properties

When growers agreed to have their citrus inspected, the MVCB provided the Survey Officer with each grower's contact details, property map and planting data. The Survey Officer then contacted each grower directly to arrange a suitable time to visit the property. The Survey Officer also used the planting data to select specific citrus patches for inspection. The aim was to include, as far as possible, a broad range of varieties, rootstocks and tree ages, with the inspected patches providing a good representation of the industry as a whole. Inspection of a broad range of varieties was also necessary to enhance the chance of detection of pest and disease symptoms, as the target pest organisms exhibit different preferences for citrus varieties.

Survey procedure

Based on the experience from past citrus surveys in the Murray Valley, this survey was conducted with the assumption that exotic pests and diseases were not present in the region. Preparations were however in place to allow for the necessary hygiene and decontamination procedures to be implemented immediately on-site in the event that a suspect exotic pest or disease was encountered.

The actual procedures followed during the survey were adapted from two earlier protocols (see CAS Biosecurity 2006 and Plant Standards Branch 2007) and are listed in Appendices 1 to 4. As detailed in Appendix 1, even though the citrus patches were assumed to be free of exotic pests and diseases, basic hygiene measures including boot and hand decontamination were applied between properties throughout the survey.

The form used to record survey patch details required under the above procedures is included in 'Appendix 5: Field Survey Sheet'. This sheet was modified to reflect the terminology used by MVCB in defining citrus properties and patches.

Sample analysis

Leaf samples collected during the survey were inspected by DPI Crop Health Services for the presence of disease organisms and invertebrate pests. The samples were specifically tested for Citrus Tristeza Virus (CTV) and citrus greening disease Candidatus Liberibacter. Other pathogens were investigated when visible symptoms indicated a need.

Resources

BV provided DPI with laminated colour photographs of exotic citrus pest and disease symptoms for referral during the survey, as well as GPS units and sample security seals. Other necessary materials (see 'Appendix 4 Equipment lists') were purchased or provided as needed by the Survey Officer.

Survey officers

Craig Murdoch (BV Knoxfield) was the Survey Coordinator for this survey. David Madge (DPI Mildura) as the Survey Officer, was responsible for the day to day planning, coordination and conduct of the survey and liaison with SunRise21, the Murray Valley Citrus Board, BV and citrus growers. Mr Madge had 27 years research experience with DPI in the Sunraysia district of the Murray Valley, including citrus IPM research and extension. He maintained good relations with the industry organisations and numerous citrus growers.

Pests and diseases targeted

Citrus canker, *Xanthomonas citri* subsp. *citri*

Varieties affected: Grapefruit > sweet orange and lemon > mandarin.

Citrus canker is caused by the bacterium Xanthomonas axonopodis pv. citri. Other common names of this disease are, bacterial canker of citrus, South American canker, false canker, canker B, citrus

bacteriosis and Asiatic canker. All aboveground parts of susceptible citrus varieties are attacked, particularly young leaves, branches and fruit (Schubert et al., 2001). The disease first appears as small, raised, watery circular spots or lesions on leaves or fruit. The lesions are initially white or yellow/dark green and later become thick, brown and corky. As the lesions grow they thicken into white, spongy scabs which darken and become corky before developing crater-like centres surrounded by a yellow halo. The lesion centres may fall out, creating a short-hole effect The ultimate size of the lesion depends on the host, on some grapefruit varieties lesions are as large as 9 mm in diameter.

Similar symptoms appear on twigs and fruit, including raised corky lesions surrounded by a water soaked margin, although twig lesions do not develop a halo. Sunken craters are particularly distinct on fruit, but the lesion does not penetrate far into the rind. Defoliation, dieback and fruit drop is common as disease severity increases (Goto and Yaguchi, 1979).

Bacterial spot (nursery leaf spot), *Xanthomonas axonopodis* pv Citrumelo

Varieties affected: Possibly all citrus

Similar symptoms to canker but lesions may have larger water-soaked margins and become less pustular and corky than canker. Possibly only obvious in nursery situations.

Mal secco, *Phoma tracheiphila*

Varieties affected: Lemon and lime > mandarin. Rare on grapefruit and sweet orange.

Symptoms appear in spring and include wilting or drying of leaves, withered shoots or suckers bearing signs of fungal infection, especially silvery-grey bark that eventually ruptures to show numerous small black spots (spore bodies). Recently infected woody tissue has a pinkish coloration and the midrib of fallen leaves may have a reddish colour. When this disease develops rapidly, the foliage can dry on the tree.

Citrus Scab (lemon or sour orange scab), *Elsinoe fawcetti*

Varieties affected: Sweet orange and lime may be less susceptible.

Trees grafted onto rough lemon rootstock are most susceptible to this fungus. Leaf, stem and fruit symptoms appear as light coloured, raised rough corky scabs, and leaves may become distorted. A yellow halo surrounds the leaf scabs on some citrus varieties. Development of the disease is favoured by warm moist conditions.

Sweet citrus scab (sweet orange scab), *Elsinoe australis*

Varieties affected: Sour orange may be less susceptible.

Affects the fruit and leaves and rarely the stems. Lesions on fruit and leaves are dark in colour, round, flattened and smooth. On younger fruits it causes a deformation of the rind, forming corky, round/irregular, protuberant lesions 2–6 mm in diameter. Leaf and twig lesions are initially funnel-shaped pockets, later scab-like, smooth and glossy, up to 2 mm diameter.

Citrus Black Spot, *Guignardia citricarpa*

Varieties affected: All citrus, particularly lemons.

Infections occur in the first 4–6 months after fruit set but the fungal mycelium is dormant just under the skin until fruit maturity when black spots appear on the fruit.

Citrus Stubborn, *Spiroplasma citri*

Varieties affected: Most citrus. Symptoms milder on lemons and limes. Many non-citrus hosts.

Trees affected when young are stunted. Leaves are shorter, broader, often cup shaped and more upright, sometimes mottled or chlorotic. Shoots may be abnormally bunched and fruit may be stunted, lopsided or acorn shaped. Fruit often don't colour fully at their stem end. This disease may reduce yield under hot, dry conditions.

CTV, Citrus Tristeza Closterovirus

Varieties affected: All citrus but symptoms worst on lime, grapefruit and some sweet orange.

Tree stunting, stem pitting, leaf yellowing and cupping, and reduced fruit size may be seen. Trees on sour orange rootstock (C. aurantium) can decline rapidly and die because of the death of the rootstock at the graft union. Mild strains of tristeza which cause symptomless infections are often used to cross-protect citrus from severe CTV.

Citrus greening/ Huanglongbing, *Candidatus Liberibacter* spp.

Varieties affected: All citrus but worst on sweet orange and mandarin.

Affected shoots are yellowed with asymmetric mottled leaves (mottling across veins); small upright chlorotic leaves, out of phase flushing and branch dieback. Unseasonal and heavy flowering may occur on diseased branches. Fruit can be small, lopsided and bitter-tasting with small, brown, aborted seeds and uneven colouring at maturity. Excessive fruit drop can occur. Numerous other diseases and nutrient deficiencies can result in superficially similar symptoms.

Powdery mildew, *Oidium tingitaninum*

Varieties affected: All citrus but mandarin, tangerine and sweet orange are most susceptible.

This disease affects leaves, stems and fruit and is more likely to occur in nurseries. Whitish powdery patches of mildew form on the upper surface of leaves, which may then shrivel and fall. May cause premature leaf and fruit drop and dieback. Older damage on leaves and fruit turns brown/grey, with remaining fruit developing brown irregular markings.

Cercospora spot, *Phaeoramularia angolensis*

Varieties affected: Possibly all citrus.

Fruits and leaves are much more susceptible than stems, on which symptoms are rare. Leaves develop initially greenish-yellow patches. At maturity, leaf spots (mainly on the underside of leaves) are 4-10 mm or more in diameter, pale-brown to brown (blackish-brown when sporulation is dense), surrounded by a dark-brown margin and a yellow halo. The centre often becomes detached resulting in a shot-hole effect. Generalised leaf death caused by joining of several lesions can result in defoliation. During wet weather the lesions sporulate and become black.

On young fruits, brown necrotic lesions form, usually circular, slightly sunken, surrounded by a raised ring giving the fruit a blistered appearance. During wet weather, the lesions sporulate and become black. In young fruits, a generalised necrosis sometimes forms, resulting in premature fruit drop. When infection of stems occurs, the lesions are dark-brown and usually occur as extensions of petiole lesions. They may join resulting in stem die-back or the formation of corky internodal regions.

Satsuma dwarf virus

Varieties affected: Most citrus, but worst on Satsuma mandarin.

On Satsuma mandarin, affected laves are typically narrower, darker in colour and spoon or boat shaped, and infected trees are stunted and produce lower yields. Other citrus types may have mild or absent symptoms.

Citrus snow scale/white louse scale, *Unaspis citri*

Varieties affected: All citrus.

The scales are mussel-shaped, 1.5-2 mm long, dull brown or grey, with grey margins and length wise median ridges. Infestations peak in autumn. Green bark, leaves, twigs and fruit are affected. On leaves, scales gather close to ribs and the midribs. Young trees (under 10 years of age) are more likely to have lighter infestations on the trunk and branches while heavier infestations spread to the twigs, leaves and fruit.

Citrus red mite, *Panonychys citri*

Varieties affected: Lemon and grapefruit > orange > tangerine.

Mild conditions favour this pest. The mites are 0.5mm long. Eggs are bright red and approximately 0.13mm in diameter. Eggs are attached to the plant surface by threads extending from a stalk at the egg tip. These mites prefer light green, maturing foliage. Infested leaves and fruit have a pale appearance. Light infestations show damage at the leaf base only. Heavy infestations result in defoliation and twig die back and may lead to reduced yields in the following season.

Glassy-winged sharp shooter, *Homalodisca vitripennis*

Varieties affected: Many plant types including citrus.

Adults are 13-14 mm long, dark brown with small yellow dots on head and thorax. Face and legs are yellow-orange in colour. The eyes are yellow with dark speckles and the abdomen has ivory and black markings under the abdomen. Wings are membranous, large, translucent and smoky-brown with reddish veins. Nymphs are wingless and grey. This pest is important as a disease vector.

Spiralling whitefly, *Aleurodicus disperses*

Varieties affected: Many plant types including citrus.

Spiralling whiteflies are small (0.2 mm long), white and exhibit a moth-like in appearance in their mode of flight. On plants with heavy infestations, whiteflies and their nymphs occur in dense populations on the undersides of the leaves of the host plant. Spiralling whitefly females produce characteristic spirals resembling fine cotton wool and mostly containing microscopic eggs. Spirals are usually found on the underside of leaves, but in heavy infestation may also be found on the upper leaf surface, fruit and on non-plant material.

Purple round scale/Florida red scale, *Chrysomphalus aonidum*

Varieties affected: Many plant types including citrus.

Adult and intermediate-stage females form flat to moderately convex, circular scales up to 2 mm in diameter, each with a slightly raised, sub-central point which is sometimes pale. If the scale is

lifted off with the point of a needle, the insect beneath is yellow and up to 1.7 mm long. Male scales are slightly paler than female scales, and are elongate-oval and half the size. First-instar nymphs are 0.3 mm long and have legs but soon settle to form circular white scales (whitecaps) up to 0.4 mm across. These become incorporated into the scales of subsequent stages, forming the paler subcentral point.

C. aonidum is a leaf-infesting species, but may spread to fruits, stems and trunks and may cause premature leaf and fruit drop and stem dieback. An infestation appears as dark-purple to reddish-brown or black spots with paler margins, on both surfaces of shaded leaves of the host plant. Heavy infestations cause yellowing of the leaves, followed by defoliation of part or all of the host. C. aonidum prefers shade and is therefore most common in the lower part of the canopy. It rarely infests green wood.

Statistical analysis

The survey design was subjected to scenario tree analysis (Martin and Sergeant 2006) to obtain surveillance system sensitivity and probability of freedom determined for the target exotic pests and diseases in Victoria for the previous two seasons and for the current survey. Baysean analysis was then used to determine the additive impact on the probability of freedom after combining the three years of survey data.

It was determined that taking into account the previous surveys and the expected number of properties for this current survey (40), the inspection of 100 trees per property would provide a suitable level of confidence in the findings of the current survey.

Results

Pest and disease observations

During the visual inspection of trees, none of the target exotic pests or diseases were observed. Nevertheless, at least one leaf sample was collected per citrus patch, with a total of 44 samples taken during the survey.

All leaf samples were submitted to DPI Crop Health Services for diagnosis for the purpose of surveillance validation. None of those samples were found to be infected with any of the target exotic diseases and no exotic pests were detected on the samples.

Samples from 34 of the 40 survey sites were found to carry citrus tristeza virus (CTV). This is not unusual given that it has been common practise to inoculate trees with a mild strain of CTV for cross-protection against severe strains. Specific testing for severe CTV strains was not carried out.

Location and characteristics of inspected citrus patches

Between June 10 and August 5 2010, 40 citrus patches were inspected. Figure 1 shows the location of Victorian citrus properties in the Murray Valley, and of those visited during the survey. Koondrook (east of Murrabit) was not included in the survey as citrus plantings in that area were found to have been removed since the previous survey. Details of the individual citrus patches that were inspected are listed in 'Appendix 6: Survey site data'.

Table 1 and Table 2 show that as intended, there was considerable variation in the size of properties visited, and in the size, age and varietal mix amongst the patches inspected. There was also considerable variation in the time required for each site inspection. This was due largely to the variation in patch size (larger patches required more walking), tree size (larger trees required more observation time), tree condition (unhealthy/pest-infested trees required more observation time) and ground condition (cultivated soil slowed progress). 'Time taken' (Table 1) relates to the on-site time required for the inspection of 100 trees, sample collection and packaging, and decontamination. It does not include travel time or grower liaison time before the field inspection.

Figure 1. Locations of citrus properties and inspection sites

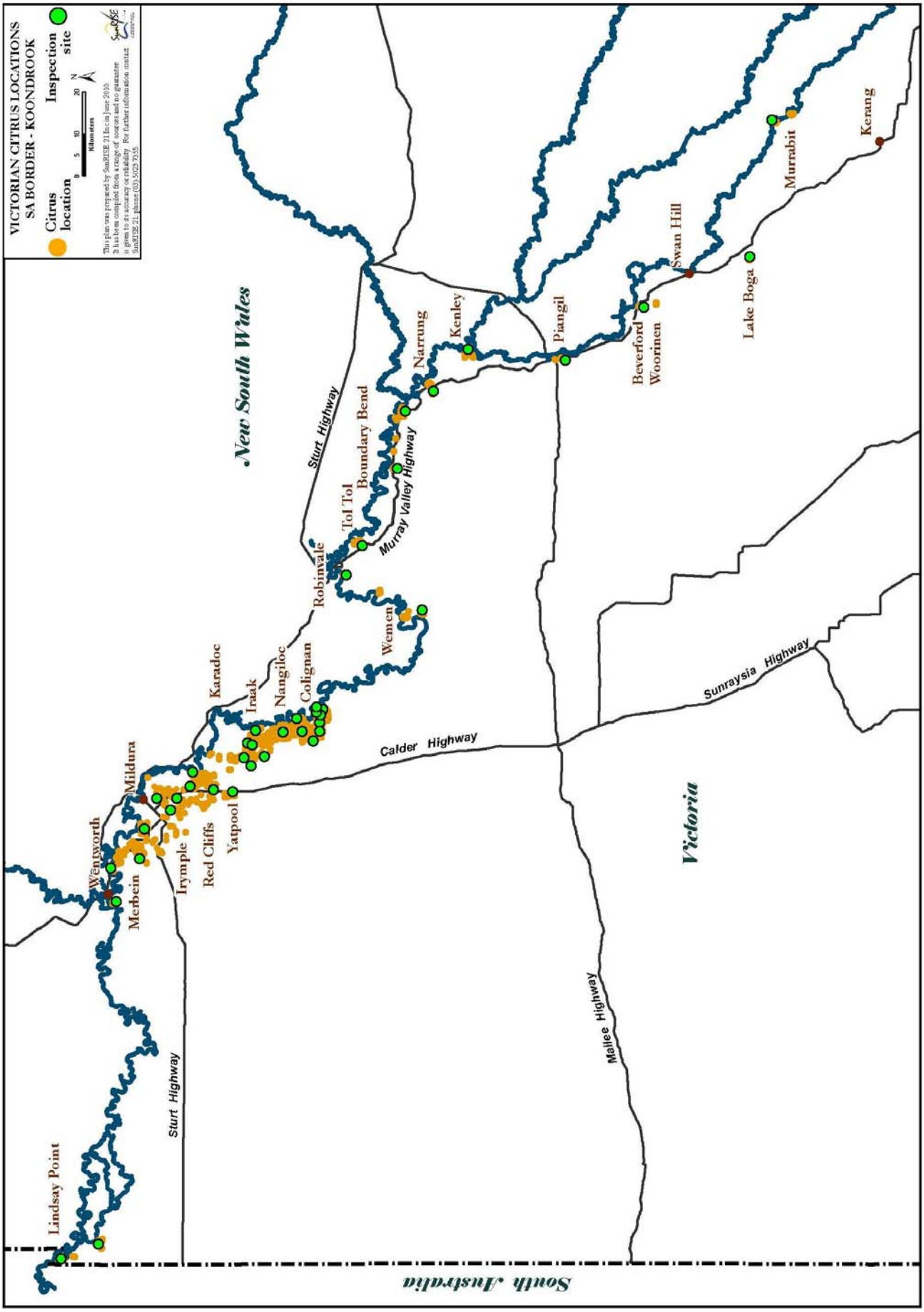

Table 1: Citrus area and tree number on properties visited and patches inspected

		Min	Max	Average	Total over all properties or patches
Properties surveyed	Ha of citrus	0.37	165.40	23.0	919.73
	No. of trees	180	98,391	11278.9	451,157
Patches inspected	Ha of citrus	0.27	8.93	2.6	104.99
	No. of trees	63	8,590	1341.0	54,982
	Tree age	4	65	20.0	
	Time taken	1hr 10m	3hr	2hr	

Table 2: Types of citrus inspected

Tree type	Variety	Rootstock
Grapefruit	Marsh, Star ruby	Citrange, Trifoliata
Lemon	Lisbon	Citrange
Mandarin	Afourer, Imperial	Citrange, Cleopatra, Trifoliata
Orange	Navels (Barnfield, Chislett, Edwards, Wiffen, Late Lane, Leng, Navelina, Pollock, Thomson and Washington), Valencia	Citrange, Citronelle, Cleopatra, Sweet Orange, Trifoliata
Pummelo	Pummelo	Sweet Orange
Tangelo	Tangelo	Sweet Orange

Only two citrus types, oranges and mandarins, made up 90% of the hectares inspected. The other types were represented by relatively small areas. As Table 3 shows, the overall mix of citrus types that were inspected reflected the industry's composition fairly closely. The age mix of trees inspected also reflected that of the industry (Table 4).

Table 3: Surveyed area by citrus type

Tree type	No. of patches	Total area (Ha)	% of total Ha surveyed	Industry composition by tree type[1]
Orange	30	79.9	76.1%	81.64%
Mandarin	5	14.6	13.9%	12.48%
Grapefruit	2	4.1	3.9%	2.53%
Lemon	1	4.9	4.6%	1.76%
Tangelo	1	0.3	0.3%	1.46%
Lime	0	0.0	0.0%	0.07%
Pummelo	1	1.3	1.2%	0.02%
Other	0	0.0	0.0%	0.04%

Table 4: Surveyed area by tree age category

Tree age (years)	% of Ha inspected	Industry composition by tree age1
>=6	91.5%	88.1%
<6	8.5%	11.9%

Statistical analysis

The statistical analysis provides a probability of freedom of the target pests and diseases, taking into account the accumulative results of the current and two previous surveys.

Based on three years of citrus canker surveys in Victoria (2004, 2006, 2010), the probability that Victoria is free of citrus canker is 99.7%2. That is, based on the survey methodology, if 1% of Victoria's citrus properties were infected with citrus canker, with an average of 5% of trees on those properties being infected, then there is a 99.7% probability that a canker infection would have been detected during the surveys.

Based on two years of citrus surveys in Victoria (2006, 2010), the probability that Victoria is free of exotic pests and diseases other than citrus canker is 98.2%2. That is, based on the survey methodology, if Victoria had 1% of its citrus properties infected with an exotic pest or disease other than citrus canker, with an average of 5% of trees on those properties being infected, then there is a 98.2% probability that the exotic pest or disease would have been detected during the surveys.

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[1] Murray Valley Citrus Board Crop Forecast 2010/2011 (April 2010)

[2] Martin Mebalds, BV (personal communication)

Industry liaison

The good relationship that existed between the Survey Officer, the MVCB and individual growers was maintained throughout this survey. MVCB did not hesitate to assist with property selection and initial grower contact, and was prompt in providing all the information necessary to undertake the survey. MVCB also assisted with industry awareness by distributing a media release about the survey directly to all citrus growers. This working relationship between DPI and the citrus industry, especially through the MVCB, was invaluable for the purpose of the survey and would be crucial in the event of management of an exotic pest or disease incursion. It should be maintained, and where possible enhanced, for future benefit for both parties.

Discussion

This survey was completed without incident and with a high level of cooperation from the citrus industry, which was greatly appreciated. The only issue encountered that was of any concern regarding planning and execution of the survey was that two citrus growers declined to be involved in the survey, citing their concern about the possible ramifications of an exotic disease being detected on their property. This concern is understandable given the citrus canker experience, but may indicate a need for education regarding the industry-wide value of surveillance for early detection of exotic pest and disease incursions.

Inspecting trees for a significant number of pests and diseases at the one time did not pose any problems. The approach taken was to keep the range of target symptoms in mind while actually scanning trees for anything unusual, i.e. not looking for specific sets of symptoms. Aiding this was the Survey Officer's experience in citrus, which allowed for the easy 'filtering' of normal/typical appearances of citrus including common pests and nutrient deficiencies. Before beginning the field work, the Survey Officer collated additional images of symptoms not included in the original field guide material. The field guide should be updated to cover all the target organisms before the next survey commences.

In preparation for this work, it was discussed whether previously inspected patches should be avoided to increase the area of citrus plantings covered by the series of surveys. Because four years had passed since the previous (2006) survey, and data from that and the 2004 survey were not on hand, it was decided that the selection of patches for the current survey wouldn't take their survey history into account. It does however seem logical that confidence in the accumulative survey findings would increase as the proportion of citrus plantings inspected grows with each survey. The selection of previously uninspected citrus patches for each new survey would require the collation of an accumulative record of previous inspections.

Within some citrus patches, trees are grown in hedgerows making it more difficult and/or time-consuming to inspect entire individual trees. An alternative and more practical approach for those sites would be to inspect twice as many half-trees, but the statistical validity of such an approach needs to be confirmed.

One of the laboratory tests applied to leaf samples from this survey, a PCR test for citrus Tristeza virus, not surprisingly detected the virus in most samples. To be of value in future surveys, tests for CTV need to be capable of differentiating between mild and severe CTV strains, as it is the severe strains that are of concern to Australia's industry.

Conclusions

The field and laboratory observations and laboratory testing performed during this survey support ongoing claims that citrus grown in the Victorian Murray Valley region is free of the following exotic pests and diseases:

Citrus Canker *Xanthomonas citri* subsp.

Bacterial spot *Xanthomonas axonopodis* pv citrumelo

Mal secco *Phoma tracheiphila*

Citrus scab *Elsinoe fawcettii*

Sweet citrus scab *Elsinoe australis*

Citrus Black Spot *Guignardia citricarpa*

Citrus Stubborn *Spiroplasma citri*

Citrus greening/ Huanglongbing *Candidatus Liberibacter asiaticus, africanus & americanus*

Powdery mildew *Oidium tingitaninum*

Cercospora spot *Phaeoramularia angolensis*

Satsuma dwarf virus

Citrus snow scale (white louse scale) *Unaspis citri*

Citrus red mite *Panonychus citri*

Glassy-winged sharp shooter *Homalodisca vitripennis*

Spiralling whitefly *Aleurodicus dispersus*

Purple round scale (Florida red scale) *Chrysomphalus aonidum*

Recommendations

Based on the experience gained from this survey, it is recommended that with regard to the next citrus survey, the Survey Coordinator and/or Survey Team:

1. Collate an accumulative list of citrus patches inspected during this and the previous two surveys and maintain such a list to allow future surveys to easily identify properties and patches that have not yet been visited.
2. Develop a more comprehensive set of identification sheets to cover all pests and diseases of interest.
3. Continue to involve local peak industry bodies for assistance in local knowledge, industry communications, relationship building and raising the profile of DPI.
4. Perform the next citrus survey in spring/early summer to increase the variation in seasonal timing of inspections.
5. Determine an appropriate approach to growers who decline to have their properties surveyed, possibly and industry-wide education/awareness exercise.
6. Determine a practical and statistically valid approach to inspection of hedge-rowed trees where the visual inspection of single entire trees is cumbersome.

It is also recommended that:

7. Tests able to detect severe strains of CTV should be used for future surveys as the mild strains currently detected are common and of little consequence.

References

CAS Biosecurity (2006) Citrus exotic pest and disease survey report - 2006

Martin, A and Sergeant, E (2006) Evaluation of Surveillance Systems: The use of non survey data sources to demonstrate freedom from disease. CRC Plant Biosecurity.

Plant Standards Branch (2007) Citrus Canker PSS-01: Surveillance procedure. Version 2.0, DPI Victoria.

Appendix 1 Field survey procedure

General hygiene

1. Wear boots that can be cleaned easily. Clean and disinfect boots (e.g. with Phytoclean) before leaving each property.
2. Wear overalls – they can be removed, bagged and disinfected in the event that any suspect P&D is encountered.
3. Carry a decontamination kit (see Table 6) and remove it from the vehicle to be ready for use, before entering the citrus patch.
4. Enter each patch on the assumption that there is unlikely to be any exotic P&D issue present. If a suspected high-risk exotic P&D is found, backoff, decontaminate as per 'Appendix 3 Full decontamination procedure', and notify the Survey Coordinator). Do not enter any other citrus properties that day.
5. Wear disposable gloves when collecting plant samples.

Post-inspection hygiene (After inspecting trees and before leaving the property)

6. Remove any loose soil from boots and bottom of field kit bucket (use screwdriver if needed).
7. Scrub outside of boots and bottom of field kit bucket with decontamination solution (Table 7) to remove all visible soil.
8. Brush and shake hat and overalls to remove dust and insects.
9. Wash hands with soap and water, then with antibacterial skin cleanser (Table 7).

Selection of blocks (properties) and patches for inspection

10. Select blocks from each citrus growing district within the region being surveyed.
11. If a citrus nursery is geographically isolated from other citrus plantings it may be included in the survey, otherwise avoid blocks planted with very young trees.
12. Where possible, select patches that appear to be less well managed.

Grower contact and site visit

13. Obtain the property owner's contact details, property map and planting details from MVCB. Where applicable, obtain the site manager's (grower's) details from the owner.
14. Contact the grower in advance and arrange a mutually acceptable date and time to visit the property. This should take into account such activities as irrigation and spraying that could impact on the ability to enter the patch or inspect the trees.

15. Ideally meet the grower at the site to discuss the survey, select a patch to inspect and find out about any hazards on the property. If this cannot be done, discuss the above issues and obtain the grower's permission to visit the site in their absence.

16. If the grower permits, the survey vehicle may be driven onto the property and to the survey site as long as it stays on designated tracks and firm ground (away from soft soil/mud) and avoids contact with citrus trees.

17. If possible, contact the grower when you have finished to let them know you are leaving the property. Some growers (especially corporate farms) may require you to sign in and out of the property for OH&S reasons.

Number and location of trees within patches

18. Regardless of patch size, inspect 100 trees per patch (scattered as much as is practical throughout the patch).

19. Patches of up to about 1,000 trees may be surveyed efficiently by inspecting every 10th tree in every row. To keep the distances walked and time taken practical, patches significantly greater than 1,000 trees may be surveyed by inspecting trees along transects that include trees within the patch as well as at its perimeter.

Targeting of varieties

20. Give highest priority to variety/rootstock combinations that are most likely to show symptoms of the widest range of target P&D.

21. If the above point involves a number of varieties, where possible select blocks representing those varieties within each district.

Inspecting trees

22. Inspect trees in an orderly manner, by walking around their perimeter examining the trunk, shoots, leaves, and fruit for symptoms of disease and pest damage.

23. Also observe leaves and fruit that have fallen to the ground.

24. Avoid unnecessary contact with suspect plants.

25. As much as is practical and safe when walking from one target tree to the next, observe the foliage and fruit of in-between trees for obvious unusual symptoms. While not officially improving the statistical power of the survey, this will increase the chance of detecting pest and disease hotspots.

Collection of plant samples

26. Symptoms vary between hosts so it is desirable to collect representative samples of all suspect material. Bacterial cultures are easiest to obtain from young symptomatic tissue.

27. Collect, handle and send samples according to the instructions in ‘Appendix 2 Sample collection, handling and transport’.

Collection of information

28. Record all information required on the survey sheet (Appendix 5: Field Survey Sheet).

29. Each Field Survey Sheet should refer to a single citrus patch.

Appendix 2 Sample collection, handling and transport

1. Any symptoms suspected of being exotic pest or disease must be sampled. If no such symptoms are found, a sample of leaves from an unthrifty tree should be collected to provide at least one sample per inspected patch. Note that some exotic diseases are difficult to differentiate from common citrus diseases without detailed laboratory analysis.

2. If symptoms consistent with exotic disease are found, advise the Survey Coordinator immediately and follow the steps in "Appendix 3 Full decontamination procedure" when exiting the survey block. Arrangements will be made for rapid transport of samples to the diagnostic laboratory. The Survey Coordinator will advise the survey team what further actions to take.

3. Avoid sample collection when the plant material is wet.

4. Trees to be sampled are to be marked prior to sample collection, with either fluorescent paint on the trunk if possible or on branches and leaves so that it is clearly visible. Alternately, tie a length of fluorescent plastic tape around the trunk or main branch and mark it in black permanent marker with the words 'P&D survey sample", then the survey patch number, the sample number (1, 2 or 3) and sample date. E.g. 'P&D survey sample CS38/1 23/6/2010'.

5. Record the GPS reading at the tree to be sampled.

6. The sample is composed of plant tissue including suspect pests, lesions or other symptoms if possible. Place the sample into a zip-lock plastic bag without paper towelling or any material that will keep the sample wet. If leaves are excessively wet, they should be shaken prior to bagging to remove excess water to reduce the risk of rot during storage. Flatten the bag to remove as much airspace as practicable and then seal the bag.

7. Label the bag using a smudge-proof permanent marking pen or pencil, or a bar-coded label. The label should include the survey patch number and the sample number (e.g. CS38/1). This label must tie the sample to the survey sheet and the GPS readings recorded. Place a DPI security seal label over the sample bag opening to prevent tampering and help with processing of samples in the laboratory.

8. Place the labelled sample bag into a second zip lock plastic bag, then flatten and seal that bag. This means the samples are double bagged and sealed.

9. Ensure the survey form is completed. A photocopy of this sheet is to accompany the sample.

10. Protect samples from direct sunlight and heat (they are best kept below 10°C during storage and transport). Once a sample has been taken, store it in a refrigerator or on thick wads of newspaper above ice or a cool pack in an insulated storage container for the rest of the day.

11. Send samples either by courier (in an esky, cool bag or styrofoam box with a cool pack) or overnight Express Post, making sure they get to Knoxfield before the weekend. If samples are collected late in the week, they should be stored in a refrigerator until Monday's post.

12. Send samples to Crop Health Services – Knoxfield, labelled clearly as shown below.

CITRUS SURVEY

Attention: Con Skyllas

Crop Health Services

DPI Knoxfield

Private Bag 15

Ferntree Gully Delivery Centre, Victoria, 3156

Notify the Survey Coordinator and Crop Health Services that a sample is on its way.

Appendix 3 Full decontamination procedure

1. Exit the patch only at the entry/decontamination station, on the suspect/infected side
2. Decontaminate (with a Phytoclean dip or equivalent) all sealed bags containing samples, equipment and survey sheets and pass to far side of decontamination sheet (the clean side)
3. Scrub outside of boots and the base of the boots thoroughly in the decontamination fluid (there should be no visible dirt)
4. Remove hat and spray with decontamination liquid, place on clean side of decontamination station
5. Remove gloves if used and overalls and place in double garbage bag on clean side of decontamination sheet
6. Walk through decontamination solution bath
7. Spray ground sheet with decontamination solution and carefully fold up plastic sheet and double-bag as waste
8. Dispose of decontamination wash
9. Spray bucket and other decontamination gear with decontamination fluid
10. All contaminated gear should be double bagged in sturdy plastic garbage bags
11. Wash exposed skin with Hibitane (or equivalent antimicrobial wash) and face with anti-bacterial wipes
12. Spray personal glasses with 70 % ethanol

Appendix 4 Equipment lists

Table 5: Field inspection kit

Mobile phone	Sealed in a plastic zip lock bag For use as emergency contact device.
GPS	Sealed in a plastic zip lock bag. To chart location of survey block and sample locations.
Clip board	Plastic, for easy washing/decontamination
3 survey sheets with map of the survey block	Where maps are not available, a map of the farm layout and the block surveyed will need to be drawn
Pencils	
Laminated P&D id sheet	
Secateurs	
3 collection kits	Each kit contained two zip lock plastic bags placed into a third zip lock plastic bag
Sample bag labels	Pre printed labels to note survey sheet and sample numbers
Sample bag security seals	Pre printed security seals
Extra zip lock plastic bags	For completed survey sheet and contaminated rubbish such as broken gloves
Spare disposable gloves	
Bucket	To carry the above items
Esky and cool bricks	For samples. Esky not to enter the inspection block
Critical comments–All items entering the block were to be considered contaminated if suspected high-risk exotic P&D (e.g. canker) were found during the inspection, even if no samples were collected at the time. In that situation, all items were to be decontaminated unless they had always been sealed in a zip lock bag.	

Table 6: Decontamination kit

Disposable gloves	
Disposable overalls	
70% Ethanol in spray pack (see below)	to decontaminate glasses
Hand brush	to brush down clothing after inspection
Skin cleanser (see below)	for hand wash
Anti-bacterial wipes	for face wask
Phytoclean solutions (see below)	for boots and other contaminated items
Screwdriver	to remove mud from boots
Scrubbing brush	to clean boots
Plastic crate or/large bucket	for washing boots and other items with Phytoclean
Sheet of plastic for ground sheet	
Garbage bags	for contaminated items
Packing Tape	to seal bags of contaminated items
Zip lock plastic bags	for contaminated items
Plastic box	to contain the above

Table 7 Decontamination materials

For decontamination of:	
footwear	one (1) part Phytoclean to ten (10) parts water, eg 1 litre Phytoclean to 10 litres water. (Alternative would be 250g/L quaternary ammonium chloride disinfectant applied at label rates for decontamination of surfaces)
equipment	one (1) part Phytoclean to fifty (50) parts water, eg 1 litre Phytoclean to 50 litres water.
personal glasses	70% ethanol, three (3) parts absolute (100%) ethanol to seven (7) parts water, eg 300ml absolute (100%) ethanol to 700ml water.
face	antibacterial wipes
other exposed skin	Microshield 2 Chlorhexidine skin cleanser. (Preferred agent was Hibitane, one (1) part Hibitane to ten (10) parts water, eg 100 mls Hibitane to 1 litre Water. Other alternatives included Microshield T Triclosan Skin Cleanser, Sapoderm Soap and Dettol Liquid Wash)

Appendix 5: Field Survey Sheet

Survey Site Number
CS _ _ _ _

State Government Victoria Department of Primary Industries

PLANT STANDARDS BRANCH

SURVEY SHEET – CITRUS

Block/Property Owner

Business Name: .....

Postal Address: Post Code:.....

Contact Person:

Telephone: Other:.....

Mobile:Fax:.....

Email:.....

Block Details

Citrus Board Block ID:

Address:

Total citrus production: .....Ha, ...trees.

Survey Patch Details

Patch ID:..... Entry GPS: S ..... E .....

Patch area:..... Ha Area surveyed: .....Ha

No. of trees in patch:..... No. of trees inspected.....

Variety/Rootstock.....

	GPS at sampled tree	Comment	Security seal #
1	S E		
2	S E		
3	S E		

Arrive:.....

Start:..... Stop:.....

Start:..... Finish:.....

Officers Name: Date of Survey ...../...../2010

Appendix 6: Survey site data

(in order of site location, west to east)

Site CS#	Location	MVCB Block ID	Block Ha of citrus	Block no. of trees	Patch ID	Patch Ha of citrus	Patch no. of trees	Citrus type	Tree age	Date surveyed
15	Lindsay Point	LIND005	52.52	19442	S	1.78	362	Star ruby on Citrange	20	7/07/2010
14	Lindsay Point	LIND001	47.10	25882	AA	2.65	870	Valencia on Cleopatra	40	7/07/2010
31	Yelta	WRAG033	20.43	11532	I	2.68	2175	Late Lane navel on Trifoliata	9	29/07/2010
17	Merbein West	MERB164	2.45	724	K	0.80	305	Late Lane navel on Citrange	36	14/07/2010
13	Yelta	YELT042	0.37	180	C	0.37	180	Washington navel on Trifoliata	50	23/06/2020
16	Merbein	MILDE28-1	40.06	15096	G	3.01	2350	Barnfield navel on Citrange	14	14/07/2010
26	Irymple	MILDF79-1	0.91	400	A	0.91	400	Late Lane navel on Citrange	9	26/07/2010
1	Irymple	MILDF37-15	5.24	2926	E	1.38	756	Navelina navel on Citrange	11	10/06/2010
2	Mildura	MILDG3-1-9	20.02	10121	J	2.82	2571	Late Lane navel on Citrange	14	10/06/2010
3	Red Cliffs	REDC0276	2.86	1709	Q	0.75	585	Imperial on Citrange	23	11/06/2010
4	Red Cliffs	REDC250	4.68	2554	D	1.83	787	Barnfield navel on Citrange	20	11/06/2010
12	Red Cliffs	REDC195	3.02	1642	F	0.77	483	Imperial on Citrange	13	22/06/2010
11	Yatpool	YATP018A	165.40	98391	3	8.37	5456	Chislett navel on Citrange	6	22/06/2010
40	Iraak	KARA019	22.08	4787	H	7.09	2500	Late Lane navel on Sweet Orange	24	5/08/2010
30	Iraak	IRAA026	14.91	6120	R	1.56	680	Barnfield navel on Citrange	8	28/07/2007

Site CS#	Location	MVCB Block ID	Block Ha of citrus	Block no. of trees	Patch ID	Patch Ha of citrus	Patch no. of trees	Citrus type	Tree age	Date surveyed
29	Iraak	IRAA025	2.28	737	J	2.05	659	Late Lane navel on Citrange	13	28/07/2010
35	Iraak	IRAA064	5.42	3881	B	1.35	1600	Navelina navel on Trifoliata	11	3/08/2010
37	Iraak	IRAA-MAIN	49.18	19828	ZH2	3.18	1814	Pollock navel on Citrange	22	4/8/2010
36	Iraak	IRAA103	38.90	29969	G	3.90	3253	Late Lane navel on Trifoliata	7	4/8/2010
33	Nangiloc	NANGFARM4A	22.87	13312	U	1.46	500	Late Lane navel on Citrange	37	30/07/2010
39	Colignan	COLI086	2.01	891	B	1.49	664	Washington navel on Sweet Orange	41	5/8/2010
38	Colignan	COLI010	32.37	14366	M	5.60	2487	Late Lane navel on Citrange	22	5/8/2010
32	Colignan	COLIFARM7	42.28	17796	F	3.38	1482	Late Lane navel on Citrange	8	30/07/2010
23	Colignan	COLIFARM8N	64.76	31696	C&D	4.21	1974	Edwards&Wiffen navel on Citrange	20	21/07/2010
22	Colignan	COLIFARM8A	16.46	15557	A	8.93	8590	Afourer on Citrange	4	21/07/2010
25	Colignan	COLI042	17.08	7791	W	2.06	890	Late Lane navel on Citrange	29	22/07/2010
27	Colignan	COLI043	24.49	10148	B	4.88	1960	Lisbon on Citrange	15	27/07/2010
24	Colignan	COLI044	27.51	11880	J	1.72	680	Marsh on Trifoliata	25	22/07/2010
28	Colignan	COLI038	27.99	14228	O	1.70	983	Imperial on Trifoliata	6	27/07/2010
18	Robinvale	ROBI_C003	3.86	941	I	2.42	943	Imperial on Cleopatra	19	15/07/2010
19	Tol Tol	TOLT015	19.48	8113	A&S	1.28	490	Pummelo on Sweet Orange	19	15/07/2010
21	Boundary Bend	BOUN013	16.97	6300	U	2.27	780	Valencia on Citrange	23	16/07/2010
20	Boundary Bend	BOUN011	10.55	2700	B	5.32	800	Washington navel on Citronelle	65	16/07/2010
34	Wemen	WEME003	6.77	2027	A	1.14	522	Valencia on Sweet Orange	10	3/08/2010

Site CS#	Location	MVCB Block ID	Block Ha of citrus	Block no. of trees	Patch ID	Patch Ha of citrus	Patch no. of trees	Citrus type	Tree age	Date surveyed
10	Narrung	NARR008	23.00	9233	H	0.63	256	Grapefruit on Citrange	30	18/06/2010
9	Kenley	KENL035	13.67	6738	L	1.79	504	Chislett navel on Sweet Orange	10	18/06/2010
7	Piangle	PIAN148	2.43	947	Q	1.42	555	Thomson navel on Citronelle	18	17/06/2010
8	Beverford	BEVE002	4.91	2672	G	2.64	1470	Chislett navel on Citrange	6	17/06/2010
6	Lake Boga	BOGA003	3.57	537	A,B,C,D	2.79	341	Valencia&Leng navel on Sweet Orange	18	16/06/2010
5	Murrabit	MURR067	38.87	17363	ZB	0.61	325	Washington navel on Trifoliata	28	16/06/2010

Appendix 7: Survey site location and contact details (see separate confidential supplement)