Subcommittee on National Plant Health Surveillance

National Surveillance Protocol

for

Citrus canker *(Xanthomonas citri)*

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Author/s	Bernie Wittwer	

Table of contents

1 Scope/rationale

Citrus canker is a contagious disease of citrus plants, caused by the bacterium *Xanthomonas citri* pv. *citri.* Both quality and yield of fruit are impacted by the infection, and establishment of this pest would have a significant impact on production and market access. This pest has been detected and eradicated in Australia previously, this protocol is to support early detection, response and pest status surveillance.

2 Background

Genus: *Xanthomonas*

Species: *citri* pv. *citri*

Previous names: reclassified from Asiatic type "A" pathotype *Xanthomonas axonopodis* pv. *citri*. Taxonomic and nomenclature review can be found in, Ference *et al.* 2018.

Xanthomonas citri pv. *citri* is the causal agent of citrus canker disease and *X. citri* infections are found in citrus production areas of the world with high rainfall and high temperatures. All above-ground plant parts of citrus including fruit, leaves, stems and thorns, are susceptible to infection. All citrus species are potential canker hosts, although there is considerable variation in susceptibility depending on the strain of the *X. citri* present.

Citrus canker may have originated in south-east Asia, spreading through other areas of Asia, South America, Oceania, Africa and the USA. Previous successful eradications have occurred in multiple countries including South Africa, New Zealand and Australia (DAWE 2021).

Lesions caused by citrus canker reduce marketability of fruit and in severe outbreaks the disease may cause twig die-back, premature fruit drop and eventual tree death. Symptoms can be exacerbated by injury caused by the feeding activity of the citrus leaf miner (*Phyllocnistis citrella*) (Gottwald *et al.* 1988), the larvae of a small moth which is widely distributed in Australia.

In Australia, the disease was first detected in the Northern Territory in 1912, though the pathogen was not identified until 1916, and eradication efforts were not successful until 1923 (Melda *et al*. 1984). Small outbreaks of canker have occurred in Australia several times since then and rapid quarantine response and destruction of hosts has ensured successful eradication. In April 2018, citrus canker was detected in plants associated within a nursery in the Northern Territory, with infections also found in Western Australia through forward traces. An eradication response was implemented, and in April 2021 Australia was declared free of citrus canker (NT Gov 2021).

Whilst citrus canker can be detected by visual surveillance, correct identification of the causal agent of canker is critical as it can be confused with similar diseases such as citrus scab (National Diagnostic Protocol 2016). Incorrect identification in the USA prompted the removal of thousands of productive citrus plants that were infected with citrus bacterial spot, a mild disease caused by the related, but non-aggressive *X. alfalfae* pv. *citrumelonis*, formerly *X. axonopodis* pv. *citrumelo* (Schaad *et al.* 2006). Given the extensive host range of the citrus canker pathogen, the high crop yield and economic value

of the host plants, along with the destructive nature of eradication, surveillance methodology must focus on early detection with a view to eradication.

3 Glossary

Table 1- Definitions and abbreviations

Term/Abbreviation	Definition
DAWE	(former) Department of Agriculture, Water and the Environment, now Department of Agriculture, Fisheries and Forestry
NAQS	Northern Australian Quarantine Strategy
NT GOV	Northern Territory Government
pv.	Pathovar

4 Pest risk profile and pathway analysis

4.1 Entry pathways

Pathway and establishment analyses conducted by the Department of Agriculture, Fisheries and Forestry are detailed in *Priority Pest Surveillance Requirements Citrus Canker*[1].

Australia only imports commercial citrus fruit and budwood from regions that are citrus canker free, or from areas with low pest prevalence and under strict phytosanitary conditions. Budwood usually enters a post-entry quarantine facility where it undergoes pathogen testing during early growth.

All non-commercial imports of citrus plants and budwood into Australia are prohibited, however entry of undeclared budwood with passengers and mail have occurred in the past, and are implicated in an incursion in Emerald, Queensland in 2004. A less likely entry pathway is the pathogen arriving on contaminated clothing or farm equipment as the bacteria is only viable for 24-72 hours, and visibly dirty equipment or clothing is likely to be intercepted at the border.

Fruit and leaves coming into Australia with passengers incidentally or intentionally through the mail is common, but establishment from these pathways is less likely than propagating material. The Torres Strait region is a historically active pathway for citrus canker and may be a plausible region for incursion into northern Australia.

4.2 Establishment and spread

Long distance spread of citrus canker within Australia can be facilitated through wind and rain events, while nursery and garden centre stock are at high-risk of disease spread even without wind assistance (Gottwald *et al.* 1989). Movement controls between states can limit spread if enacted early enough. If the bacteria remain viable, contaminated clothing, equipment and infected plant material moving from an outbreak area can increase the opportunity for localised spreading.

[1] Available on request through the *Subcommittee on National Plant Health Surveillance*

Illegal budwood stock has a high chance of establishment and most previous incursions are thought to have occurred from imports of illegal planting stock. Young plants are particularly at threat for infection, especially those damaged by the feeding behaviour of leaf miners.

5 Pest biology and ecology

5.1 Detection in the field

Quality photographs supported by symptom descriptions allows for suspect citrus canker to be identified by surveillance practitioners as well as by most people in the community.

Symptoms are described in Section 7 – Disease expression.

5.2 Identification

For diagnostic analysis, refer to the *National Diagnostic Protocol for Asiatic Citrus Canker* which contains specific diagnostic advice for molecular tests, symptom evaluation, bacterial isolation and characterisation, and pathogenicity assays.

There are five identified types of citrus canker:

- The causative agent for strain A (including Asian canker and Oriental canker) is *Xanthomonas citri* pv*. citri*.
- *Xanthomonas fuscans* pv. *aurantifolii* strain B causes canker B and *Xanthomonas fuscans* pv. *aurantifolii* strain C causes canker C (or South American canker).
- Group D strains, causing citrus bacteriosis, and group E strains, causing citrus bacterial spot have also been identified (Moreira et al. 2010; CABI 2021).

For confirmation, *X. citri* may be officially distinguished from other *Xanthomonas* pathogens via DNA-based assays and serological tests. Molecular methods are able to detect the presence of *X. citri* prior to the eruption of lesions, and if PCR is required, specific primers have been developed and recent advancements in amplification protocols has increased accuracy and efficiency of testing for *X. citri* (Ference *et al.* 2018).

Commercial serological tests, also known as an enzyme-linked immunosorbent assay (ELISA) are available, however currently only detect strain A – not A* or A^w, and positive testing is not reliable until 4 days post-eruption. Benefits of serological testing are that they can be performed with a kit in the field, and no particular training is required for the user, but their current use is limited to delimiting surveys where the isolate has been identified as strain A. Current detection and identification methods, including a full description of serological tests, primers and older techniques is reviewed in Ference *et al.* (2018).

5.3 Lifecycle and transmission

On leaves and fruit, *X. citri* propagates in wounds and lesions particularly those caused by the feeding behaviour of leaf miners or pruning (**Figure 2**), and new infections commonly occur during vegetative

flushes. The occurrence of citrus canker lesions on root systems in soil has not been confirmed (Reddy and Naidu 1986).

The optimum temperature for infection is between 20 and 30°C (Koizumi 1977). Under these conditions, bacteria may multiply 3 – 4 log units per lesion and cells may emerge from stomatal openings in as little as 5 days, increasing infection potential to neighbouring trees.

Wind-driven rain is the most common natural dispersal agent, and wind speeds ≥ 8 m/s (29 km/h) aid in the penetration of bacteria through the stomatal pores or wounds made by thorns, insects and blowing sand. Practices such as overhead irrigation, hedging and pruning increase susceptibility and can promote the transmission of disease on equipment.

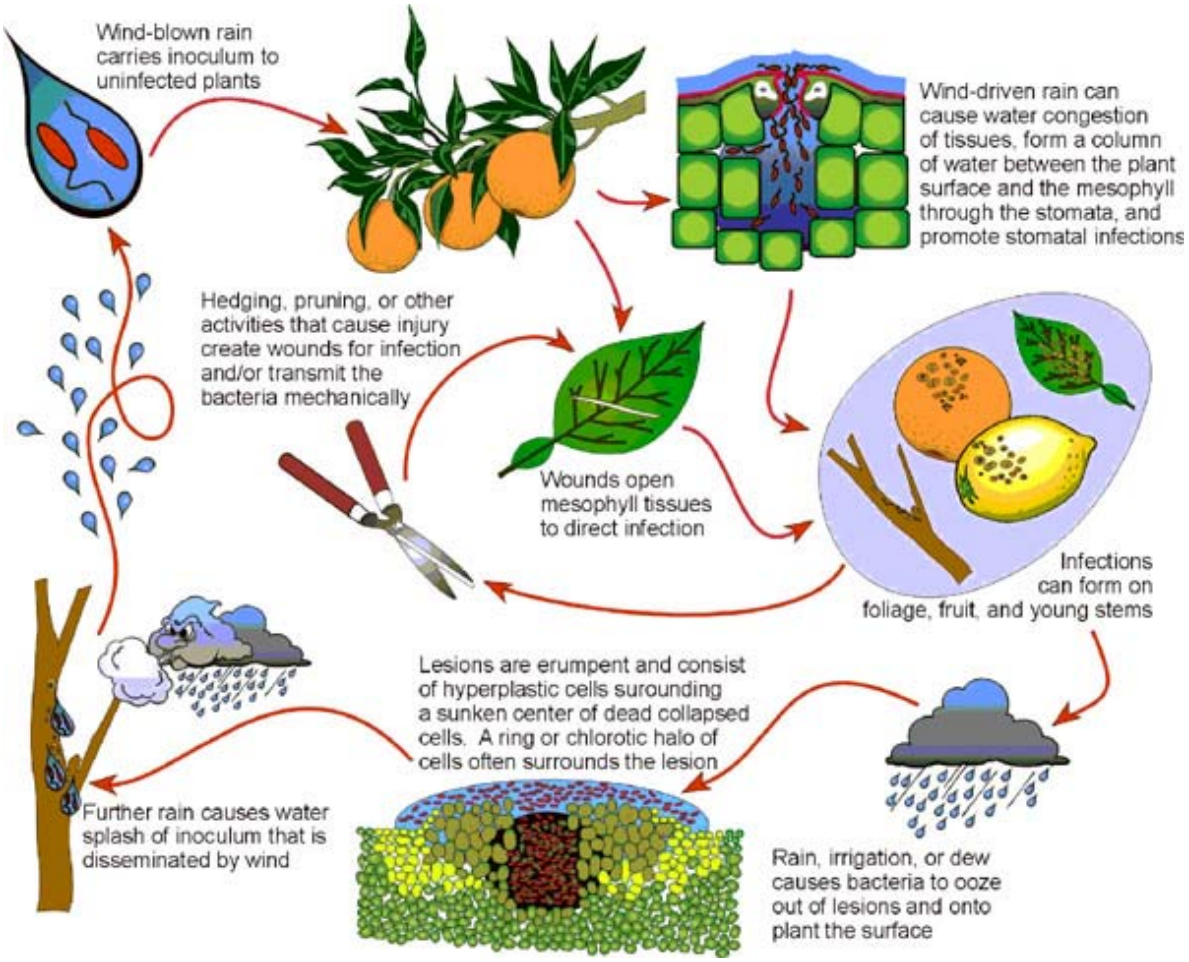

Figure 2 - Disease Cycle and Epidemiology *[Source: Gottwald* et al. *2002]*

5.4 Habitat

Xanthomonas citri survives in diseased plant tissues from season to season and is the primary inoculum source. It is possible for bacteria to survive for a number of years on infected tissue (including woody branches) if they are dry and free of soil. Bacterial cells can also survive in the margins of leaf and fruit lesions, until they decompose. *X. citri* has been reported surviving as an epiphyte on host and non-host plants including a number of invasive weeds in Australia, and as a

saprophyte on straw mulch or in soil. Exposure to direct sunlight accelerates death of the bacteria, even on plant surfaces. Low temperatures also have a significant negative effect on survivability (Gottwald *et al.* 2002).

Survival on inanimate objects, including clothing and machinery is limited to 24-72 hours (Graham *et al.* 2000).

5.5 Vectors

Citrus feeding insects can cause wounds and enhance disease susceptibility but are not vectors of *X. citri*. Potential movement of infected fruit and plant parts and bacterial cells by herbivorous animals, including flying insects, may assist infection spread, although this requires confirmation.

6 Host range and part of host affected

All above ground tissues of citrus cultivars can be affected, and young trees and those undergoing leaf flush are more susceptible than older trees. Among commercial citrus varieties and rootstocks, citrus canker is most severe on grapefruit (*C. x paradisi*), West Indian lime (*C. aurantiifolia*), lemon (C. limon), sweet orange (*C. sinensis*) and trifoliate orange (*Poncirus trifoliata*) and their hybrids (Ference *et al*. 2018).

It should be noted that variety, hybridisation of host plants along with strain of *X. citri* affect susceptibility and no host list is exhaustive. While *Citrus*, *Poncirus*, *Fortunella* and their hybrids are thought to be the most common natural hosts, other rutaceous genera have demonstrated infection after artificial inoculation and response planning should consider wider susceptibility.

Citrus canker can also affect some native Australian Rutaceae species, such as desert lime (*Citrus glauca)* lemon aspen (*Acronychia acidula*), lime berry (*Micromelum minutum*) and native mock orange (*Murraya paniculata var. ovatifoliolata)*, although the ornamental mock orange is not considered a host. Other plants such as wampee (*Clausena lansium*), white sapote (*Casimiroa edulis*) and elephant apple (*Feronia limonia*) are also known hosts (NT Government 2018).

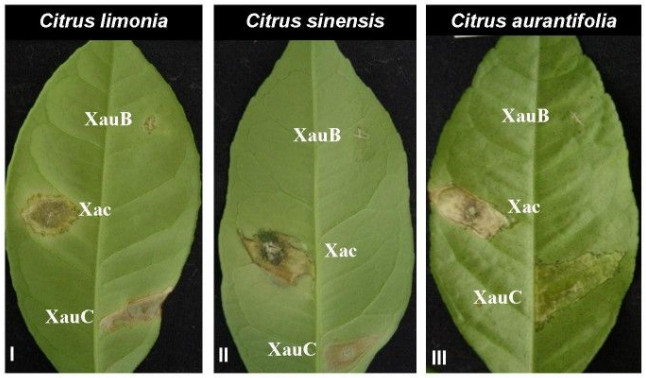

Figure 3 - Disease symptoms caused by Group A (XAC), Group B (XauB) and Group C (XauC) strains on leaves of three different citrus species. The lesions caused by A and B strains are similar but differ in size. Strain C

causes a hypersensitive response in *C. limonia* (I) and *C. sinensis* (II), with *C. aurantifolia* (III) as a true host. The pictures were taken 21 days after inoculation. [*Source: Moreira* et al*. 2010*].

While it had been reported that goat weed, *Ageratum conyzoides* L. - an invasive weed in Australia - could serve as a host of *X. citri*., further research is required to confirm. A study in Iran (Zarei *et al.* 2018) demonstrated survivability on a number of non-host plats, including Bindweed (*Convolvulus arvensis*) an invasive weed species found in Australia (Parsons and Cuthbertson 2001).

7 Disease expression

- Symptoms of the disease first appear as tiny, slightly raised spots or lesions, beginning as pinpoint spots and attaining a maximum size of 2 to 10 mm in diameter (**Figure 4**). They may be accompanied by leaf miner damage (**Figure 5**).
- Lesions become visible about 7 to 10 days after infection commonly on the underside of leaves, soon thereafter on the upper leaf surface and on fruit, and as they age, lesions change colour from tan through brown to grey (**Figures 6 and 7**).
- The eventual size of the lesions depends mainly on the age of the host tissue at the time of infection, and on the citrus cultivar.
- After infection of the leaves, symptoms typically spread onto twigs and eventually to branches (**Figure 8**).
- The lesions expand and become thick and spongy or corky and can be surrounded by a characteristic chlorotic halo, although the halo is not always visible on fruit or stems. With frequent rain the lesions may flatten and develop a water-soaked/oily margin. Both the halo and water-soaked margins are useful diagnostic symptoms of citrus canker and are more easily detected with transmitted light (Gottwald and Graham 2000).
- Citrus canker is mostly a leaf-spotting and fruit rind-blemishing disease, but when conditions are highly favourable for infection, infections can cause defoliation, discoloured bark, shoot dieback and fruit drop (Graham 2001).

Figure 4 – Citrus canker infection visible on mandarin (left) and lime (right) leaves *[Source: NSW DPI]*

Figure 5 - Citrus canker and leaf miner damage on lime leaves *[Source: NSW DPI]*.

Figure 6 – Canker symptoms on lime (left) and tangelo (right) fruit. Note the difference in visibility of the chlorotic halo. [*Source: NSW DPI*].

Figure 7 – Older canker symptoms on grapefruit, which are darker in colour and lack the halo. *[Source: NSW DPI].*

Figure 8 – Canker symptoms on the twig of a Tangelo plant *[Source: NSW DPI].*

Along with strain differences, the appearance of citrus canker lesions can vary depending on:

- Citrus variety
- Host growth stage
- Plant part - lesions on the one leaf are often similar in size because the short time frame for susceptibility only allows for only one infection period. Lesions can vary in size on fruit because fruit rind is susceptible for a longer period than leaf tissue allowing more than one infection period to occur.
- Age of the lesions – the margin may disappear as the lesions age and can be irregular in shape and appear atypical if found in association with a wound site (such as leaf miner wounds) or if trees are water stressed through drought or reduced irrigation.
- Processing - lesions on fruit that have been through the packing shed appear less corky and erumpent than lesions found on un-waxed fruit as during processing the top of the lesion is shaved off leaving a smooth, slightly raised dark spot, still with an irregular margin.

Similar pathologies

Citrus canker can be confused with the disease lemon scab (*Elsinoë fawcettii*) which occurs in coastal areas of Australia. Lemon scab lesions are drier than those of citrus canker, only appear on one side of the leaf and lack the characteristic yellow halo (Hardy and Donovan 2007). Citrus bacterial spot (*Xanthomonas alfalfa* pv. *citrumelonis*), also has similar leaf symptoms to citrus canker, however the lesions are flat and rarely form on citrus fruit.

8 Surveillance methodology

8.1 Survey locations

Citrus canker is generally found in hot and humid areas, and if present, disease expression is highest in these areas during the wet season. As a consequence, surveys should be concentrated in residential areas and community gardens of citrus growing regions, peri-urban areas, nurseries and commercial orchards in Australia's north tropical and sub-tropical areas, including the Torres Strait. Areas which remain cooler in the south may be monitored less frequently, with targeted surveillance when the weather is warm and humid (e.g. coastal areas) particularly where overhead irrigation is used.

8.2 Surveillance methods

Visual surveys

Visual surveillance for all disease symptoms provides the best means to detect citrus canker early enough to eradicate it. Sampling asymptomatic plants is not recommended as diagnostic tests for asymptomatic plants have not been validated and the probability of detection is likely to be reduced significantly in the absence of symptoms.

Due to the wide variety of citrus plants that may be kept in residential areas and community gardens, it is recommended surveyors talk with landholders and walk around the property to ascertain the presence of all hosts including potted and interior plants, at the beginning of the survey.

Nurseries predominantly carry young plants and are a major establishment node for citrus canker, *every* host plant at each nursery should be included in each survey.

In large orchards, surveyors should sample blocks on the external boundaries of the orchard, as well as those blocks closest to facilities such as packing or machinery sheds, houses or staff amenities. At all sites there should be a particular focus on young trees and trees that face prevailing wind and rain.

Surveillance methods proposed here are derived from Benham (2008). At each survey there are four examination stages to complete a visual survey:

1 **Initial assessment** – to stand back and examine each plant to determine health status and phenology. The initial assessment will also allow the surveyor to determine the survey effort required given the number and size of trees.

2 **Systematic visual scanning** – sectioning, scanning and detecting – visually dividing each tree into sections allows the viewer to carefully examine each area of the canopy specifically, and gain a high confidence that each host has been thoroughly surveyed.

3 **Detailed inspection** – of any suspect plant parts. All above ground tissues of each host should be inspected thoroughly for characteristic lesions.

4 **Peripheral inspection** – any detached litter in the area around each host should be examined thoroughly for symptoms as well.

If blemishes and lesions are found, and citrus canker cannot be confidently discounted, then a sample should be collected. If citrus canker is suspected, all details regarding the movement (source and destination) of host plants, including rootstock seedlings and budwood should be recorded for traceability.

8.3 Survey timing and frequency

Surveys should be undertaken across the year, although disease expression is most prominent during periods of wet weather (i.e. wet season in tropical and sub-tropical regions), during leaf flushes or where overhead irrigation is used. An increase in survey frequency during times of high detection is recommended. Surveys can be scheduled for 60-120 days after a suitable infection event where there is high temperature rain and wind.

8.4 Sample handling

By law everyone must comply with biosecurity legislation when moving any suspect exotic plant pest sample, including when sending samples for identification.

In developing a surveillance program, all participants must be clear about their obligations regarding what to do if suspect samples need to be moved.

If movement obligations are not understood, contact the Emergency Plant Pest hotline on 1800 084 881, to obtain instructions to collect and move samples safely.

General diagnostic laboratory contact, preparation and sample submission information is provided below in **Table 2**.

All laboratories should be contacted before sample submission to determine if they have suitable diagnostic capability for the pest (including the life stage being sampled) and have appropriate accreditation to receive biosecurity material. In some cases, specimens may need to be collected as live samples for diagnostic reasons and the laboratory must meet jurisdictional requirements to handle live specimens.

Table 2 – State and territory diagnostic contacts for submission of suspect plant pest samples.

Jurisdiction	Contact details
Queensland	13 25 23 Submitters will be advised what to do with samples through this service.
Western Australia	08 9368 3080 Photos of samples can also be submitted through MyPestGuide app or website Preparation and submission: https://www.agric.wa.gov.au/livestock-biosecurity/sending-specimens-identification
South Australia	(08) 8429 2249 Preparation: https://pir.sa.gov.au/__data/assets/pdf_file/0020/236234/Packaging_Brochure_low.pdf Submission: https://pir.sa.gov.au/research/services/crop_diagnostics/insect_diagnostic_service
New South Wales	1800 680 244 biosecurity@dpi.nsw.gov.au Preparation and submission: https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/plant-health/collecting-and-submitting-plant-or-insect-samples
Northern Territory	(08) 8999 2118 Submission: https://nt.gov.au/industry/agriculture/food-crops-plants-and-quarantine/plant-diseases-and-pests/plant-pathology-and-entomology-contacts
Victoria	(03) 9032 7515 Submission: https://agriculture.vic.gov.au/support-and-resources/services/diagnostic-services
Tasmania	(03) 6165 3777 plantdiagnosticservices@nre.tas.gov.au Preparation and submission: https://nre.tas.gov.au/biosecurity-tasmania/plant-biosecurity/plant-diagnostic-services

All samples should be sorted by tree and kept cool prior to identification. Transport times should be minimised, and packages should not be sent on a Friday to avoid samples deteriorating over weekends. Where possible a laboratory submission form should be included with the sample, contact the diagnostic laboratory prior to sending the sample for specific instructions.

Any paperwork should be separately bagged from the samples to avoid damage.

All samples require three levels of packaging, each sample is double bagged and then placed in padded bag, tough bag, corrugated cardboard box or Biobottle. All bags should then be placed in postal bag or box.

Leaves

Regardless of diagnostic technique, all samples of suspect citrus leaves should be double-bagged, and both the inner bag and hands disinfected with quaternary ammonium before placing in the outer bag. If the leaves are moist include absorbent towel in the bag with them.

Fruit

As above, fruit should be double bagged, any soft or rotten fruit should be wrapped in paper towel before being bagged separately to the rest of the sample. The secondary bag should also contain absorbent material in case of leakage.

9 Record keeping

Surveillance data captured for use in the plant health surveillance system in Australia should be collected using the Pest Record Specification. This biosecurity specific data standard is endorsed for use by the Sub-committee on National Plant Health Surveillance and Plant Health Committee and is maintained by the Commonwealth. Surveillance planning should include the development of a program data standard, based on the Pest Record Specification, and utilising any relevant pest-specific data protocols. Information on using the Pest Record Specification and Data Protocols is available on the Plant Surveillance Network Australasia-Pacific (PSNAP) website.

A pest specific data protocol for Citrus Canker has been developed and is available on the PSNAP website.

When undertaking surveillance, the data fields to be collected must be considered for individual pests and surveillance methods and the data protocol describes the mandatory, required and optional data fields. A number of data fields have specific controlled vocabulary from which they must be filled. Controlled vocabulary lists are included in the data protocol and tabled below, based on the methods and technology described in this protocol.

Table 3 – Controlled vocabulary lists for citrus canker

scientificName	**inspectionMethod**	**hostMaterial**	**protocolID**
Xanthomonas citri *(*If known use pathovar name e.g. *Xanthomonas citri* **pv.** *citri)*	Visual surveillance	Taxa within: Rutaceae	Citrus canker

10 Research and Development

10.1 Triggers for NSP document review

The National Diagnostic Protocol for Asiatic Citrus Canker, *Xanthomonas citri* pv. *citri* will be reviewed and re-released in 2024. This protocol should be reviewed once the NDP is released to ensure diagnostic links and information is consistent.

11 Contact and further information

Nerida Donovan - Citrus Pathologist

DPI NSW Elizabeth Macarthur Agricultural Institute Woodbridge Rd, Menangle NSW 2568

Mail: Private Bag 4008, Narellan NSW 2567. Phone: +61 2 4640 6333 Fax: +61 2 4640 6300

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