Plant Pest and Disease Survey on Norfolk Island

Final Project Report

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Acknowledgment

We acknowledge and respect Victorian Traditional Owners as the original custodians of Victoria's land and waters, their unique ability to care for Country and deep spiritual connection to it. We honour Elders past and present whose knowledge and wisdom has ensured the continuation of culture and traditional practices.

We are committed to genuinely partner, and meaningfully engage, with Victoria's Traditional Owners and Aboriginal communities to support the protection of Country, the maintenance of spiritual and cultural practices and their broader aspirations in the 21st century and beyond

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Executive Summary

Background

Agriculture Victoria Research (AVR) was contracted by the Department of Infrastructure, Transport, Regional Development, Communications and the Arts (DITRDCA) to conduct a Plant Pest and Disease Survey on Norfolk Island. This happened approximately 10 years after the last survey of this kind (Maynard *et al.* 2018) was conducted on the island. Surveillance was undertaken for a range of pests and diseases of plants that could impact Norfolk Island or mainland Australia. The surveillance program was developed in collaboration with DITRDCA, the Department of Agriculture, Fisheries and Forestry (DAFF) and other stakeholders. It built on the past survey by updating data and addressing gaps in our knowledge, specifically for nematodes, bacteria, viruses, and phytoplasmas.

Survey team expertise

AVR assembled a team of highly experienced entomology, weed science and microbiology (viruses, phytoplasmas, bacteria, fungi, nematodes) diagnosticians and taxonomists. Some of the team members are part of Crop Health Services (CHS), the largest government plant diagnostic laboratory in Australia. CHS is a world class diagnostic service, with National Association of Testing Authorities (NATA) accreditation for quality assurance and provides diagnostic support and capability to many other state and Commonwealth government agencies. The team have extensive experience in pest and pathogen surveillance and diagnostics that underpins plant biosecurity activities for the state of Victoria and have the capability to provide high throughput diagnostics and surveillance testing for pests and pathogens (e.g., outbreaks, area-of-freedom surveys). The team utilises a combination of epidemiology, morphological taxonomic assignment, and modern molecular methods (PCR, LAMP, metabarcoding, metagenomics, genomics) to inform diagnoses.

Surveys

The team conducted two plant pest and disease surveys on Norfolk Island, in March and October 2022, referred to as the summer and spring survey, respectively. The summer survey was undertaken from 10 to 19 March 2022. A total of 46 locations were surveyed across Norfolk Island, focusing predominantly on agricultural areas. Given the focus on agricultural hosts the majority of sites were on private land, however, reserves, public areas (e.g., carparks, streets, sport fields) and the Norfolk Island National Park were also included. The spring survey was undertaken from 10 to 23 October (insects, nematodes, bacteria, viruses, fungi) and from 1 to 6 November (weeds). More than 40 locations were surveyed across Norfolk Island. Additional properties were also included, and targeted visits were conducted when Norfolk Island community members requested specific plants/crops be examined.

Overall, more than 1,000 samples were collected and tested between the two surveys:

- Fungi, bacteria, viruses: more than 500 samples (leaf, stem, trunk, root or fruit).
- Insects: more than 400 samples (individual or mixtures of insects).
- Nematodes: approximately 180 samples (soil).
- Weeds: more than 20 samples (pressed specimens).

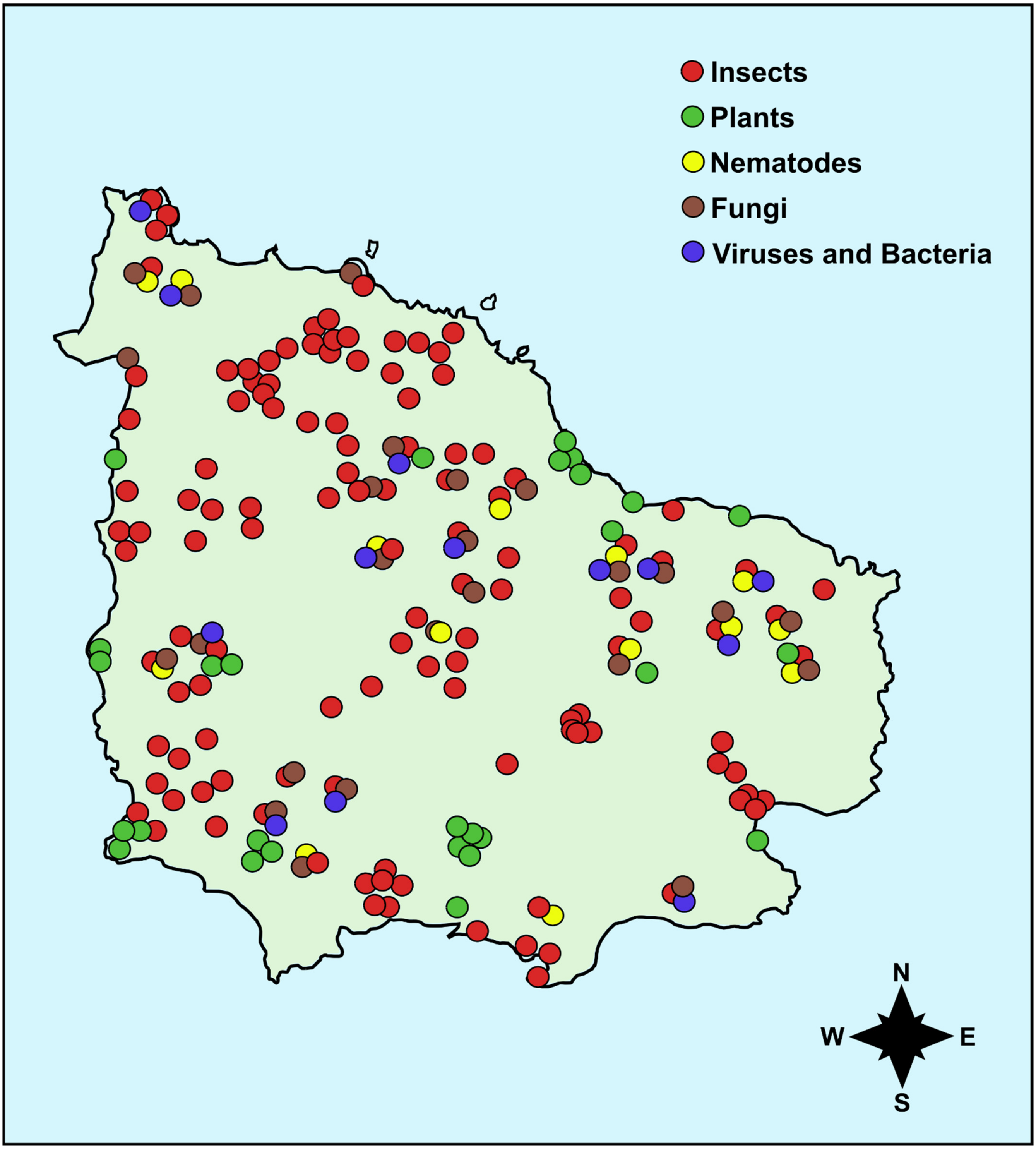

Figure 1: Sampling locations on Norfolk Island. Different coloured dots correspond to the different organisms collected.

Results

Significant findings and highlights of the survey are summarised here:

- **No record of any newly identified significant high-priority pests or pathogens on Norfolk Island was made during the survey.**

- Confirmed presence for a number of known pest insect species present on Norfolk Island, including the tomato potato psyllid (TPP; *Bactericera cockerelli*), the 28-spotted ladybird beetle (*Henosepilachna vigintioctopunctata*), the Banana root borer weevil (*Cosmopolites sordidus*), the Argentine ant (*Linepithema humile*), and at least two of the grass armyworms (*Spodoptera* spp.).

- Record of at least six insect pests previously not reported on Norfolk Island, including aphids, psyllids, and Thrips. Additionally, we recorded some insects that had been previously reported on the island, but not recorded during the most recent survey (Maynard *et al.* 2018). For example, this is the case of the Macadamia nut borer moth (*Cryptophlebia ombrodelta*), a serious pest of lychee and longan worldwide. Overall, these new or poorly recorded pest insect species were generally recorded at very low numbers. Their impact should be assessed by further studies.

- Records of many insect species not previously reported in Norfolk Island, including introduced and endemic (i.e. found no where else) psyllid species (a group scarcely studied), as well as beneficial insect species, including parasitoids of known pests.

- No exotic fungal pathogen was detected as part of this survey. Overall, a total of 527,368 high quality, full-length fungal rRNA gene sequences were generated from fungal samples, representing taxa including known plant pathogens, species that are closely related to known pathogens, and novel fungal species that haven't been previously sequenced.

- Records of 12 species of plant-parasitic nematodes in Norfolk Island soils, including one species of cyst nematode, *Heterodera trifolii* (clover cyst nematode), and two species of root knot nematodes (*Meloidogyne* spp.). All these species had previously been recorded on the island.

- No record of the previously reported potato cyst nematode (PCN, *Globodera rostochiensis*) on Norfolk Island, despite analysing 14 different potato soil samples. This may suggest the cyst nematode is not currently present on Norfolk Island.

- No record of exotic plant pathogenic bacteria.

- Records of 16 virus species previously not reported in Norfolk Island including one virus that was not previousy reported in Australia: southern tomato virus (STV). STV is not associated with disease, and both have a limited host range. Therefore, neither virus is considered an emergency plant pest on Norfolk Island. The remaining 14 virus species are not part of the NPPP list and their impact on Norfolk Island plants should not be a cause of concern.

- Seven new records of weeds or plants naturalised (i.e., established) on Norfolk Island were reported, including the invasive weed species bitou bush (*Osteospermum moniliferum*) at one site. Of these, bitou bush and agapanthus are known to be troublesome weeds in other parts of the world. Both of these species are limited to a single location on Norfolk Island, and it is prudent to attempt eradication of these naturalised populations. Of the remaining five new plant species, only Indian siris has weedy potential, although it seems that the single specimen present does not produce seed.

- Key organisms (including weeds) collected as a part of the survey will be deposited within the Victorian Pest and Pathogen Herbarium (VPRI), the Victorian Agricultural Insect Collection (VAIC) and other insect collections, as well as the National Herbarium of Victoria (NHV) to create a resource and database of Norfolk Island pests and pathogens for future reference.

- Genetic sequencing data generated from this survey has been uploaded on the GenBank public database providing novel information on the organisms living on Norfolk Island.

- The AVR team (Figure 2) engaged extensively with the Norfolk Island community, with presentation to the public and to the biosecurity teams, a visit to the school, attendance to the 2022 Norfolk Island Agricultural Fair, and daily meetings with community members.

Figure 2: AVR team members during the spring Survey (October 2022). From left to right, Reannon Smith, Francesco Martoni, Elisse Nogarotto, Saidi Achari, Geoff Kelly, Mark Blacket, John Wainer.

Abbreviations and glossary

APVMA: Australian Pesticides and Veterinary Medicines Authority.

ASV: Amplicon Sequence Variant. A DNA read that differs from another of at least one base.

AVR: Agriculture Victoria Research.

DNA barcoding: a method of specimen identification using short, standardized segments of DNA. Every species has its own barcode, just as every person has their own fingerprint. These DNA barcodes can be compared to a reference library to provide an ID.

DNA metabarcoding: this technique applies DNA barcoding to a sample containing multiple specimens (e.g., multiple insects collected by a trap). It provides a rapid means of biodiversity assessment by identifying multiple species simultaneously in a sample using DNA sequencing. These may include samples of bulk tissue from whole organisms or environmental samples of water or soil with residual or bound DNA.

COI: Subunit I of the Cytochrome c oxidase mitochondrial gene.

DAFF: Department of Agriculture, Forestry and Fisheries.

DITRDCA: Department of Infrastructure, Transport, Regional Development, Communications and the Arts.

DNA: Deoxyribonucleic acid.

Endemic species: Organism present only in one country or a particular area.

Exotic species: Organism that has been introduced / has arrived in a country or a particular area.

HTS: High throughput sequencing.

LAMP: Loop-mediated Isothermal Amplification.

Native species: Organism that is from a country but also present elsewhere.

NDP: National Diagnostic Protocols.

NHV: National Herbarium of Victoria.

NIRC: Norfolk Island Regional Council.

NPPP: National Plant Priority Pests.

NSW: New South Wales (Australian State).

OTU: Operational Taxonomic Unit. Grouping of reads or ASVs within a certain threshold of genetic similarity.

PCN: Potato Cyst Nematode, *Globodera rostochiensis*. Nematode pest.

PCR: Polymerase chain reaction.

QLD: Queensland (Australian State).

Read(s): DNA sequence(s) generated by a HTS technology.

SWD: Spotted Wing Drosophila, *Drosophila suzukii.* Insect pest.

TAS: Tasmania (Australian State).

TPP: Tomato Potato Psyllid, *Bactericera cockerelli.* Insect pest.

USA: United States of America (Country).

VAIC: Victorian Agricultural Insect Collection.

VIC: Victoria (Australian State).

VPRI: Victorian Pest and Pathogen Herbarium.

Introduction

Background

Norfolk Island is a sub-tropical island located at 29.03° South and 167.95° East, approximately 1,600 km north-east of Sydney and 1,100 km north-west of Auckland. Norfolk Island is 3,455 hectares in area and 5 x 8 km in size. Norfolk Island is a 2.5-hour flight from Sydney and Brisbane (Australia).

The Department of Infrastructure, Transport, Regional Development, Communications and the Arts (DITRDCA) is responsible for the provision of state type functions for Norfolk Island, including biosecurity functions that are typically delivered by a state or territory government. The Department of Agriculture, Forestry and Fisheries (DAFF) is responsible for managing and mitigating pest incursions at the first ports of entry under the *Biosecurity Act 2015*.

The Norfolk Island Plant Pest and Disease Survey will provide DITRDCA and DAFF with additional data on the current plant pest and disease status of the island, to fill gaps in knowledge, determine changes in risks since the baseline survey was conducted in 2012-2014 (Maynard *et al.* 2018) and infrom management actions.

Key stakeholders involved in the survey include DITRDCA, the Administrator of Norfolk Island, Norfolk Island Regional Council (NIRC), Norfolk Island businesses and residents, DAFF, Parks Australia and the Norfolk Island Flora & Fauna Society.

Project objectives

- Prepare a survey design.
- Conduct spring and summer plant pest and disease surveys.
- Report new detections of pests and diseases on Norfolk Island.
- Collect and lodge pest and disease specimens in reference collections held by insect collections and herbaria.
- Gather citizen-science data on plants and pests and further investigate and analyse.
- Provide a final report including recommendations on the outcomes of the pest and disease survey.

Key Research Findings

Across all disciplines, the main focus was to determine if **known exotic priority pests and pathogens** could be recorded for the first time on Norfolk Island. This refers to species that have been reported elsewhere in the world (but never on Norfolk Island) and are known to be harmful to agriculture. In particular, the list of Australian National Priority Plant Pests (NPPP) was used to determine which priority pest species could be of higher introduction risk. These pests are known to have severe consequences on common agricultural crops around the world.

Based on previous surveys (Maynard *et al.* 2018), a number of **non-native pests previously recorded on Norfolk Island**, were also targeted to determine if their presence could still be recorded. These are not considered priority pests and pathogens due to the fact they are already known to be on the island. Nonetheless, since these species have been introduced to Norfolk Island, assessing their presence/absence across the island is still considered a valuable information. In the context of Norfolk Island's unique biodiversity and environment, it is also important to highlight how some organisms known as pests elsewhere may not behave in the same manner on the island, and their "pest status" may not be reflective of their behaviour and impact on the local plants.

Finally, the techniques used during this survey enabled us to record also non-pest organisms, including **endemic species** (present only on Norfolk Island), **native species** (originally on Norfolk Island but also present elsewhere) and **novel species**.

Novel species reported here are species considered new to science, which implies no information on these species is available anywhere in the literature. This is because no scientific record of these species has been previously obtained. Based on the biogeographical position of Norfolk Island and considering the history of isolated evolution of most of the organisms there, most of the novel species recorded there can be hypothesised to be endemic. The genetic analysis conducted cannot determine whether or not the novel species recorded have a pathogenic impact on plants. The record of novel species is a first step to enable further research on these organisms, especially when these species are recorded in association with plants of agricultural value.

The key research findings for this project are divided across a number of disciplines. The findings are summarised here. Additional information on the methods and results can be found in the respective appendices:

1. Entomology (appendix A).
2. Fungal plant pathogens (appendix B).
3. Nematology (appendix C).
4. Bacterial and viral plant pathogens (appendices D and E).
5. Weeds (appendix F).

Entomology summary

The main aim of the entomological survey was to identify insect species belonging to the Australian National Priority Plant Pests (NPPP) list previously unreported on Norfolk Island.

A total of 113 samples (each containing multiple insect specimens belonging to different species) were processed using metabarcoding, with an additional 120 samples (individual insects) processed using COI DNA barcoding. DNA sequences were generated for more than 500 arthropod species, including insects, spiders, mites, and even ticks. Insects recorded here belonged to 14 orders, encompassing the major groups of insects. These included Blattodea (cockroaches), Coleoptera (beetles), Diptera (flies), Dermaptera (earwigs), Diptera (flies/mosquitoes), Hemiptera (aphids, psyllids), Hymenoptera (wasps, ants), Lepidoptera (moths, butterflies), Neuroptera (antlions), Orthoptera (crickets), Psocoptera (barklice), Termitoidea (termites), Thysanoptera (Thrips) and Trichoptera (caddisflies).

No new NPPP insects were recorded on Norfolk Island. However, 8 insect species that are known to be pests of agricultural plants elsewhere were recorded during this survey. These are four aphids (Dysaphis aucupariae, Hyperomyzus carduellinus, Macrosiphoniella sanborni, Melanaphis sacchari), a psyllid (Heteropsylla cubana), a thrips (Frankliniella occidentalis), a moth (Cryptophlebia ombrodelta) and a termite (Coptotermes acinaciformis). However, due to the low number of individuals recorded, we are unsure whether or not these insects are having a damaging impact on plants on Norfolk Island, and as such whether or not they warrant a 'pest status' in this context.

Amongst the more than 500 insect species recorded (including pests, native, and beneficial insects) a number of target pest species were identified (Figure 3A-C). These target species had already been identified in the 2012-2014 Quarantine Survey (Maynard *et al.* 2018). This includes the 28-spotted ladybird (pest of potatoes), the banana weevil (pest of banana plantations), the Argentine ant, a number of aphid (Aphididae) and Thrips (Thysanoptera) species, the tomato potato psyllid (TPP, pest of tomatoes), and other arthropods, such as the Citrus Rust Mite. Overall, the vast majority of the taxa recorded here were not agricultural pests. Not only native and endemic species, but also non-pest introduced species composed the bulk of insect species recorded across the surveys. This can be considered a factor representing a healthy environment rich in biodiversity, showing different groups of insects co-existing across the island, both in the native forests and in the agricultural landscape.

All specimens were retained as vouchers and no insect specimens were destroyed for DNA extraction.

Fungal plant pathogens summary

Overall, a total of 527,368 high quality, full-length fungal rRNA gene sequences were generated from fungal samples collected from surveys 1 and 2, representing taxa including known plant pathogens, species that are closely related to known pathogens, and novel fungal species that haven't been previously sequenced. These sequences represent an invaluable resource for future biosecurity, fungal pathogen diagnostics and fungal taxonomy. The higher taxonomic resolution resulted in an increased sensitivity of the method used by this survey, hence more fungal species were identified when compared to that of the last survey. Importantly, no exotic fungal pathogen was detected as part of this survey.

Based on the collection permit obtained, fungal plant pathogens samples consisted of plant material preserved in solutions which enabled AVR to sequence their DNA, but not to culture them. Therefore, samples were collected when symptoms could be observed on the plants (yellowing of the leaves, death of branches, etc).

From the 87 samples analysed during the spring field work, 424 DNA sequences were generated, belonging to 208 different fungal taxa (i.e. species; details in appendix B). Sixty-five samples had fungal taxa that are known to cause the same symptoms that were observed (highlighted in yellow in appendix B), including 22 taxa that were reported in the 2012 survey. This result suggests that the sequenced pathogen was indeed the agent causing the observed symptom. These taxa included known pathogens and their closely related species (e.g. *Colletotrichum aenigma* for avocado leaf spot and *Podosphaera xanthii* for pumpkin powdery mildew). Novel fungi were also identified.

Of the 177 symptomatic samples analysed during the summer survey, there were 252 different fungal taxa represented across 883 DNA sequences (details in appendix B). Ninety-eight samples had fungal taxa that are known to cause the observed symptoms (highlighted in yellow in appendix B), including 11 taxa previously reported in the 2012 survey. These taxa included known pathogens and their closely related species (e.g., *Austropuccinia psidii* for myrtle rust and *Exserohilum turcica* for maize leaf blight). Novel fungi were also identified.

Sequences that were generated from asymptomatic samples (49) didn't generate any positive result. Thirty-three samples were not analysed due to unsuccessful amplification.

Nematology summary

The survey recorded 13 species of plant-parasitic nematodes in Norfolk Island soils. No nematode pests on the list of Australian National Priority Plant Pests (NPPP) were detected. Three species of plant-parasitic nematodes detected in this survey are new records for Norfolk Island, viz., *Helicotylenchus pseudorobustus* (spiral nematode), *Paratylenchus* sp. (pin nematode), and *Rotylenchulus reniformis* (reniform nematode). No conspicuous evidence of damage symptoms of host plants was detected.

Although a previous record indicated the occurrence of the introduced potato cyst nematode (PCN, *Globodera rostochiensis*) on Norfolk Island, this survey found no evidence of its presence on island, despite a total of 14 soil samples collected from potato-cultivated areas. Although seven species of plant-parasitic nematodes were detected in association with roots of potato, none were present in high numbers and no overt evidence of root or tuber damage by nematodes was noticed in the field.

Six species of nematodes were found in golf course and bowling green turfgrass. All three golf course soil samples and one of the two in the bowling green samples had a high nematode hazard index. This was particularly the case for the bowling green sample, where root lesion nematode (*Pratylenchus* sp.) was detected in relatively high numbers. Nevertheless, the turfgrass appeared in relatively good condition, with no obvious nematode-caused symptoms of dieback, thinning, wilting, or discolouring, suggesting that its good maintenance may be adequate to sustain high quality greens.

No NPPP cyst nematodes (*Heterodera* spp.) were detected during the survey. Only one species of cyst nematode, *Heterodera trifolii* (clover cyst nematode), was recorded. No conspicuous root galling of vegetables was observed, even though two species of root knot nematodes (*Meloidogyne*) were frequently detected. This suggests that the situation may have improved over the past 40 years since the first and only other nematode survey on Norfolk Island.

Bacterial and viral plant pathogens summary

The presence of exotic bacterial and viral pathogens was tested for using conventional molecular testing following National Diagnostic Protocols (NDP) and High Throughput Sequencing (HTS). These tests are available for all the major bacterial and viral pathogens of concern, and are widely considered the best approach to determine presence/absence of pathogens. In the summer and spring surveys, 126 samples were tested for bacterial pathogens and 199 samples were tested for viral pathogens.

The bacterial samples were tested for *Xylella fastidiosa*, *Candidatus* Liberibacter *solanacearum*, Huanglongbing (*Candidatus* Liberibacter africanus, americanus and asiaticus), *Erwinia amylovora*, *Xanthomonas citri* subsp. *citri, Clavibacter michiganensis* subsp. s*epedonicus, Xanthomonas fragariae and Pantoea stewartii subsp. stewartii* following procedures outlined in the NDPs.

No positive detections were observed through molecular and HTS screening methods. The lack of detection of bacterial pathogens some of which have been previously reported in Norfolk Island (Maynard *et al*. 2018) could be associated with prevailing weather conditions during the surveys which can have an impact on bacterial diseases manifestations and also populations of insects that vector some bacterial pathogens.

The virology samples were tested for plum pox virus, phytoplasma and begomoviruses following the protocols outlined in the NDPs. No detections were noted for any of these exotic viruses. Using HTS for virus screening, 39 virus species including two exotic viruses: southern tomato virus and watermelon crinkle leaf-associated virus 2 were detected.

There were 16 virus species that are endemic to Australia but have not been detected by previous Norfolk Island surveys in 2013. Detection of these additional endemic virus species could be due to the sensitivity of the HTS tool used for virus diagnostic. The virus species detected by this Norfolk Island survey are not part of the NPPP list and their impact on Norfolk Island plants can be controlled by standard virus management strategies.

Weeds summary

Eight new records of weeds or plants naturalised on Norfolk Island were made during the summer and spring surveys: Duckweed (*Lemna disperma*), Indian siris (*Albizia lebbeck*) and Bitou bush (*Chrysanthemoides monilifera var. rotundata*), Agapanthus (*Agapanthus praecox*; Figure 3D), Nelson's slime lily (*Albura nelsonii*), Bromeliad (*Billbergia pyramidalis*), Angel-wing begonia (*Begonia coccinea*), and Ti (pink ornamental variety; *Cordyline fruticosa*).

Additionally, two plants collected were the first herbarium press of species previously recorded as naturalised by Mills (2010), but for which no herbarium voucher existed: Water cress (*Nasturtium officinale*) and Ivy-leaved Violet (*Viola hederacea*).

The record of these species highlights the need for a transparent weed prioritisation process to consider what species are the most important to manage, what management interventions are the most useful, and how these vary depending on different species. Such tools exist in New South Wales and Victoria and could be modified for use on Norfolk Island.

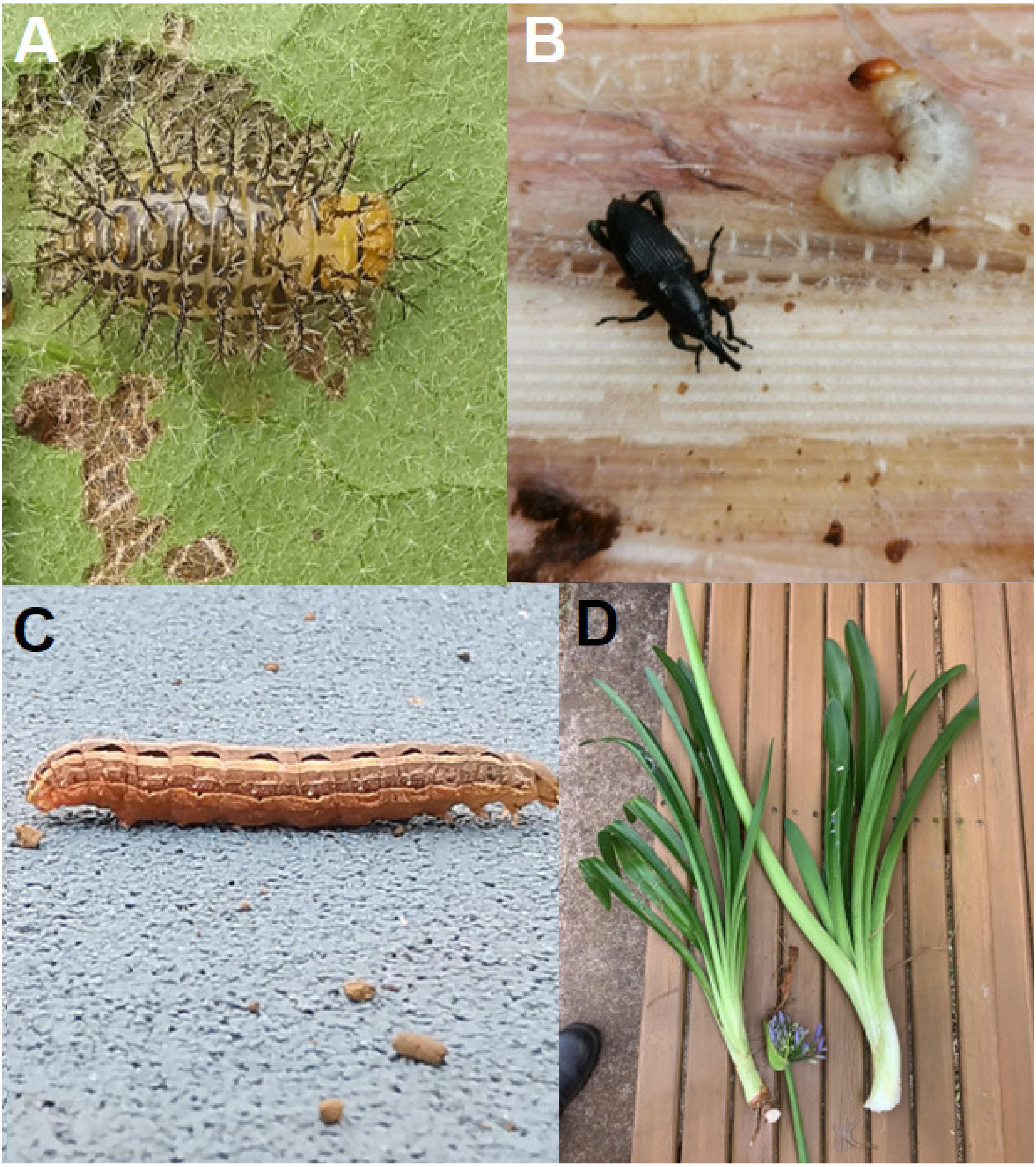

Figure 3: examples of pests and weeds recorded on Norfolk Island. The immature of a 28-spotted ladybird beetle, pest of potatoes (**A**), the immature and adult of the Banana borer weevil, pest of bananas (**B**), the immature of an armyworm moth, pest of pastures (**C**), and the *Agapanthus* weed recorded in this survey (**D**).

Project Outputs

This project generated a number of outputs aimed at benefiting the Norfolk Island community, informing biosecurity management, the general scientific community and our understanding of Norfolk Island biodiversity (Table 1).

Firstly, the main output of the survey was to generate an updated list of organisms present on Norfolk Island. This was achieved thanks to the extensive sampling (Figure 1) across more than 35 properties and public locations across the island. The analysis of the samples enabled the discovery of a number of organisms previously not reported on the island, including both endemic and introduced species. This output will provide an important baseline of the species present on the island, establishing a dataset for future surveys and/or ongoing survillence.

Additionally, the use of molecular techniques throughout the project enabled generation of DNA sequences for a number of organisms, from insects to plant pathogens. These sequences provide a novel means to approach Norfolk Island biodiversity. Thanks to this data, future studies and surveys will be able to compare the genetic information linked to Norfolk Island organisms to that available for species distributed elsewhere in the world. Ultimately, this will accelerate the understanding of Norfolk Island biodiversity, enabling data access from any part of the world.

Survey outputs also include a number of presentations and discussions with the Norfolk Island community, often mediated by the Flora & Fauna Society, in which the project, its objectives and results were presented (Table 1). This enabled the AVR team to develop relationships with property owners and therefore obtain access to additional sampling locations and a more exhaustive sample pool. The members of the AVR team shared their knowledge on a number of topics, from entomology to nematology, from plant pathogens to weed control, and learnt from community members about their observations and knowledge of Norfolk Island. Members of the AVR team also presented their work at the local school and to the local biosecurity team.

Table 1: List of the outputs generated during this project, their description and impact.

Output	Description	Impact
Updates to the lists of organisms found on Norfolk Island	The main output of this survey was to update the current lists of organisms known to be present on Norfolk Island.	Biosecurity, surveillance, science
New genetic data generated for Norfolk Island organisms.	Submission of more than 100 genetic sequences representing organisms present on Norfolk Island. A total of 102 COI sequences have been uploaded on the public database GenBank (Accession numbers OR050315-OR050416). These sequences represent a number of species (both endemic and introduced) collected on Norfolk Island, including pests and beneficial insects, both native and introduced.	Biosecurity, surveillance, science
Preservation of voucher specimens from Norfolk Island.	Key organisms (including weeds) collected as a part of the survey will be deposited within the Victorian Pest and Pathogen Herbarium (VPRI), the Victorian Agricultural Insect Collection (VAIC) and the National Herbarium of Victoria (NHV) to create a resource and database of Norfolk Island pests and pathogens for future reference.	Biosecurity, surveillance, science
Community presentation	On island presentations provided an overview of survey techniques, methods and aims, as well as (in spring) results. This	Community engagement

summer and spring	engagement with the Norfolk Island community built greater understanding of plant biosecurity. In addition, community members provided the team with information useful for the survey.	
DAFF biosecurity team meeting	A meeting was conducted between the Agriculture Victoria team and the Norfolk Island DAFF biosecurity team, to showcase possible applications of Loop-mediated isothermal amplification (LAMP) assays on Norfolk Island. This meeting with technical staff enabled discussions of the scope and potential needs of the biosecurity team on the island.	Technical engagement
School visit	A series of talks were presented at the Norfolk Island Central School during the spring survey. This engagement with the Norfolk Island school enabled communication of the importance of biosecurity to the students.	Community engagement

Recommendations

Based on the results of this survey, there are a number of recommendations:

1. **Undertake periodical pest surveillance for Norfolk Island.** Surveillance is of paramount importance to protecting agricultural and environmental values, especially in the context of islands and isolated communities. A continuous surveillance program can provide timely information on early detection of pest species, enabling a quick response to an incursion, therefore allowing pest eradication. On the other hand, surveys conducted every 10 years are more limited to a presence/absence recording, with scarce chances to intercept the early stages of an incursion. This may result in very costly management programs over a long period of time. The Agriculture Victoria team recommends that periodical surveys, possibly every year (but ideally with monthly collected samples, to cover all seasons), are conducted across Norfolk Island, providing a continuous series of records that would enable the timely record of the early phases of an incursion. Such yearly surveys could be conducted by trained teams of citizens, empowering the Norfolk Island community to manage their own biosecurity, with planning and technical advice from research scientist teams. Once collected, samples could be sent to diagnostic facilities and analysed. Such a surveillance plan would be both time and cost-effective, limiting the requirement of research teams travelling to and from Norfolk Island.

2. **Develop a database of the Norfolk Island genetic diversity.** Norfolk Island is a unique place moulded by a rich history of biogeographic isolation as well as new species introductions following the socio-political changes of the past few centuries. With the advancement of molecular techniques for the identification of various organisms, including fungi, nematodes, bacteria, viruses and insects, he vast majority of specdatabasing and “collecting” the genetic diversity recorded on Norfolk Island should be a priority. Ties present on Norfolk Island are endemic (present only on the island) or at least native (have always been present on the island, but are also present elsewhere), with many organisms that have never been characterised genetically (i.e., no DNA sequence has ever been generated). Generating a database of genetic sequences with the aim of characterising all the organisms present on the archipelago would provide a number of advantages. For example, recording what organisms are already present on the island would facilitate the identification of exotic or introduced species. Furthermore, a complete catalogue of the Norfolk Island species would provide ecological information (e.g., additional distribution for species that have been recorded elsewhere) that could prove extremely

useful for biological control of pests or integrated pest management. Such a database should be developed as a collaboration across industries and institutions, so that comprehensiveness can be ensured. Ideally, anyone conducting collections of arthropods on Norfolk Island should be required to provide genetic sequences in exchange or providing samples for such endeavour. This would ensure that any future collection of material from the island (i.e. by research scientists, museum bioblitz, private collectors) would contribute to the development of such a database.

3. **Development of a morphological voucher/tissue collection of Norfolk Island diversity.** Currently, organisms collected on Norfolk Island are scattered in museums and collections around the world. While this provides a very important record for some of the species collected in the past, it makes examinations and assessments quite challenging, with most of the specimens not databased and therefore in unknown locations. An insect collection (which could include plant material, too) located on Norfolk Island would provide a number of advantages for the community and for any future researcher interested in a specific group of organisms. Voucher specimens for all the species recorded on Norfolk Island could be housed in the same place, making examination of such materials straightforward and providing a compendium of Norfolk Island diversity. Additionally, a voucher collection (hosting preserved specimens) could be complemented by a tissue collection (hosting DNA samples), too.

4. **Involvement of the Norfolk Island community to support biosecurity activities.** During the project, the AVR team was often led and helped by members of the community. It was very clear that members of the community are undoubtedly the most experienced in identifying changes in the environment they are so familiar with. In the context of Norfolk Island, while most of the community may lack specific technical knowledge on biosecurity activities, the community definitely have a deep understanding of biogeographical changes, including presence/absence of specific organisms. This advantage should be harnessed, as it has been in the past, to facilitate surveillance activities. Training on specific topics, such as how to identify priority pests and pathogens, how to collect specimens for further analyses, how to source material for collection, who to contact in case of potential exotic incursions, should be frequent and widely promoted. A variety of training (including workshops, public talks and presentations, hand-on activities) could provide additional strength to the biosecurity activities conducted on the island, by substantially increasing the sampling and surveillance power, which would otherwise weigh solely on biosecurity personnel.

5. **Future fungal plant pathogen surveys should include permits to collect live specimens.** The associations between fungal species identified by DNA sequences and recorded disease symptoms still require further assessment for some samples. Based on the collection permit obtained, this survey could analyse fungal DNA present in dead preserved plant samples but not conduct sequencing from soil samples or *in planta* experiments. While this enabled us to isolate fungal sequences and match many of these to known records on databases, it did not allow us to further explore the genetic diversity of novel species or the correlation between fungal species and observed symptoms. To improve our understanding of such associations (between disease symptoms and fungal species), the fungal strains could be isolated from the diseased tissues for further assays such as whole genome sequencing and *in planta* infection. This warrants future surveys of Norfolk Island, with permits allowing the return of isolated fungi to quarantine labs, which would allow further investigation of specific plant/fungi/disease symptom systems. While we don't suggest this survey to be immediately repeated, we strongly recommend the next survey (indeally within the next two years) works towards this aim.

6. **Weed management prioritisation process.** The record of multiple weed species presented here highlights the need for a transparent weed management prioritisation process. Such a process is required to understand and evaluate what plant species are the most important to manage, what management interventions are the most useful, and how these vary depending on different aspects (i.e., plant species, time of the year recorded, distribution on the island). Such tools are generally specific to different locations, since they take into account the general environment and how it impacts the management of introduced weeds. Similar tools exist in New South Wales and Victoria and could be modified for use on Norfolk Island.

Acknowledgements

The Agriculture Victoria team would like to thank the Norfolk Island community, and any person who welcomed us on their property and shared their knowledge with us. We would like to thank the Norfolk Island Flora & Fauna Society, Norfolk Island National Park Rangers, the DAFF team, and the DITRDCA team. Many thanks to the editors of the Norfolk Islander and the hosts of Radio Norfolk, for their interest in this survey and their help in sharing relevant information.

Appendices

Table 2: List of appendices attached to this report.

Appendix	Topic
Appendix A	Entomology report
Appendix B	Fungal plant pathogens report
Appendix C	Nematology report
Appendix D	Bacterial plant pathogens report
Appendix E	Viral plant pathogens report
Appendix F	Weeds report
Appendix G	References

Appendix A: Entomology report

Norfolk Island Plant Pest and Disease Survey – Arthropods

Background and Introduction

The entomology component of this survey targeted agricultural pests, searching for both pests known to be present on the island, such as the Tomato Potato Psyllid (TPP), and pests not known to be present there, such as the Spotted Wing Drosophila. As part of this survey, a literature review was conducted to determine which insect species have been recorded from Norfolk Island in the past (Holloway 1977; Smithers 1998; Maynard *et al.* 2018). The surveys were conducted in different seasons (spring and summer) in order to maximise chances of recording a higher level of diversity, taking into account the different biological cycles of as many insects as possible. Collection methods included a variety of methodologies (see below), from the use of nets for sweeping, to the deployment of lured traps, but also targeted hand collection and the use of lamp sheets at night.

Methods

Collection methods

Insects were collected using a variety of techniques, from approximately 60 different locations on the island, including private properties and areas of interest in the Norfolk Island National Park and public reserves.

Collection methods aimed to target a variety of different insect groups, including diurnal/nocturnal, flyers/non-flyers, native/introduced. In order to collect has many insects as possible, collection techniques included:

- Hand collection from known host plants, based on information obtained from the literature (Figure 1A).
- Sweeping agricultural areas using an entomological net, and collecting the insects from it using an entomological aspirator (Figure 1B).
- Spot lighting and using lamps to attract insects at night (Figure 1C).
- Deployment of different traps, including pitfall traps, yellow sticky traps and lured traps (Figure 1D).

Figure 1: Dr Francesco Martoni and Dr Mark Blacket using some of the entomological collection techniques adopted during the survey. Hand collection (**A**), net sweeping (**B**), night collection (**C**) and trapping (**D**).

DNA Barcoding

DNA barcoding is a method of specimen identification using short, standardized segments of DNA. Every species has its own barcode, just as every person has their own fingerprint. These DNA barcodes can be compared to a reference library to provide an ID.

Non-destructive DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen, Germany) with an overnight incubation period (~ 17 hours) at 56 °C (as previously described in Martoni *et al.* (2021). The volume of ATL+proteinaseK (ratio 9:1) used depended on the size of insect or insect part. For larger insects, only a leg or part of a leg was used. A subsample of 200 µl lysate per sample was purified on the DNeasy spin columns, following the manufacturer's instructions. After the non-destructive DNA extraction, the insects contained in each sample were preserved in high grade ethanol (100%) for further morphological examination, if required.

Polymerase chain reactions (PCRs) were performed in targeting a ~ 700 bp fragment of the standard barcode region (Hebert *et al.* 2003) within the *Cytochrome c Oxidase* subunit I gene (COI) using the LCO1490 – HCO2148 (Folmer 1994) primer pairs. The PCR was conducted in 25 µL reactions consisting of 14.7 µL Bovine Serum Albumin (New England Biolabs, MA, USA), 5 µL of 5x Bioline MyFi reaction buffer (Meridian Bioscience, OH, USA), 1 µL of each primer (10 µM), 0.5 µL MyFi DNA polymerase (Meridian Bioscience, OH, USA) and 2.5 µL of template DNA. The PCR cycle started with 2 minutes at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 49 °C for 45 seconds and extension at 72 °C for 45 seconds. Finally, the reaction ended with an extension phase of 2 minutes at 72 °C. Amplification was verified by gel electrophoresis (1% w/v agarose). Sequencing was conducted on a Sanger sequencer, outsourced to Macrogen Inc.

The software MEGA X (Kumar *et al.* 2018) was used to manually examine the electropherograms and align the sequences. Sequences were blasted against the NCBI database to obtain a taxonomical ID (Table 1).

DNA Metabarcoding

DNA metabarcoding applies DNA barcoding to a sample containing multiple specimens (e.g, multiple insects collected by a trap). It provides a rapid means of biodiversity assessment by identifying multiple species simultaneously in a sample using DNA sequencing. These may include samples of bulk tissue from whole organisms or environmental samples of water or soil with residual or bound DNA.

Non-destructive DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen, Germany) with an overnight incubation period (~ 17 hours) at 56 °C (as previously described in Martoni *et al.* (2021). The volume of ATL+proteinaseK (ratio 9:1) used depended on the number and size of insects present in each sample, ranging from 600 µL to 1 mL. A subsample of 200 µl lysate per sample was then purified on the DNeasy spin columns, following the manufacturer's instructions. After the non-destructive DNA extraction, the insects contained in each sample were preserved in high grade ethanol (100%) for further morphological examination, if required.

Polymerase chain reactions (PCRs) were performed in duplicate targeting a ~ 200 bp fragment of the standard barcode region (Hebert *et al.* 2003) within the *Cytochrome c Oxidase* subunit I gene (COI) using the FwhF2 – FwhR2n (Vamos *et al.* 2017; Marquina *et al.* 2018) primer pairs. Partial Illumina adapter sequences (in bold italic) were incorporated into the primers for use in 2-step PCR (FwhF2: 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGDACWGGWTGAACWGTWTAYCCHCC-3'; FwhR2n: 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTRATWGCHCCDGCTARWACWGG-3'). The initial PCR was conducted in 25 µL reactions consisting of 14.7 µL Bovine Serum Albumin (New England Biolabs, MA, USA), 5 µL of 5x Bioline MyFi reaction buffer (Meridian Bioscience, OH, USA), 1 µL of each primer (10 µM), 0.5 µL MyFi DNA polymerase (Meridian Bioscience, OH, USA) and 2.5 µL of template DNA. The PCR cycle started with 2 minutes at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 49 °C for 45 seconds and extension at 72 °C for 45 seconds. Finally, the reaction ended with an extension phase of 2 minutes at 72 °C. Amplification was verified by gel electrophoresis (1% w/v agarose).

After the initial PCR amplification, the amplicons were diluted 1/10 and run through a second 6–8 cycles of real-time PCR (rtPCR) to attach unique dual indexes and the remainder of the Illumina adapter sequence. Each indexing rtPCR reaction (50 µL volume) contained 32.5 µL of ddH2O, 10 µL of 5 x Phusion HF Buffer (Thermo Fisher Scientific, MA, USA), 1 µL of dNTP mix (10 mM), 1 µL of SYBR Green I Mix (Thermo Fisher Scientific, MA, USA) diluted 1/1000 in ddH2O, 0.5 µL Phusion DNA polymerase (Thermo Fisher Scientific, MA, USA), 4 µL of sample-specific indexing primers (2.5 µM) and 1 µL of the diluted PCR product. rtPCR cycling conditions were 98 °C for 30 s, followed by 6–8 cycles of 98 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s, with fluorescence measurement conducted in the 72 °C phase. The amplification curve was visually inspected in real time and stopped while still in the exponential phase to prevent over-amplification artefacts.

Amplicons were purified and normalised using the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, MA, USA) following the manufacturer's protocol, but eluting the final product in 15 µl instead of 20 µl. Normalised and cleaned rtPCR amplicons were then pooled together and the resulting library was quality checked, sized and quantified using a High Sensitivity D1000 ScreenTape assay performed on a 2200 TapeStation (Agilent Technologies, CA, USA). The final pooled library was diluted to a concentration of 7 pM, spiked with 15% PhiX and sequenced using V3 chemistry (2 x 250 bp reads) across four flow cells on an Illumina MiSeq system (Illumina, CA, USA).

Bioinformatic analysis

Bioinformatic analysis followed the pipeline generated for the iMapPESTS project and available here: https://alexpiper.github.io/iMapPESTS/local_metabarcoding.html. Most of the pipeline was run on R software (R Core Team 2023). Raw sequence reads were demultiplexed using bcl2fastq allowing for a single mismatch in the indexes, then trimmed of PCR primer sequences using BBDuK v.38 (Bushnell *et al.* 2017). Sequence quality profiles were used to remove reads with more than one expected error (Edgar and Flyvbjerg 2015) or those containing ambiguous 'N' bases, then all remaining sequences were truncated to 205 bp and analysed using DADA2 v.1.16 (Callahan *et al.* 2016). Following denoising, amplicon sequence variants (ASVs), inferred separately from each sequencing run, were combined into a single table and chimeras were detected and removed de-novo using the removeBimeraDenovo function in DADA2. To further filter any non-specific amplification products and pseudogenes, the ASVs were aligned to a profile hidden Markov model (PHMM) (Eddy 1998) of the full-length COI barcode region (Piper *et al.* 2021) and then checked for frame shifts and stop codons that commonly indicate pseudogenes (Roe and Sperling 2007). Taxonomy was assigned using the IDTAXA algorithm of Murali *et al.* (2018) implemented in the DECIPHER v.2.22.0 R package, trained on an in-house COI database created for the iMapPESTS project (Piper *et al.* 2021), accepting only assignments with a bootstrap confidence threshold of 60% or above. To increase classification to species level, we also incorporated a BLASTn v.2.13.0 (Altschul *et al.* 1990) search against the same in-house database and, to reduce the risk of over-classification, we only accepted BLAST species assignments if the BLAST search agreed with IDTAXA at the Genus rank. Finally, all retained ASVs assigned to the same insect species were merged, while ASVs that could not be assigned to species, but only to a higher taxonomic rank (i.e., genus, family, order), were manually compared against the GenBank nucleotide collection (nt/nr) using the Megablast algorithm on the NCBI BLAST web server (Sayers *et al.* 2022), following the procedure described in Martoni *et al.* 2023. This enabled us to match or partially match the unassigned ASVs to sequences present in GenBank that may have been uploaded more recently than the reference database was created or did not pass the stringent filtering parameters defined in Piper *et al.* (2021). ASVs matching sequences with a similarity between 99% and 100% were labelled using the accession number of the GenBank sequence they matched (e.g., Diptera sp. XX00000). ASVs partially matching sequences, with a similarity between 96% and 98.99%, were labelled as "near" the accession number of the GenBank sequence they matched (e.g., Diptera sp. nr XX00000). Following this procedure, ASVs with a genetic similarity < 96% to any given sequence present in GenBank were manually aligned using Geneious Prime 2022.0.2 (www.geneious.com) and MEGA X (Kumar *et al.* 2018) and grouped into a single operational taxonomic unit (OTU) when their divergence was < 5%.

Results

More than 400 samples were collected on Norfolk Island and morphologically examined. Based on the initial examination, a total of 233 samples were processed using molecular techniques. Of these, a total of 113 samples (each containing multiple insect specimens belonging to different species) were processed using metabarcoding, with an additional 120 samples (individual insects) processed using COI DNA barcoding. DNA sequences were generated for more than 500 arthropod species, including insects, spiders, mites, and even ticks. These sequences matched or partially matched sequences available on public databases and resulted in records belonging to 14 orders, encompassing the major groups of insects. These included Blattodea (cockroaches), Coleoptera (beetles), Diptera (flies), Dermaptera (earwigs), Diptera (flies/mosquitoes), Hemiptera (aphids, psyllids), Hymenoptera (wasps, ants), Lepidoptera (moths, butterflies), Neuroptera (antlions), Orthoptera (crickets), Psocoptera (barklice), Termitoidea (termites), Thysanoptera (Thrips) and Trichoptera (caddisflies) (Figure 2, Tables 1 and 2). Additionally, other arthropods were also recorded from the Arachnid Class (Figure 3, Table 2). These included mostly spiders (Araneae, in red in Figure 3), followed by trombidiform mites (light green in Figure 3), Mesostigmata mites (dark green in Figure 3), ixodid ticks (blue in Figure 3) and sarcoptiform mites (purple in Figure 3).

Importantly, a number of insects that are known elsewhere to be pests of plants were recorded. These are mostly aphids (*Dysaphis aucupariae, Hyperomyzus carduellinus, Macrosiphoniella sanborni, Melanaphis sacchari*), a psyllid (*Heteropsylla cubana*), a thrips (*Frankliniella occidentalis*), and a termite (*Coptotermes acinaciformis*).

A total of 102 sequences were sub.mitted to NCBI's GenBank database, with accession numbers OR050315-OR050416.

Discussion

The results showed a number of important findings.

Firstly, a number of exotic pest species previously not reported (in Maynard *et al.* 2018) were recorded. However, these are not part of the NPPP list, suggesting that their impact on Norfolk Island plants should not be a cause of concern. Of the pests recorded, Plantain aphid, Asian Sowthistle Aphid, Chrysanthemum Aphid are pests of weeds or ornamentals. The Yellow sugarcane aphid is considered a serious pest of sugar cane, however this plant is not an important crop on the island. Similarly, the impact of the Western Flower Thrips is not likely to be as damaging to garden flowers as it is for the cut flower industry. While the Leucaena psyllid is considered a serious pest of *Leucaena* plants, *Leucaena* is an introduced plant on Norfolk Island and the role of this psyllids could be argued to be more similar to a biological control than to a nuisance.

An interesting record was that of the Macadamia nut borer moth (*Cryptophlebia ombrodelta*), a serious pest of lychee and longan worldwide. This species was not recorded during the previous survey (Maynard *et al.* 2018) but had been reported previously on Norfolk Island (Holloway 1977). On hatching, larvae bore into the green fruit searching for the seed, leading to the fruit dropping. The larva will then develop in the fallen fruit. If the larva attacks ripening fruit, on the other hand, this generally does not fall, and the larva will drown in the juice (Queensland Government 2022). During this survey we recorded a single incidence of this pest, suggesting the population number are currently not of concern. However, the fact this species was not found during the 2012-2014 survey may suggest that populations vary in size across years, making it challenging to record. Should this be the case for other species, smaller surveys conducted more often may enable to record a higher number of species, without risking to have collection expeditions falling during years with smaller populations.

Of the DNA sequences obtained, only ~250 matched a species identification level (between 97-100% similarity) with a known species present on a public database. For example, 10 beetles, almost 60 flies, 6 bugs, 10 butterflies/moths could only be identified to the order level (Table 2). This suggests that more than half of the DNA sequences obtained here represent either species new to science or species that have been formally described but have never been genetically characterised. While this was not unexpected considering the high level of endemism on Norfolk Island, it certainly highlights the need of further molecular characterisation of the insect species present in the archipelago, and the need of species descriptions for the groups that are present only on Norfolk Island.

Overall, the vast majority of the taxa recorded here were not agricultural pests. Not only native and endemic species, but also non-pest introduced species composed the bulk of insect species recorded across the surveys. This can be considered a factor representing a healthy environment rich in biodiversity, showing different groups of insects co-existing across the island, both in the native forests and in the agricultural landscape.

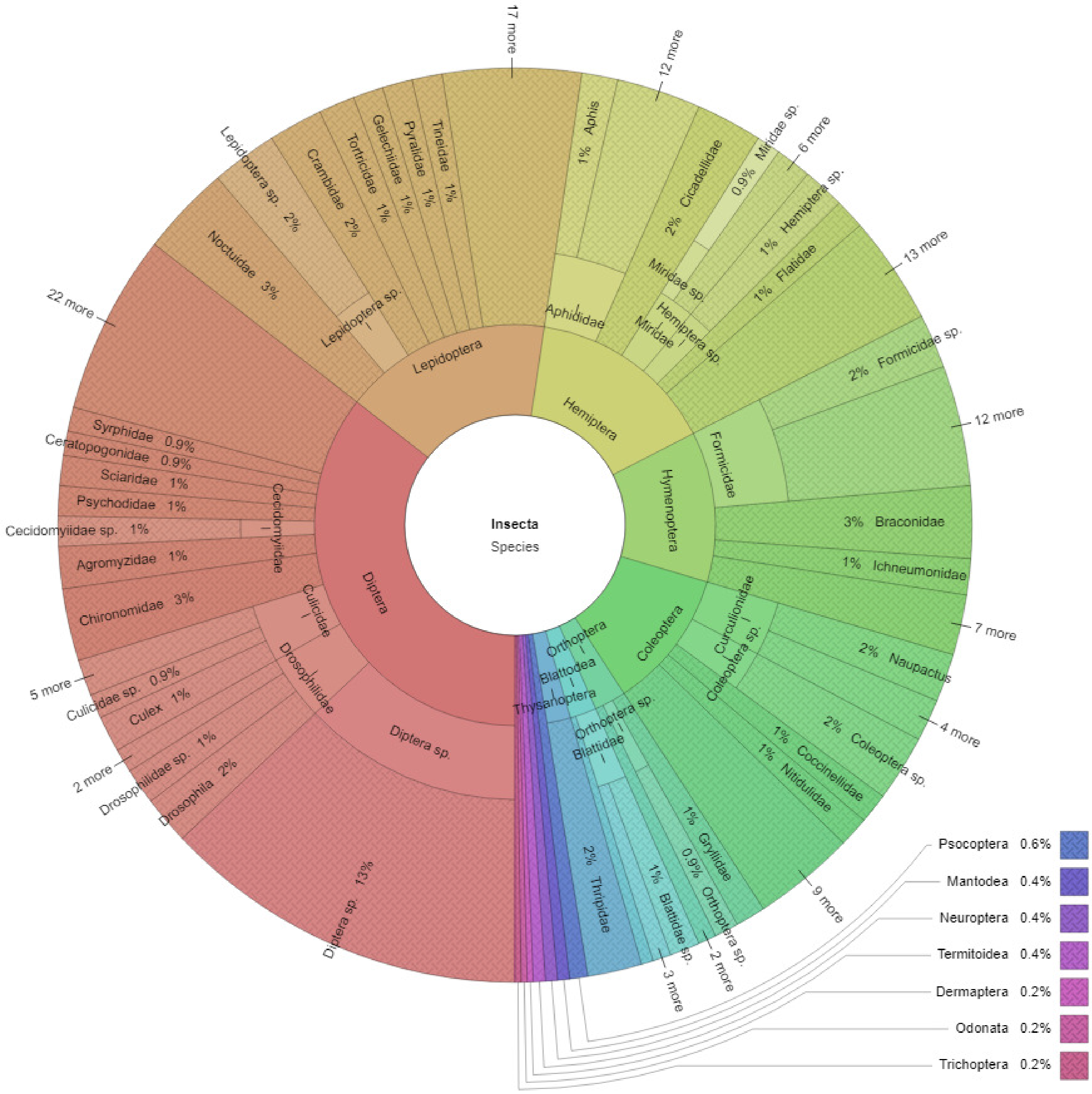

Figure 2: Krona plot showing the species of insect recorded during this survey, colour-coded by order. The order showing the highest number of species recorded was Diptera (in red), followed by Lepidoptera (orange), Hemiptera (yellow), Hymenoptera (light green), Coleoptera (dark green), Orthoptera, Blattodea, Thysanoptera, Psocoptera, Mantodea, Neuroptera, Dermaptera, Odonata, Termitoidea and Trichoptera. This interactive graphic will be made available on DITRDCA website.

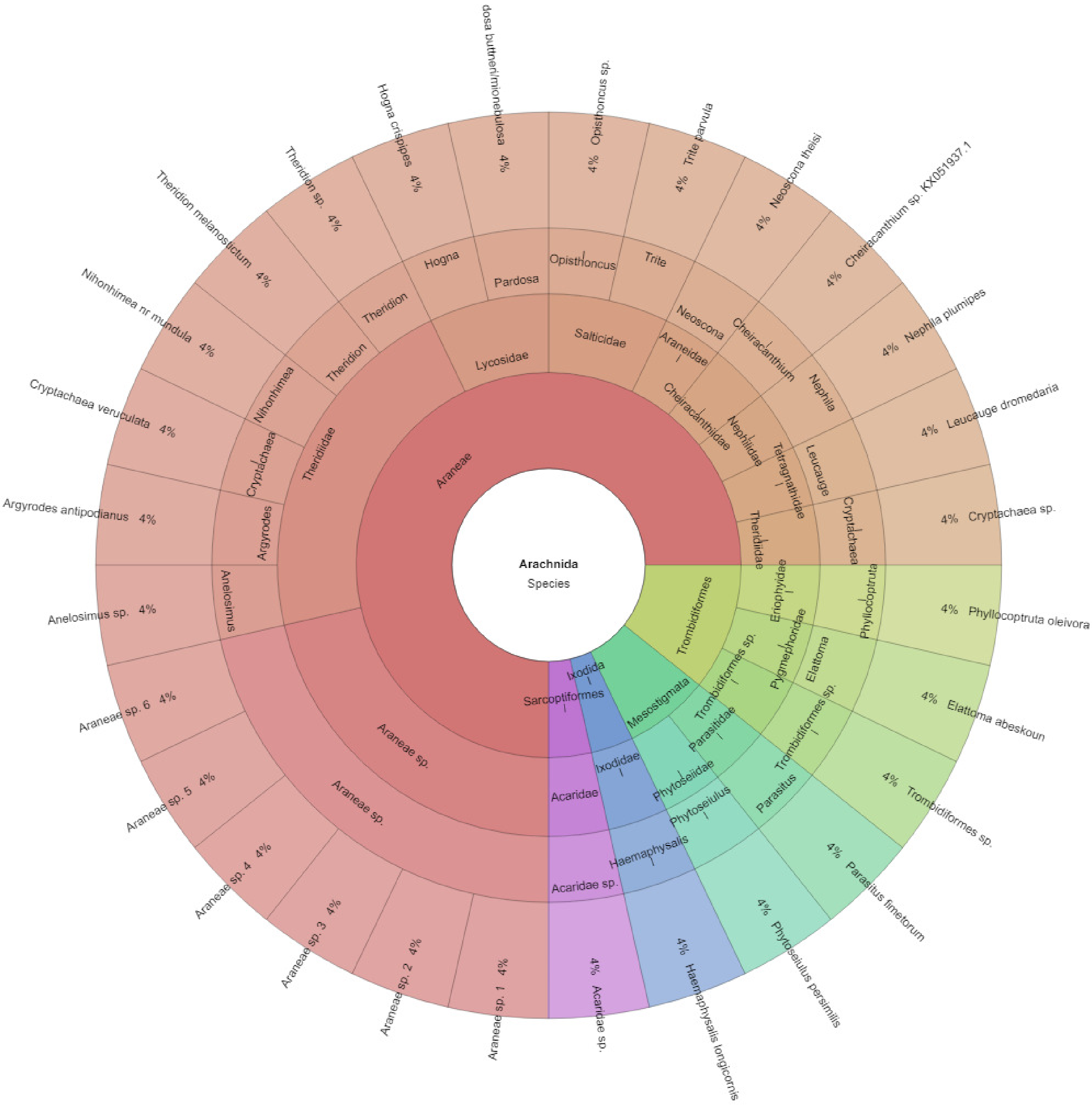

Figure 3: Krona plot showing the species of arachnids recorded during this survey, colour-coded by order. The order showing the highest number of species recorded was spiders (Araneae, in red), followed by trombidiform mites (light green), Mesostigmata mites (dark green), ixodid ticks (blue) and sarcoptiform mites (purple). This interactive graphic will be made available on DITRDCA website.

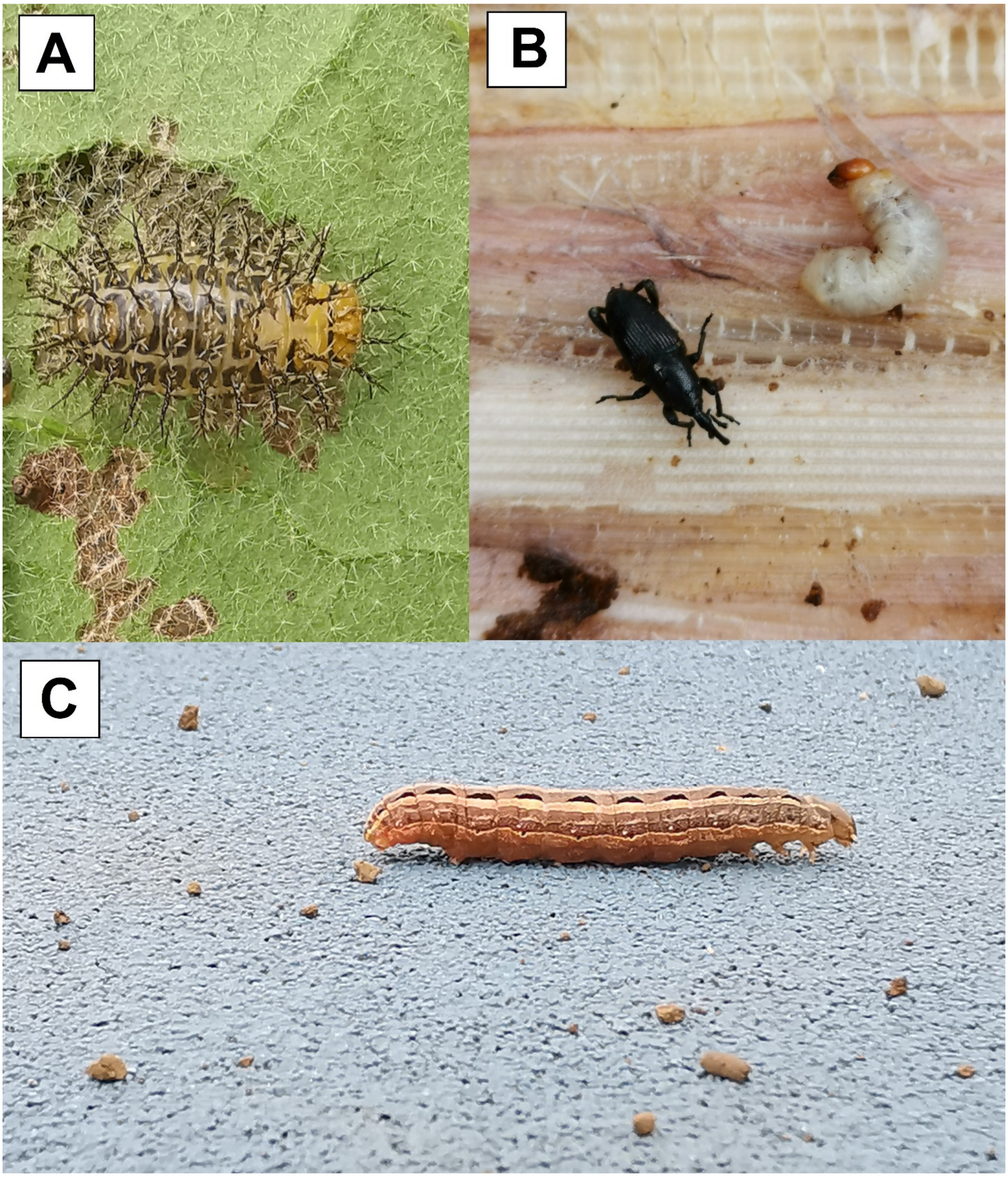

Figure 4: Examples of pest insects recorded on Norfolk Island. The 28-spotted ladybird beetle (A; immature stage), the grub and adult of the Banana borer (B) and the immature of one of the armyworm moths (C; *Spodoptera* sp.). All pests were recorded in the previous survey (Maynard *et al.* 2018).

Table 1: list of insect species that matched a DNA sequence obtained using COI barcoding analysis. The table includes Order, Family, Genus and Species for each sequence obtained. The "notes" section highlights native species and beneficials (in green), known pests (in red) and sequences that could not be identified to species which are therefore considered potential native records.

Order	Family	Genus	Species	Notes
Blattodea	Blattidae	Blattidae sp.	Blattidae sp.	Cockroach
Blattodea	Blattidae	*Panesthia*	*Panesthia* nr *cribrata*	Cockroach
Blattodea	Blattidae	*Periplaneta*	*Periplaneta americana*	Pest cockroach
Blattodea	Blattidae	*Pycnoscelus*	*Pycnoscelus* sp.	Cockroach
Coleoptera	Cerambycidae	*Enicodes*	*Enicodes* sp.	Native
Coleoptera	Coccinellidae	*Coelophora*	*Coelophora inaequalis*	Predator
Coleoptera	Coccinellidae	*Henosepilachna*	*Henosepilachna vigintioctopunctata*	28-spotted ladybird
Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 1	Beetle
Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 2	Beetle
Coleoptera	Curculionidae	*Asynonychus*	*Asynonychus cervinus*	Fuller's Rose weevil (garden pest)
Coleoptera	Curculionidae	*Cosmopolites*	*Cosmopolites sordidus*	Banana root borer
Coleoptera	Curculionidae	Curculionidae sp.	Curculionidae sp.	Weevil
Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp.	Weevil
Coleoptera	Scarabeidae	*?Digitonthophagus*	*?Digitonthophagus* sp.	Beetle
Coleoptera	Scarabeidae	*Heteronychus*	*Heteronychus arator*	African Black Beetle
Diptera	Ceratopogonidae	Ceratopogonidae sp.	Ceratopogonidae sp.	Fly
Diptera	Dolichopodidae	Dolichopodidae sp.	Dolichopodidae sp.	Matches 99.85% KX052907.1
Diptera	Drosophilidae	Drosophilidae sp.	Drosophilidae sp.	Vinegar fly
Diptera	Lauxaniidae	*Steganopsis*	*Steganopsis* nr *melanogaster*	Native
Diptera	Lauxaniidae	*Steganopsis*	*Steganopsis* sp.	Fly
Diptera	Stratiomyidae	*Exaireta*	*Exaireta* nr *spinigera*	Garden soldier fly
Hemiptera	Aphididae	*Aphis*	*Aphis gossypii*	Aphid
Hemiptera	Aphididae	*Aphis*	*Aphis* nr *aurantii*	Aphid
Hemiptera	Cicadellidae	Cicadellidae sp.	Cicadellidae sp.	Hopper
Hemiptera	Flatidae	*Siphanta*	*Siphanta acuta*	Hopper
Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp.	Bug
Hemiptera	Phacopteronidae	*Pseudophacopteron*	*Pseudophacopteron* sp.	Native psyllid
Hymenoptera	Braconidae	*Meteorus*	*Meteorus pulchricornis*	Parasitoid wasp
Hymenoptera	Crabronidae	Crabronidae sp.	Crabronidae sp.	Wasp
Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp.	Ant
Hymenoptera	Formicidae	*Iridomyrmex*	*Iridomyrmex anceps/suchieri*	Ant
Hymenoptera	Formicidae	*Iridomyrmex*	*Iridomyrmex* sp.	Ant
Hymenoptera	Formicidae	*Nylanderia*	*Nylanderia tasmaniensis*	Ant
Hymenoptera	Formicidae	*Ochetellus*	*Ochetellus glaber*	Ant
Hymenoptera	Formicidae	*Tapinoma*	*Tapinoma melanocephalum*	Ant
Hymenoptera	Ichneumonidae	*Echthromorpha*	*Echthromorpha agrestoria*	Parasitoid wasp
Hymenoptera	Ichneumonidae	*Ichneumon*	*Ichneumon promissorius*	Parasitoid wasp

Hymenoptera	Ichneumonidae	*Lissopimpla*	*Lissopimpla excelsa*	orchid dupe wasp
Hymenoptera	Vespidae	Polistes	*Polistes chinensis*	Paper wasp
Lepidoptera	Cosmopterigidae	Cosmopterigidae sp.	Cosmopterigidae sp.	Butterflies/Moths
Lepidoptera	Erebidae	*Achea*	*Achea janata*	Butterflies/Moths
Lepidoptera	Erebidae	*Mocis*	*Mocis frugalis*	Sugarcane looper
Lepidoptera	Immidae	Immidae sp.	Immidae sp.	Butterflies/Moths
Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp.	Butterflies/Moths
Lepidoptera	Noctuidae	*Condica*	*Condica illecta*	Butterflies/Moths
Lepidoptera	Noctuidae	*Ctenoplusia*	*Ctenoplusia albostriata*	Butterflies/Moths
Lepidoptera	Noctuidae	*Leucania*	Leucania stenographa	Sugar Cane Armyworm
Lepidoptera	Noctuidae	*Spodoptera*	*Spodoptera litura*	Armyworm
Lepidoptera	Noctuidae	*Spodoptera*	*Spodoptera mauritia*	Armyworm
Lepidoptera	Nymphalidae	*Junonia*	*Junonia villida*	Meadow argus
Lepidoptera	Papilionidae	*Papilio*	*Papilio amynthor*	Norfolk Island Swallowtail
Lepidoptera	Pyralidae	*Endotricha*	*Endotricha mesenterialis*	Butterflies/Moths
Lepidoptera	Sphingidae	*Agrius*	*Agrius convolvuli*	Hawk moth
Lepidoptera	Tineidae	*Opogona*	*Opogona omoscopa*	Butterflies/Moths
Lepidoptera	Tineidae	Tineidae sp.	Tineidae sp.	Butterflies/Moths
Lepidoptera	Tortricidae	*Cryptophlebia*	*Cryptophlebia ombrodelta*	Macadamia nut borer
Lepidoptera	Tortricidae	*Strepsicrates*	*Strepsicrates semicanella*	Butterflies/Moths
Mantodea	Mantidae	Mantidae sp.	Mantidae sp.	Mantis
Neuroptera	Chrysopidae	*Mallada*	*Mallada basalis*	Predator
Odonata	Coenagrionidae	*Ischnura*	*Ischnura aurora*	Damselfly
Orthoptera	Orthoptera sp.	Orthoptera sp.	Orthoptera sp.	Possible native cricket
Termitodea	Termitodea sp.	Termitodea sp.	Termitodea sp.	Possible termite pest

Table 2: list of arthropods taxa (including spiders, mites and ticks) that matched a DNA sequence detected using metabarcoding analysis. The table includes Class, Order, Family, Genus and Species for each ASV sequences obtained. The list includes 447 species, belonging to 19 orders and 118 families.

Class	**Order**	**Family**	**Genus**	**Species**
Arachnida	Araneae	Araneae sp.	Araneae sp.	Araneae sp. 1
Arachnida	Araneae	Araneae sp.	Araneae sp.	Araneae sp. 2
Arachnida	Araneae	Araneae sp.	Araneae sp.	Araneae sp. 3
Arachnida	Araneae	Araneae sp.	Araneae sp.	Araneae sp. 4
Arachnida	Araneae	Araneae sp.	Araneae sp.	Araneae sp. 5
Arachnida	Araneae	Araneae sp.	Araneae sp.	Araneae sp. 6
Arachnida	Araneae	Araneidae	*Neoscona*	*Neoscona theisi*
Arachnida	Araneae	Cheiracanthiidae	*Cheiracanthium*	*Cheiracanthium* sp. KX051937.1
Arachnida	Araneae	Lycosidae	*Hogna*	*Hogna crispipes*
Arachnida	Araneae	Lycosidae	*Pardosa*	*Pardosa buttneri/mionebulosa*
Arachnida	Araneae	Nephilidae	*Nephila*	*Nephila plumipes*
Arachnida	Araneae	Salticidae	*Opisthoncus*	*Opisthoncus* sp.
Arachnida	Araneae	Salticidae	*Trite*	*Trite parvula*
Arachnida	Araneae	Tetragnathidae	*Leucauge*	*Leucauge dromedaria*

Arachnida	Araneae	Theridiidae	*Anelosimus*	*Anelosimus* sp.
Arachnida	Araneae	Theridiidae	*Argyrodes*	*Argyrodes antipodianus*
Arachnida	Araneae	Theridiidae	*Cryptachaea*	*Cryptachaea veruculata*
Arachnida	Araneae	Theridiidae	*Nihonhimea*	*Nihonhimea* nr *mundula*
Arachnida	Araneae	Theridiidae	*Theridion*	*Theridion melanostictum*
Arachnida	Araneae	Theridiidae	*Theridion*	*Theridion* sp.
Arachnida	Araneae	Theridiidae	*Cryptachaea*	*Cryptachaea* sp.
Arachnida	Ixodida	Ixodidae	*Haemaphysalis*	*Haemaphysalis longicornis*
Arachnida	Mesostigmata	Parasitidae	*Parasitus*	*Parasitus fimetorum*
Arachnida	Mesostigmata	Phytoseiidae	*Phytoseiulus*	*Phytoseiulus persimilis*
Arachnida	Sarcoptiformes	Acaridae	*Acaridae* sp.	*Acaridae* sp.
Arachnida	Trombidiformes	Eriophyidae	*Phyllocoptruta*	*Phyllocoptruta oleivora*
Arachnida	Trombidiformes	Pygmephoridae	*Elattoma*	*Elattoma abeskoun*
Insecta	Blattodea	Blattidae	Blattidae sp.	Blattidae sp. 1
Insecta	Blattodea	Blattidae	Blattidae sp.	Blattidae sp. 2
Insecta	Blattodea	Blattidae	Blattidae sp.	Blattidae sp. 3
Insecta	Blattodea	Blattidae	Blattidae sp.	Blattidae sp. 4
Insecta	Blattodea	Ectobiidae	*Balta*	*Balta scripta*
Insecta	Blattodea	Ectobiidae	Ectobiidae sp.	Ectobiidae sp.
Insecta	Coleoptera	Cerambycidae	*Enicodes*	*Enicodes* sp.
Insecta	Coleoptera	Cerambycidae	*Prionus*	*Prionus insularis*
Insecta	Coleoptera	Chrysomelidae	*Chaetocnema*	*Chaetocnema confinis*
Insecta	Coleoptera	Chrysomelidae	*Chrysomelidae* sp.	*Chrysomelidae* sp. MH926741.1
Insecta	Coleoptera	Coccinellidae	*Coelophora*	*Coelophora inaequalis*
Insecta	Coleoptera	Coccinellidae	*Halmus*	*Halmus chalybeus*
Insecta	Coleoptera	Coccinellidae	*Henosepilachna*	*Henosepilachna vigintioctopunctata*
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 1
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 2
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 3
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 4
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 5
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 6
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 7
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 8
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 9
Insecta	Coleoptera	Coleoptera sp.	Coleoptera sp.	Coleoptera sp. 10
Insecta	Coleoptera	Corylophidae	Corylophidae sp.	Corylophidae sp. 1
Insecta	Coleoptera	Corylophidae	Corylophidae sp.	Corylophidae sp. 2
Insecta	Coleoptera	Corylophidae	*Sericoderus*	*Sericoderus* sp.
Insecta	Coleoptera	Curculionidae	*Asynonychus*	*Asynonychus cervinus*
Insecta	Coleoptera	Curculionidae	*Cosmopolites*	*Cosmopolites sordidus*
Insecta	Coleoptera	Curculionidae	Curculionidae sp.	Curculionidae sp.
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus leucoloma*
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus peregrinus*
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 1
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 2

Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 3
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 4
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 5
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 6
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 7
Insecta	Coleoptera	Curculionidae	*Naupactus*	*Naupactus* sp. 8
Insecta	Coleoptera	Elateridae	*Alaus*	*Alaus melanops*
Insecta	Coleoptera	Elateridae	*Conoderus*	*Conoderus exsul*
Insecta	Coleoptera	Latridiidae	Latridiidae sp.	Latridiidae sp. 1
Insecta	Coleoptera	Latridiidae	Latridiidae sp.	Latridiidae sp. 2
Insecta	Coleoptera	Monotomidae	*Monotoma*	*Monotoma picipes*
Insecta	Coleoptera	Mycetophagidae	*Typhaea*	*Typhaea stercorea*
Insecta	Coleoptera	Nitidulidae	*Aethina*	*Aethina concolor*
Insecta	Coleoptera	Nitidulidae	*Brachypeplus*	*Brachypeplus* sp. 1
Insecta	Coleoptera	Nitidulidae	*Carpophilus*	*Carpophilus* sp. 1
Insecta	Coleoptera	Nitidulidae	*Carpophilus*	*Carpophilus* sp. 2
Insecta	Coleoptera	Nitidulidae	*Urophorus*	*Urophorus humeralis*
Insecta	Coleoptera	Silvanidae	*Cryptamorpha*	*Cryptamorpha desjardinsii*
Insecta	Dermaptera	Forficulidae	*Apterygida*	*Apterygida media*
Insecta	Diptera	Agromyzidae	Agromyzidae sp.	Agromyzidae sp.
Insecta	Diptera	Agromyzidae	*Cerodontha*	*Cerodontha australis*
Insecta	Diptera	Agromyzidae	*Ophiomyia*	*Ophiomyia phaseoli*
Insecta	Diptera	Agromyzidae	*Phytomyza*	*Phytomyza plantaginis*
Insecta	Diptera	Agromyzidae	*Pseudonapomyza*	*Pseudonapomyza* nr EF104731.1
Insecta	Diptera	Agromyzidae	*Pseudonapomyza*	*Pseudonapomyza* sp.
Insecta	Diptera	Agromyzidae	*Pseudonapomyza*	*Pseudonapomyza* sp. EF104731.1
Insecta	Diptera	Anisopodidae	*Sylvicola*	*Sylvicola dubius*
Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp.	Cecidomyiidae sp. 1
Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp.	Cecidomyiidae sp. 2
Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp.	Cecidomyiidae sp. 3
Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp.	Cecidomyiidae sp. 4
Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp.	Cecidomyiidae sp. 5
Insecta	Diptera	Ceratopogonidae	*Atrichopogon*	*Atrichopogon* sp. nr MG172104.1
Insecta	Diptera	Ceratopogonidae	Ceratopogonidae sp.	Ceratopogonidae sp.
Insecta	Diptera	Ceratopogonidae	Ceratopogonidae sp.	Ceratopogonidae sp. KX051919.1
Insecta	Diptera	Ceratopogonidae	*Dasyhelea*	*Dasyhelea ludingensis*
Insecta	Diptera	Chironomidae	*Camptocladius*	*Camptocladius stercorarius*
Insecta	Diptera	Chironomidae	*Chironomus*	*Chironomus cloacalis*
Insecta	Diptera	Chironomidae	*Chironomus*	*Chironomus* sp. KC750311.1
Insecta	Diptera	Chironomidae	*Chironomus*	*Chironomus tepperi*
Insecta	Diptera	Chironomidae	*Paratanytarsus*	*Paratanytarsus kathleenae*
Insecta	Diptera	Chironomidae	*Polypedilum*	*Polypedilum nubifer*
Insecta	Diptera	Chironomidae	*Procladius*	*Procladius paludicola*
Insecta	Diptera	Chironomidae	*Procladius*	*Procladius villosimanus*
Insecta	Diptera	Chironomidae	*Riethia*	*Riethia stictoptera*
Insecta	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus bispinosus*

Insecta	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus fuscithorax*
Insecta	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus semibarbitarsus*
Insecta	Diptera	Chloropidae	Chloropidae sp.	Chloropidae sp.
Insecta	Diptera	Chloropidae	Chloropidae sp.	Chloropidae sp.
Insecta	Diptera	Culicidae	*Aedes*	*Aedes notoscripta/notoscriptus*
Insecta	Diptera	Culicidae	*Aedes*	*Aedes* sp.
Insecta	Diptera	Culicidae	*Culex*	*Culex annulirostris*
Insecta	Diptera	Culicidae	*Culex*	*Culex australicus*
Insecta	Diptera	Culicidae	*Culex*	*Culex molestus/quinquefasciatus*
Insecta	Diptera	Culicidae	*Culex*	*Culex* sp. 1
Insecta	Diptera	Culicidae	*Culex*	*Culex* sp. 2
Insecta	Diptera	Culicidae	*Culex*	*Culex* sp. 3
Insecta	Diptera	Culicidae	Culicidae sp.	Culicidae sp. 1
Insecta	Diptera	Culicidae	Culicidae sp.	Culicidae sp. 2
Insecta	Diptera	Culicidae	Culicidae sp.	Culicidae sp. 3
Insecta	Diptera	Culicidae	Culicidae sp.	Culicidae sp. 4
Insecta	Diptera	Culicidae	*Mansonia*	*Mansonia uniformis*
Insecta	Diptera	Culicidae	*Neocellia*	*Anopheles splendidus*
Insecta	Diptera	Culicidae	*Neomyzomyia*	*Anopheles annulipes*
Insecta	Diptera	Culicidae	*Ochlerotatus*	*Ochlerotatus notoscriptus*
Insecta	Diptera	Diptera sp.	*Atherigona*	*Atherigona nr orientalis*
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 1
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 2
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 3
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 4
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 5
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 6
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 7
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 8
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 9
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 10
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 11
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 12
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 13
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 14
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 15
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 16
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 17
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 18
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 19
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 20
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 21
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 22
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 23
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 24
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 25

Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 26
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 27
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 28
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 29
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 30
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 31
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 32
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 33
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 34
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 35
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 36
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 37
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 38
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 39
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 40
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 41
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 42
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 43
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 44
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 45
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 46
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 47
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 48
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 49
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 50
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 51
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 52
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 53
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 54
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 55
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 56
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 57
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. 58
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. JF872591.1
Insecta	Diptera	Diptera sp.	Diptera sp.	Diptera sp. JN298847.1
Insecta	Diptera	Dolichopodidae	*Amblypsilopus*	*Amblypsilopus connexus*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila busckii*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila buzzatii/koepferae*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila funebris*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila hydei*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila immigrans*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila melanogaster*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila repleta*
Insecta	Diptera	Drosophilidae	*Drosophila*	*Drosophila simulans*
Insecta	Diptera	Drosophilidae	Drosophilidae sp.	Drosophilidae sp. 1
Insecta	Diptera	Drosophilidae	Drosophilidae sp.	Drosophilidae sp. 2

Insecta	Diptera	Drosophilidae	Drosophilidae sp.	Drosophilidae sp. 3
Insecta	Diptera	Drosophilidae	Drosophilidae sp.	Drosophilidae sp. 4
Insecta	Diptera	Drosophilidae	Drosophilidae sp.	Drosophilidae sp. 5
Insecta	Diptera	Drosophilidae	*Scaptodrosophila*	*Scaptodrosophila kirki*
Insecta	Diptera	Drosophilidae	*Scaptodrosophila*	*Scaptodrosophila lattivitata*
Insecta	Diptera	Drosophilidae	*Scaptomyza*	*Scaptomyza australis*
Insecta	Diptera	Drosophilidae	*Scaptomyza*	*Scaptomyza nr australis*
Insecta	Diptera	Ephydridae	*Hydrellia*	*Hydrellia tritici*
Insecta	Diptera	Limonidae	Limonidae sp.	Limonidae sp.
Insecta	Diptera	Limoniidae	*Symplecta*	*Symplecta pilipes*
Insecta	Diptera	Lonchaeidae	*Lamprolonchaea*	*Lamprolonchaea brouniana*
Insecta	Diptera	Milichiidae	*Desmometopa*	*Desmometopa varipalpis*
Insecta	Diptera	Muscidae	*Stomoxys*	*Stomoxys calcitrans*
Insecta	Diptera	Pipunculidae	*Tomosvaryella*	*Tomosvaryella* sp. HM421623.1
Insecta	Diptera	Psychodidae	*Clogmia*	*Clogmia albipunctata*
Insecta	Diptera	Psychodidae	*Clogmia*	*Clogmia* nr *albipunctata*
Insecta	Diptera	Psychodidae	*Psychoda*	*Psychoda cinerea*
Insecta	Diptera	Psychodidae	*Psychoda*	*Psychoda* sp. JF872464.1
Insecta	Diptera	Psychodidae	Psychodidae sp.	Psychodidae sp.
Insecta	Diptera	Rhiniidae	*Stomorhina*	*Stomorhina discolor*
Insecta	Diptera	Sarcophagidae	Sarcophagidae sp.	Sarcophagidae sp.
Insecta	Diptera	Sarcophagidae	*Oxysarcodexia*	*Oxysarcodexia varia*
Insecta	Diptera	Scatopsidae	*Coboldia*	*Coboldia fuscipes*
Insecta	Diptera	Scatopsidae	*Holoplagia*	*Holoplagia guamensis*
Insecta	Diptera	Sciaridae	*Cosmosciara*	*Cosmosciara perniciosa*
Insecta	Diptera	Sciaridae	*Leptosciarella*	*Leptosciarella* sp.
Insecta	Diptera	Sciaridae	*Pseudolycoriella*	*Pseudolycoriella cavatica*
Insecta	Diptera	Sciaridae	Sciaridae sp.	Sciaridae sp. 1
Insecta	Diptera	Sciaridae	Sciaridae sp.	Sciaridae sp. 2
Insecta	Diptera	Sepsidae	*Lasionemopoda*	*Lasionemopoda hirsuta*
Insecta	Diptera	Sepsidae	*Sepsis*	*Sepsis dissimilis*
Insecta	Diptera	Simuliidae	*Simulium*	*Simulium ornatipes*
Insecta	Diptera	Sphaeroceridae	*Pullimosina*	*Pullimosina heteroneura*
Insecta	Diptera	Sphaeroceridae	*Rachispoda*	*Rachispoda* sp. KY834990.1
Insecta	Diptera	Syrphidae	*Melanostoma*	*Melanostoma apicale*
Insecta	Diptera	Syrphidae	*Simosyrphus*	*Simosyrphus grandicornis*
Insecta	Diptera	Syrphidae	*Sphaerophoria*	*Sphaerophoria macrogaster*
Insecta	Diptera	Syrphidae	Syrphidae sp.	Syrphidae sp.
Insecta	Diptera	Tephritidae	*Dioxyna*	*Dioxyna bidentis*
Insecta	Diptera	Tephritidae	*Procecidochares*	*Procecidochares utilis*
Insecta	Diptera	Trichoceridae	*Nothotrichocera*	*Nothotrichocera cranstoni*
Insecta	Diptera	Ulidiidae	*Physiphora*	*Physiphora clausa*
Insecta	Hemiptera	Aleyrodidae	*Trialeurodes*	*Trialeurodes vaporariorum*
Insecta	Hemiptera	Alydidae	*Leptocorisa*	*Leptocorisa acuta*
Insecta	Hemiptera	Aphalaridae	*Blastopsylla*	*Blastopsylla occidentalis*
Insecta	Hemiptera	Aphalaridae	*Cryptoneossa*	*Cryptoneossa triangula*

Insecta	Hemiptera	Aphididae	*Acyrthosiphon*	*Acyrthosiphon caraganae/churchillense/kondoi*
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis aurantii*
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis lichtensteini*
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis nerii*
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis gossypii*
Insecta	Hemiptera	Aphididae	*Aulacorthum*	*Aulacorthum solani*
Insecta	Hemiptera	Aphididae	*Brachycaudus*	*Brachycaudus helichrysi*
Insecta	Hemiptera	Aphididae	*Dysaphis*	*Dysaphis aucupariae*
Insecta	Hemiptera	Aphididae	*Hyperomyzus*	*Hyperomyzus carduellinus*
Insecta	Hemiptera	Aphididae	*Hysteroneura*	*Hysteroneura setariae*
Insecta	Hemiptera	Aphididae	*Lipaphis*	*Lipaphis pseudobrassicae / erysimi*
Insecta	Hemiptera	Aphididae	*Macrosiphoniella*	*Macrosiphoniella sanborni*
Insecta	Hemiptera	Aphididae	*Macrosiphum*	*Macrosiphum euphorbiae*
Insecta	Hemiptera	Aphididae	*Melanaphis*	*Melanaphis sacchari*
Insecta	Hemiptera	Aphididae	*Pentalonia*	*Pentalonia nigronervosa*
Insecta	Hemiptera	Aphididae	*Rhopalosiphum*	*Rhopalosiphum maidis*
Insecta	Hemiptera	Aphididae	*Rhopalosiphum*	*Rhopalosiphum padi*
Insecta	Hemiptera	Aphididae	*Rhopalosiphum*	*Rhopalosiphum rufiabdominalis*
Insecta	Hemiptera	Cicadellidae	*Austroagallia*	*Austroagallia torrida*
Insecta	Hemiptera	Cicadellidae	*Balclutha*	*Balclutha incisa*
Insecta	Hemiptera	Cicadellidae	*Batracomorphus*	*Batracomorphus angustatus*
Insecta	Hemiptera	Cicadellidae	Cicadellidae sp.	Cicadellidae sp. nr KY843731.1
Insecta	Hemiptera	Cicadellidae	*Empoasca*	*Empoasca distinguenda*
Insecta	Hemiptera	Cicadellidae	*Exitianus*	*Exitianus plebeius*
Insecta	Hemiptera	Cicadellidae	*Macrosteles*	*Macrosteles laevis*
Insecta	Hemiptera	Cicadellidae	*Maiestas*	*Maiestas knighti*
Insecta	Hemiptera	Cicadellidae	*Orosius*	*Orosius argentatus*
Insecta	Hemiptera	Cicadellidae	*Planicephalus*	*Planicephalus flavicosta*
Insecta	Hemiptera	Corixidae	*Agraptocorixa*	*Agraptocorixa parvipunctata*
Insecta	Hemiptera	Delphacidae	*Sogatella*	*Sogatella kolophon*
Insecta	Hemiptera	Flatidae	*Colgar*	*Colgar rufostigmatum*
Insecta	Hemiptera	Flatidae	Flatidae sp.	Flatidae sp. 1
Insecta	Hemiptera	Flatidae	Flatidae sp.	Flatidae sp. 2
Insecta	Hemiptera	Flatidae	Flatidae sp.	Flatidae sp. 3
Insecta	Hemiptera	Flatidae	*Siphanta*	*Siphanta acuta*
Insecta	Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp. 1
Insecta	Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp. 2
Insecta	Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp. 3
Insecta	Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp. 4
Insecta	Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp. 5
Insecta	Hemiptera	Hemiptera sp.	Hemiptera sp.	Hemiptera sp. 6
Insecta	Hemiptera	Lygaeidae	*Nysius*	*Nysius caledoniae*
Insecta	Hemiptera	Lygaeidae	*Nysius*	*Nysius caledoniae/plebeius*
Insecta	Hemiptera	Lygaeidae	*Nysius*	*Nysius graminicola*
Insecta	Hemiptera	Meenoplidae	*Phaconeura*	*Phaconeura froggatti*

Insecta	Hemiptera	Miridae	*Campylomma*	*Campylomma* sp. KY682767.1
Insecta	Hemiptera	Miridae	*Coridromius*	*Coridromius chenopoderis*
Insecta	Hemiptera	Miridae	*Creontiades*	*Creontiades dilutus*
Insecta	Hemiptera	Miridae	Miridae sp.	Miridae sp. 1
Insecta	Hemiptera	Miridae	Miridae sp.	Miridae sp. 2
Insecta	Hemiptera	Miridae	Miridae sp.	Miridae sp. 3
Insecta	Hemiptera	Miridae	Miridae sp.	Miridae sp. 4
Insecta	Hemiptera	Miridae	*Nesidiocoris*	*Nesidiocoris tenuis*
Insecta	Hemiptera	Miridae	*Taylorilygus*	*Taylorilygus apicalis*
Insecta	Hemiptera	Miridae	*Tytthus*	*Tytthus chinensis*
Insecta	Hemiptera	Pemphigidae	*Tetraneura*	*Tetraneura nigriabdominalis*
Insecta	Hemiptera	Pentatomidae	*Dictyotus*	*Dictyotus caenosus*
Insecta	Hemiptera	Pentatomidae	*Glaucias*	*Glaucias amyota*
Insecta	Hemiptera	Pentatomidae	*Rhacognathus*	*Rhacognathus americanus*
Insecta	Hemiptera	Rhyparochromidae	*Pseudopachybrachius*	*Pseudopachybrachius guttus*
Insecta	Hemiptera	Tingidae	*Teleonemia*	*Teleonemia scrupulosa*
Insecta	Hemiptera	Triozidae	*Bactericera*	*Bactericera cockerelli*
Insecta	Hymenoptera	Apidae	*Apis*	*Apis mellifera*
Insecta	Hymenoptera	Apidae	*Eufriesea*	*Eufriesea anisochlora*
Insecta	Hymenoptera	Apidae	*Tetragonula*	*Tetragonula carbonaria*
Insecta	Hymenoptera	Bethylidae	*Goniozus*	*Goniozus jacintae*
Insecta	Hymenoptera	Braconidae	*Apanteles*	*Apanteles* sp. HM394867.1
Insecta	Hymenoptera	Braconidae	*Apanteles*	*Apanteles* sp. MH138500.1
Insecta	Hymenoptera	Braconidae	*Aphaereta*	*Aphaereta aotea*
Insecta	Hymenoptera	Braconidae	*Aphidius*	*Aphidius colemani*
Insecta	Hymenoptera	Braconidae	*Asobara*	*Asobara persimilis*
Insecta	Hymenoptera	Braconidae	*Cotesia*	*Cotesia ruficrus*
Insecta	Hymenoptera	Braconidae	*Dolichogenidea*	*Dolichogenidea* sp.
Insecta	Hymenoptera	Braconidae	*Dolichogenidea*	*Dolichogenidea tasmanica*
Insecta	Hymenoptera	Braconidae	*Homolobus*	*Homolobus* sp. MW056205.1
Insecta	Hymenoptera	Braconidae	*Meteorus*	*Meteorus pulchricornis*
Insecta	Hymenoptera	Braconidae	*Microplitis*	*Microplitis* sp. MH138960.1
Insecta	Hymenoptera	Figitidae	*Leptopilina*	*Leptopilina victoriae*
Insecta	Hymenoptera	Formicidae	*Cardiocondyla*	*Cardiocondyla emeryi*
Insecta	Hymenoptera	Formicidae	*Cardiocondyla*	*Cardiocondyla minutior*
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 1
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 2
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 3
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 4
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 5
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 6
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 7
Insecta	Hymenoptera	Formicidae	Formicidae sp.	Formicidae sp. 8
Insecta	Hymenoptera	Formicidae	*Iridomyrmex*	*Iridomyrmex anceps*
Insecta	Hymenoptera	Formicidae	*Linepithema*	*Linepithema humile*
Insecta	Hymenoptera	Formicidae	*Monomorium*	*Monomorium rothsteini*

Insecta	Hymenoptera	Formicidae	*Nylanderia*	*Nylanderia braueri/glabrior/tasmaniensis*
Insecta	Hymenoptera	Formicidae	*Nylanderia*	*Nylanderia* sp.
Insecta	Hymenoptera	Formicidae	*Ochetellus*	*Ochetellus glaber*
Insecta	Hymenoptera	Formicidae	*Paratrechina*	*Paratrechina longicornis*
Insecta	Hymenoptera	Formicidae	*Plagiolepis*	*Plagiolepis alluaudi*
Insecta	Hymenoptera	Formicidae	*Polyrhachis*	*Polyrhachis vigilans*
Insecta	Hymenoptera	Formicidae	*Technomyrmex*	*Technomyrmex jocosus*
Insecta	Hymenoptera	Formicidae	*Tetramorium*	*Tetramorium altivagans*
Insecta	Hymenoptera	Formicidae	*Tetramorium*	*Tetramorium bicarinatum*
Insecta	Hymenoptera	Formicidae	*Tetramorium*	*Tetramorium simillimum*
Insecta	Hymenoptera	Halictidae	Halictidae sp.	Halictidae sp.
Insecta	Hymenoptera	Hymenoptera sp.	Hymenoptera sp.	Hymenoptera sp.
Insecta	Hymenoptera	Ichneumonidae	*Anacis*	*Anacis* sp. KY447103.1
Insecta	Hymenoptera	Ichneumonidae	*Diadromus*	*Diadromus collaris*
Insecta	Hymenoptera	Ichneumonidae	*Diplazon*	*Diplazon laetatorius*
Insecta	Hymenoptera	Ichneumonidae	*Lissopimpla*	*Lissopimpla excelsa*
Insecta	Hymenoptera	Vespidae	*Polistes*	*Polistes chinensis*
Insecta	Hymenoptera	Vespidae	*Ropalidia*	*Ropalidia romandi*
Insecta	Lepidoptera	Choreutidae	*Tebenna*	*Tebenna micalis*
Insecta	Lepidoptera	Coleophoridae	*Coleophora*	*Coleophora crypsineura*
Insecta	Lepidoptera	Cosmopterigidae	*Anatrachyntis*	*Anatrachyntis badia*
Insecta	Lepidoptera	Crambidae	*Achyra*	*Achyra affinitalis*
Insecta	Lepidoptera	Crambidae	*Culladia*	*Culladia cuneiferellus*
Insecta	Lepidoptera	Crambidae	*Hellula*	*Hellula hydralis*
Insecta	Lepidoptera	Crambidae	*Metoeca*	*Metoeca foedalis*
Insecta	Lepidoptera	Crambidae	Crambidae sp.	Crambidae sp.
Insecta	Lepidoptera	Crambidae	Crambidae sp.	Crambidae sp.
Insecta	Lepidoptera	Crambidae	*Nomophila*	*Nomophila corticalis/noctuella*
Insecta	Lepidoptera	Crambidae	*Pyrausta*	*Pyrausta augustalis*
Insecta	Lepidoptera	Crambidae	*Spoladea*	*Spoladea recurvalis*
Insecta	Lepidoptera	Elachistidae	*Elachista*	*Elachista synethes*
Insecta	Lepidoptera	Gelechiidae	*Anaptilora*	*Anaptilora ephelotis/homoclera*
Insecta	Lepidoptera	Gelechiidae	*Ephysteris*	*Ephysteris subdiminutella*
Insecta	Lepidoptera	Gelechiidae	*Hypatima*	*Hypatima baliodes*
Insecta	Lepidoptera	Gelechiidae	*Phthorimaea*	*Phthorimaea operculella*
Insecta	Lepidoptera	Gelechiidae	*Stegasta*	*Stegasta variana*
Insecta	Lepidoptera	Geometridae	*Chloroclystis*	*Chloroclystis insigillata*
Insecta	Lepidoptera	Geometridae	*Phrissogonus*	*Phrissogonus laticostata*
Insecta	Lepidoptera	Geometridae	*Scopula*	*Scopula rubraria*
Insecta	Lepidoptera	Gracillariidae	*Macarostola*	*Macarostola ida*
Insecta	Lepidoptera	Hesperiidae	*Bolla*	*Bolla atahuallpai*
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 1
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 2
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 3
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 4

Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 5
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 6
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 7
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 8
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 9
Insecta	Lepidoptera	Lepidoptera sp.	Lepidoptera sp.	Lepidoptera sp. 10
Insecta	Lepidoptera	Lycaenidae	Lycaenidae sp.	Lycaenidae sp.
Insecta	Lepidoptera	Lycaenidae	*Zizina*	*Zizina otis*
Insecta	Lepidoptera	Noctuidae	*Athetis*	*Athetis tenuis*
Insecta	Lepidoptera	Noctuidae	*Chrysodeixis*	*Chrysodeixis eriosoma*
Insecta	Lepidoptera	Noctuidae	*Condica*	*Condica illecta*
Insecta	Lepidoptera	Noctuidae	*Ctenoplusia*	*Ctenoplusia albostriata*
Insecta	Lepidoptera	Noctuidae	*Ctenoplusia*	*Ctenoplusia limbirena*
Insecta	Lepidoptera	Noctuidae	*Helicoverpa*	*Helicoverpa armigera/assulta*
Insecta	Lepidoptera	Noctuidae	*Leucania*	*Leucania stenographa*
Insecta	Lepidoptera	Noctuidae	*Mocis*	*Mocis proverai*
Insecta	Lepidoptera	Noctuidae	*Mythimna*	*Mythimna convecta*
Insecta	Lepidoptera	Noctuidae	*Mythimna*	*Mythimna separata*
Insecta	Lepidoptera	Noctuidae	*Proteuxoa*	*Proteuxoa cinereicollis*
Insecta	Lepidoptera	Noctuidae	*Spodoptera*	*Spodoptera litura*
Insecta	Lepidoptera	Noctuidae	*Spodoptera*	*Spodoptera mauritia*
Insecta	Lepidoptera	Noctuidae	*Thysanoplusia*	*Thysanoplusia orichalcea*
Insecta	Lepidoptera	Nymphalidae	*Junonia*	*Junonia vestina*
Insecta	Lepidoptera	Nymphalidae	*Junonia*	*Junonia villida*
Insecta	Lepidoptera	Pterophoridae	*Exelastis*	*Exelastis pumilio*
Insecta	Lepidoptera	Pyralidae	*Endotricha*	*Endotricha approximalis/mesenterialis*
Insecta	Lepidoptera	Pyralidae	*Epicrocis*	*Epicrocis metallopa*
Insecta	Lepidoptera	Pyralidae	*Morosaphycita*	*Morosaphycita oculiferella*
Insecta	Lepidoptera	Pyralidae	*Pyralidae* sp.	*Pyralidae* sp.
Insecta	Lepidoptera	Saturniidae	*Archaeoattacus*	*Archaeoattacus edwardsii*
Insecta	Lepidoptera	Tineidae	*Edosa*	*Edosa xystidophora*
Insecta	Lepidoptera	Tineidae	*Monopis*	*Monopis ochroptila*
Insecta	Lepidoptera	Tineidae	*Opogona*	*Opogona omoscopa/sacchari*
Insecta	Lepidoptera	Tortricidae	*Capua*	*Capua dura*
Insecta	Lepidoptera	Tortricidae	*Crocidosema*	*Crocidosema plebejana*
Insecta	Lepidoptera	Tortricidae	*Epiphyas*	*Epiphyas dotatana*
Insecta	Lepidoptera	Tortricidae	*Merophyas*	*Merophyas divulsana*
Insecta	Lepidoptera	Xyloryctidae	*Scieropepla*	*Scieropepla serina*
Insecta	Lepidoptera	Yponomeutidae	*Prays*	*Prays nephelomima*
Insecta	Mantodea	Mantodea sp.	Mantodea sp.	Mantodea sp.
Insecta	Neuroptera	Chrysopidae	*Mallada*	*Mallada basalis*
Insecta	Neuroptera	Hemerobiidae	*Micromus*	*Micromus tasmaniae*
Insecta	Orthoptera	Acrididae	*Ecphantus*	*Ecphantus quadrilobus*
Insecta	Orthoptera	Gryllidae	Gryllidae sp.	Gryllidae sp.
Insecta	Orthoptera	Gryllidae	*Teleogryllus*	*Teleogryllus commodus*
Insecta	Orthoptera	Gryllidae	*Trigonidium*	*Trigonidium sjostedti*

Insecta	Orthoptera	Gryllidae	*Trigonidium*	*Trigonidium* sp. 1
Insecta	Orthoptera	Gryllidae	*Trigonidium*	*Trigonidium* sp. 2
Insecta	Orthoptera	Orthoptera sp.	Orthoptera sp.	Orthoptera sp. 1
Insecta	Orthoptera	Orthoptera sp.	Orthoptera sp.	Orthoptera sp. 2
Insecta	Orthoptera	Orthoptera sp.	Orthoptera sp.	Orthoptera sp. 3
Insecta	Orthoptera	Tettigoniidae	*Euconocephalus*	*Euconocephalus nasutus*
Insecta	Orthoptera	Tettigoniidae	*Euconocephalus*	*Euconocephalus pallidus*
Insecta	Psocoptera	Ectopsocidae	*Ectopsocus*	*Ectopsocus californicus*
Insecta	Psocoptera	Lepidopsocidae	*Echmepteryx*	*Echmepteryx madagascarensis*
Insecta	Psocoptera	Psocopetera sp.	Psocopetera sp.	Psocopetera sp.
Insecta	Termitoidae	Rhinotermitidae	*Coptotermes*	*Coptotermes acinaciformis*
Insecta	Thysanoptera	Thripidae	*Arorathrips*	*Arorathrips mexicanus*
Insecta	Thysanoptera	Thripidae	*Frankliniella*	*Frankliniella occidentalis*
Insecta	Thysanoptera	Thripidae	*Frankliniella*	*Frankliniella schultzei*
Insecta	Thysanoptera	Thripidae	*Hercinothrips*	*Hercinothrips femoralis*
Insecta	Thysanoptera	Thripidae	*Tenothrips*	*Tenothrips frici*
Insecta	Thysanoptera	Thripidae	Thripidae sp.	Thripidae sp.
Insecta	Thysanoptera	Thripidae	*Thrips*	*Thrips* sp.
Insecta	Thysanoptera	Thripidae	*Thrips*	*Thrips tabaci*
Insecta	Thysanoptera	Thripidae	*Thrips*	*Thrips trehernei*
Insecta	Trichoptera	Calamoceratidae	*Phylloicus*	*Phylloicus priapulus*

Table 3: Pest species for which a DNA match was recorded in this survey. The table includes the insect species (with their order, family and common names) and information on previous records. Some of the insects were previously recorded under a different name that has been recently synonymised ("Synonym"). While these names may appear to be new, the insect had already been recorded under a different name.

Order	**Family**	**Species**	**Common name**	**Reported in prev. surveys?**
Blattodea	Blattidae	*Periplaneta americana*	American cockroach	Y
Coleoptera	Coccinellidae	*Henosepilachna vigintioctopunctata*	28-spotted ladybird beetle	Y
Coleoptera	Chrysomelidae	*Chaetocnema* sp.	Flea beetle	Y
Coleoptera	Curculionidae	*Asynonychus cervinus*	Fuller's Rose weevil	Y (synonym)
Coleoptera	Curculionidae	*Cosmopolites sordidus*	Banana root borer	Y
Coleoptera	Scarabeidae	*Heteronychus arator*	African Black Beetle	Y
Hemiptera	Aphididae	*Acyrthosiphon kondoi*	Bluegreen aphid	Y
Hemiptera	Aphididae	*Aphis aurantii*	Black Citrus aphid	Y (synonym)
Hemiptera	Aphididae	*Aphis gossypii*	Cotton aphid	Y
Hemiptera	Aphididae	*Aphis nerii*	Oleander aphid	Y
Hemiptera	Aphididae	*Aulacorthum solani*	Glasshouse potato aphid	Y
Hemiptera	Aphididae	*Brachycaudus helichrysi*	Plum aphid	Y
Hemiptera	Aphididae	*Dysaphis aucupariae*	Plantain aphid (Wild service)	N
Hemiptera	Aphididae	*Hyperomyzus carduellinus*	Asian Sowthistle Aphid	N

Hemiptera	Aphididae	*Hysteroneura setariae*	Rusty Plum Aphid	Y
Hemiptera	Aphididae	*Lipaphis pseudobrassicae*	Turnip aphid	Y
Hemiptera	Aphididae	*Macrosiphoniella euphorbiae*	Potato Aphid	Y
Hemiptera	Aphididae	*Macrosiphoniella sanborni*	Chrysanthemum Aphid	N
Hemiptera	Aphididae	*Melanaphis sacchari*	Yellow sugarcane aphid	N
Hemiptera	Aphididae	*Pentalonia nigronervosa*	Banana aphid	Y
Hemiptera	Aphididae	*Rhopalosiphum padi*	Cherry-Oat aphid	Y
Hemiptera	Aphididae	*Rhopalosiphum maidis*	Corn aphid	Y
Hemiptera	Aphididae	*Rhopalosiphum rufiabdominalis*	Rice root aphid	Y
Hemiptera	Psyllidae	*Heteropsylla cubana*	Leucaena psyllid	N
Hemiptera	Triozidae	*Bactericera cockerelli*	Tomato Potato Psyllid	Y
Hymenoptera	Formicidae	*Linepithema humile*	Argentine ant	Y
Hymenoptera	Vespidae	*Polistes chinensis*	Asian Paper Wasp	Y
Lepidoptera	Crambidae	*Spoladea recurvalis*	Beet webworm moth	Y
Lepidoptera	Erebidae	*Mocis frugalis*	Sugarcane looper	Y
Lepidoptera	Noctuidae	*Spodoptera litura*	Armyworm	Y
Lepidoptera	Noctuidae	*Spodoptera mauritia*	Armyworm	Y
Lepidoptera	Noctuidae	*Chrysodeixis eriosoma*	Green garden looper	Y
Lepidoptera	Noctuidae	*Leucania stenographa*	Sugar Cane Armyworm	Y
Lepidoptera	Tortricidae	*Cryptophlebia ombrodelta*	Macadamia nut borer	Y
Termitoidea	Rhinotermitidae	*Coptotermes acinaciformis*	Termite	N
Thysanoptera	Thripidae	*Thrips tabaci*	Tobacco/Onion thrips	Y
Thysanoptera	Thripidae	*Frankliniella schultzei*	Cotton thrips	Y
Thysanoptera	Thripidae	*Frankliniella occidentalis*	Western Flower Thrips	N
Thysanoptera	Thripidae	*Hercinothrips femoralis*	Greenhouse thrips	Y

Appendix B: Fungal plant pathogens report

Norfolk Island Plant Pest and Disease Survey – Fungal plant pathogens

Background and Introduction

The plant pathogen component of this survey targeted symptomatic plants. This was mostly due to the collection permit that was released to the Agriculture Victoria team, enabling us to collect plant material preserved in solutions for DNA sequencing. Samples were therefore collected when symptoms could be observed on the plants (yellowing of the leaves, death of branches, etc). The surveys were conducted in different seasons (spring and summer) in order to maximise chances of recording a higher level of diversity. This also gave members of the community the opportunity to observe changes and symptoms in the plants and to report these during the second field collection. As a result, most of the samples were collected on private properties, upon request from members of the community.

Methods

A total of 98 samples were collected from 54 plant hosts in the summer survey on Norfolk Island (details in Table 1). Samples were stored in CATB upon collection and were stored under 4℃ until total nucleic acid extraction, which was done using a Qiagen DNeasy Plant Mini Kit. To profile the fungal strains in these samples, the extracted nucleic acid was used as template and the unique molecule identifier (UMI)-tagging primers were used to amplify the full length fungal rRNA genes. Amplicons were sequenced using ONT long-read sequencing platforms and were processed using an in-house pipeline to generated consensus sequences. These sequences were further analysed using BLASTn to assign taxonomy, including an initial assignment based on the NCBI nt database to identify the top five abundant taxa, which were subject to another round of assignment based on the NCBI nt database and fungal WGS database to improve accuracy.

During the spring survey, a total of 22 sites were visited, including 18 farms where several vegetable plants and fruit trees were surveyed (details in Table 1). Two hundred and fifty-nine samples were collected and brought back to CHS in CTAB for processing. These included 59 asymptomatic plant tissues. The rest (200) had typical fungal infection symptoms such as streaking, necrotic lesions, leaf spots, rust, etc. Most of the samples were of leaves with a few fruits, flowers and phloem tissues. These samples were processed for total nucleic acid extraction, UMI-based full-length fungal rRNA genes amplification, and ONT sequencing and data analysis using the methods described for the summer survey.

Figure 1: Dr Saidi Achari collecting fungal pathogen samples in Norfolk Island.

Results

Of the 87 samples analysed, there are 208 different fungal taxa represented across 424 sequences analysed as the 'top five' taxa per sample (see materials and methods, details in Table 2). Eleven sequences are 100% identical in ten different samples representing eight different taxa, and 194 different sequences are >99% identical over >99% of the query length in BLASTn analysis, representing 85 taxa. These taxa included known pathogens and their closely related species (e.g. *Colletotrichum aenigma* for avocado leaf spot and *Podosphaera xanthii* for pumpkin powdery mildew). Novel fungi were also identified. Sixty-five samples had fungal taxa that are known to cause the observed symptoms (highlighted in yellow in Table 2), including 22 taxa that have been reported in the 2012 survey. No exotic fungal pathogen was detected from samples from the first survey. Eleven samples were not analysed due to lack of metadata (three) or unsuccessful amplification (eight).

Of the 177 symptomatic samples analysed, there are 252 different fungal taxa represented across 883 sequences analysed as the 'top five' taxa per sample (details in Table 3). Seven sequences are 100% identical in 7 different samples representing 7 different taxa, and 470 different sequences are >99% identical over >99% of the query length in BLASTn analysis, representing 115 taxa. These taxa included known pathogens and their closely related species (e.g., *Austropuccinia psidii* for myrtle rust and *Exserohilum turcica* for maize leaf blight). Novel fungi were also identified. Ninety-eight samples had fungal taxa that are known to cause the observed symptoms (highlighted in yellow in Table 3), including 11 taxa that have been reported in the 2012 survey. No exotic fungal pathogen was detected from samples from the second survey. Thirty-three samples were not analysed due to unsuccessful amplification.

Discussion

Overall, a total of 527,368 high quality, full-length fungal rRNA gene sequences were generated from fungal samples collected from the summer and spring surveys. These represent taxa including known plant pathogens, species that are closely related to known pathogens, and novel fungal species that haven't been previously sequenced. No exotic fungal species (for Norfolk Island) were recorded.

These sequences represent an invaluable resource for biosecurity, fungal pathogen diagnostics and fungal taxonomy as these full-length fungal rRNA gene sequences have a much greater taxonomic resolution when compared to traditional Internal Transcribed Spacer based methods that are based on partial rRNA sequences. Such higher taxonomic resolutions led to a comprehensive profiling of fungi, including pathogens, that are present on the Norfolk Island. This increased resolution enables us to differentiate closely related species of the same genus (e.g., *Fusarium* spp. and *Colletotrichum* spp.) and to associate the identified species to the recorded disease symptoms. The higher taxonomic resolution results in an increased sensitivity of the method used by this survey, hence more fungal species were identified when compared to that of the last survey. Moreover, as the number of available fungal genomes and gene sequences from public databases (e.g. NCBI) is increasing, the higher taxonomic resolutions provided by the full-length fungal rRNA gene sequences could help us to make informed decisions when novel pathogens are reported, leading to improved preparedness and responsiveness to exotic diseases. For example, if a novel disease-causing fungus is reported and genome sequenced overseas, the genome sequence can be used to query the database of Norfolk Island rRNA sequences to see if it is already present on the Island. This would inform a response to this pathogen.

The associations between fungal species identified by sequences and the recorded disease symptoms still require further assessment for some samples. This includes cases where known pathogens were recorded but didn't match the disease symptoms (e.g. banana black sigatoka), as well as cases where possible disease-causing fungal species were not identified. One possible reason for such results was that the disease development stage was unknown when the samples were collected. It is possible that those samples were collected at the early stage of disease, when the abundance of the pathogen is low, which consequently may not be represented by the top 5 most abundant taxa present on the samples. It is also possible that the fungi identified were colonisers on lesions initially caused by bacteria and/or insects. To improve our understanding of such associations between disease symptoms and fungal species, the fungal strains could be isolated from the diseased tissues for further assays such as whole genome sequencing and in planta infection. This warrants future surveys on Norfolk Island, with permits allowing the return of isolated fungi to quarantine labs, which would allow further investigation of specific plant/fungi/disease symptom systems.

Table 1: Details of the samples collected during the spring and summer surveys.

Date	Sample No	Tube Number	Host Plant (common name)	Symptoms
16-Mar	50	108	Avocado	Leaf spot
16-Mar	51	109	Passion fruit	Leaf spot
16-Mar	42	113	Pohutukawa	Root (diseased plant)
16-Mar	47	114	Inga edulis	Leaf scorch
16-Mar	45	115	Banana	trunk rot (next to soft rot)
	52	118	Apple	Leaf spot
16-Mar	41	121	Pohutukawa	Branch (diseased plant)
16-Mar	46	124	Banana	Trunk rot (fungal hyphae)
16-Mar	49	126	Banana	Black sigatoka
15-Mar	35	127	Capsicum	Leaf spot
15-Mar	38	129	Carrot	Leaf spot
15-Mar	39	130	Prunus	Yellow leaf spot
16-Mar	48	133	Commonball passion fruit	Leaf spot
16-Mar	44	135	Pohutukawa	Thick root (diseased plant)
15-Mar	37	136	Mushroom	Healthy
15-Mar	34	137	Apple	Powdery Mildew
15-Mar	36	139	Pear	Leaf Spot
16-Mar	40	143	Pohutukawa	Leaf spot (secondary infection)
16-Mar	43	146	Pohutukawa	Thin root (diseased plant)
14-Mar	29	201	Carrot	Stem lesion
13-Mar	7	202	Pandanus	Leaf spot
14-Mar	19	205	Mango	Anthracnose
14-Mar	20	206	Lime	Rot around tip of lime
14-Mar	28	207	Potato	Healthy
13-Mar	13	208	Citrus	Stem lesion
13-Mar	9	211	buffalo grass	Leaf spot
14-Mar	26	213	Beet root	No symptoms healthy
14-Mar	21	216	Avocado	Lesion on fruit
13-Mar	15	218	Mushroom (red)	Fruiting body
14-Mar	27	220	Potato	Tuber warts
14-Mar	22	222	Tomato	Leaf spot

14-Mar	25	224	Plant hopper	
13-Mar	17	226	Peach	Bracket fungi on trunk
13-Mar	10	228	Norfolk Island Palm	Leaf spit
13-Mar	8	230		Rust on leaves (only on gully)
13-Mar	16	232	Mandarin	black sooty mould
14-Mar	24	235	Nectarine (White)	Trunk disease
13-Mar	12	237	Fig	Leaf spot
13-Mar	14	238	Tobacco	Powdery Mildew
14-Mar	23	239	Broomstick (weed)	Stem lesion
13-Mar	18	247	Peach (golden queen)	Bracket fungi on trunk
15-Mar	33	248	Potato	Root (healthy)
13-Mar	11	249	Norfolk Island Pine	Tip die back
17-Mar	61	321	Bioluminescent fungi	Mushroom
16-Mar	57	322	Carrot	Leaf spot
16-Mar	54	323	Apple	Leaf spot
16-Mar	55	325	Avocado	Leaf spot
16-Mar	53	335	Passion fruit (Kiwi gold)	Leaf spot
16-Mar	59	336	Tomato	Root lesion
16-Mar	58	338	Zucchini	Crown rot
16-Mar	56	340	Avocado	Small stones in flesh
17-Mar	60	346	Avocado	Pit/stone in flesh
15-Mar	32	403	Frangipani	Rust
15-Mar	30	408	Strawberry	crown
15-Mar	31	417	Rockmelon	Leaf spot
12-Mar	5	424	Paw Paw	Leaf spot (cercospora?)
12-Mar	1	440	Apple	Canker
12-Mar	4	442	Grapevine	Canker
12-Mar	6	444	Paw Paw	Fruit spot
12-Mar	2	446	Banana	Leaf spot (black sigatoka)
12-Mar	3	447	Tangerine	Leaf spot (black)
17-Mar	74	451	Wisteria	Leaf spot
17-Mar	67	452	Strawberry	Leaf spots / petiole
17-Mar	73	453	Peach	Leaf spot

17-Mar	63	454	Potato	Root (Poor growth)
18-Mar	87	456	Capsicum	Leaf spot
17-Mar	70	457	Banana	Root
17-Mar	72	458	Banana	Spots on fruits
17-Mar	66	459	Japanese pumpkin	Powdery Mildew
17-Mar	62	460	Banana	Fusarium? Lesions
18-Mar	75	462	Bell pepper	Leaf spot
17-Mar	69	464	Banana	Skin discoloration
18-Mar	86	465	Paw Paw	Fruit lesion
18-Mar	80	466	Avocado	Spot/rot
17-Mar	64	467	Bean	Vine (mottling)
17-Mar	71	468	Lorn	Leaf spot
18-Mar	89	469	Pear	Pear malformation
18-Mar	85	471	Jacaranda	Dieback (*Philaenus*)
18-Mar	78	472	Monstera	Leaf spot
17-Mar	65	473	Banana	Black sigatoka
18-Mar	77	475	Apple	Diseased
18-Mar	76	479	Pepino	Leaf spot
17-Mar	68	481	Pear	Lesion on fruit
18-Mar	88	484	Fig	Leaf spot
18-Mar	92	485	Zucchini	Collar rot
18-Mar	95	486	Carnation	Powdery Mildew
18-Mar	84	487	Lemon	Leaf spot
18-Mar	83	490	Paspaley	Seed
18-Mar	82	491	Apple	Virus
18-Mar	94	493	Pear	Pear malformation
18-Mar	90	495	Avocado	Skin/leaf rot
18-Mar	93	496	Zucchini	Powdery Mildew
18-Mar	91	497	Lapsilum	Leaf spot / virus
18-Mar	79	498	Avocado	Skin splits
18-Mar	81	500	Apple	Powdery Mildew

Table 2: The fungal taxa on each sample collected during the summer survey as indicated by HTS. The taxon highlighted in yellow is most likely the disease-causing fungi. For each sample, only the top one to five fungal taxa are reported.

Sample ID	Host	Symptom	Final ID	Pesent in NI survey 2012?
230	N/A	Rust on leaves (only on gully)	*Plenodomus* sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
230	N/A	Rust on leaves (only on gully)	Paramyrothecium foliicola	N
118	Apple	Leaf spot	Pestalotiopsis sp.	Y
118	Apple	Leaf spot	Diaporthe nobilis	N
118	Apple	Leaf spot	Cladosporium cladosporioides	Y
118	Apple	Leaf spot	Elsinoe sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
118	Apple	Leaf spot	Phaeosphaeria sp.	Y
137	Apple	Powdery Mildew	Podosphaera cerasi (P. clandestina)	N
137	Apple	Powdery Mildew	Ampelomyces sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
137	Apple	Powdery Mildew	Meira sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
137	Apple	Powdery Mildew	Papiliotrema terrestris	N
137	Apple	Powdery Mildew	Kalmanozyma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
323	Apple	Leaf spot	Cladosporium cladosporioides	Y
323	Apple	Leaf spot	Alternaria arborescens	N
323	Apple	Leaf spot	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
323	Apple	Leaf spot	Pestalotiopsis sp.	Y
440	Apple	Canker	Phaeoacremonium minimum	N
440	Apple	Canker	Fusarium solani	Y
440	Apple	Canker	Plectosphaerella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
440	Apple	Canker	Nigrospora oryzae	N
475	Apple	Diseased	Cladosporium cladosporioides	Y
475	Apple	Diseased	Cercospora zeina	N
475	Apple	Diseased	Phaeosphaeria sp.	Y
475	Apple	Diseased	Cercospora zeina	N
475	Apple	Diseased	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
491	Apple	Virus	Cladosporium cladosporioides	Y
491	Apple	Virus	Quambalaria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
491	Apple	Virus	Diaporthe eres	N

491	Apple	Virus	Toxicocladosporium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
500	Apple	Powdery Mildew	Moesziomyces antarcticus (Pseudozyma antarctica)	N
500	Apple	Powdery Mildew	Podosphaera xanthii	N
500	Apple	Powdery Mildew	Podosphaera cerasi (P. clandestina)	N
500	Apple	Powdery Mildew	Cladosporium cladosporioides	Y
500	Apple	Powdery Mildew	Pseudozyma hubeiensis	N
108	Avocado	Leaf spot	Colletotrichum aenigma	N
108	Avocado	Leaf spot	Talaromyces sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
108	Avocado	Leaf spot	Penicillium brevicompactum	N
108	Avocado	Leaf spot	Aspergillus sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
216	Avocado	Lesion on fruit	Colletotrichum aenigma	N
216	Avocado	Lesion on fruit	Ceraceosorus sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
216	Avocado	Lesion on fruit	Classicula sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
216	Avocado	Lesion on fruit	Neocamarosporium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
216	Avocado	Lesion on fruit	Vishniacozyma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
325	Avocado	Leaf spot	Colletotrichum siamense	Y
325	Avocado	Leaf spot	Talaromyces sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
325	Avocado	Leaf spot	Penicillium brevicompactum	N
325	Avocado	Leaf spot	Aspergillus sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
340	Avocado	Small stones in flesh	Colletotrichum acutatum	N
340	Avocado	Small stones in flesh	Cladosporium cladosporioides	Y
340	Avocado	Small stones in flesh	Vishniacozyma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
340	Avocado	Small stones in flesh	Purpureocillium lilacinum	N
346	Avocado	Pit/stone in flesh	Colletotrichum aenigma	N
346	Avocado	Pit/stone in flesh	Nigrospora oryzae	N
346	Avocado	Pit/stone in flesh	Melanodothis sp.	N
346	Avocado	Pit/stone in flesh	Diaporthe nobilis	N
466	Avocado	Spot/rot	Colletotrichum aenigma	N
466	Avocado	Spot/rot	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N

466	Avocado	Spot/rot	Fibularhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
466	Avocado	Spot/rot	Moesziomyces antarcticus	N
466	Avocado	Spot/rot	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
495	Avocado	Skin/leaf rot	Colletotrichum fructicola	N
495	Avocado	Skin/leaf rot	Jaminaea lanaiensis	N
495	Avocado	Skin/leaf rot	Quambalaria	N
495	Avocado	Skin/leaf rot	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
495	Avocado	Skin/leaf rot	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
498	Avocado	Skin splits	Fusarium solani	Y
498	Avocado	Skin splits	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
498	Avocado	Skin splits	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
498	Avocado	Skin splits	Cladosporium cladosporioides	Y
498	Avocado	Skin splits	Vishniacozyma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
115	Banana	trunk rot (next to soft rot)	Ceratobasidium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
115	Banana	trunk rot (next to soft rot)	Armillaria gallica	N
115	Banana	trunk rot (next to soft rot)	Podosphaera sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
115	Banana	trunk rot (next to soft rot)	Pyrrhoderma noxium	N
124	Banana	trunk rot (fungal hyphae)	Purpureocillium lilacinum (Paecilomyces lilacinus)	N
126	Banana	Black sigatoka	Pyricularia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
126	Banana	Black sigatoka	Phaeosphaeria sp.	Y
126	Banana	Black sigatoka	Microdochium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
446	Banana	Leaf spot (black sigatoka)	Pyricularia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
446	Banana	Leaf spot (black sigatoka)	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
446	Banana	Leaf spot (black sigatoka)	Microdochium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y

446	Banana	Leaf spot (black sigatoka)	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
457	Banana	Root	Podosphaera sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
457	Banana	Root	Acremonium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
457	Banana	Root	Rhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
457	Banana	Root	Rhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
458	Banana	Spots on fruits	Sclerotiophoma versabilis	N
458	Banana	Spots on fruits	Didymella glomerata	N
458	Banana	Spots on fruits	Colletotrichum siamense	Y
458	Banana	Spots on fruits	Cladosporium cladosporioides	Y
458	Banana	Spots on fruits	Farysia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
460	Banana	Fusarium? Lesions	Fusarium sacchari	N
460	Banana	Fusarium? Lesions	Purpureocillium lilacinum	N
460	Banana	Fusarium? Lesions	Digitaria exilis	N
460	Banana	Fusarium? Lesions	Clonostachys sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
460	Banana	Fusarium? Lesions	Nigrospora sphaerica	Y
464	Banana	Skin discoloration	Farysia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
464	Banana	Skin discoloration	Phaeophleospora eucalypticola	N
464	Banana	Skin discoloration	Taphrina sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
464	Banana	Skin discoloration	Nigrospora oryzae	N
473	Banana	Black sigatoka	Pyricularia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
473	Banana	Black sigatoka	Phaeosphaeria sp.	Y
473	Banana	Black sigatoka	Microdochium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
473	Banana	Black sigatoka	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
467	Bean	vine (mottling)	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
467	Bean	vine (mottling)	Plectosphaerella plurivora	N
467	Bean	vine (mottling)	Diaporthe ampelina	N
467	Bean	vine (mottling)	Parafenestella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
467	Bean	vine (mottling)	Nigrospora oryzae	N

213	Beet root	No symptoms healthy	Papiliotrema terrestris	N
213	Beet root	No symptoms healthy	Moesziomyces antarcticus	N
213	Beet root	No symptoms healthy	Curvularia kusanoi	N
213	Beet root	No symptoms healthy	Cryomyces sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
213	Beet root	No symptoms healthy	Rhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
462	Bell pepper	Leaf spot	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
462	Bell pepper	Leaf spot	Taphrina	N
462	Bell pepper	Leaf spot	Stagonosporopsis chrysanthemi	N
462	Bell pepper	Leaf spot	Colletotrichum nicotianae	N
321	Bioluminescent fungi	Mushroom	Mycena chlorophos	N
321	Bioluminescent fungi	Mushroom	Scytalidium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
239	Broomstick (weed)	Stem lesion	Elsinoe sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
239	Broomstick (weed)	Stem lesion	Ganoderma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
239	Broomstick (weed)	Stem lesion	Papiliotrema sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
239	Broomstick (weed)	Stem lesion	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
239	Broomstick (weed)	Stem lesion	Stagonosporopsis chrysanthemi	N
211	buffalo grass	Leaf spot	Pyricularia oryzae (Magnaporthe grisea)	N
211	buffalo grass	Leaf spot	Clonostachys sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
211	buffalo grass	Leaf spot	Phaeosphaeria sp.	Y
211	buffalo grass	Leaf spot	Ramichloridium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
211	buffalo grass	Leaf spot	Parafenestella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
127	Capsicum	Leaf spot	Dothistroma septosporum	N
127	Capsicum	Leaf spot	Stagonosporopsis chrysanthemi	N
127	Capsicum	Leaf spot	Meira sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
127	Capsicum	Leaf spot	Erysiphe necator	N
127	Capsicum	Leaf spot	Cladosporium cladosporioides	Y

456	Capsicum	Leaf spot	Parafenestella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
456	Capsicum	Leaf spot	Cladosporium cladosporioides	Y
456	Capsicum	Leaf spot	Albifimbria verrucaria	N
456	Capsicum	Leaf spot	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
456	Capsicum	Leaf spot	Nigrospora oryzae	N
486	Carnation	Powdery Mildew	Golovinomyces sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
486	Carnation	Powdery Mildew	Kalmanozyma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
486	Carnation	Powdery Mildew	Moesziomyces antarcticus	N
486	Carnation	Powdery Mildew	Cladosporium cladosporioides	Y
486	Carnation	Powdery Mildew	Papiliotrema sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
129	Carrot	Leaf spot	Alternaria alternata	Y
129	Carrot	Leaf spot	Paracamarosporium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
129	Carrot	Leaf spot	Podosphaera sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
129	Carrot	Leaf spot	Cladosporium cladosporioides	Y
129	Carrot	Leaf spot	Cercospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
201	Carrot	Stem lesion	Ganoderma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
201	Carrot	Stem lesion	Cercospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
201	Carrot	Stem lesion	Purpureocillium lilacinum	N
201	Carrot	Stem lesion	Parastagonospora nodorum (species ID to be confirmed, 95%< BLAST identity <99%)	N
201	Carrot	Stem lesion	Epicoccum sorghinum	N
322	Carrot	Leaf spot	Stemphylium lycopersici	N
322	Carrot	Leaf spot	Cercospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
322	Carrot	Leaf spot	Plectosphaerella cucumerina	N
322	Carrot	Leaf spot	Podosphaera sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
322	Carrot	Leaf spot	Cladosporium cladosporioides	Y
208	Citrus	Stem lesion	Drechslerella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N

208	Citrus	Stem lesion	Cyphellophora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
208	Citrus	Stem lesion	Colletotrichum trifolii	N
208	Citrus	Stem lesion	Toxicocladosporium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
133	Commonball passion fruit	Leaf spot	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
133	Commonball passion fruit	Leaf spot	Jaminaea lanaiensis	N
133	Commonball passion fruit	Leaf spot	Cercospora zeina	N
468	Corn	Leaf spot	Exserohilum turcica Et28A (Setosphaeria turcica)	N
468	Corn	Leaf spot	Didymella glomerata	N
468	Corn	Leaf spot	Moesziomyces antarcticus	N
468	Corn	Leaf spot	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
468	Corn	Leaf spot	Aureobasidium melanogenum	N
237	Fig	Leaf spot	Classicula	N
237	Fig	Leaf spot	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
237	Fig	Leaf spot	Podosphaera cerasi	N
237	Fig	Leaf spot	Didymella glomerata	N
484	Fig	Leaf spot	Colletotrichum fructicola	N
484	Fig	Leaf spot	Uredo sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
484	Fig	Leaf spot	Aureobasidium melanogenum	N
484	Fig	Leaf spot	Diaporthe longicolla (Phomopsis longicolla)	N
403	Frangipani	Rust	Coleosporium asterum	N
403	Frangipani	Rust	Rhodofomes sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
403	Frangipani	Rust	Acaromyces ingoldii	N
403	Frangipani	Rust	Colletotrichum aenigma	N
403	Frangipani	Rust	Cladosporium colombiae	N
442	Grapevine	Canker	Tetrapyrgos sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
114	Inga edulis	Leaf scorch	Diaporthe eres	N
114	Inga edulis	Leaf scorch	Fibularhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
114	Inga edulis	Leaf scorch	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
114	Inga edulis	Leaf scorch	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N

471	Jacaranda	Dieback (Philaenus)	Diaporthe ampelina	N
471	Jacaranda	Dieback (Philaenus)	Stagonosporopsis chrysanthemi	N
459	Japanese pumpkin	Powdery Mildew	Podosphaera xanthii (Podosphaera fusca)	Y
459	Japanese pumpkin	Powdery Mildew	Papiliotrema sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
459	Japanese pumpkin	Powdery Mildew	Phaeosphaeria sp.	Y
459	Japanese pumpkin	Powdery Mildew	Phaeosphaeria sp.	Y
459	Japanese pumpkin	Powdery Mildew	Erysiphe necator	N
497	Lapsilum ??	Leaf spot / virus	Cladosporium cladosporioides	Y
497	Lapsilum ??	Leaf spot / virus	Paraphaeosphaeria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
497	Lapsilum ??	Leaf spot / virus	Phaeosphaeria sp.	Y
497	Lapsilum ??	Leaf spot / virus	Microdochium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
497	Lapsilum ??	Leaf spot / virus	Curvularia kusanoi	N
487	Lemon	Leaf spot	Alternaria arborescens	N
487	Lemon	Leaf spot	Colletotrichum siamense	Y
487	Lemon	Leaf spot	Fusarium camptoceras	N
487	Lemon	Leaf spot	Podosphaera aphanis	N
487	Lemon	Leaf spot	Cladosporium cladosporioides	Y
206	Lime	Rot around tip of lime	Fusarium stilboides	N
206	Lime	Rot around tip of lime	Colletotrichum siamense	Y
206	Lime	Rot around tip of lime	Papiliotrema terrestris	N
206	Lime	Rot around tip of lime	Aspergillaceae sp.	N
206	Lime	Rot around tip of lime	Neofusicoccum parvum	Y
232	Mandarin	black sooty mould	Leptoxyphium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
232	Mandarin	black sooty mould	Microcyclospora sp	N
232	Mandarin	black sooty mould	Pseudocercospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
205	Mango	Anthracnose	Melanodothis sp.	N
205	Mango	Anthracnose	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
205	Mango	Anthracnose	Symmetrospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
205	Mango	Anthracnose	Phaeophleospora eucalypticola	N
472	Monstera	Leaf spot	Colletotrichum nicotianae (Colletotrichum tabacum,Colletotrichum gloeosporioides)	Y
472	Monstera	Leaf spot	Ramichloridium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N

472	Monstera	Leaf spot	Zasmidium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
136	Mushroom	Healthy	Agaricus sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
136	Mushroom	Healthy	Fusarium equiseti	Y
136	Mushroom	Healthy	Cladosporium cladosporioides	Y
136	Mushroom	Healthy	Papiliotrema terrestris	N
136	Mushroom	Healthy	Curvularia eragrostidis	N
218	Mushroom (red)	Fruiting body	Coprinopsis cinerea (species ID to be confirmed, 95%< BLAST identity <99%)	N
218	Mushroom (red)	Fruiting body	Clonostachys sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
218	Mushroom (red)	Fruiting body	Peziza sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
235	Nectarine (White)	Trunk disease	Fomitopsis palustris	N
228	Norfolk Island Palm	Leaf spit	Neogloea sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
228	Norfolk Island Palm	Leaf spit	Pseudocercospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
228	Norfolk Island Palm	Leaf spit	Diaporthe ampelina (species ID to be confirmed, 95%< BLAST identity <99%) (synonym Phomopsis viticola)	N
228	Norfolk Island Palm	Leaf spit	Elsinoe sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
228	Norfolk Island Palm	Leaf spit	Dactylella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
202	Pandanus	Leaf spot	Ganoderma sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
202	Pandanus	Leaf spot	Cladosporium cladosporioides	Y
202	Pandanus	Leaf spot	Aureobasidium melanogenum	N
202	Pandanus	Leaf spot	Papiliotrema terrestris	N
202	Pandanus	Leaf spot	Zasmidium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
490	Paspaley	Seed	Claviceps paspali	N
490	Paspaley	Seed	Curvularia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
490	Paspaley	Seed	Cladosporium cladosporioides	Y
490	Paspaley	Seed	Paraphaeosphaeria sp.	N
424	Paw Paw	Leaf spot (cercospora?)	Clonostachys sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N

424	Paw Paw	Leaf spot (cercospora?)	Suillus sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
424	Paw Paw	Leaf spot (cercospora?)	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
424	Paw Paw	Leaf spot (cercospora?)	Dothistroma septosporum	N
424	Paw Paw	Leaf spot (cercospora?)	Colletotrichum aenigma	N
444	Paw Paw	Fruit spot	Dothistroma septosporum	N
444	Paw Paw	Fruit spot	Stagonosporopsis chrysanthemi	N
444	Paw Paw	Fruit spot	Colletotrichum siamense	Y
444	Paw Paw	Fruit spot	Dothistroma septosporum	N
444	Paw Paw	Fruit spot	Fusarium luffae	N
465	Paw Paw	Fruit lesion	Stagonosporopsis chrysanthemi	N
465	Paw Paw	Fruit lesion	Colletotrichum acutatum	N
465	Paw Paw	Fruit lesion	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
465	Paw Paw	Fruit lesion	Cladosporium austrohemisphaericum	N
465	Paw Paw	Fruit lesion	Fusarium tricinctum	N
226	Peach	Bracket fungi on trunk	Ganoderma boninense	N
226	Peach	Bracket fungi on trunk	Ganoderma boninense (species ID to be confirmed, 95%< BLAST identity <99%)	N
226	Peach	Bracket fungi on trunk	Nectriaceae sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
226	Peach	Bracket fungi on trunk	Hortaea werneckii	N
226	Peach	Bracket fungi on trunk	Purpureocillium lilacinum	N
453	Peach	Leaf spot	Tranzschelia discolor	Y
453	Peach	Leaf spot	Cladosporium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
453	Peach	Leaf spot	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
453	Peach	Leaf spot	Fibularhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
453	Peach	Leaf spot	Pseudocercospora crystallina	N
247	Peach (golden queen)	Bracket fungi on trunk	Ganoderma boninense	N
247	Peach (golden queen)	Bracket fungi on trunk	Papiliotrema terrestris	N
247	Peach (golden queen)	Bracket fungi on trunk	Cladosporium cladosporioides	N

247	Peach (golden queen)	Bracket fungi on trunk	Phaeosphaeria sp	N
139	Pear	Leaf Spot	Colletotrichum fioriniae	N
139	Pear	Leaf Spot	Neopestalotiopsis sp.	N
139	Pear	Leaf Spot	Rutstroemia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
139	Pear	Leaf Spot	Neogloea sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
139	Pear	Leaf Spot	Fibularhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
469	Pear	Pear malformation	Pestalotiopsis sp.	Y
469	Pear	Pear malformation	Acremonium sp.	N
469	Pear	Pear malformation	Fusarium oxysporum	Y
469	Pear	Pear malformation	Cladosporium cladosporioides	Y
469	Pear	Pear malformation	Phaeosphaeria sp.	Y
481	Pear	Lesion on fruit	Colletotrichum acutatum	N
481	Pear	Lesion on fruit	Quambalaria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
481	Pear	Lesion on fruit	Cladosporium cladosporioides	Y
481	Pear	Lesion on fruit	Farysia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
493	Pear	Pear malformation	Penicillium expansum	N
493	Pear	Pear malformation	Aureobasidium pullulans	N
493	Pear	Pear malformation	Pichia kluyveri	N
493	Pear	Pear malformation	Cladosporium cladosporioides	Y
493	Pear	Pear malformation	Alternaria alternata	Y
479	Pepino	Leaf spot	Colletotrichum nicotianae (Colletotrichum tabacum,Colletotrichum gloeosporioides)	Y
479	Pepino	Leaf spot	Pseudopyrenochaeta sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
479	Pepino	Leaf spot	Stagonosporopsis chrysanthemi	N
479	Pepino	Leaf spot	Parastagonospora sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
479	Pepino	Leaf spot	Cladosporium cladosporioides	Y
224	Plant hopper	N/A	Cordycipitaceae sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
224	Plant hopper	N/A	Clonostachys rosea	N
224	Plant hopper	N/A	Simplicillium sp.	N
224	Plant hopper	N/A	Lecanicillium sp.	N
113	Pohutukawa	Root (diseased plant)	Pyrrhoderma noxium	N
113	Pohutukawa	Root (diseased plant)	Neofusicoccum ribis	N

135	Pohutukawa	Thick root (diseased plant)	Phanerodontia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
135	Pohutukawa	Thick root (diseased plant)	Pyrrhoderma noxium	N
135	Pohutukawa	Thick root (diseased plant)	Pseudophaeomoniella oleicola	N
207	Potato	Healthy	Rhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
207	Potato	Healthy	Fusarium oxysporum	Y
207	Potato	Healthy	Plectosphaerella plurivora	N
207	Potato	Healthy	Cladosporium cladosporioides	N
207	Potato	Healthy	Stagonosporopsis chrysanthemi	N
220	Potato	Tuber warts	Pseudopyrenochaeta sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
220	Potato	Tuber warts	Phaeosphaeria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
220	Potato	Tuber warts	Helminthosporium solani	N
220	Potato	Tuber warts	Purpureocillium lilacinum	N
248	Potato	Root (healthy)	Tetracladium sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
248	Potato	Root (healthy)	Nectriaceae sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
248	Potato	Root (healthy)	Diaporthe sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
248	Potato	Root (healthy)	Fusarium nematophilum	N
248	Potato	Root (healthy)	Chaetomella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
454	Potato	Root (Poor growth)	Fusarium solani	Y
454	Potato	Root (Poor growth)	Arthrobotrys sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
454	Potato	Root (Poor growth)	Plectosphaerella plurivora	N
454	Potato	Root (Poor growth)	Pyxidiophora arvernensis	N
130	Prunus	Yellow leaf spot	Tranzschelia discolor	N
130	Prunus	Yellow leaf spot	Fusarium stilboides	N
130	Prunus	Yellow leaf spot	Cladosporium cladosporioides	N
130	Prunus	Yellow leaf spot	Colletotrichum nicotianae (Colletotrichum tabacum,Colletotrichum gloeosporioides)	Y
130	Prunus	Yellow leaf spot	Fibularhizoctonia sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
417	Rockmelon	Leaf spot	Paramyrothecium foliicola	N
417	Rockmelon	Leaf spot	Phaeosphaeria sp.	Y
417	Rockmelon	Leaf spot	Sclerotiophoma versabilis	N
417	Rockmelon	Leaf spot	Cladosporium cladosporioides	Y

417	Rockmelon	Leaf spot	Podosphaera xanthii (Podosphaera fusca)	Y
447	Tangerine	Leaf spot (black)	Colletotrichum sp. (species ID to be confirmed, 95%< BLAST identity <99%)	Y
447	Tangerine	Leaf spot (black)	Diaporthe citri	Y
238	Tobacco	Powdery Mildew	Golovinomyces cichoracearum (Erysiphe cichoracearum)	N
238	Tobacco	Powdery Mildew	Erysiphe necator	N
238	Tobacco	Powdery Mildew	Meira sp.	N
238	Tobacco	Powdery Mildew	Moesziomyces antarcticus	N
238	Tobacco	Powdery Mildew	Calonectria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
222	Tomato	Leaf spot	Stemphylium lycopersici	N
222	Tomato	Leaf spot	Plectosphaerella cucumerina (Plectosporium tabacinum)	N
222	Tomato	Leaf spot	Stagonosporopsis chrysanthemi	N
222	Tomato	Leaf spot	Cladosporium cladosporioides	Y
222	Tomato	Leaf spot	Moesziomyces antarcticus	N
336	Tomato	Root lesion	Podosphaera aphanis	N
336	Tomato	Root lesion	Rhizoctonia solani	N
336	Tomato	Root lesion	Fusarium equiseti	N
336	Tomato	Root lesion	Stemphylium lycopersici	N
336	Tomato	Root lesion	Albifimbria verrucaria	N
451	Wisteria	Leaf spot	Colletotrichum aenigma	N
451	Wisteria	Leaf spot	Annulohypoxylon sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
451	Wisteria	Leaf spot	Erysiphe sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
451	Wisteria	Leaf spot	Diaporthe sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
451	Wisteria	Leaf spot	Mycosphaerelloides sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
338	Zucchini	Crown rot	Fusarium equiseti	N
338	Zucchini	Crown rot	Podosphaera aphanis	N
338	Zucchini	Crown rot	Sporobolomyces sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
338	Zucchini	Crown rot	Thelonectria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
338	Zucchini	Crown rot	Stemphylium lycopersici	N
485	Zucchini	Collar rot	Stagonosporopsis chrysanthemi	N
485	Zucchini	Collar rot	Plectosphaerella cucumerina (Plectosporium tabacinum)	N
485	Zucchini	Collar rot	Fusarium equiseti	Y
485	Zucchini	Collar rot	Paramyrothecium foliicola	N

485	Zucchini	Collar rot	Hannaella sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
496	Zucchini	Powdery Mildew	Podosphaera xanthii	N
496	Zucchini	Powdery Mildew	Moesziomyces antarcticus	N
496	Zucchini	Powdery Mildew	Beauveria sp. (species ID to be confirmed, 95%< BLAST identity <99%)	N
496	Zucchini	Powdery Mildew	Cladosporium cladosporioides	Y
496	Zucchini	Powdery Mildew	Stagonosporopsis chrysanthemi	N

Table 3: The fungal taxa on each sample collected during the spring survey as indicated by HTS. The taxon highlighted in yellow is most likely the disease-causing fungi. For each sample, only the top one to five fungal taxa are reported.

Sample ID	Host	Symptom	Final ID	Present in previous survey
1	Myrtaceae	rust	*Austropuccinia psidii*	N
			Farysia itapuensis	N
			Podosphaera xanthii	Y
			Chrysoporthe cubensis	N
			Melanodothis sp.	N
3	Norfolk Island pine	necrosis on the leaflets	*Neofusicoccum ribis*	N
			Pseudopithomyces maydicus	N
			Stemphylium lycopersici	N
			Hortaea werneckii	N
			Umbilicaria sp.	N
5	Yucca spp.	rust	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Dioszegia hungarica	N
			Diaporthe ampelina	N
7	Veronica spp.	downy mildew	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Dioszegia hungarica	N
			Diaporthe ampelina	N
8	Veronica spp.	leaf necrosis	*Stemphylium lycopersici*	N
			Podosphaera xanthii	Y
			Puccinia striiformis	N
			Passalora sequoiae	N
			Golubevia sp.	N
10	Plectranthus spp	leaf spot	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N

			Pseudocercospora crystallina	N
12	Oleaceae	leaf necrosis	*Diaporthe eres*	N
			Pestalotiopsis sp.	Y
			Elsinoe arachidis	N
			Parastagonospora nodorum	N
			Taphrina deformans	Y
14	Pawpaw	black leaf spot underneath the leaf	*Dothistroma septosporum*	N
			Stagonosporopsis sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N
15	Banana	greyish blackish spot on the banana fruit skin	*Cladosporium austrohemisphaericum*	N
			Podosphaera aphanis	N
			Meira miltonrushii	N
			Tremellales sp.	N
			Phaeosphaeria sp.	Y
16	Banana	yellowish blackish streak on the banana leaf	*Microdochium nivale*	N
			Stagonosporopsis sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
17	Banana	black and yellow leaf streak on banana leaf	*Cladosporium cladosporioides*	Y
			Colletotrichum cliviicola	N
			Stagonosporopsis sp. YZ-2020a	N
			Dioszegia hungarica	N
			Mycosphaerelloides madeirae	N
19	English cabbage	yellow/brown v-shaped lesion	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Dioszegia hungarica	N
20	Tomato	greyish leaf spot	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
			Dioszegia hungarica	N
22	Banana	black spots (freckles) on leaf	*Taphrina deformans*	Y
			Microdochium nivale	N
			Phaeophleospora eucalypticola	N
			Musa acuminata var. zebrina	N
			Kalmanozyma brasiliensis	N
23	Pumpkin	yellow spots on leaf	*Colletotrichum fioriniae*	N
			Stagonosporopsis sp. YZ-2020a	N

			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
			Dioszegia hungarica	N
25	Lettuce	spots on leaf	*Cladosporium cladosporioides*	Y
			Colletotrichum cliviicola	N
			Stagonosporopsis sp. YZ-2020a	N
			Diaporthe ampelina	N
			Taphrina deformans	Y
29	Beetroot	greyish leaf spot	*Golubevia* sp.	N
			Quambalaria eucalypti	N
			Cryptococcus sp.	N
			Silene stockenii	N
			Cladosporium sp.	Y
31	Banana	yellow/black streak on leaf	*Colletotrichum aenigma*	N
			Umbilicaria grisea	N
			Phaeosphaeria sp.	Y
			Peltaster fructicola	N
			Microdochium nivale	N
32	Castor plant	rust	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N
			Diaporthe ampelina	N
34	Mango	necrosis of the flower	*Colletotrichum asianum*	Y
			Papiliotrema terrestris	N
			Puccinia striiformis	N
			Talaromyces sp.	N
			Sclerotiophoma versabilis	N
36	Parsely	brownish greyish spots on leaf	*Colletotrichum cliviicola*	N
			Stagonosporopsis sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
			Dioszegia hungarica	N
38	Mulberry	yellowish brownish spots on leaf	*Dioszegia hungarica*	N
			Stagonosporopsis sp. YZ-2020a	N
			Fusarium avenaceum	N
			Colletotrichum aenigma	N
			Dioszegia hungarica	N
40	Mulberry	brownish black spots on leaf	*Neophloeospora maculans*	N
			Sclerotiophoma versabilis	N
			Mrakia psychrophila	N
			Trophis scandens	N
			Phaeophleospora eucalypticola	N
42	Guava	necrosis along the leaf margin	*Tremellales* sp.	N

			Papiliotrema terrestris	N
			Tremellales sp.	N
			Cladosporium cladosporioides	Y
			Stagonosporopsis sp. YZ-2020a	N
44	Citrus	black leaf spot	*Colletotrichum viniferum*	N
			Rhinocladiella similis	N
			Simplicillium aogashimaense	N
			Golubevia sp.	N
			Naematelia aurantialba	N
46	Banana	yellow/black streak on leaf	*Microdochium nivale*	N
			Stagonosporopsis sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Phaeosphaeria sp.	N
			Mycosphaerelloides madeirae	N
47	Psidium spp.	brownish spot on leaf	*Austropuccinia psidii*	N
			Sclerotiophoma versabilis	N
			Neofusicoccum ribis	N
			Microdochium trichocladiopsis	N
			Aureobasidium sp.	Y
49	Pitch Fox	necrosis along the leaf margin	*Papiliotrema laurentii*	N
			Bidens alba var. radiata	N
			Stemphylium lycopersici	N
			Kalmanozyma brasiliensis	N
			Cladosporium sp.	Y
51	Purple flax lilly	reddish-brownish streaks on leaves	*Colletotrichum aenigma*	N
			Chaetomium globosum	N
			Mycosphaerelloides madeirae	N
			Phyllosticta citricarpa	N
			Diplodia mutila	N
53	Tomato	yellow halo around necrotic spots	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium sp.	Y
			Colletotrichum cliviicola	N
			Dioszegia hungarica	N
			Diaporthe ampelina	N
54	Prunus	fruit drop- young fruits were dying on the tree and becoming mummified	*Aureobasidium pullulans*	N
			Prunus dulcis	N
			Melanodothis sp.	N
			Umbilicaria deusta	N
			Symmetrospora coprosmae	N
56	Celery	light brown circular to angulr lesions	*Cladosporium cladosporioides*	Y
			Colletotrichum cliviicola	N
			Stagonosporopsis sp. YZ-2020a	N
			Dioszegia hungarica	N

			Diaporthe ampelina	N
57	Celery	Orangish leaf spot	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Golubevia sp.	N
			Diaporthe ampelina	N
58	Jackfruit	necrosis along the leaf margin	*Colletotrichum fructicola*	N
			Cladosporium cladosporioides	Y
			Stagonosporopsis sp. YZ-2020a	N
			Diaporthe ampelina	N
			Ustilago trichophora	N
60	Mango	greyish blotches/spots on leaf surface	*Xylaria* sp.	N
			Phyllosticta citricarpa	N
			Phaeophleospora eucalypticola	N
			Zasmidium angulare	N
			Colletotrichum asianum	Y
61	Mango	leaf necrotic spots surrounded by yellow halo	*Colletotrichum asianum*	Y
			Phyllosticta citricarpa	N
			Symmetrospora coprosmae	N
			Meira miltonrushii	N
			Mycosphaerelloides madeirae	N
62	Norfolk Island pine	Phloem and leaflet samples taken from unhealthy looking plants. In the same spot there were a lot of dead trees, including Oak.	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Dioszegia hungarica	N
64	Norfolk Island pine	Phloem and leaflet samples taken from unhealthy looking plants. In the same spot there were a lot of dead trees, including Oak.	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Dioszegia hungarica	N
			Diaporthe ampelina	N
65	Norfolk Island pine	Phloem and leaflet samples taken from unhealthy looking plants. In the same spot there were a lot of dead trees, including Oak.	*Zymoseptoria tritici*	N
			Neocatenulostroma microsporum	N

			Scolecobasidium constrictum	N
			Corynespora cassiicola	N
			Colletotrichum siamense	Y
66	Norfolk Island pine	Phloem and leaflet samples taken from unhealthy looking plants. In the same spot there were a lot of dead trees, including Oak.	*Colletotrichum siamense*	Y
			Zymoseptoria tritici	N
			Ophiosphaerella narmari	N
			Stagonosporopsis sp. *YZ-2020a*	N
			Parastagonospora nodorum	N
67	Norfolk Island pine	Phloem and leaflet samples taken from unhealthy looking plants. In the same spot there were a lot of dead trees, including Oak.	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Fusarium equiseti	N
			Diaporthe ampelina	N
69	Passionfruit	yellow and brown necrotic spots	*Colletotrichum gloeosporioides*	Y
			Stagonosporopsis sp. YZ-2020a	N
			Diaporthe ampelina	N
			Cladosporium cladosporioides	Y
			Golubevia sp.	N
71	Pumpkin	yellowish brownish spots on leaf	*Dioszegia hungarica*	N
			Stagonosporopsis sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
72	Parsley	tiny brownish spots on leaf	*Dioszegia hungarica*	N
			Saitozyma podzolica	N
			Sporobolomyces roseus	N
			Filobasidium sp. 1	N
			Filobasidium sp .2	N
74	Corn	greyish brownish blotches on leaf	*Exserohilum turcica* Et28A	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Stagonosporopsis sp. YZ-2020a	N
			Diaporthe ampelina	N
77	Sweet potato	yellow spots on leaf	*Stagonosporopsis* sp. YZ-2020a	N
			Ipomoea trifida	N
			Purpureocillium lilacinum	N
			Taphrina deformans	Y

			Epicoccum sorghinum	N
78	Sweet potato	yellow blotches on leaf	*Stagonosporopsis* sp. YZ-2020a	N
			Ipomoea trifida	N
			Symmetrospora coprosmae	N
			Phaeosphaeria sp.	Y
			Taphrina sp.	N
81	Peas	brownish black spots on leaf	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Didymella pinodes	N
			Ustilago bromivora	N
83	Citrus	yellow spots with necrotic centres	*Elsinoe fawcettii*	N
			Mucor circinelloides	N
			Cladonia uncialis	N
			Taphrina deformans	Y
85	Chocolate Sapote	black raised lessions on leaf	*Dioszegia hungarica*	N
			Colletotrichum aenigma	N
			Elsinoe fawcettii	N
			Knufia petricola	N
			Dioszegia hungarica	N
87	Custard apple	yellow blotches on leaf	*Colletotrichum aenigma*	N
			Phyllosticta citricarpa	N
			Diaporthe nobilis	N
			Cyanodermella asteris	N
			Ramichloridium luteum	N
89	Apple	black spots/lessions on leaf	*Naganishia albida*	N
			Podosphaera xanthii	Y
			Aureobasidium sp.	Y
			Quambalaria eucalypti	N
			Tremellales sp.	N
91	Soursop	yellowish black lessions on leaves	*Colletotrichum gloeosporioides*	Y
			Diaporthe nobilis	N
			Teratoramularia kirschneriana	N
			Phyllosticta citricarpa	N
			Cystobasidium benthicum	N
93	Mulberry	yellow halo around necrotic spots on leaves	*Colletotrichum fioriniae*	N
			Neophloeospora maculans	N
			Dioszegia hungarica	N
			Cladosporium cladosporioides	Y
			Tremellales sp.	N
94	Passionfruit	yellowish brownish spots on leaf	*Colletotrichum gloeosporioides*	Y
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N

			Plectosphaerella plurivora	N
			Sclerotiophoma versabilis	N
95	Grapes	yellowish blotches	*Sporobolomyces roseus*	N
			Podosphaera aphanis	N
			Leucosporidium creatinivorum	N
			Filobasidium floriforme	N
			Podosphaera xanthii	Y
97	Large garlic	orangish lesion in the midrib of the leaf	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
			Quambalaria eucalypti	N
99	Prunus	brownish reddish spots	*Puccinia hordei*	N
			Suhomyces pyralidae	N
			Cladosporium cladosporioides	Y
			Quambalaria eucalypti	N
			Podosphaera xanthii	Y
102	Coffee	yellowing and necrosis of leaf margins	*Colletotrichum tropicale*	N
			Phyllosticta citricarpa	N
			Diaporthe longicolla	N
			Passalora sp. CPC 12319	N
			Pallidocercospora acaciigena	N
104	Avocado	very unhealthy avocado tree	*Caloplaca camptidia*	N
			Mariannaea sp.	N
			Elsinoe fawcettii	N
			Flagelloscypha sp.	N
			Neocatenulostroma microsporum	N
105	Avocado	very unhealthy avocado tree	*Papiliotrema terrestris*	N
			Acaromyces ingoldii	N
			Dioszegia hungarica	N
			Quambalaria eucalypti	N
			Aureobasidium sp.	Y
106	Avocado	very unhealthy avocado tree	*Phyllosticta citricarpa*	N
			Colletotrichum tropicale	N
			Neofusicoccum ribis	N
			Corynespora cassiicola	N
			Diaporthe nobilis	N
107	Avocado	very unhealthy avocado tree	*Colletotrichum siamense*	Y
			Phyllosticta citricarpa	N
			Neofusicoccum ribis	N
			Cryomyces antarcticus	N
			Mycosphaerelloides madeirae	N

108	Avocado	very unhealthy avocado tree	*Colletotrichum tropicale*	N
			Neofusicoccum ribis	N
			Phyllosticta citricarpa	N
			Papiliotrema terrestris	N
			Diaporthe nobilis	N
110	Pawpaw	brownish spots/lesions on the fruit	*Stagonosporopsis chrysanthemi*	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N
			Fusarium citri	N
			Umbilicaria subpolyphylla	N
111	Citrus	black spots on fruit	*Elsinoe fawcettii*	N
			Exophiala lecanii-corni	N
			Elsinoe australis	N
			Phialocephala fluminis	N
			Elsinoe australis	N
112	Citrus	scabby look on the fruit	*Elsinoe fawcettii*	N
			Cladosporium cladosporioides	Y
			Mycosphaerelloides madeirae	N
			Meira miltonrushii	N
			Umbilicaria grisea	N
113	Staghorn	brownish, blackish leaf spots	*Kalmusia* sp.	N
			Cyanodermella asteris	N
			Naematelia aurantialba	N
			Quambalaria eucalypti	N
			Puccinia hordei	N
115	Onion	white sunken leisions on leaves	*Ustilago trichophora*	N
			Stagonosporopsis sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Ampelomyces quisqualis	N
118	Strawberry	dark brown lesions on leaf	*Colletotrichum gloeosporioides*	Y
			Juglanconis oblonga	N
			Pseudocercospora macadamiae	N
			Naganishia albida	N
			Dothistroma septosporum	N
121	Apple	blackish greyish spots on leaf	*Taphrina deformans*	Y
			Dioszegia hungarica	N
			Colletotrichum siamense	Y
			Tremellales sp.	N
			Quambalaria eucalypti	N
123	Chillies	yellowish spots with brown centres on leaf	*Dothistroma septosporum*	N
			Taphrina deformans	Y
			Phaeosphaeria sp.	N

			Dioszegia hungarica	N
			Microdochium nivale	N
122	Apple	yellowing along the leaf margins	*Boeremia exigua*	N
			Papiliotrema terrestris	N
			Amorphotheca resinae	N
			Colletotrichum scovillei	N
			Podosphaera cerasi	N
125	Grape	yellow spots with brown necrotic spots	*Neofusicoccum ribis*	N
			Dioszegia hungarica	N
			Taphrina deformans	Y
			Dioszegia hungarica	N
			Diaporthe nobilis	N
126	Orchid	black sunken lesions on leaf	*Microcyclospora pomicola*	N
			Teratosphaeria destructans	N
			Myrmaecium sp.	N
			Colletotrichum sp.	Y
			Knufia petricola	N
127	Palm fronds	yellowish brownish lesions	*Colletotrichum horii*	N
			Diaporthe caulivora	N
			Mycosphaerelloides madeirae	N
			Cystobasidium benthicum	N
			Phyllosticta citricarpa	N
131	Hibiscus	black lesions on the leaf	*Podosphaera xanthii*	Y
			Scolecobasidium constrictum	N
			Gossypium turneri	N
			Quambalaria eucalypti	N
			Cystobasidium slooffiae	N
133	Eucalyptus	yellowish blotche on leaf	*Phyllosticta citricarpa*	N
			Mycosphaerelloides madeirae	N
			Juglanconis oblonga	N
			Teratosphaeria gauchensis	N
			Pseudocercospora crystallina	N
135	Palm fronds	yellowish brownish lesions	*Diaporthe nobilis*	N
			Colletotrichum siamense	Y
			Neofusicoccum ribis	N
			Stagonosporopsis sp. YZ-2020a	N
			Zasmidium angulare	N
136	Palm fronds	yellowish and dark brown lesions on leaf	*Diaporthe nobilis*	N
			Colletotrichum siamense	Y
			Sclerotiophoma versabilis	N
			Didymella glomerata	N
			Taphrina deformans	Y
137	Lilly plant	rust on the underside of the leaf	*Puccinia coronata*	Y

			Leptosphaeria biglobosa 'thlaspii' group	N
			Taphrina deformans	Y
			Sclerotiophoma versabilis	N
			Ramichloridium luteum	N
139	Zucchini	powdery mildew	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Dioszegia hungarica	N
140	Pumpkin	powdery mildew	*Cladosporium* sp.	Y
			Stagonosporopsis sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Dioszegia hungarica	N
141	Basidiomycete	orangish red fungi on the ground under the trees	*Coprinopsis cinerea*	N
			Fusarium equiseti	Y
			Cladosporium cladosporioides	Y
			Podosphaera aphanis	N
			Clonostachys rosea	N
142	Basidiomycete	greyish blackish fungi on the ground under the tree	*Lepista sordida*	N
			Stagonosporopsis sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Dioszegia hungarica	N
143	Basidiomycete	big greyish ball kind fungi on the ground under the tree	*calvatia gigantea*	N
			Fimicolochytrium jonesii	N
			Penicillium sp.	Y
			Purpureocillium lilacinum	N
			Lepista sordida	N
144	Cucurbit	necrosis on leaf margin	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
			Dioszegia hungarica	N
146	Citrus	yellowish spots with brown centres and scabby looking leaf	*Elsinoe fawcettii*	N
			Stagonosporopsis sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Fusarium stilboides	N
147	Citrus	black blotches under leaf surface	*Colletotrichum gloeosporioides*	Y
			Neofusicoccum ribis	N
			Fusarium stilboides	N

			Zasmidium angulare	N
			Ramularia collo-cygni	N
148	Avocado	blackish growth under leaf surface	*Colletotrichum tropicale*	N
			Diaporthe nobilis	N
			Neofusicoccum ribis	N
			Phyllosticta citricarpa	N
			Mycosphaerelloides madeirae	N
149	Mango	black lesion underneath the leaf	*Phyllosticta citricarpa*	N
			Colletotrichum gloeosporioides	Y
			Passalora sp. CPC 12319	N
			Venturia carpophila	N
			Macrophomina phaseolina	N
150	Myrtaceae	black sooty kind growth underneath leaf surface and bromnish greyish lesions on leaf surface	*Neocatenulostroma microsporum*	N
			Pseudocercospora macadamiae	N
			Schizothyrium pomi	N
			Pseudopyrenochaeta lycopersici	N
			Lasallia pustulata	N
151	Citrus	whitish greyish spots on leaf surface	*Colletotrichum gloeosporioides*	Y
			Knufia petricola	N
			Leptoxyphium fumago	N
			Rhinocladiella similis	N
			Cladonia uncialis	N
152	Citrus	yellowish blotches with brownish scabby growth on leaf	*Knufia petricola*	N
			Colletotrichum gloeosporioides	Y
			Leptoxyphium fumago	N
			Rhinocladiella similis	N
			Cladonia uncialis	N
153	Citrus	yellowish black lesions on leaf surface	*Colletotrichum gloeosporioides*	Y
			Leptoxyphium fumago	N
			Leptoxyphium fumago	N
			Allomyces macrogynus	N
			Cladosporium cladosporioides	Y
154	Citrus	black spots on leaf surface	*Colletotrichum aenigma*	N
			Exophiala lecanii-corni	N
			Mycosphaerelloides madeirae	N
			Diaporthe citri	Y
			Phyllosticta citricarpa	N
155	Passionfruit	necrotic lesion on leaf surface	*Colletotrichum fructicola*	N
			Cladosporium cladosporioides	Y
			Stagonosporopsis sp. YZ-2020a	N

			Dioszegia hungarica	N
			Diaporthe ampelina	N
156	Jackfruit	brownish lesions on leaf surface	*Colletotrichum gloeosporioides*	Y
			Phyllosticta citricarpa	N
			Phialocephala fluminis	N
			Teratosphaeria gauchensis	N
			Rhinocladiella similis	N
157	Eggplant	brown necrotic spots surrounded by yellow halo	*Diaporthe ampelina*	N
			Stagonosporopsis sp. YZ-2020a	N
			Parafenestella ontariensis	N
			Golubevia sp.	N
			Dioszegia hungarica	N
158	Avocado	brownish lesions on leaf surface	*Teratosphaeria gauchensis*	N
			Trophis scandens	N
			Cystobasidium benthicum	N
			Saitozyma podzolica	N
			Phialocephala fluminis	N
159	Palm fronds	brown necrotic spots	*Colletotrichum siamense*	Y
			Diaporthe ampelina	N
			Stagonosporopsis sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Phyllosticta citricarpa	N
160	Carrot	powdery mildew on leaf surface	*Erysiphe pisi*	N
			Quambalaria eucalypti	N
			Papiliotrema laurentii	N
			Golubevia sp.	N
			Dioszegia hungarica	N
161	Carrot	orangish spots on leaf	*Colletotrichum gloeosporioides*	Y
			Erysiphe pisi	N
			Quambalaria eucalypti	N
			Dioszegia hungarica	N
			Microbotryum scabiosae	N
162	Carrot	greyish white growth underneath the leaf	*Erysiphe pisi*	N
			Papiliotrema laurentii	N
			Dioszegia hungarica	N
			Quambalaria eucalypti	N
			Golubevia sp.	N
163	Carrot	prangish brown lesions/spots on leaf surface	*Stagonosporopsis* sp. YZ-2020a	N
			Septoria linicola	N
			Erysiphe pisi	N
			Cladosporium cladosporioides	Y
			Plectosphaerella plurivora	N

164	Avocado	black spots on leaf	*Colletotrichum gloeosporioides*	Y
			Teratosphaeria destructans	N
			Knufia petricola	N
			Acidiella bohemica	N
			Mycosphaerelloides madeirae	N
165	Mango	black spot on leaf	*Phyllosticta citricarpa*	N
			Colletotrichum aenigma	N
			Pseudocercospora crystallina	N
			Cladosporium cladosporioides	Y
			Passalora sp. CPC 12319	N
166	Parsnip	necrotic lesion on leaf surface with yellow halo	*Colletotrichum gloeosporioides*	Y
			Cercospora cf. *flagellaris*	N
			Stagonosporopsis sp.	Y
			Dothistroma septosporum	N
			Dioszegia hungarica	N
168	Beans	necrotic lesions along leaf margin	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Dioszegia hungarica	N
169	Beans	brownish spot on leaf surface	*Dioszegia hungarica*	N
			Phakopsora pachyrhizi	Y
			Stagonosporopsis sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Verticillium klebahnii	N
171	Pineapple	yellowish halo with brown centre on leaf	*Phialocephala fluminis*	N
			Kockovaella imperatae	N
			Exophiala lecanii-corni	N
			Pseudocercospora crystallina	N
			Cladosporium sp.	Y
175	Broad bean	brownish black spots on leaf	*Dioszegia hungarica*	N
			Cladosporium sp.	Y
			Stagonosporopsis sp.	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
176	Broad bean	brown blotches on leaf	*Dioszegia hungarica*	N
			Botrytis cinerea	N
			Papiliotrema terrestris	N
			Stagonosporopsis sp.	Y
			Vicia sativa subsp. *nigra*	N
180	Pawpaw	brownish greyish spots on leaf	*Dothistroma septosporum*	N
			Plectosphaerella cucumerina	N
			Stagonosporopsis sp.	Y

			Phaeophleospora eucalypticola	N
			Dothistroma septosporum	N
181	Chillies	frog eye spots on leaf	*Stagonosporopsis* sp.	N
			Cladosporium sp.	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Dioszegia hungarica	N
183	Macadamia	necrotic lesions along leaf margins and on the surface	*Colletotrichum tropicale*	N
			Diaporthe nobilis	N
			Neofusicoccum ribis	N
			Phyllosticta citricarpa	N
			Passalora sp. CPC 12319	N
184	Grapes	reddish brown lesion on leaf surface (included petiole)	*Diaporthe ampelina*	N
			Papiliotrema terrestris	N
			Dioszegia hungarica	N
			Cladosporium cladosporioides	Y
			Erysiphe necator	N
185	Native hibiscus	necrotic lesion along margin	*Colletotrichum gloeosporioides*	Y
			Diaporthe nobilis	N
			Venturia inaequalis	N
			Cystobasidium slooffiae	N
			Peltaster fructicola	N
186	Rosaceae	necrotic lesion on leaf surface	*Colletotrichum aenigma*	N
			Phyllosticta citricarpa	N
			Tremellales sp.	N
			Neofusicoccum ribis	N
			Passalora sp. CPC 12319	N
188	Jackfruit	orangish brown spots and necrotic lesions on leaf	*Colletotrichum siamense*	Y
			Stagonosporopsis sp. YZ-2020a	N
			Trophis scandens	N
			Teratosphaeria pseudoeucalypti	N
			Cladosporium cladosporioides	Y
189	Galangal	brown necrotic spots surrounded by yellow halo	*Teratosphaeria* sp.	N
			Teratosphaeria destructans	N
			Corynespora cassiicola	N
			Phyllosticta citricarpa	N
			Dioszegia hungarica	N
190	Cassava	necrotic lesions on leaf (large)	*Colletotrichum siamense*	Y
			Stagonosporopsis sp. YZ-2020a	N
			Diaporthe nobilis	N
			Phyllosticta citricarpa	N

			Lecanosticta acicola	N
191	Cassava	brownish lesions on leaf (small)	*Lecanosticta acicola*	N
			Oidium heveae	N
			Stagonosporopsis sp. YZ-2020a	N
			Neopestalotiopsis clavispora	N
			Diaporthe nobilis	N
192	Pessimon	brownish black spot on leaf surface	*Phyllosticta citricarpa*	N
			Colletotrichum gloeosporioides	Y
			Quambalaria eucalypti	N
			Cladosporium cladosporioides	Y
			Saitozyma podzolica	N
193	Meryta latifolia (endemic to NI)	dark brown spot on leaf	*Phyllosticta citricarpa*	N
			Zasmidium angulare	N
			Mycosphaerelloides madeirae	N
			Colletotrichum siamense	Y
			Diaporthe nobilis	N
194	Malaysian apple	black spots and greyish growth on leaf	*Umbilicaria deusta*	N
			Rhinocladiella similis	N
			Umbilicaria deusta	N
			Exophiala dermatitidis	N
			Xenosphaeropsis pyriputrescens	N
195	Apple	darl brown blotches on leaf	*Phaeosphaeria* sp.	Y
			Toxicocladosporium irritans	N
			Diaporthe nobilis	N
			Teratosphaeria gauchensis	N
			Elsinoe fawcettii	N
196	unknown host	leaf covered with black spots on the surface	*Golubevia* sp.	N
			Quambalaria eucalypti	N
			Phialocephala fluminis	N
			Mycosphaerelloides madeirae	N
			Exophiala spinifera	N
197	starfruit	brown spot with whitish center on leaf	*Colletotrichum scovillei*	N
			Phaeophleospora eucalypticola	N
			Macrophomina phaseolina	N
			Taphrina deformans	Y
			Leptoxyphium fumago	N
198	unknown host	necrotic lesions on leaf surface	*Colletotrichum siamense*	Y
			Cladosporium cladosporioides	Y
			Stagonosporopsis sp. YZ-2020a	N
			Fusarium solani	N
			Dioszegia hungarica	N

200	Banana	yellow/black Sigatoka (took leaf stalk sample)	*Mycosphaerelloides madeirae*	N
			Ramichloridium luteum	N
			Pyricularia grisea	N
			Phaeophleospora eucalypticola	N
			Taphrina deformans	Y
202	Passionfruit	brownish lesions on leaf (took leaf and stalk sample)	*Colletotrichum cliviicola*	N
			Septoria linicola	N
			Golubevia sp.	N
			Mycosphaerelloides madeirae	N
			Passiflora cincinnata	N
203	Avocado	big black spots and necrosis on leaf	*Colletotrichum aenigma*	N
			Diaporthe nobilis	N
			Phyllosticta citricarpa	N
			Phaeophleospora eucalypticola	N
			Neofusicoccum ribis	N
204	Avocado	small black spots and necrosis on leaf	*Colletotrichum tropicale*	N
			Diaporthe nobilis	N
			Phaeophleospora eucalypticola	N
			Umbilicaria grisea	N
			Stagonosporopsis sp. YZ-2020a	N
205	Potato	necrosis along leaf margin	*Dioszegia hungarica*	N
			Didymella glomerata	N
			Saitozyma podzolica	N
			Puccinia striiformis	N
			Stagonosporopsis chrysanthemi	N
207	Potato	frog eye spots and necrosis on leaf	*Didymella glomerata*	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N
			Didymella glomerata	N
			Alternaria alternata	Y
208	Avocado	blackish necrotic spot with greenish halo	*Colletotrichum gloeosporioides*	Y
			Diaporthe ampelina	N
			Phyllosticta citricarpa	N
			Neofusicoccum parvum	Y
			Taphrina deformans	Y
209	Grapevine	leaf with necrotic margins (included leaf stalk)	*Diaporthe nobilis*	N
			Cladosporium cladosporioides	Y
			Aureobasidium melanogenum	N
			Quambalaria eucalypti	N
			Hortaea werneckii	N

210	Grapevine	This is a very old vine-leaf with necrotic lesions on the leaf	*Didymella glomerata*	N
			Cladosporium sp.	Y
			Chrysoporthe cubensis	N
			Corynespora cassiicola	N
			Hortaea werneckii	N
211	Pelagonium spp. (ornamental)	yellowish spots on the surface and brownish spots (mostly in circles) at the back	*Puccinia graminis*	N
			Neofusicoccum ribis	N
			Puccinia arachidis	N
			Podosphaera xanthii	Y
				N
213	Pohutukawa	greyish blackish growth on the underneath of the leaf	*Neocatenulostroma* sp. 1	N
			Neocatenulostroma sp. 2	N
			Dioszegia hungarica	N
			Podosphaera xanthii	Y
			Cryomyces antarcticus	N
215	Pohutukawa	3 trees had suddenly died. This was not associated with any rainy period. The shoots are coming out of the dead trunk. Sample was taken by Ross Mann and it was identified as bracket fungi (this is a secondary coloniser and cannot be the causal agent for tree death. I took some more sample from the base of the new shoot and the dead tree. When I scraped the bark, there was slight reddish coloured bark which was not anywhere else on the bark of the shoot) (don't know if this is natural or has some association with the cause)	*Parafenestella ontariensis*	N
			Corynespora cassiicola	N
			Purpureocillium lilacinum	N
			Nectria cinnabarina	N
			Atractiella rhizophila	N
216	Pohutukawa	3 trees had suddenly died. This was not associated with any rainy period. The shoots are coming out of the dead trunk. Sample was taken by Ross Mann and it was identified as	*Suillus alpinus*	N

		bracket fungi (this is a secondary coloniser and cannot be the causal agent for tree death. I took some more sample from the base of the new shoot and the dead tree. When I scraped the bark, there was slight reddish coloured bark which was not anywhere else on the bark of the shoot) (don't know if this is natural or has some association with the cause)		
			Puccinia graminis	N
			Puccinia sp.	Y
			Nectria cinnabarina	N
			Tinctoporellus epimiltinus	N
217	Pohutukawa	3 trees had suddenly died. This was not associated with any rainy period. The shoots are coming out of the dead trunk. Sample was taken by Ross Mann and it was identified as bracket fungi (this is a secondary coloniser and cannot be the causal agent for tree death. I took some more sample from the base of the new shoot and the dead tree. When I scraped the bark, there was slight reddish coloured bark which was not anywhere else on the bark of the shoot) (don't know if this is natural or has some association with the cause)	*Atractiella rhizophila*	N
			Puccinia graminis	N
			Nectria cinnabarina	N
			Pseudocercospora macadamiae	N
			Rhodotorula sphaerocarpa	N
219	Citrus	greyish circular lesions on fruit skin	*Phialocephala fluminis*	N
			Peltaster fructicola	N
			Exophiala lecanii-corni	N
			Dioszegia hungarica	N
			Puccinia triticina	N
220	Citrus	blackish spots all over the fruit skin	*Quambalaria eucalypti*	N
			Peltaster fructicola	N
			Fonsecaea erecta	N
			Phialocephala fluminis	N

			Teratosphaeria gauchensis	N
222	Sugarcane	reddish blotches and black spots on the covering of the peduncle	*Microdochium trichocladiopsis*	N
			Epicoccum sorghinum	N
			Phaeosphaeria sp.	Y
			Didymella glomerata	N
			Elsinoe ampelina	N
225	Squash	necrotic lesion on leaf	*Stagonosporopsis* sp.	N
			Cladosporium sp.	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Parastagonospora nodorum	N
227	Corn	whitish greyish on lecrotic lesion on leaf surface	*Pyricularia oryzae*	N
			Didymella glomerata	N
			Ustilago tritici	N
			Neocatenulostroma microsporum	N
				N
229	Mango	black spots on the underneath of the leaf	*Papiliotrema terrestris*	N
			Dioszegia hungarica	N
			Colletotrichum asianum	Y
			Cladosporium cladosporioides	Y
			Aureobasidium sp.	Y
230	Prunus	yellow spots on the leaf surface with brown spots underneath with necrotic lesion	*Quambalaria eucalypti*	N
			Prunus dulcis	N
			Taphrina deformans	Y
			Puccinia hordei	N
			Podosphaera xanthii	Y
231	Sugarcane	yellowish brown spots on leaf surface	*Stagonosporopsis* sp. YZ-2020a	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Dioszegia hungarica	N
			Diaporthe ampelina	N
232	Sugarcane	purplish blotch in the midvein with brown and yellow spots on the leaf surface	*Stagonosporopsis* sp. YZ-2020a	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Diaporthe ampelina	N
			Kalmanozyma brasiliensis	N
233	Kentia Palm seedling	greyish brown lesion on the seedling (stem)	*Talaromyces marneffei*	N
			Exophiala lecanii-corni	N

			Trichoderma simmonsii	N
			Spencermartinsiella europaea	N
			Thielavia terrestris	N
234	Kentia Palm seedling	brownish lesions with slight yellow halo on the leaf and necrotic leaf tip	*Talaromyces trachyspermus*	N
			Colletotrichum cliviicola	N
			Cladosporium sp.	Y
			Stagonosporopsis sp.	Y
			Exophiala lecanii-corni	N
235	Banana	black spots on fruit with clear halo	*Cladosporium cladosporioides*	Y
			Tremellales sp.	N
			Plectosphaerella cucumerina	N
			Hortaea werneckii	N
			Colletotrichum aenigma	N
236	Banana	greyish, whitish, blackish lesion on fruit skin	*Sclerotiophoma versabilis*	N
			Phaeophleospora eucalypticola	N
			Tremellales sp.	N
			Colletotrichum aenigma	N
			Symmetrospora coprosmae	N
237	Mango	brown necrotic spots with yellow halo and necrotic lesions at the tip of the leaf	*Saitozyma podzolica*	N
			Colletotrichum asianum	Y
			Diaporthe nobilis	N
			Phyllosticta citricarpa	N
			Phaeophleospora eucalypticola	N
238	Mango	majority of the flowers were dead	*Colletotrichum asianum*	Y
			Mycosphaerelloides madeirae	N
			Fusarium secorum	N
			Microcyclosporella mali	N
			Phaeosphaeria sp.	Y
239	Prunus	gumming and formation of small tumor on the tree branch- took a twig sample which had gumminess. Not a healthy tree, just had a single branch while part of the tree had rot. Had discoloured xylem vessel.	*Neofusicoccum parvum*	Y
			Pseudevernia furfuracea	N
			Elsinoe batatas	N
			Pseudocercospora crystallina	N
			Exophiala spinifera	N
241	Banana	black/yellow sigatoka symptoms- plant very	*Articulospora proliferata*	N

		unhealthy. Took leaf stalk sample		
			Microdochium nivale	N
			Epicoccum sorghinum	N
			Parastagonospora nodorum	N
			Plectosphaerella plurivora	N
240	Citrus	black sooty mould like growth	*Exophiala lecanii-corni*	N
			Saitozyma podzolica	N
			Leptoxyphium fumago	N
			Neofusicoccum ribis	N
			Diaporthe longicolla	N
242	White sapote	yellowish near leaf margins with brownish spots and necrosis	*Colletotrichum shisoi*	N
			Phialocephala fluminis	N
			Knufia petricola	N
			Mrakia psychrophila	N
			Epicoccum sorghinum	N
243	White sapote	yellowish and brownish lesion on leaf surface	*Phialocephala fluminis*	N
			Cladosporium sp.	Y
			Aesculus assamica	N
			Mrakia psychrophila	N
			Dioszegia hungarica	N
246	Broad bean	necrotic lesions on leaf margins	*Dioszegia hungarica*	N
			Cladosporium cladosporioides	Y
			Sclerotiophoma versabilis	N
			Mrakia psychrophila	N
			*Vicia sativa sub*sp. *nigra*	N
247	Broad bean	flower necrosis- flower stalk sample taken	*Stagonosporopsis* sp. *YZ-2020a*	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N
			Diaporthe ampelina	N
248	Broad bean	blackish greyish sunken lesion on beans	*Stagonosporopsis* sp. *YZ-2020a*	N
			Colletotrichum cliviicola	N
			Cladosporium cladosporioides	Y
			Dioszegia hungarica	N
			Annulohypoxylon stygium	N
249	Lettuce	brown necrotic spots with circular halo	*Entyloma ficariae*	N
			Septoria petroselini	Y
			Mrakia psychrophila	N
			Cryptococcus sp.	Y
			Coniferiporia weirii	N

250B	Passionfruit	necrotic lesion with yellow patches (took leaf stalk sample)	*Cladosporium cladosporioides*	Y
			Moesziomyces antarcticus	N
			Passiflora cincinnata	N
			Tremellales sp.	N
			Plectosphaerella plurivora	N
252	Sugarcane	brown necrotic lesion with yellow halo	*Stagonosporopsis* sp. *YZ-2020a*	N
			Cladosporium cladosporioides	Y
			Colletotrichum cliviicola	N
			Diaporthe ampelina	N
			Sporobolomyces roseus	N
253	Avocado	stunted, unhealthy looking tree. Scarce feeder root. Lumpiness on the roots. Took phloem and root sample	*Paraphaeosphaeria minitans*	N
			Phialophora chinensis	N
			Scytalidium lignicola	N
			Tricharina praecox	N
			Sarocladium brachiariae	N
254	Avocado	stunted, unhealthy looking tree. Scarce feeder root. Lumpiness on the roots. Took phloem and root sample	*Didymella arachidicola*	N
			Leptodontidium sp.	N
			Sarocladium brachiariae	N
			Exophiala lecanii-corni	N
			Macrophomina phaseolina	N
255	Coffee	large necrotic spots with yellow halo on leaves front and back	*Cercospora cf. flagellaris*	N
			Pseudopithomyces maydicus	N
			Papiliotrema terrestris	N
			Zasmidium angulare	N
			Zymoseptoria tritici	N
256	Coffee	orangish brown spots with yellow halo front and back of the leaf	*Phyllosticta citricarpa*	N
			Neofusicoccum parvum	Y
			Cladosporium sp.	Y
			Diaporthe ampelina	N
			Botryosphaeria dothidea	N
257	Broad bean	dark brown small spots on leaf surface	*Dioszegia hungarica*	N
			Stagonosporopsis sp. *YZ-2020a*	N
			Cladosporium sp.	Y
			Colletotrichum cliviicola	N
			Stenocarpella maydis	N

258	Guava	black spots on the underneath of the leaf and brownish leaf colour	*Phyllosticta citricarpa*	N
			Mrakia psychrophila	N
			Lasiodiplodia theobromae	N
			Phialocephala fluminis	N
			Phialophora chinensis	N
259	Passionfruit	greyish brown sunken spots on small fruit	*Cladosporium cladosporioides*	Y
			Septoria linicola	N
			Plectosphaerella plurivora	N
			Dioszegia hungarica	N
			Alternaria solani	Y

Appendix C: Nematology report

Norfolk Island Plant Pest and Disease Survey – Nematodes

Background and Introduction

The only published plant-parasitic nematode survey of Norfolk Island (Khair 1982) listed 39 species of economically important nematodes on Norfolk Island, ten of which were identified to genus level only. The Norfolk Island Quarantine Survey 2012–2014 (Anonymous 2014, Maynard *et al.* 2018) did not survey for nematodes. The CABI Invasive Species Compendium lists only four nematode species, viz., *Globodera rostochiensis*, *Meloidogyne hapla*, *M. incognita* and *Radopholus similis* on Norfolk Island (CABI 2019). The APPD (Australian Plant Pest Database) lists only five plant-parasitic nematode species (8 specimens) from Norfolk Island, viz., *Helicotylenchus dihystera*, *Macroposthonia ornata*, *Scutellonema brachyurus*, *Xiphinema elongatum* and *X. enciculiferum*, all presumably collected from turfgrass at the Norfolk Island golf course in 2002 and 2003.

In this survey, particular attention was directed toward sampling for two of the three groups of nematode species that are included in the list of Australia's 42 National Priority Plant Pests (NPPPs), viz., two species of PCN (*Globodera* spp.) (NPPP No. 19) and six species of cyst nematodes (*Heterodera* spp.) (NPPP No. 23).

The two wood-feeding species of *Bursaphelenchus* (*B. xylophilus* and *B. cocophilus*, NPPP No. 33) were not actively searched. Hosts of *B. xylophilus* (pine wilt nematode, PWN) are all conifers of the families Pinaceae and Cupressaceae. The only conifers present on Norfolk Island are the endemic Norfolk Island pine (*Araucaria heterophylla*, family Araucariaceae) and the naturalised *Cupressus lusitanica*, neither of which are regarded as potential hosts of *B. xylophilus* (e.g., Dwinell and Mota 2001, Cram and Hanson 2006). The lack of suitable hosts, and the fact that species of *Bursaphelenchus* dwell within tree wood and therefore require unique extraction methods (e.g., driving augers into tree trunks) that would not be readily acceptable to tree owners or managers, meant that *B. xylophilus* could be excluded from active sampling. Similarly, *B. cocophilus* (red ring nematode, coconut palm nematode, RRN) was excluded from active sampling. Hosts of RRN are likely to include most members of the family Arecaceae (palms) (Giblin-Davis *et al.* 2013, Kanzaki and Giblin-Davis 2016), of which three species occur on Norfolk Island, viz., the indigenous *Rhopalostylis baueri* and the naturalised *Howea forsteriana* and *Phoenix canariensis*. There is no definitive method for detection of RRN during visual inspection of suspect trees. For conclusive evidence, palm tree tissue must be collected and brought to a laboratory for extraction of nematodes, a process requiring either tree destruction or the taking of deep trunk core samples. Additionally, RRN requires the presence of beetle vectors, primarily the South American palm weevil (*Rhynchophorus palmarum*), very large insects up to 5 cm in length, that could not readily evade notice if present on Norfolk Island and which was not found in this survey.

The CABI Invasive Species Compendium datasheet (CABI 2019) and the EPPO Global Database (EPPO 2022) for *Globodera rostochiensis* (potato cyst nematode, PCN) list this species as present on Norfolk Island. Both sources give as reference Marshall (1998) and state that they reflect the 'current pest situation evaluated by EPPO on the basis of information dated 1998', but that reference makes no comment about Norfolk Island. On the other hand, a different chapter in the same book, by Turner and Evans (1998), does list PCN from Norfolk Island but confusingly gives EPPO as the information source.

Only one species of cyst nematode, *Heterodera trifolii* (clover cyst nematode), has been recorded from Norfolk Island (Khair 1982) prior to this survey, and this species is not listed as an Australian NPPP. There appears to be no cereal grown on island, so there is little opportunity to search for some of the cyst nematode species that represent the species that is number 23 on the NPPP list (i.e., cereal cyst nematodes of the genus *Heterodera*), although others have a relatively broad host range.

Figure 1: Dr John Wainer collecting soil samples on Norfolk Island.

Methods

Soil sampling and processing

Soil samples were collected during the periods of 14–19 March and 10–23 October 2022. The seasonal separation between field trips allowed for sampling of different annual crops. In the summer survey, 83 nematode soil samples were collected from 50 different species of host plants from 19 properties. A further 102 soil samples were collected in the spring survey, from 59 different host plant species and 22 properties. The total number of soil samples collected from the two field trips was 185, the total number of host plant species sampled was 81, and the total number of properties sampled was 31.

Soil samples of approximately 500 g were collected from the root zone of potentially infected host plants to a depth of about 15 cm. Living nematodes were extracted from soil using the Whitehead tray method (Whitehead and Hemming 1965, Hooper 1986, Stirling *et al.* 2002). Additionally, Fenwick cans were employed to extract any PCN cysts from soil samples (Fenwick 1940, EPPO 2013).

Nematode identification

Plant-parasitic nematodes were microscopically examined and morphologically identified to genus and occasionally to species level with the use of a broad range of pertinent literature that included keys and descriptions (e.g., Decraemer 1995, Hunt 1993, Index to CIH Descriptions of Plant-Parasitic Nematodes 1972–1985, Mai and Mullin 1996, Siddiqi 2000).

A few specimens of each taxon of plant-parasitic nematodes from each soil sample were selected for molecular species determinations.

DNA extraction

DNA was extracted using the DNeasy® Blood and Tissue Kit (Qiagen®) following the manufacturer's instructions with a few modifications. The tissue lysate was incubated for 3 hours at 56 ℃ for 800 rpms in a thermomixer. The isolated DNA was stored at -20℃ till further use.

Extracted DNA was checked for amplifiable DNA using polymerase chain reaction (PCR). CO1 gene sequence was targeted for amplification. A master mix was prepared by mixing the ingredients as mentioned in Table 1. The master mix was pipetted out in a 96-well PCR plate for the desired samples and 5 µl of DNA template was added into each well.

The plate was spun down before loading into the PCR machine. PCR conditions were programmed for CO1 (Table 2) region, and the total reaction volume was stated as 25 µl. A portion of the CO1 gene was amplified with the following primers: forward primer (JB3) 5’-TTTTTTGGGCATCCTGAGGTTTAT-3' and reverse primer (JB5) 5’-AGCACCTAAACTTAAAACATAATGAAAATG-3’ (Bowles *et al.* 1992; Derycke *et al.* 2005).

Table 1. Master mix for one PCR reaction.

Ingredients	Quantity (µl)
Water (ddH_2O)	5.5
One*Taq*® DNA Polymerase	12.5
Forward Primer 10 µM	1
Reverse Primer 10 µM	1
DNA	5

Table 2. PCR cycle programme for *Heterodera* spp. CO1 region detection.

Cycles	Temperature ℃	Duration
x1	94	4 mins
x35	94	1 min
	55	1.5 mins
	72	2 mins
x1	72	10 mins

Gel run and photograph

A 0.8 % agarose gel solution (0.8 g agarose in 100 ml of 0.5 TBE buffer) was prepared by heating in a microwave until agarose was melted and cooled down to about 70℃. 2 µl of SYBR™ Safe was added to the melted agarose. The solution was gently swirled and poured into a gel casting tray containing two combs and was allowed to set (1 hour). 7 µl of the PCR product was pipetted into the wells along with 6 µl of the DNA ladder. The PCR product was run on the agarose gel for 50 minutes at 100V. The gel was visualised and photographed under UV light.

Sanger sequencing and molecular identification

PCR products were sent for purification and sequencing at Macrogen (Seoul, Rep. of Korea). All resulting sequences were trimmed, aligned, and analysed using Geneious Prime® 2023.0.4 (www.geneious.com). Sequence identity was checked using NCBI BLAST.

Results

Comparison of Norfolk Island plant-parasitic nematode identifications using traditional microscope morphology examination and molecular (sanger sequencing) techniques is presented in Table 3.

Table 3. Comparison of Norfolk Island plant-parasitic nematode identifications using traditional microscope morphology examination and molecular (sanger sequencing) techniques.

Sample No.	Morphological identity determination	BLAST match	% Identity	Query cover %	Accession	Host plant soil sample
72	*Criconema* sp.	*Criconema mutabile*	100	90	MN710709.1	*Cynodon dactylon* (Bermuda grass)

Sample No.	Morphological identity determination	BLAST match	% Identity	Query cover %	Accession	Host plant soil sample
72	*Helicotylenchus* sp.	*Helicotylenchus pseudorobustus*	84.18	86	MG663105.1	*Cynodon dactylon* (Bermuda grass)
179	*Helicotylenchus* sp.	No result in database				*Trifolium repens* (white clover)
166	*Heterodera* sp.	*Heterodera trifolii*	99.53	100	MZ882166.1	*Phaseolus vulgaris* (string beans)
189	*Heterodera* sp.	*Heterodera trifolii*	99.51	100	MK621904.1	*Trifolium repens* (white clover)
178	*Heterodera* sp.	*Heterodera trifolii*	99.49	85	MW345296.1	*Trifolium repens* (white clover)
110	*Meloidogyne* sp.	*Meloidogyne hapla*	99.53	100	KM887149.1	*Daucus carota* (carrot)
112	*Meloidogyne* sp.	*Meloidogyne hapla*	100	100	JX683718.1	*Daucus carota* (carrot)
112	*Meloidogyne* sp.	*Meloidogyne hapla*	97.39	100	JX683718.2	*Daucus carota* (carrot)
189	*Meloidogyne* sp.	*Meloidogyne hapla*	99.53	100	KM887149.1	*Trifolium repens* (white clover)
189	*Meloidogyne* sp.	*Meloidogyne hapla*	85.58	78	MH128578.1	*Trifolium repens* (white clover)
185	*Meloidogyne* sp.	*Meloidogyne hapla*	99.32	99	KM887149.1	*Zea mays* (corn)
157	*Meloidogyne* sp.	*Meloidogyne incognita*	100	100	MH743221.1	*Capsicum annuum* (chilli)
178	*Meloidogyne* sp.	*Meloidogyne incognita*	99.76	100	MH743221.1	*Trifolium repens* (white clover)
111	*Paratylenchus* sp.	No result in database				*Phaseolus vulgaris* (string beans)
157	*Rotylenchulus* sp.	*Rotylenchulus reniformis*	99.49	89	KT003727.1	*Capsicum annuum* (chilli)
127	*Rotylenchulus* sp.	*Rotylenchulus reniformis*	99.24	89	KT003727.1	*Daucus carota* (carrot)
131	*Rotylenchulus* sp.	*Rotylenchulus reniformis*	99.28	100	OM985019.1	*Pisum sativum* (sweet pea)
181	*Rotylenchulus* sp.	*Rotylenchulus reniformis*	99.04	100	OM985019.1	*Solanum lycopersicum* (tomato)
185	*Rotylenchulus* sp.	*Rotylenchulus reniformis*	99.49	91	KT003727.1	*Zea mays* (corn)
93	*Scutellonema* sp.	*Scutellonema* sp.	95.7	88	JX472099.1	*Daucus carota* (carrot)
189	*Xiphinema* sp.	*Ophidascaris filaria*	85.41	95	OM867304.1	*Trifolium repens* (white clover)

All 14 potato (*Solanum tuberosum*) soil samples, from eleven properties across Norfolk Island, were found to be free of potato cyst nematode (*Globodera* sp.) cysts and larvae. Seven species of plant-parasitic nematodes detected in these 14 potato soil samples are listed in Table 4.

Table 4. Nematodes detected in Norfolk Island potato (*Solanum tuberosum*) soil samples.

Sample No.	Nematode species
10	no nematodes present
27	no nematodes present
56	no nematodes present
89	*Criconema mutabile*
	Helicotylenchus sp.
	Meloidogyne sp.
	Rotylenchulus reniformis
	Tylenchulus semipenetrans
106	*Paratylenchus* sp.
108	*Rotylenchulus reniformis*
115	*Criconema mutabile*
	Rotylenchulus reniformis
124	*Scutellonema* sp.
137	*Helicotylenchus* sp.
152	no nematodes present
174	no nematodes present
183	*Helicotylenchus* sp.
	Meloidogyne sp.
	Rotylenchulus reniformis
184	*Helicotylenchus* sp.
	Meloidogyne sp.
	Rotylenchulus reniformis
187	*Criconema mutabile*
	Meloidogyne sp.
	Rotylenchulus reniformis

Six species of plant-parasitic nematode species, and their incidence per standard quantity of soil (200 cc), detected in five turf (Bermuda grass, *Cynodon dactylon*) soil samples from greens at Norfolk Island's golf course and lawn bowling club are listed in Table 5.

Table 5. Nematodes detected in turf (*Cynodon dactylon*) soil at the Norfolk Island golf course and Norfolk Island lawn bowling club.

Sample number	Nematode species	No. of nematodes per 200 cc of soil
Sample no. 72 (golf course)	*Criconema mutabile*	9
	Helicotylenchus pseudorobustus	111
	Meloidogyne sp.	153
	Xiphinema (2 spp.)	204
Sample no. 75 (golf course)	*Criconema mutabile*	207

Sample number	Nematode species	No. of nematodes per 200 cc of soil
	Helicotylenchus pseudorobustus	198
	Meloidogyne sp.	45
	Xiphinema sp.	45
Sample no. 74 (golf course)	*Criconema mutabile*	36
	Helicotylenchus pseudorobustus	18
	Meloidogyne sp.	54
	Pratylenchus sp.	18
	Xiphinema sp.	126
Sample no. 73 (bowling club)	*Pratylenchus* sp.	18
Sample no. 76 (bowling club)	*Pratylenchus* sp.	372

Plant-parasitic nematodes detected in some potential *Heterodera* host plant soil samples are listed in Table 6.

Table 6. Nematodes detected in Norfolk Island soil samples from potential host plants of NPPP *Heterodera* species.

Sample No.	Host plant	Nematode species
157	chilli (*Capsicum annuum*)	*Helicotylenchus pseudorobustus*
		Meloidogyne incognita
		Rotylenchulus reniformis
104	pumpkin (*Cucurbita pepo*)	*Meloidogyne* sp.
162	pumpkin (*Cucurbita pepo*)	*Xiphinema* sp.
159	zucchini (*Cucurbita pepo*)	*Xiphinema* sp.
93	carrot (*Daucus carota*)	*Scutellonema* sp.
110	carrot (*Daucus carota*)	*Helicotylenchus pseudorobustus*
		Meloidogyne hapla
112	carrot (*Daucus carota*)	*Meloidogyne hapla*
		Rotylenchulus reniformis
127	carrot (*Daucus carota*)	*Rotylenchulus reniformis*
161	carrot (*Daucus carota*)	no nematodes present
111	string beans (*Phaseolus vulgaris*)	*Paratylenchus* sp.
166	string beans (*Phaseolus vulgaris*)	*Helicotylenchus pseudorobustus*
		Heterodera trifolii
		Pratylenchus sp.
131	sweet pea (*Pisum sativum*)	*Rotylenchulus reniformis*
160	tomato (*Solanum lycopersicum*)	*Criconema mutabile*
		Xiphinema sp.
181	tomato (*Solanum lycopersicum*)	*Paratrichodorus* sp.
		Pratylenchus sp.

Sample No.	Host plant	Nematode species
		Rotylenchulus reniformis
178	white clover (*Trifolium repens*)	*Helicotylenchus pseudorobustus*
		Heterodera trifolii
		Meloidogyne incognita
179	white clover (*Trifolium repens*)	*Helicotylenchus pseudorobustus*
189	white clover (*Trifolium repens*)	*Helicotylenchus pseudorobustus*
		Heterodera trifolii
		Meloidogyne hapla
		Xiphinema sp.
194	white clover (*Trifolium repens*)	*Helicotylenchus* sp.
		Heterodera trifolii
		Meloidogyne sp.
128	corn (*Zea mays*)	no nematodes present
155	corn (*Zea mays*)	*Criconema mutabile*
		Helicotylenchus pseudorobustus
		Meloidogyne sp.
		Pratylenchus sp.
		Rotylenchulus reniformis
185	corn (*Zea mays*)	*Helicotylenchus pseudorobustus*
		Meloidogyne hapla
		Rotylenchulus reniformis

Summary and Discussion

- This survey recorded 13 species of plant-parasitic nematodes in Norfolk Island soils. No nematode pests on the list of Australian National Priority Plant Pests (NPPP) were detected.

- Three species of plant-parasitic nematodes detected in the survey are new records for Norfolk Island, viz., *Helicotylenchus pseudorobustus* (spiral nematode), *Paratylenchus* sp. (pin nematode), and *Rotylenchulus reniformis* (reniform nematode). No conspicuous evidence of damage symptoms of host plants was detected.

- Rapid morphological nematode taxonomic diagnosis usually resulted in identification only to genus level, whereas molecular diagnosis was generally able to provide species identification; in most cases generic identification was consistent using both techniques.

- Although a literature record has indicated the occurrence of the introduced potato cyst nematode (PCN, *Globodera rostochiensis*) on Norfolk Island, the present survey of potato soils found no evidence of its presence on island.

- Six species of nematodes were found in golf course and bowling green turfgrass. All three golf course soil samples and one of the two in the bowling green had a high nematode hazard index. This was particularly the case for the bowling green sample, where root lesion nematode (*Pratylenchus* sp.) was detected in relatively high numbers. Nevertheless, the turfgrass appeared in relatively good condition, with no obvious nematode-caused symptoms of dieback, thinning, wilting, or discolouring, suggesting that its good maintenance may be adequate to sustain high quality greens.

- No NPPP cyst nematodes (*Heterodera* spp.) were detected during the survey. Only one species of cyst nematode, *Heterodera trifolii* (clover cyst nematode), was recorded, and this species is not listed as an Australian NPPP.

- No conspicuous root galling of vegetables was observed, even though two species of root knot nematodes (*Meloidogyne*) were frequently detected. This suggests that the situation may have improved over the past 40 years since the first and only previous nematode survey on Norfolk Island.

Morphological examination of living nematodes using a binocular dissecting microscope is generally adequate to achieve identification to the genus level relatively swiftly and accurately, whereas it is often very difficult to confidently identify specimens to the species level (see Table 3). Species determination usually requires a high-power compound microscopic examination and preparing good quality fixed and preserved specimens for microscope slide mounting can be a labour intensive and time-consuming process. Moreover, in the literature there is a dearth of global taxonomic generic revisions and easily usable species morphology keys of the often very speciose genera of plant-parasitic nematodes. On the other hand, molecular species determination has largely become a relatively swift and reliable procedure. The concordance of morphological generic determinations and molecular species determinations (Table 3) supports confidence that the molecular species determinations are probably almost all reliable, except that for sample number 50, where *Ophidascaris filaria*, an ascaridoid intestinal parasite of pythons in India and Sri Lanka (Sprent 1988), is unequivocally in error.

Although a literature record has indicated the occurrence of the introduced potato cyst nematode (PCN, *Globodera rostochiensis*) on Norfolk Island, the present survey of potato soils found no evidence of its presence on island. This literature record, Turner and Evans (1998), lists PCN from Norfolk Island but gives EPPO as the information source. Confusingly, the EPPO Global Database (EPPO 2022) for *G. rostochiensis*, as well as the CABI Invasive Species Compendium datasheet (CABI 2019), list this species from Norfolk Island but give the source reference as Marshall (1998). In fact, Marshall (1998) makes no reference to Norfolk Island, but the paper occurs as a chapter in the same book as the contributed chapter by Turner and Evans (1998). This record therefore appears to be untraceable and may well be spurious, as there are no confirmed literature records of PCN from Norfolk Island.

Although seven species of plant-parasitic nematodes were detected in association with roots of potato, none were present in high numbers and no overt evidence of root or tuber damage by nematodes was noticed in the field.

As plant-parasitic nematodes can be serious pests of turfgrass and their damage can be severe and expensive to resolve, due attention was given to assessing the nematode load on greens at Norfolk Island's golf course and lawn bowling club. Precise information about nematode economic threshold or damage threshold levels in turfgrass is scarce, as explained by Stirling *et al.* (2002): 'Lack of experimental work and the complexity of the nematode fauna mean that damage thresholds for nematodes on turf are not well defined. Also, factors such as grass species, mowing height, nutritional status, soil compaction and presence of other root pathogens influence the extent of losses from nematodes.' Nevertheless, they provided hazard index measures for the total complex of plant-parasitic nematode species in turf per 200 ml of soil as low (<50), moderate (50–200), and high (>200). In that case, all three golf course soil samples and one of the two in the bowling green have a high hazard index. This is potentially cause for concern and may warrant some form of remedial attention. Moreover, Dickerson *et al.* (2000) provided action levels for a few plant-parasitic nematodes in turfgrass, *Cynodon dactylon*. They indicated that numbers of *Pratylenchus* spp. (root lesion nematode) over 300 per 200 cc of soil are likely to cause a problem. Based on this figure, one of the bowling club's soil samples (no. 76), with a level of *Pratylenchus* at 372 nematodes per 200 cc of soil, may require corrective action. Comment about application of chemical agents as a management option is outside the purview of this report, but information about appropriate nematicide treatment for control of plant-parasitic nematodes in turf should be readily available from pertinent agronomists. Notwithstanding the somewhat high incidence of root lesion nematodes, the turfgrass appeared in relatively good condition, with no obvious nematode-caused symptoms of dieback, thinning, wilting, or discolouring, suggesting that its good maintenance may be adequate to sustain high quality greens.

Dickerson *et al.* (2000) provided action levels for a few other plant-parasitic nematodes in turfgrass. They indicated that a problem was likely to occur when *Helicotylenchus* spp. (spiral nematode) numbers were over 2000 per 200 cc of soil, and when numbers of *Meloidogyne* spp. (root knot nematode) and *Xiphinema americanum* (dagger nematode) each were over 600–700 per 200 cc of soil. They also indicated that for *Criconemella ornata*, a species of ring nematode relatively closely related to *Criconema mutabile*, when numbers were over 2000 per 200 cc of soil a problem was likely to occur. These figures suggest that the numbers of these nematodes in the golf course and bowling green are not each specifically high enough to elicit control measures.

Six species of cyst nematodes (*Heterodera* spp.) are listed within Australia's 42 National Priority Plant Pests (NPPP No. 23), viz., *H. carotae* (carrot cyst nematode), *H. filipjevi* (Filipjev's cereal cyst nematode), *H. glycines* (soybean cyst nematode), *H. latipons* (Mediterranean cereal cyst nematode), *H. sorghi* (sorghum cyst nematode) and *H. zeae* (maize cyst nematode). Of these, *H. latipons* is most unlikely to be encountered on Norfolk Island as its narrow host range includes wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*) and rye (*Secale cereale*) (Subbotin *et al.* 2010), crops not grown on island. On the other hand, there may be good potential for the other species to become established, as the only host of *H. carotae*, i.e., carrot (*Daucus carota*), is grown abundantly, and the remaining four species have a relatively broad host range including food plants and weeds that occur on the island. No NPPP *Heterodera* species were detected; the only detected species of the genus was *Heterodera trifolii*, the clover cyst nematode, a nematode with a range of host plants in the family Fabaceae as well as several other families.

Three species of plant-parasitic nematodes detected in the present survey are new records for Norfolk Island, viz., *Helicotylenchus pseudorobustus* (spiral nematode), *Paratylenchus* sp. (pin nematode), and *Rotylenchulus reniformis* (reniform nematode). It is possible that these three species had been present on Norfolk Island for a very long time and may have been inadvertently overlooked or undetermined during Khair's (1982) survey. For example, Khair (1982) listed four species of *Helicotylenchus* from Norfolk Island, three identified to species (*H. dihystera*, *H. erythrinae*, and *H. multicinctus*) and one undetermined. It is possible that this undetermined species was *Helicotylenchus pseudorobustus* as Khair (1982) recorded it in association with roots of beetroot, corn, pepper, and strawberry, while in the present survey *Helicotylenchus pseudorobustus* was detected with roots of corn and pepper as well as Bermuda grass, carrot, string beans, and white clover.

The symptoms of *Helicotylenchus pseudorobustus* are more subtle than those of certain other nematodes, such as root-knot or sting nematodes (Crow 2017). While it is a parasite of many economically important plants (e.g., CABI PlantwisePlus 2022, Ferris 2020), *Helicotylenchus pseudorobustus* is seldom considered a major pest on most of them. Its pathogenicity has been tested by many workers on several plants, showing that only very high population levels can cause damage to crops (CABI PlantwisePlus 2022). To reflect the observation that these nematodes are mild plant pathogens, the California Department of Food and Agricultural (CDFA) designated for *Helicotylenchus* spp. a 'C' action-oriented pest rating, i.e., 'an organism subject to no state enforced action outside of nurseries except to retard spread' (Chitambar 2016a).

Although Khair (1982) recorded no *Paratylenchus* on the island, internationally they are a common component of the fauna of cultivated crops, plantations and natural vegetation (Ghaderi 2019). As *Paratylenchus* species are generally considered mild and common pathogens of plants, so that even in relatively high numbers they are of little concern in most economic crops (Talavera and Navas 2002, Chitambar 2017, Ghaderi 2019, Ferris 2023a), the CDFA designated for them a 'C' action-oriented pest rating (Chitambar 2017). Moreover, they were invariably observed during the present survey in very low numbers.

It is surprising that Khair (1982) did not recognise the ubiquitous *Rotylenchulus reniformis* as a component of the Norfolk Island nematode fauna, given that the present survey detected it broadly across a range of crops, including carrot, chilli, corn, potato, sweet pea, and tomato. *Rotylenchulus reniformis* has a very broad global distribution and has an extremely wide host range covering most plant families (e.g., CABI Compendium 2022, Ferris 2023b). Stirling (2023) noted that this species and root-knot nematodes are the most widespread and damaging nematode pests in tropical and subtropical climates. *Rotylenchulus reniformis* is recognized as an economically important damaging pest particularly on cotton, pineapple, sweet potato and soyabean. Other reported important crop host plants include cowpea, banana, aubergine, cabbage, okra, melon, pigeon pea, tea and tobacco (CABI Compendium 2022, Ferris 2023b). The CDFA designated for *Rotylenchulus reniformis* an 'A' action-oriented pest rating, i.e., 'an organism of known economic importance subject to state … enforced action' (Chitambar 2016b). Nevertheless, during the present survey there was no apparent evidence of unacceptable above or below ground symptoms in host plants, which may suggest that the incidence of this nematode was relatively low.

The Pacific islands have a diverse range of food and cash crops and correspondingly have a broad variety of significant nematode problems (e.g., Kirby 1978, Bridge 1988, Orton-Williams 1980, Khurma *et al.* 2008, Grandison *et al.* 2009, Singh *et al.* 2010, 2012). Correspondingly, Khair (1982) found three species of root knot nematodes (*Meloidogyne*) in soils across Norfolk Island that were particularly damaging to food plants, and he noted that: 'Vegetables and especially root crops were most seriously affected. In some situations, galls were up to 60 mm diameter and most plants showed signs of unthriftiness, and exhibited symptoms relating to root damage'. He went so far as to warn that: '… root-knot nematode could be considered a major problem in the soils of Norfolk Island' and also suggested that: 'The widespread occurrence of infected wild Tobacco and Lantana bushes on the outskirts of cultivated land appears to provide a constant source of inoculum for new crops.' Fortunately, the situation may have improved over the past 40 years as the present survey found no conspicuous nematode root galling of vegetables even though two species of *Meloidogyne* were detected, infecting carrot (*Daucus carota*), corn (*Zea mays*), chilli (*Capsicum annuum*), potato (*Solanum tuberosum*), clover (*Trifolium repens*) and turf (*Cynodon dactylon*). Reasons for any changes in severity of plant damage by pest nematodes on Norfolk Island are unclear, although further field survey work may contribute to elucidation of the issue.

Appendix D: Bacterial plant pathogens report

Norfolk Island Plant Pest and Disease Survey – plant pathogenic bacteria

Background and Introduction

The plant pathogenic bacteria component of this survey targeted symptomatic plants. This was mostly due to the collection permit that was released to the Agriculture Victoria team, enabling the team to collect plant material preserved in solutions to sequence their DNA. Samples were therefore collected when symptoms could be observed on the plants (yellowing of the leaves, death of branches, etc). The testing of samples focused on determining the presence of exotic bacteria using conventional molecular testing following National Diagnostic Protocols (NDP) and High Throughput Sequencing (HTS). These tests are available for all the major bacterial and viral pathogens of concern, and are widely considered the best approach to determine the presence/absence of pathogens. The surveys were conducted in different seasons (spring and summer) to maximise the chances of recording a higher level of diversity, and to give the community the opportunity to observe changes and symptoms in plants between surveys, and to report these during the second field collection. As a result, most of the samples were collected on private properties, upon request of members of the community who had observed symptoms on their plants.

Figure 1: Elisse Nogarotto collecting plant samples on Norfolk Island.

Methods

Summer survey

The 72 samplesfor bacterial testing were pre-processed on island: tissues were selected for processing based on the presence of symptoms, such as bacterial spots or by relevant tissue type, such as collection of petioles and veins for vascular limited bacteria. Tissue samples were stored 15 mL screw cap tubes that had been prefilled with 5 mL CTAB buffer (Green et al 1999) and transported back to the AgriBio laboratories for DNA extraction and bacterial testing. Seventy-two samples were tested for a range of exotic bacterial pathogens using PCR assays. The bacteria that were tested included *Xylella spp*, *Candidatus* Liberibacter solanacearum, Huanglongbing (*Candidatus* Liberibacter africanus, americanus and asiaticus), *Erwinia amylovora, Xanthomonas citri* subsp. *citri* and *Clavibacter michiganensis* subsp. *sepedonicus*.

Spring survey

Fifty-four samples were processed on-island, this included selecting/cutting out appropriate tissue for testing (material was not weighed as no scale available) and grinding the tissue in 5mL of CTAB buffer (Green et al 1999) in universal extraction bags (Bioreba). After processing, 2 mL of the the macerates were transferred to 2mL centirgfuge tubes and then frozen. For transport back to AgriBio all tubes were sealed with parafilm and placed in an insulated lunch box containing icepacks to keep the contents cool. Upon arrival samples were stored in the freezer at -20℃ prior to DNA extraction and bacterial testing. The spring samples were tested for a range of bacteria including *Xylella* spp. *Candidatus* Liberibacter solanacearum, Huanglongbing (*Candidatus* Liberibacter africanus, americanus and asiaticus), *Erwinia amylovora, Xanthomonas citri* subsp. *citri*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Xanthomonas fragariae* and *Pantoea stewartii* subsp. *stewartii*.

DNA extraction

DNA extraction was done using a DNeasy Plant Mini Kit (Qiagen) with modifications (Green et al 1999).

Polymerase chain reaction (PCR)

DNA extracts were initially screened with a quality control (QC; housekeeping) real-time PCR (qPCR) to ensure DNA was of sufficient quality and quantity for testing using molecular methods, including PCR and high throughput sequencing (Table 1). A GoTaq® Probe qPCR kit (Promega) was used for all QC and pathogen qPCR assays A Platinum™ Taq DNA Polymerase kit (Invitrogen) was used for all endpoint PCR assays , except for the *Xylella* generic PCR which used the MyTaq™ HS Mix (Bioline) kit. All qPCR and PCR kits were used as per manufacturer's instructions. All qPCR and PCRassays used 5 µL of template in each reaction.

Testing panels of samples were prepared for each of the target pathogens based on host. Testing for exotic bacteria was done using PCR assays described in Australian endorsed or draft National Diagnostic Protocols (NDP) or other international method. A list of all primers and probes used for target bacteria is given in Table1, which also incudes primer/probe sequence (including fluorophore for probes), final concentrations, amplification or PCR cycling conditions, expected product size for PCRs and the reference.

High throughput sequencing

Ten samples from the summer sampling and all 54 samples from the spring sampling were also analysed by metagenomic high throughput sequencing. The same DNA used for PCR testing was also used for HTS. Libraries were prepared for metagenomic HTS using the NEXTFLEX® Rapid XP DNA-Seq Kit (PerkinElmer) with the NEXTFLEX 384 Unique Dual Index Barcodes version 19.06 (PerkinElmer) and run on the NovaSeq 6000 System (Illumina) (Rodrirgues Jardim et al 2022).

The ten summer samples analysed by metagenomiv HTS were also analysed using an AVR developed hybridization capture HTS method for multiplexed detection of specific bacteria in a single sample (Brohier N, unpublished). The hybridization capture HTS uses biotinylated oligonucleotide probes to capture targeted DNA regions within the genomes of Xylella spp., *Erwinia amylovora, Xanthomonas citri* subsp *citri, Candidatus* Liberibacter asiaticus, *Candidatus*

Liberibacter africanus, *Candidatus* Liberibacter americanus, *Candidatus* Liberibacter solanacearum, and *'Candidatus* phytoplasma' species in the 16SrI group.

Bioinformatics analysis

Metagenome assemblies: sequence reads were *de novo* assembled using SPAdes 3.15.2 (Prjibelski et al., 2020).

Databases used for analysis: A custom database was created that contained the reference genomes of a range of exotic bacteria that were downloaded from the NCBI; it includes reference genomes of *Xylella* spp. *Pseudomonas syringae* pv. *actinidiae* (Psa), *Xanthomonas citri* pv. *citri*, *Liberibacter*, *Erwinia amylovora*, *Candidatus* Phytoplasma spp., *Xanthomonas fragariae*, *Pectobacterium* spp., *Pantoea* spp. and *Dickeya* spp. A local offline database (abfvhp_22022022) comprising of NCBI sequences from archaea, bacteria, fungus, viruses, human and phytoplasma was alos created to enable a more in-depth analysis of the data. The raw and assembled sequence data of each sample was compared to both databases to enable detection of matching sequences to the exotic and other bacteria, if they were present.

Blastn: Raw reads and assembled contigs were compared against both databases using Blastn, keeping the Blastn parameters as -max_target_seqs 1 -evalue 1e-10 to ensure results were significant.

Kraken2 analysis: Kraken2 was used to analyse the raw reads from the samples against the abfvhp_22022022 database (comprising of NCBI sequences from archaea, bacteria, fungus, viruses, human and phytoplasma) to identify any exotic bacteria. For a closer look at the kraken2 output, KrakenTools-1.2/extract_kraken_reads.py was used to extract any reads corresponding to the exotic bacteria sequences contained in the custom database. Extracted reads from each sample matching to each exotic bacteria were checked manually and positive indication of the presence of a bacterium required a minimum of 0.02% or 1000 reads.

Results

PCR screening

All DNA extracts tested passed the initial QC step (housekeeping qPCR). *Xylella fastidiosa*, *Candidatus* Liberibacter solanacearum, Huanglongbing (*Candidatus* Liberibacter africanus, americanus and asiaticus), *Erwinia amylovora, Xanthomonas citri* subsp. *citri* and *Clavibacter michiganensis* subsp. *sepedonicus* were not detected in the most summer and spring samples that were tested using the endpoint PCR or qPCR assays. *Xanthomonas fragariae* and *Pantoea stewartii* subsp. *stewartia* were not detected in the spring samples that were tested using the endpoint PCR or qPCR assays. Seven summer samples gave indeterminate results for one or more PCR assays (Table 2).

High throughput sequencing

Summer sampling

Seven of the 72 samples gave indeterminate PCR results, i.e., they could not be considered either positive or negative. These seven samples and three additional samples, which gave negative PCR results but had suspect symptoms or due to the age of the plant, (bolded samples, Table 2), were analysed by by metagenomic and targeted HTS for bacteria. The HTS results indicated that there were no exotic bacterial pathogens present in the samples.

Spring sampling

No exotic bacterial pathogens were detected in the 54 bacterial samples from the spring surveillance using PCR and metagenomic HTS (Table 3).

Table 1. The endpoint or real-time PCR assays used for bacterial testing. Information includes reference to a National Diagnostic Protocol (NDP) or other international protocol, target pathogen, reference to literature, primer/probe names and sequence (including fluorophore for probes), final concentrations in each assay, amplification or PCR cycling conditions and expected product size for PCRs.

NDP number or other international protocol used	Target and reference	Primer name	Primer Sequence (5' – 3'). If probe, fluorophore stated in brackets	Final concentration	Amplification or cycling conditions, expected product size (PCR only)
N/A – housekeeper detection for extracts (QC)	Housekeeper qPCR (Weller *et al.* 2000)	COX-F	CGT CGC ATT CCA GAT TAT CCA	0.25 µM	50 ℃ 2 min, 95 ℃ 2 mins, (95 ℃ 15s, 60 ℃ 30s) x40
		COX-R	CAA CTA CGG ATA TAT AAG AGC CAA AAC TG	0.25 µM	
		COX-p	TGC TTA CGC TGG ATG GAA TGC CCT (HEX)[1]	0.1 µM	
NDP 18	Candidatus *Liberibacter* solanacearum qPCR (Li *et al.* 2009)	LsoF	GTC GAG CGC TTA TTT TTA ATA GGA	0.25 µM	50 ℃ 2 min, 95 ℃ 2 mins, (95 ℃ 10s, 58 ℃ 30s) x40
		HLBr	GCG TTA TCC CGT AGA AAA AGG TAG	0.25 µM	
		HLBp	AG ACG GGT GAG TAA CGC G (FAM)	0.1 µM	
NDP 25	Candidatus *Liberibacter* africanus qPCR (Li *et al.* 2006)	HLBaf	CGA GCG CGT ATT TTA TAC GAG CG	0.25 µM	50 ℃ 2 min, 95 ℃ 2 mins, (95 ℃ 10s, 58 ℃ 30s) x40
		HLBr	GCG TTA TCC CGT AGA AAA AGG TAG	0.25 µM	
		HLBp	AG ACG GGT GAG TAA CGC G (FAM)	0.1 µM	
NDP 25	Candidatus *Liberibacter* americanus qPCR (Li *et al.* 2006)	HLBam	GAG CGA GTA CGC AAG TAC TAG	0.25 µM	50 ℃ 2 min, 95 ℃ 2 mins, (95 ℃ 10s, 58 ℃ 30s) x40
		HLBr	GCG TTA TCC CGT AGA AAA AGG TAG	0.25 µM	
		HLBp	AG ACG GGT GAG TAA CGC G (FAM)	0.1 µM	
NDP 25	Candidatus *Liberibacter* asiaticus qPCR (Li *et al.* 2006)	HLBas	TCG AGC GCG TAT GCA ATA CG	0.3 µM	50 ℃ 2 min, 95 ℃ 2 mins, (95 ℃ 10s, 58 ℃ 30s) x40
		HLBr	GCG TTA TCC CGT AGA AAA AGG TAG	0.3 µM	
		HLBp	AG ACG GGT GAG TAA CGC G (FAM)	0.1 µM	
NDP 6 2022 draft	*Xylella fastidiosa* qPCR (Harper *et al.* 2010)	XF-F	GGG TTG CGT GGT GAA ATC AAG	0.3 µM	50 ℃ 2 min, 95 ℃ 2 mins, (95 ℃ 10s, 62 ℃ 30s) x40
		XF-R	CAC GGC TGG TAA CGG AAG A	0.3 µM	
		XF-P	TC GCA TCC CGT GGC TCA GTC C (FAM)	0.1 µM	
IPPC DP 25	*Xylella fastidiosa* PCR (Minsavage *et al.* 1994)	RST31	GCGTTAATTTTCGAAGTGATTCGATTGC	0.4 µM	95 ℃ 2 min, (95 ℃ 30s, 55 ℃ 30s, 72 ℃ 45s) x35, 72 ℃ 10 min Expected product size: 733 bp
		RST33	CACCATTCGTATCCCGGTG	0.4 µM	
NDP 6 2022 draft[2]	*Xylella* generic PCR	Xgen-ComEC-F	AGTCATGCTGATAGTGATCACGT	0.4 µM	95 ℃ 2 min, (95 ℃ 30s, 60 ℃ 30s, 72 ℃ 30s) x40, 72 ℃ 5 min Expected product size: 650 bp

		Xgen-ComEC-650-R	CAGCATGTCTCGTTTCTCCGA	0.4 µM	
NDP 9 2018 draft	*Xanthomonas citri* subsp. *citri* PCR(Cubero and Graham, 2002)	J-pth 1	CTTCAACTCAAACGCCGGAC	0.8 µM	95 °C 2 min, (95 °C 30s, 58 °C 30s, 72 °C 30s) x35, 72 °C 5 min Expected product size: 197 bp
		J-pth 2	CATCGCGCTGTTCGGGAG	0.8 µM	
NDP 9 2018 draft[3]	*Xanthomonas citri* subsp. *citri* multiplex PCR	R8-PP1F	GACACCGGCTATCTGCATCA	0.8 µM	95 °C 2 min, (95 °C 30s, 58 °C 30s, 72 °C 30s) x35, 72 °C 5 min Expected product size: R8 = 150 bp, R22 = 400 bp
		R8-PP1R	AAATCCTTTGATCGGCGGGT	0.8 µM	
		R22-1F	ACCACCACTAACATCCGGAG	0.4 µM	
		R22-1R	TTCTCAAAAGAGGCTACAATGAAGA	0.4 µM	
NDP draft (no number) written 2018[4]	*Erwinia amylovora* PCR	Ea1F	TTTCCGTATGCCTGCTGTCCGC	0.4 µM	95 °C 2 min, (95 °C 30s, 64 °C 30s, 72 °C 30s) x35, 72 °C 7 min Expected product size: 235 bp
		Ea1R	TCAGTTATGCGCCACCCCATGC	0.4 µM	
NDP draft (no number) written 2018[4]	*Erwinia amylovora* PCR	Ea2F	AACGCATCGTCCGGGCAAATGT	0.4 µM	95 °C 2 min, (95 °C 30s, 64 °C 30s, 72 °C 30s) x35, 72 °C 7 min Expected product size: 388 bp
		Ea2R	AGCGCTGTCTGGAAACCCATGC	0.4 µM	
EPPO 2010[5]	*Clavibacter sepedonicus* qPCR	CMS 50-2F	CGGAGCGCGATAGAAGAGGA	0.4 µM	50 °C 2 min, 95 °C 2 mins, (95 °C 15s, 60 °C 1 min) x40
		CMS 133R	GGCAGAGCATCGCTCAGTACC	0.8 µM	
		Cms 50-53T	AAGGAAGTCGTCGGATGAAGATGCG (FAM)	0.2 µM	
EPPO 2016	*Pantoea stewartii* pv. *stewartii* (Tambong *et al.* 2008)	cps-RT74F	TGC TGA TTT TAA GTT TTG CTA	0.4 µM	50 °C 2 min, 95 °C 2 mins, (95 °C 15s, 60 °C 1 min) x50
NDP draft (no number) written 2020		cps-177R	AAG ATG AGC GAG GTC AGG ATA	0.8 µM	
		cps-133	TCG GGT TCA CGT CTG TCC AAC (FAM)	0.2 µM	
EPPO 2016 NDP draft (no number) written 2020	*Pantoea stewartii* pv. *stewartii*	PST-1	CCT CAC ACC ATC GGA TGT G	1 µM	95 °C 2 min, (95 °C 30s, 58 °C 30s, 72 °C 30s) x40, 72 °C 7 min Expected product size: 360
		PST-R	ATG AGG TTA TTA ACC TCA CCA	1 µM	
NDP draft (no number) written 2022	*Xanthomonas fragariae* (Weller *et al.* 2007)	Xf gyrB-F	CCG CAG CGA CGC TGA TC	0.25	50 °C 2 min, 95 °C 2 mins, (95 °C 15s, 60 °C 30s) x40
		Xf gyrB-R	ACG CCC ATT GGC AAC ACT TGA	0.25	
		Xf gyrB-P	TCC GCA GGC ACA TGG GCG AAG AAT TC (JOE)	0.1	

Notes: [1] HEX labelled probe read on VIC channel (Quantstudio3, Applied Biosystems). [2] Note: NDP draft has not been published. *Xylella* generic primer sequences are not yet publicly available. [3] NDP draft has not been published. *Xanthomonas citri* subsp. *citri* primer sequences are not yet publicly available. [4] NDP draft has not been published. *Erwinia amylovora* primer sequences are not yet publicly available.

[5] Note: *Clavibacter sepedonicus* testing material was not ideal. Only leaf material only was able to be obtained, unable to sample potato tubers (i.e., testing of this material may be insignificant). There was an update to the EPPO method in 2022 after this testing was completed. Validations of additional confirmation methods are underway in the laboratory at AgriBio.

Table 2: The 72 samples collected during the summer 2022 Norfolk Island survey for bacterial testing. Molecular (PCR and high throughput sequencing (HTS)) screening results shown for bacterial pathogens. The samples selected for bacterial detection using HTS are shown in bold front. A '-' indicates that the samples was tested but the bacteria was not detected. NA indicates that the sample was not tested for a specific pathogen by PCR. IND in dicates that an indetermineate result was obtained. CLso = Candidatus *Liberibacter* solanacearum; CLaf = Candidatus *Liberibacter* africanus; Clam = Candidatus *Liberibacter* americanus; Clas = Candidatus *Liberibacter* asiaticus; Cms = *Clavibacter michigensis sepedonicus*; Xcc = *Xanthomonas citri* subsp. *citri*; Lib = *Liberibacter*.

ID	**Host Plant**	**Notes**	**CLso**	**CLaf**	**Clam**	**Clas**	***Xylella***	**Cms**	**Xcc**	**Lib**
104	Celery	Mottle (virus) necrosis (fungal)	-	NA	NA	NA	NA	NA	NA	NA
105	*Prunus*	Leaf spots, yellowing (mottle), shot hole	NA	NA	NA	NA	-	NA	NA	NA
106	Tomato	Leaf curling, distortion, necrosis	-	NA	NA	NA	NA	NA	NA	NA
107	Sweet potato	Necrosis along veins	-	NA	NA	NA	NA	-	NA	NA
110	Sweet potato	Mottle, mosaic	-	NA	NA	NA	NA	-	NA	NA
111	grapefruit	Yellowing	NA	NA	NA	NA	-	NA	NA	NA
112	Peach	Mottle	NA	NA	NA	NA	-	NA	NA	NA
117	Carrot	Leaf and stem lesions	-	NA	NA	NA	NA	NA	NA	NA
119	**Norfolk lemon**	**No symptoms**	**NA**	**IND**	**-**	**IND**	**-**	**NA**	**-**	**-**
120	Avocado (Hass)	Leaf mottle	NA	NA	NA	NA	-	NA	NA	NA
123	Grapevine (grown from seed)	Mottle	NA	NA	NA	NA	-	NA	NA	NA
128	Teddy celery	Yellowing, leaf spots	-	NA	NA	NA	NA	NA	NA	NA
132	Macadamia	Leaf scorch	NA	NA	NA	NA	-	NA	NA	NA
134	Plum	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
144	China Pear	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
145	Grape	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
147	Tomato	Small leaves, purpling, some yellowing	-	NA	NA	NA	NA	NA	NA	NA
148	*Prunus*	Leafspots, mottle	NA	NA	NA	NA	-	NA	NA	NA
149	*Prunus*	Mottle, yellowing	NA	NA	NA	NA	-	NA	NA	NA
203	**Mandarin**	**Mottling**	**NA**	**-**	**-**	**-**	**-**	**NA**	**-**	**-**
204	Capsicum (near Citrus)	Mottling leaves	-	-	-	-	NA	NA	NA	-
210	Potato (10 plants)	No symptoms	-	NA	NA	NA	NA	-	NA	NA
212	Stone fruit	Budwood for virus	NA	NA	NA	NA	-	NA	NA	NA
215	Potato	No symptoms	-	NA	NA	NA	NA	-	NA	NA
217	Tomato	No symptoms	-	NA	NA	NA	NA	NA	NA	NA

219	**Bush lemon**	**Mottling, yellowing**	**NA**	**-**	**-**	**IND**	**-**	**NA**	**-**	**-**
221	tomato leaf	No symptoms	-	NA	NA	NA	NA	NA	NA	NA
227	Yam	Bacterial spot	-	NA	NA	NA	NA	NA	NA	NA
229	Potato	Swollen nodes	-	NA	NA	NA	NA	-	NA	NA
233	Tomato	Little leaf	-	NA	NA	NA	NA	NA	NA	NA
234	Potato	No symptoms	-	NA	NA	NA	NA	-	NA	NA
240	Fig	Mottle	NA	NA	NA	NA	-	NA	NA	NA
243	**Navel orange (Citrus)**	**Mild mottle**	**NA**	**-**	**-**	**IND**	**-**	**NA**	**-**	**-**
245	Very old Tomato	Discolouration	-	NA	NA	NA	NA	NA	NA	NA
246	Prunus	Yellowing along veins, mottle	NA	NA	NA	NA	-	NA	NA	NA
250	Stone fruit	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
401	**Apple**	**Check Fire blight**	**NA**	**NA**	**NA**	**NA**	**IND**	**NA**	**NA**	**NA**
402	Good apple	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
404	Peach golden queen	Shot hole + Crinkle	NA	NA	NA	NA	-	NA	NA	NA
405	Capsicum	Leaf distortion, yellowing, mottle	-	NA	NA	NA	NA	NA	NA	NA
406	Nectarine	Shot hole and virus symptoms on leaves	NA	NA	NA	NA	-	NA	NA	NA
409	Plum	Old history	NA	NA	NA	NA	-	NA	NA	NA
411	Tobacco	Suspect virus symptom	NA	NA	NA	NA	-	NA	NA	NA
412	Peach	Crinkle and vein bonding	NA	NA	NA	NA	-	NA	NA	NA
414	Satsuma plum	BLS/ shot hole symptoms	NA	NA	NA	NA	-	NA	NA	NA
416	Pear	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
418	**Citrus, old lemon**	**No symptoms**	**NA**	**-**	**-**	**-**	**-**	**NA**	**-**	**-**
423	Hibiscus	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
425	Native Oleander – Pittosporum	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
426	Apple	Apple mosaic virus-like symptoms	NA	NA	NA	NA	-	NA	NA	NA
427	Sweet potato like vine	Young leaves with mosaic like symptoms	-	NA	NA	NA	NA	NA	NA	NA
430	Lime tree	Lime/branch die back, HLB like	NA	NA	NA	NA	-	NA	-	NA
433	Peach	Classic shot hole	NA	NA	NA	NA	-	NA	NA	NA
434	*Prunus* tree (likely peach)	Shot hole	NA	NA	NA	NA	-	NA	NA	NA
435	Apple	No symptoms	NA	NA	NA	NA	-	NA	NA	NA

436	Unknown Tree	Leaf spot, leaf crinkle, leaf senescence	NA	NA	NA	NA	-	NA	NA	NA
437	**Citrus**	**Ring spot on leaves,**	**NA**	**-**	**-**	**-**	**-**	**NA**	**-**	**-**
438	**Old Citrus – Mandarin**	**Yellow new growth and spots on fruits**	**NA**	**-**	**-**	**IND**	**-**	**NA**	**-**	**-**
439	Tomato	Bacterial wilt, but not classic	-	NA	NA	NA	NA	NA	NA	NA
441	Apple	Tip dieback, No classic FB	NA	NA	NA	NA	-	NA	NA	NA
443	Tomato	Back leaf spot	-	NA	NA	NA	NA	NA	NA	NA
445	Peach	Classic shot hole	NA	NA	NA	NA	-	NA	NA	NA
449	Grapevine	Descended from Pitcornie immigration	NA	NA	NA	NA	-	NA	NA	NA
450	Grapevine	Possible GLRV symptoms	NA	NA	NA	NA	-	NA	NA	NA
470	Pear	Witches' broom	NA	NA	NA	NA	-	NA	NA	NA
476	Chilli	Leaf yellowing	-	NA	NA	NA	NA	NA	NA	NA
477	Grape – golden	No symptoms	NA	NA	NA	NA	-	NA	NA	NA
483	**Orange**	**Yellowing leaves**	**NA**	**-**	**-**	**IND**	**-**	**NA**	**-**	**-**
488	Pepino	Virus like growth	-	NA	NA	NA	NA	NA	NA	NA
492	**Lemon**	**Spotting, curling, yellowing**	**NA**	**-**	**-**	**-**	**IND**	**NA**	**-**	**-**
494	Norfolk Island Pine	Yellowing	NA	NA	NA	NA	-	NA	NA	NA
499	Potato	Yellowing, poor growth,	-	NA	NA	NA	NA	-	NA	NA

Table 3: The 54 samples collected during the spring 2022 survey on Norfolk Island. They were tested using PCR assays and metagenomic high throughput sequencing for *Xylella* spp.,*Candidatus* Liberibacter solanacearum, *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus. *Candidatus* Liberibacter asiaticus, *Erwinia amylovora, Xanthomonas citri* subsp. *citri*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Xanthomonas fragariae* and *Pantoea stewartia* subsp. *stewartia.* A '-' indicates a negative result. No exotic or endemic pathogenic bacteria were detected.

Sample #	Host Plant	Symptoms	CLso	CLaf	Clam	Clas	*Xylella*	Cms	Xcc	Lib	HTS
1	Potato	Crinkling	-	-	-	-	-	-	-	-	-
2	Potato	Crinkling	-	-	-	-	-	-	-	-	-
3	Macadamia	Scorch	-	-	-	-	-	-	-	-	-
4	Tomato	Stunted, crinkled	-	-	-	-	-	-	-	-	-
5	Tomato	Stunted, crinkled	-	-	-	-	-	-	-	-	-
6	Avocado	Scorched, dieback - only older leaves	-	-	-	-	-	-	-	-	-
7	Wild cape goose berry	Crinkled	-	-	-	-	-	-	-	-	-
8	Mulberry	Leaf spots	-	-	-	-	-	-	-	-	-
9	Lilly	Streak into main vein	-	-	-	-	-	-	-	-	-
10	Strawberry	Phyllody	-	-	-	-	-	-	-	-	
11	Lemon	Scabs, blisters with yellow halo	-	-	-	-	-	-	-	-	-
12	Lemon	Chlorosis	-	-	-	-	-	-	-	-	-
13	Potato	Purpling	-	-	-	-	-	-	-	-	-
14	Potato	Leaf rolling	-	-	-	-	-	-	-	-	-
15	Corn	Stripes	-	-	-	-	-	-	-	-	-
16	Carrot	Curling, some purpling	-	-	-	-	-	-	-	-	-
17	Norfolk lemon	Blistery	-	-	-	-	-	-	-	-	-
18	Grapefruit	Chlorosis	-	-	-	-	-	-	-	-	-
19	*Prunus* sp	Reddening of stems, red spots	-	-	-	-	-	-	-	-	-
20	White peach	Red spots	-	-	-	-	-	-	-	-	-
21	Tomato	Purpling and curling.	-	-	-	-	-	-	-	-	-
22	*Prunus* sp	Shot hole-ish	-	-	-	-	-	-	-	-	
23	Avocado	Edges of leaves scorched, tree not healthy	-	-	-	-	-	-	-	-	-
24	Frangipanni	Limp leaves, nectrotic margins	-	-	-	-	-	-	-	-	-
25	Strawberry	Underside dry spots, no ooze	-	-	-	-	-	-	-	-	-
26	Carrot	Purpling	-	-	-	-	-	-	-	-	-
27	Apple	Spot, no grafting on this tree	-	-	-	-	-	-	-	-	-

28	Apple	Crook tips on one branch	-	-	-	-	-	-	-	-	-
29	Tomatoes	Crinkle tops, upward curl	-	-	-	-	-	-	-	-	-
30	Lettuce	Yellowing crop, veins darkened	-	-	-	-	-	-	-	-	-
31	Carrot	Yellowing tips, maybe some purpling	-	-	-	-	-	-	-	-	-
32	Tomato	Kinda mottlish, chlorotic in spots	-	-	-	-	-	-	-	-	-
33	Hawaiian passionfruit	Some darn veins (looks like just dead tissue)	-	-	-	-	-	-	-	-	-
34	Citrus	Fasciation	-	-	-	-	-	-	-	-	-
35	Citrus sp.	Curl and fasciation	-	-	-	-	-	-	-	-	-
36	Citrus, navel orange	Chlorotic areas	-	-	-	-	-	-	-	-	
37	Citrus	Yellow area, blotches	-	-	-	-	-	-	-	-	-
38	Tomato	Sad and bits of yellowing, droopy	-	-	-	-	-	-	-	-	-
39	Citrus	Yellowing, scaly fruit, some blisters	-	-	-	-	-	-	-	-	-
40	Orange	Chlorosis and spots	-	-	-	-	-	-	-	-	-
41	Citrus	Spots	-	-	-	-	-	-	-	-	-
42	Grapevine (old)	Scorch	-	-	-	-	-	-	-	-	
43	Avocado	Scorch	-	-	-	-	-	-	-	-	-
44	Potato	Crinkles and yellowing - might be the variety	-	-	-	-	-	-	-	-	-
45	Sweet potato	Discoloured, not sure	-	-	-	-	-	-	-	-	-
46	Potato	Purpling	-	-	-	-	-	-	-	-	-
47	Avacado	Dieback	-	-	-	-	-	-	-	-	-
48	Pohutakaw	Dieback (cut to stumps), curly leaves	-	-	-	-	-	-	-	-	-
49	Parsley sp.	Intesnse curling	-	-	-	-	-	-	-	-	-
50	Banana	Sick, dieback. Some discolouration	-	-	-	-	-	-	-	-	-
51	Citrus	Blotchy and spots	-	-	-	-	-	-	-	-	-
52	*Prunus*	Dieback and sap	-	-	-	-	-	-	-	-	-
53	Broad bean	Soft almost bruised looking beans	-	-	-	-	-	-	-	-	-
54	Capsicum	Yellowing	-	-	-	-	-	-	-	-	-

Discussion

There were no exotic bacterial pathogens detected through molecular and HTS screening of the Norfolk Island plant samples collected in summer and spring of 2022. The testing provided evidence of absence on Norfolk Island for bacteria occurring in the Australian top 42 National Plant Priority Pests, including *Xylella* spp. *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus, *Candidatus* Liberibacter asiaticus, *E. amylovora, X. c.* subsp. *citri*, and *Candidatus* Phytoplasma species (16SrI and those associated with banana diseases). Although the bacteria *Candidatus* Liberibacter solanacearum and its insect vector the tomato potato psyllid (TPP; *Bactericera cockerelli*) have been reported on previous Norfolk Island surveys (Maynard *et al*. 2018), the bacterial pathogen was not detected in this survey. The lack of detection of bacterial pathogens some of which have been previously reported in Norfolk Island (Maynard *et al*. 2018) could be associated with prevailing weather conditions during the surveys. This can have an impact on bacterial disease manifestations and also populations of insects that vector some of these bacterial pathogens. Further surveys are recommended across multiple seasons to be able to capture the plant bacterial diversity in Norfolk Island.

Norfolk Island Plant Pest and Disease Survey – Viral plant pathogens

Background and Introduction

The plant pathogenic virus component of this survey targeted symptomatic plants. This was mostly due to the collection permit that was released to the Agriculture Victoria team, enabling the team to collect plant material preserved in solutions for DNA sequencing. Samples were therefore collected when symptoms could be observed on the plants (yellowing of the leaves, death of branches, etc). The testing of samples aimed to determine the presence of exotic virues using conventional molecular testing following National Diagnostic Protocols (NDP) and High Throughput Sequencing (HTS). The virology samples were tested for plum pox virus, phytoplasma and begomoviruses following the protocols outlined in the NDPs.

These tests are available for all the major viral pathogens of concern and are widely considered the best approach to determine presence/absence of pathogens. The surveys were conducted in different seasons (spring and summer) to maximise chances of recording a higher level of viral diversity. This also provided members of the community the opportunity to observe changes and symptoms in plants and to report these during the second field collection. As a result, most of the samples were collected on private properties, upon request of members of the community who had observed symptoms on their plants.

Methods

Summer survey

Samples were pre-processed on island: tissues were selected for processing based on the presence of virus-like symptoms or by relevant tissue type, such as collection of petioles and veins for vascular limited viruses and phytoplasmas. Tissue samples were stored 15 mL screw cap tubes that had beenin prefilled with 5 mL CTAB buffer (Green et al 1999), One-hundred and eight samples were tested a range of exotic viruses and virus-like organisms including plum pox virus (PPV), phytoplasma, tobamoviruses, potyviruses, closterovirirdae, begomoviruses and pospiviroids with using PCR assays.

Spring survey

Ninety-one samples were processed on-island, this included selecting/cutting out appropriate tissue for testing (material was not weighed as no scale available) and grinding the tissue in 5mL of CTAB buffer (Green et al 1999) in universal extraction bags (Bioreba). After processing, 2 mL of each macerate was transferred to 2mL centirgfuge tube and then frozen. For transport back to AgriBio all tubes were sealed with parafilm and placed in an insulated lunch box containing icepacks to keep the contents cool. Upon arrival samples were stored in the freezer at -20℃ prior to nuclic acid extraction and virus, viroid or phytoplasma testing.

Nucelic acid extraction

Total nucleic acid was extracted from each sample using a Rneasy II Plant Mini Kit (Qiagen), following the manufacturer's instructions, except the CTAB was used as the lysis buffer.

Molecular testing

Total nucleic acid extracts were initially screened with a quality control (QC; housekeeping) endpoint RT-PCR or PCR (Table 1) to ensure RNA and DNA was of sufficient quality and quantity for testing using molecular methods, including PCR and high throughput sequencing.

RT-qPCR assays were done using the AgPath-ID™ One-Step RT-PCR Reagents (Invitrogen). All endpoint RT-PCR assays were done using the Superscript III Platinum™ *Taq* DNA Polymerase kit (Invitrogen). All kits were used as per manufacturer's instructions. Phytoplasma and closteroviridae rd2 (DNA) testing was undertaken using Platinum™ *Taq* DNA Polymerase kit (Invitrogen). All molecular methods (RT-qPCR, RT-PCR and PCR) methods used 4 µL of template in each reaction, except the nested closteroviridae rd2 (DNA) which used 1 µL of the first round PCR in the nested PCR step. Testing panels were prepared for each of the target pathogens based on host.

Testing for exotic viruses, viroids and phytoplasmas was done using PCR assays described in Australian endorsed or draft National Diagnostic Protocols (NDP) or other international method. A list of all primers and probes used for target virus, viroids and phytoplasma testing is given in Table1, which also incudes primer/probe sequence (including fluorophore for probes), final concentrations, amplification or PCR cycling conditions, expected product size for PCRs and the reference.

High throughput sequencing

Five µl of each of the total nucleic acid extracts were used for HTS. Libraries were prepared using NEBNext® Ultra™ II RNA library prep kit, following the manufacturer's instructions. The size range and concentration of the libraries were determined using the 2200 TapeStation® system (Agilent Technologies) and Qubit® Fluorometer 2.0 (Invitrogen), respectively, and the resulting quantification values were used to pool the libraries. The resulting library was finally sequenced using the NovaSeq 6000 system (Illumina) with a paired read length of 2 × 150 bp.

The raw sequence data was quality filtered, adapters were trimmed, and the generated sequence read pairs were validated using Fastp (version 0.20.0) with default parameters. SPAdes (version 3.13.0; Bankevich et al 2012) was used to d*e novo* assemble the trimmed sequence reads into contigs. The resulting *de novo* assembled contigs were analysed against the NCBI nucleotide database using the alignment search tool BLASTn for the presence of viruses and voirds in the samples. Reference mapping of the sequence reads for each sample was also done using Bowtie2 (version 2.3.4.2) using the most similar genome identified in the previous BLASTn search.

Table 1. The endpoint or real-time PCR or RT-PCR assays used for used for virus, viroid and phytoplasma testing. Information includes reference to a National Diagnostic Protocol (NDP) or other international protocol, target pathogen, reference to literature, primer/probe names and sequence (including fluorophore for probes), final concentrations in each assay, amplification or RT-PCR cycling conditions and expected product size for RT-PCRs.

NDP number or other international protocol used	Target and reference	Primer name	Primer Sequence (5' – 3'). If probe, fluorophore stated in brackets	Final concentration	Amplification or cycling conditions, expected product size (RT-PCR only)
N/A – housekeeper detection for extracts (QC)	Housekeeper RT-PCR (Thompson *et al* 2003)	Nad2.1a	GGACTCCTGACGTATACGAAGGATC	0.5 µM	48˚C for 45 min, 1 cycle 94˚C for 2 min, 40 cycles 94˚C for 30 sec, 50 for 30 sec, 72˚C for 30 sec, 1 cycle of 72˚C for 10 min.
		Nad2.1b	AGCAATGAGATTCCCCAATATCAT	0.5 µM	
	Housekeeper 16s PCR (Wesiberg *et al* 1991)	fD1	AGAGTTTGATCCTGGCTCAG	0.5 µM	95 °C 1 min, (95 °C 1 min, 56 °C 1 min, 72 °C 90s) x 35, 72 °C 5 min
		rP1	ACGGTTACCTTGTTACGACTT	0.5 µM	
NDP 2	Plum pox virus RT-qPCR (Schneider *et al.* 2004)	PPV-qF	CCAATAAAGCCATTGTTGGATC	0.5 µM	48 °C 30 min, 94 °C 5 mins, (94 °C 10s, 60 °C 45s) x40
		PPV-qR	TGAATTCCATACCTTGGCATGT	0.5 µM	
		PPV-qP	[6FAM]-CTTCAGCCACGTTACTGAAATGTGCCA-[TAMRA]	0.25 µM	
NDP 17	Phytoplasma Rd 1 (Deng and Hiruki 1991)	P1	AAGAGTTTGATCCTGGCTCAGGATT	0.5 µM	95 °C 1 min, (95 °C 1 min, 56 °C 1 min, 72 °C 90s) x 35, 72 °C 5 min
		P7	CGTCCTTCATCGGCTCTT	0.5 µM	
	Phytoplasma Rd 2	R16F2n	GAAACGACTGCTAAGACTGG	0.5 µM	95 °C 1 min, (95 °C 1 min, 56 °C 1 min, 72 °C 90s) x 35, 72 °C 5 min
	(Schneider *et al.* 1995).	R16R2	TGACGGGCGGTGTGTACAAACCCCG	0.5 µM	Expected size: 1.6Kb
Draft NDP	Begomoviruses	Homer	CCNMRDGGHTGTGARGGNCC	0.5 µM	94 °C 1 min, (94 °C 30s, 55 °C 30s, 72 °C 30s) x 35, 72 °C 10 min
	(Revill *et al.* 2003)	Krusty	SVDGCRTGVGTRCANGCCAT	0.5 µM	Expected size: 580bp
N/A	Tobamoviruses	F-5476	GAAGAAGTTGTTGATGAGTTCAT	0.5 µM	48˚C for 45 min, 1 cycle 94˚C for 2 min, 40 cycles 94˚C for 45s, 60 for 45s, 72˚C for 45s, 1 cycle of 72˚C for 10 min.
	(Levitzky *et al.* 2019)	R-6287	GATTTAAGTGGAGGGAAAAACAC	0.5 µM	Expected size: 811bp

N/A	Potyviruses (Zheng *et al.* 2010)	NIB2F	GTITGYGTIGAYGAYTTYAAYAA	0.5 µM	48˚C for 45 min, 1 cycle 94˚C for 2 min, 40 cycles 94˚C for 45s, 45 for 45s, 72˚C for 45s, 1 cycle of 72˚C for 5 min.
		NIB3RN	TCIACIACIGTIGAIGGYTGNCC	0.5 µM	Expected size: 350bp
N/A	Closteroviridae Rd1	dHSP up1	GGIHTIGAITTYGGIACIACITT	1 µM	48˚C for 30 min, 1 cycle 50˚C for 2 min, 1 cycle 94˚C for 4 min, 5 cycles (94˚C for 30s, 43 for 10s, 38˚C for 5s, 72˚C for 15s), 35 cycles (94˚C for 30s, 43 for 10s, 72˚C for 30s) 1 cycle of 72˚C for 2 min.
	(Dovas & Katis, 2003)	dHSP up1G	AGTTYGGGACGACGTT	0.4 µM	
		dHSP do2	GTICCICCICCNAARTC	0.5 µM	
		dHSP do2C	GTICCICCCCCNAARTC	0.5 µM	
	Closteroviridae Rd2	dHSP nest1	TTYGGGACGACGTTYAGYAC	1 µM	94˚C for 3 min, 1 cycle 48˚C for 15s, 1 cycle 72˚C for 15s, 39 cycles (94˚C for 30s, 54 for 30s, 72˚C for 10s), 1 cycle of 72˚C for 2 min.
	(Dovas & Katis, 2003)	dHSP nest2	TYGGGACGACGTTYTCNAC	1 µM	Expected size: 500-535bp
		dHSP nest3	SCIGCIGMISWIGGYTCRTT	1 µM	
		dHSP nest3G	GCGGMGSWGGGNTCRTT	1 µM	
N/A	Pospiviroid (Verhoeven *et al.* 2004)	Pospi-FW	GGGATCCCCGGGGAAAC	0.5 µM	48˚C for 45 min, 1 cycle 94˚C for 2 min, 15 cycles of 94˚C for 30 sec, 62 for 90 sec, 72˚C for 45 sec, 30 cycles of 94˚C for 30 sec, 59 for 90 sec, 72˚C for 45 sec, 1 cycle of 72˚C for 10 min Expected product size: 200 bp

Results

RT-PCR and PCR screening

The total nucleic acid of the 107 samples collected during the summer sampling passed the initial QC step for suitableinty in the RNA and DNA based asays. All samples were tested for plum pox virus (PPV), phytoplasma, tobamoviruses, potyviruses, viruses in the family *Closterovirirdae*, begomoviruses and pospivoids, even if they were not a known host. The molecular results showed viruses endemic to the Australian mainland such as tomato mosaic virus and tomato chlorotic dwarf viroid were present in a few samples (Table 2). No exotic viruses that are Australian National Plant Priority Pests were detected, including plum pox virus and exotic tobamoviruses (Table 2).

The samples collected during spring were not test using PCR and RT-PCR assays for viruses or virus-like organism.

High throughput sequencing

Summer sampling

Seventy-six samples were selected for metagenomic HTS because their RNA was of sufficient quality for sequencing. Viruses snd vioids were detected in 30/76 samples and multiple viruses were detected in 7/30 samples (Table 3). Three of the viruses were repored in the previous 2012-2014 survey (Maynard et al 2018). The remaining 22 virus or viroid species that were found on Norfolk Island by HTS in the summer samples are known to occur in Australia. Southern tomato virus (STV) was not previously reported on Norfolk Island nor on mainland Australia.

Spring sampling

The HTS results of the 91 virology samples from the spring surveillance confirmed the presence of STV (Sample 5) on Norfolk Island (Table 4). Viruses or viroids were detected in 27/91 samples that were tested by HTS. Six virus species were recorded in the 2012-2014 Norfolk Island survey, and two of these six viruses were also found in the summer survey samples. There were 22 viruses or viroids that had not been recorded in the 2012-2014 survey and 7/16 had also not been reported from the summer survey. A summary of virus detections is detailed in table 4.

Discussion

The specific PCR testing and HTS analyses provided evidence of absence on Norfolk Island for viruses occurring on the top 42 list of National Plant Priority Pests, including plum pox virus and exotic tobamoviruses and begomoviruses.

HTS detected a total of two viroids and 37 virus species from 29 virus genera across the summer and spring samples. There were 35 virus species that are endemic to the Australian mainland and one virus that was not previously reported in Australia or on Norfolk Island: STV in summer sample 106. STV is a cryptic virus which is transmitted through seed, has not directly been associated with any symptoms in tomato plants and is not known to infect other plant species (Gonzalez et.al 2021), therefore this virus is not considered to be a significant threat.

In the two surveys in 2022, there were 31 virus species that were detected on Norfolk Island that are endemic to mainland Australia but have not been detected during previous Norfolk Island surveys (by Maynard *et al*. 2018). Several of these species are primarily transmitted though vegetative propagation or grafting, such as many of the viruses detected in the citrus, pome and stone fruit sampels, and may have been introduced with the planting material used to produce the plants. It is likely that these viruses have been present on Norfolk Island for some time.
Several viruses, such as ToMV, maybe seed borne and transmitted mechanically and therefore could have been introduced by seed followed by subsequent spread from plant to plant through contact or on equipment and hands. Tobamoviruses such as ToMV may persist in the environment on alternative hosts or debris in soil, which can lead to infections in subsequent crops. Therefore, these virus species could represent new virus introductions, or they may have been present on the island but not detected by the diagnostic tools used in previous surveys.

HTS is a sensitive and unbiased diagnostic tool and the application of HTS for plant virus surveillance Norfolk Island enabled an extensive in-depth characterisation and detection of viruses that could not have been easily achieved using more traditional virology disagnostic tools such as electron microscopy, serological assays and PCR. The additional endemic and exotic virus species detected by this Norfolk Island survey are not part of the NPPP list and their impact on Norfolk Island plants should not be a cause of concern.

Plants cannot be cured once infected by a virus and virus disease management is required to prevent further spread to uninfected plants. Management of plant viruses require the application of three components: exclusion/prevention, reduction of virus sources, and protection of host plant. For exclusion/prevention, crops should be cultivated in areas where there has been low disease incidence or during periods when the virus or its vector such as aphids, thrips, mealybugs etc, are at a low activity level. Using certified healthy and virus free plants also prevents introduction of viruses into new crops. Control of weeds and other virus hosts and insect vectors, destroying old crops promptly, and separating new crops from maturing crops, are critical management strategies for reducing virus sources. Chemical or biological control for insect vectors that can transmit plant viruses is also an important management strategy to protect crops from insect vectored viruses.

Table 2. The 107 samples, collected during the Norfolk Island summer 2022 survey that were tested for virus, viroids and phytoplasma using PCR or RT-PCR molecular testing in summer 2022. A'-' indicates that the pathogens was not detected. AIMV= Alstroemeria mosaic virus; ToMV= Tomato mosaic virus; PMMoV= Pepper mild mottle virus and TCDVd= Tomato chlorotic dwarf viroid.

ID	Host Plant	Notes	Plum pox virus (PPV)	Phytoplasma	*Tobamovirus*	*Potyvirus*	*Closteroviridae*	*Begomovirus*	*Pospiviridae*
101	Pumpkin	Mottling, spots, necrosis	-	-	-	-	-	-	-
102	*Physalis*	Yellowing, mottling	-	-	-	-	-	-	TCDVd (Pospiviroid +ve)
103	Weed? (unknown)	Distorted, yellowing leaves	-	-	-	-	-	-	-
104	Celery	Mottle (virus) necrosis (fungal)	-	-	-	-	-	-	-
105	*Prunus*	Leaf spots, yellowing (mottle), shot hole (related to leaf spots)	-	-	-	-	-	-	-
106	Tomato	Leaf curling, distortion, necrosis	-	-	-	-	-	-	-
107	Sweet potato	Necrosis along veins	-	-	-	-	-	-	-
110	Sweet potato	Mottle	-	-	-	-	-	-	-
111	Grapefruit	Yellowing	-	-	-	-	-	-	(Pospiviroid +ve)
112	Peach	Mottle	-	-	-	-	-	-	-
116	Weed (Astercaceae)	New leaves slightly distorted	-	-	-	-	-	-	-
117	Carrot	Leaf and stem lesions	-	-	-	-	-	-	-
119	Norfolk lemon	No symptoms	-	-	-	-	-	-	-
120	Avocado (Hass)	Leaf mottle	-	-	-	-	-	-	-
122	Watermelon	Crinkle, mottle	-	-	-	-	-	-	-
123	Grapevine (grown from seed)	Mottle	-	-	-	-	-	-	-
125	Watermelon	No symptoms, may be yellowing	-	-	-	-	-	-	-
128	Celery	Yellowing, leaf spots	-	-	-	-	-	-	-
132	Macadamia	Leaf scorch	-	-	-	-	-	-	-
134	Plum	No symptoms, mottle?	-	-	-	-	-	-	-
138	Toadstool, uknowm species	No symptoms	-	-	-	-	-	-	-
140	Tomato	Distorted leaves, mottling, necrosis	-	-	ToMV	-	-	-	-
141	Edible mushroom	No symptoms	-	-	-	-	-	-	-
142	French Alfalfa	Mottle	-	-	-	-	-	-	-

144	China Pear	NBacterial spots	-	-	-	-	-	-	-
145	Grape (var. Isabel, black grape)	No symptoms	-	-	-	-	-	-	-
147	Tomato	Small leaves, purpling, some yellowing	-	-	ToMV	-	-	-	-
148	Prunus	Leafspots, mottle	-	-	-	-	-	-	-
149	Prunus	Mottle, yellowing	-	-	-	-	-	-	-
150	Lily sp.	Mottle	-	-	-	AlMV	-	-	-
203	Mandarin	Mottling	-	-	-	-	-	-	-
204	Capsicum	Mottling (near Citrus)	-	-	-	-	-	-	-
209	Unknown	Mottling, yellowing, had green leaf hoppers	-	-	-	-	-	-	-
210	Potato (10 plants)	No symptoms	-	-	-	-	-	-	-
212	Stone fruit	No symptoms	-	-	-	-	-	-	-
214	Leek	Yellowing and spots	-	-	-	-	-	-	-
215	Potato	No symptoms	-	-	-	-	-	-	-
217	Tomato	No symptoms	-	-	-	-	-	-	-
219	Bush lemon	Mottling, yellowing	-	-	-	-	-	-	-
221	Tomato self sown	Leafspot	-	-	-	-	-	-	-
223	Amaranthus	Mild mottle	-	-	-	-	-	-	-
225	Hibiscus	Mottling	-	-	-	-	-	-	-
227	Yam	Bacterial spot	-	-	-	-	-	-	-
229	Potato	Swollen nodes	-	-	-	-	-	-	TCDVd (Pospiviroid +ve)
233	Tomato	Little leaf	-	-	-	-	-	-	-
234	Potato	Swollen nodes	-	-	-	-	-	-	-
236	Agave	Bacterial lesion	-	-	-	-	-	-	-
240	Fig	Mottle	-	-	-	-	-	-	-
242	Melon	Mild mottle	-	-	-	-	-	-	-
243	Navel orange	Mild mottle	-	-	-	-	-	-	-
244	Commelina	Mottling	-	-	PMMoV	-	-	-	-
245	Very old tomato	Slight vascular discolouration	-	-	-	-	-	-	-
246	*Prunus*	Yellowing along veins, mottle	-	-	-	-	-	-	-

250	Peach	Virus like symptoms	-	-	-	-	-	-	-
401	Apple	Check Fire blight	-	-	-	-	-	-	-
402	Good apple	No symptoms	-	-	-	-	-	-	-
404	Peach golden queen	Shot hole and leaf crinkle	-	-	-	-	-	-	-
405	Capsicum	Leaf distortion, yellowing, mottle	-	-	-	-	-	-	-
406	Nectarine	Shot hole and virus-like symptoms on leaves	-	-	-	-	-	-	-
407	Commelina	Virus glike symptoms	-	-	-	-	-	-	-
409	Plum	Old historic tree	-	-	-	-	-	-	-
410	Native grass	Leaf lesions	-	-	-	-	-	-	-
411	Tobacco	Suspect virus symptom	-	-	-	-	-	-	-
412	Peach	Crinkle and vein banding	-	-	-	-	-	-	-
413	Coleus	Virus symptoms, interveinal chlorosis, mosaic, crinkle	-	-	-	-	-	-	-
414	Satsuma plum	Bacterial leafspot/shot hole symptoms	-	-	-	-	-	-	-
415	Rockmelon	Spots	-	-	-	-	-	-	-
416	Pear	No symptoms	-	-	-	-	-	-	-
418	Citrus, old lemon	Yellowing	-	-	-	-	-	-	-
419	"Bluebell" -like plant with "lantern" flower	Bacteria symptoms	-	-	-	-	-	-	-
420	Tropical clover	White clover mosaic virus like symptoms	-	-	-	-	-	-	-
421	Lantana sp	Leaf spot	-	-	-	-	-	-	-
422	Amaranthaceae	Lesions on leaves	-	-	-	-	-	-	-
423	Hibiscus	Not recorded	-	-	-	-	-	-	-
425	Native Oleander - Pittosporum	Xylella-like symptoms	-	-	-	-	-	-	-
426	Apple	Apple mosaic symptoms	-	-	-	-	-	-	-
427	Sweet potato like vine	Young leaves with mosaic like symptoms	-	-	-	-	-	-	-
428	Iron wood	Interveined chlorosis on leaves of lower branches	-	-	-	-	-	-	-
429	Tropical grass	Leaf lesions	-	-	-	-	-	-	-
430	Lime tree	Lime/branch die back	-	-	-	-	-	-	-
431	Broad leaf weed	Classic leaf lesion	-	-	-	-	-	-	-

432	Leaves from a brassica plant	Nobacto symptoms on veggie, despite wet season	-	-	-	-	-	-	-
433	Peach	Classic shot hole	-	-	-	-	-	-	-
434	Prunus tree (likely peach)	Shot hole	-	-	-	-	-	-	-
435	Apple	Fireblight-like symptoms	-	-	-	-	-	-	-
436	Tree – unknwon species	Leaf spot, leaf crinkle, leaf senescence	-	-	-	-	-	-	-
437	Citrus	Ring spot on leaves,	-	-	-	-	-	-	-
438	Old Citrus - Mandarin	Yellow new growth, zig zag and possible spots on fruits	-	-	-	-	-	-	-
439	Tomato	Bacterial wilt, but not classic	-	-	-	-	-	-	-
441	Apple	Tip dieback, No classic firblights	-	-	-	-	-	-	-
443	Tomato	Back leaf spot	-	-	-	-	-	-	-
445	Peach	Classic shot hole	-	-	-	-	-	-	-
448	Commelina	Wet lesions on leaf	-	-	-	-	-	-	-
449	Grapevine	Descended from Pitcairnie immigration	-	-	-	-	-	-	-
450	Grapevine	Possible GLRV symptoms	-	-	-	-	-	-	-
470	Pear	Witches' broom	-	-	-	-	-	-	-
476	Chilli	Leaf yellowing	-	-	-	-	-	-	-
477	Grape - golden	No symptoms	-	-	-	-	-	-	-
478	Asteraceae weed ?	Crinkle, green islands, mottle	-	-	-	-	-	-	-
480	Banana - 18 months	Stunted	-	-	-	-	-	-	-
482	Strawberry	Small fruit not productive	-	-	-	-	-	-	-
483	Orange	Yellowing leaves	-	-	-	-	-	-	-
488	Pepino	Virus like growth	-	-	-	-	-	-	-
489	Banana	Weak growth, yellowing	-	-	-	-	-	-	-
492	Lemon	Spotting, curling, yellowing	-	-	-	-	-	-	-
494	Norfolk Island pine	Yellowing	-	-	-	-	-	-	-
499	Potato	Yellowing, poor growth	-	-	-	-	-	-	-

Table 3: Summary of the plant virus sequences that were detected in the summer 2022 virology survey samples using high throughput sequencing. Viruses indicated in red are pathogens that have not been previously report in Australia, including Norfolk Isalnd. Virus species detected on previous Norfolk Island surveys (Maynard *et al*. 2018) are in green font. N/A indicates no viruses were detected through HTS. The blast identity indicates the nucleotide (nt) similarity to the closest matching strain of the same virus species.

Sample number	Host Plant (common name)	Notes / symptoms	Virus species detected	Genus	Blast identity (percentage (%) nt similarity)	Largest contig	Genome completeness (% genome coverage)
NI.101	Pumpkin	Mottling, spots, necrosis	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.102	*Physalis*	Yellowing and mottling	Dahlia mosaic virus	Caulimovirus	80%	1290	Several partials
NI.103	Weed (unknown)	Distorted, yellowing leaves	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.104	Celery	Mottle (virus) necrosis (fungal)	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.105	*Prunus*	leaf spots, yellowing (mottle), shot hole (related to leaf spots)	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.106	Tomato	Leaf curling, distortion, necrosis	Southern tomato virus	*Amalgavirus*	99%	3432	Full genome
			Tomato matilda virus	*Tomavirus*	98%	8560	Full genome
NI.110	Sweet potato	Mottle, mosaic	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.111	Grapefruit	Yellowing test for liberibacter, phyto, virus	Citrus tristeza virus	*Closterovirus*	99%	19254	Full genome
NI.112	Peach	Mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.116	Weed (*Asleaceae*)	Sonchus New leaves slightly distorted	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.119	Norfolk lemon	No symptoms	Citrus tristeza virus	*Closterovirus*	99%	19325	Full genome
NI.120	Avocado (Hass)	Leaf mottle					
NI.122	Watermelon	Crinkle, mottle	Cucumis melo cryptic virus	*Amalgavirus*	99%	1641 & 1723	Full genome
NI.125	*Physalis peruviana*	No symptoms, may be yellowing	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.128	Celery	Yellowing, leaf spots					
NI.134	Plum	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.138	Unknown species		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.140	Tomato	Distorted leaves, mottling, necrosis	Tomato matilda virus	*Tomavirus*	98%	8657	Full genome
			Tomato mosaic virus	*Tobamovirus*	99%	6504	Full genome

NI.141	Edible mushroom	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.142	French Alfalfa	Mottle	Bean common mosaic virus	*Potyvirus*	98%	10310	Full genome
NI.145	French Alfalfa	Mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.148	Grape (table)	No symptoms, black grape var. isabel	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.149	*Prunus*	Leafspots, mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.150	Lily (unknown sp.)	Mottle	Alstroemeria mosaic virus	*Potyvirus*	99%	9471	Full genome
NI.203	Mandarin	Mottling	Citrus tristeza virus	*Closterovirus*	99%	19507	Full genome
NI.204	Capsicum (near Citrus)	Stunted mostly discoloured leaves	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.209	*Hibiscus cannabinus*	Mottling, yellowing, had green leaf hoppers	Datura yellow vein virus	*Nucleorhabdovirus*	90%	5870	Several partials (75%)
NI.210	Potato (10 plants)	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.212	Stone fruit	Budwood for virus	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.214	leek	Yellow spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.215	Potato	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.217	Tomato	No vascular disease, bacto (leaf spot) + virus	Tomato fruit blotch virus	*Blunervirus*	98%	3285	Several partials (80%)
NI.219	Bush lemon	Mottling, yellowing	Citrus tristeza virus	*Closterovirus*	98%	13060	Full genome
NI.221	Tomato leaf	No symptom	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.223	*Amaranthus*	Mild mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.225	Hibiscus	Mottling	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.229	Potato	Swollen nodes	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.233	Tomato	Small leaves stunting Little leaf phyto, virus	Tomato matilda virus	*Tomavirus*	985	8661	Full genome
NI.240	Fig	Mottle	Fig mosaic virus	*Emaravirus*	95%	6994	Several partials (80%)
NI.242	Melon	Mild mottle	Watermelon crinkle leaf-associated virus 1	*Coguvirus*	995	1690	Several partials (90%)
NI.243	Navel orange (Citrus)	Mild mottle	Citrus tristeza virus	*Closterovirus*	99%	19503	Full genome
NI.244	Commelina	Mottling	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.246	*Prunus* species	Yellowing along veins, mottle	Plum bark necrosis stem pitting-associated virus	*Ampelovirus*	975	13608	Full genome

NI.250	Stone fruit	Peach test for virus	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.402	Good apple	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.404	Peach golden queen	Shot hole + Crinkle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.405	Capsicum	Leaf distortion, yellowing, mottle	Capsicum chlorosis virus	*Othotospovirus*	99%	8916	Full genome
NI.406	Nectarine	Shot hole and virus symptoms on leaves	Plum bark necrosis stem pitting-associated virus	*Ampelovirus*	985	9302	Partial
NI.407	Commelina	Virus generic ….	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.409	Plum	Old history	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.411	Tobacco	Suspect virus symptom	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.412	Peach	Crinkle and vein bonding	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.413	Coleus	Virus symptoms, interveinal chlorosis, mosaic, crinkle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.415	Rock Mellon	Spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.416	Pear	No symptoms	Citrus virus A	*Closterovirus*	98%	2273	Several partials (60%)
			Apple stem pitting virus	*Foveavirus*	86%	2421	Several partials (80%)
			Apple rubbery wood virus 2		99%	1440	Several partials (60%)
NI.420	Tropical clover	Mosaic - like symptoms	Bean common mosaic virus	*Potyvirus*	99%	10022	Full genome
NI.421	Lantana sp?	leaf spot	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.423	Hibiscus	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.426	Apple	Mosaic symptoms	Apple stem grooving virus	*Capillovirus*	98%	4963	Several partials (90%)
			Apple stem pitting virus	*Foveavirus*	86%	4940	Several partials (90%)
			Citrus concave gum-associated virus	*Coguvirus*	99%	4285	Several partials (90%)
			Apple rubbery wood virus 2	*Rubodvirus*	98%	3822	Several partials (60%)
			Apple green crinkle associated virus	*Foveavirus*	85%	2696	Several partials (60%)
			Apple chlorotic leaf spot virus	*Trichovirus*	93%	2348	Several partials (40%)
NI.427	Sweet potato like vine	Young leaves with mosaic like symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.428	Iron wood	Interveined chlorosis on leaves of lower branches	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

NI.449	Grapevine	Descended from Pitcarinie immigration	Grapevine red globe virus	*Maculavirus*	91%	2543	Several partials (90%)
			Hop stunt viroid	*Hostuviroid*	99%	424	Full genome
NI.450	Grapevine	Possible leaf roll symptoms	Grapevine yellow speckle viroid 1	*Apscaviroid*	99%	494	Full genome
			Hop stunt viroid	*Hostuviroid*	99%	428	Full genome
NI.470	Pear	Witches' broom	Citrus virus A	*Closterovirus*	98%	6914	Several partials (60%)
			Apple stem pitting virus	*Foveavirus*	87%	2097	Several partials (50%)
			Apple rubbery wood virus 2	*Rubodvirus*	99%	3776	Several partials (80%)
NI.476	Chilli	Leaf yellowing	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.477	Grape - golden	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.478	*Asteraceae* weed	Crinkle, green islands, mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.480	Banana - 18 month	Stunted, Reddening. Possislby abiotic	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.482	Strawberry	Small fruit not productive	Strawberry mottle virus	*Stramovirus*	83%	2538	Several partials (90%)
NI.483	Orange	Yellowing	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.488	Pepino	Virus like growth	Potato leafroll virus	*Polerovirus*	97%	2678	Several partials (90%)
NI.489	Banana	Weak growth, yellowing	Banana mild mosaic virus	*Betaflexiviridae*	96%	5916	Several partials (90%)
NI.492	Lemon	Spotting, curling, yellowing, galls underneath	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.494	Unknown	Yellowing	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
NI.499	Potato	Yellowing, poor growth	Potato virus X	*Potexvirus*	97%	6644	Full genome

Table 4: Summary of the plant virus sequences that were detected spring 2022 virology survey samples using high throughput sequencing. Viruses indicated in red are pathogens that have not been previously report in Australia, including Norfolk Island. Virus species detected on previous Norfolk Island surveys (Maynard *et al.* 2018) are in green font. N/A indicates no viruses were detected through HTS. The blast identity indicates the nucleotide (nt) similarity to the closest matching strain of the same virus species.

Sample ID	**Host Plant**	**Symptoms**	**Virus species detected**	**Genus**	**Blast identity (percentage (%) nt similarity)**	**Largest contig (nt)**	**Genome completeness (% genome coverage)**

1	Potato	Crinkling	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
2	Potato	Crinkling	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
3	Pumpkin (local hybrid)	Yellow mosaic	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
4	Pumpkin (butternut cross)	Grey spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
5	Tomato	Stunted/dead	Southern tomato virus	*Amalgavirus*	99%	2173	Partial (80%)
			Tobacco vein clearing virus	*Solendovirus*	80%	1660	Several Partials (80%)
6	Tomato	Stunted	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
7	Avocado	Spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
8	Lilly	Spots, streak	Chickpea chlorotic dwarf	*Mastrevirus*	87%	1060	Severa Partials (60%)
9	Lilly	Stripe, spotty	Alstroemeria mosaic virus	*Potyvirus*	99%	9770	Full genome
10	Lemon	Pimples/indents on fruit and leaves	Citrus tristeza virus	*Closterovirus*	99%	19254	Full genome
11	Dianella	Yellow spots, necrotic lesions	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
12	Pumpkin	Necrotic spots / lesions	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
13	Sweet Potato	Distortion, epinasty - insect pupae present	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
14	Watermelon	Mild mottle, marginal chlorosis / spotting	Watermelon crinkle leaf-associated virus 2	*Coguvirus*	99%	4294	Several partials (80%)
15	Potato	Leaf curl	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
16	Pumpkin	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
17	Norfolk Lemon	No symptoms	Citrus tristeza virus	*Closterovirus*	99%	19214	Full genome
18	3rd Citrus @ house	No symptoms	Citrus tristeza virus	*Closterovirus*	99%	17539	Full genome
19	Grapefruit - row furthest from house	No symptoms	Citrus tristeza virus	*Closterovirus*	99%	10320	Several Partials (90%)

20	Parsely	Distortion, interveinal chlorosis	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
21	Chilli / capsicum	Faint mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
22	Beans	Leaf hopper feeding	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
23	Tobacco bush	Mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
24	Pear - near house	Leaves	Citrus virus A	*Closterovirus*	98%	3905	Several partials
			Apple stem pitting virus	*Foveavirus*	86%	10202	Full genome
			Apple stem grooving virus	*Capillovirus*	98%	2464	Several partials (80%)
			Apple rubbery wood virus 2	*Rubodvirus*	99%	1440	Several partials (90%)
25	Pear - near house	Stem	Citrus virus A	*Closterovirus*	98%	6667	Full genome
			Apple stem pitting virus	*Foveavirus*	88%	9241	Full genome
			Apple stem grooving virus	*Capillovirus*	98%	1828	Several partials (60%)
			Apple rubbery wood virus 2	*Rubodvirus*	99%	6936	Full genome
26	Lemons	Stipple mottle distortion	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
27	Nectarine	Shothole	Plum bark necrosis stem pitting-associated virus	*Ampelovirus*	97%	8688	Several partials (90%)
28	Grapevine	Leaves - mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
29	Grapevine	Stem	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
30	Giant Garlic	Leaf streak / break	Leek yellow stripe virus	*Potyvirus*	94%	10257	Full genome
			Garlic latent virus	*Carlavirus*	97%	8519	Full genome

			Garlic common latent virus	*Carlavirus*	97%	7911	Full genome
31	Grapevine	Mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
32	Pawpaw / papaya	Mottle / distortion /scorch	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
33	Tomato	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
34	Potato	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
35	Chilli /nightshade	Stunt / yellowing and distortion	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
36	Bush tobacco	Vein netting / mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
37	Strawberry	Slight edge chlorosis	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
38	Nectarine	Mottle	Plum bark necrosis stem pitting-associated virus	*Ampelovirus*	97%	14180	Full genome
39	Self-seed tomato - cherry	Upward leaf curl, chlorotic break	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
40	Self-seed tomato - yellow cherry	Distortion. Mutant	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
41	Corn	Streak / break / chlorotic	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
42	Potato	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
43	Potato	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

44	Cabbage	Chlorotic spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
45	*Physalis*	Stunt / yellowing and distortion	Cucumber mosaic virus	*Cucomovirus*	99%	3600	Full genome
46	Pumpkin	Mottle	Cucumber mosaic virus	*Cucomovirus*	99%	3460	Full genome
47	Lettuce	Necrosis / necrotic spots	Lettuce mosaic virus	*Potyvirus*	99%	10184	Full genome
48	Broccoli	Mottle, break, distortion	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
49	Carrot	Yellow spots, mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
50	Capsicum / chilli	Distortion, chlorosis	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
51	*Physalis*	Green vein netting	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
52	Self-seed tomato	Leaf mottle	Tomato mosaic virus	*Tobamovirus*	99%	6383	Full genome
53	Grapevine - row 2	Red blotch & spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
54	Grapevine	Red leaf	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
55	*Potatoes* -front of property	Small, stunt and Distort.	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
56	Physalis- next to potatoes	Yellow mottle, distortion	Alfalfa mosaic virus	*Alfamovirus*	99%	3870	Full genome
57	Tomatoes near house	Epinasty	Tomato matilda virus	*Tomavirus*	98%	8690	Full genome

58	Lemon	Distorted leaves, poor growth	Citrus tristeza virus	*Closterovirus*	99%	7864	Several Partials (90%)
59	Citrus	Chlorosis	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
60	Tomatoes	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
61	Plum	No symptoms	Apricot vein clearing associated virus	*Prunevirus*	98%	8344	Full genome
			Prunus necrotic ringspot virus	*Ilarvirus*	99%	3317	Full genome
			Apple chlorotic leaf spot virus	*Trichovirus*	91%	3116	Several partials (80%)
62	Plum	No symptoms	Apricot vein clearing associated virus	*Prunevirus*	98%	5881	Several partials (80%)
			Prunus necrotic ringspot virus	*Ilarvirus*	99%	3007	Full genome
			Apple chlorotic leaf spot virus	*Trichovirus*	91%	7013	Full genome
63	Pumpkin	Mottle / blotch	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
64	Broad bean	Leaf mottle mosaic	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
65	Chilli / capsicum	Mosaic / variegation	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
66	Chilli / capsicum	Blotch / yellow	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
67	Grapevine	Leaf sample - mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

68	Grapevine	Cane sample	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
69	Packham pear near house	No symptoms	Apple stem pitting virus	*Foveavirus*	88%	9262	Full genome
			Apple chlorotic leaf spot virus	*Trichovirus*	91%	2577	Several partials (80%)
70	Weed *solanaceous nightshade*	Chlorotic patterns	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
71	Native Lemon	Strange shoot tip	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
72	Potato - second row from bottom	Distorted / stunt	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
73	Potatoes	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
74	Potatoes	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
75	Potatoes	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
76	Grapevine	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
77	Old grapevine in courtyard	No symptoms	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
78	Geranium at driveway entrance	Mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
79	Old grapevine in courtyard	No symptoms	Hop stunt viroid	*Hostuviroid*	100%	425	Full genome
80	Squash	Mottle	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
81	Potatoes - back paddock	Random sample	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
82	Potato	Chlorotic - surrounded by healthy	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
83	Potato in beans	Leaf hopper and aphids	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
84	Apple - front near avos	Unhealthy appearance	Apple stem pitting virus	*Foveavirus*	88%	1726	Several partials (60%)
85	Pear	Unhealthy appearance	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
86	Zucchini - front yard	Spots/ necrosis	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
87	Squash - front yard	Chlorotic spots	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
88	*Apiaceae* weed	Chlorotic break	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

89	Banana	Expressed concerned about health	Banana streak virus	*Badnavirus*	99%	7378	Full genome
90	Banana	Expressed concerned about health	Banana streak virus	*Badnavirus*	99%	7538	Full genome
			Sugarcane mild mosaic virus	*Potyvirus*	60%	13204	Full genome
91	Aster weed	Twisted distorted growth habit	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

Appendix F: Weeds report

Norfolk Island Plant Pest and Disease Survey – Weeds

Background and Introduction

The approach to the weed survey of Norfolk Island was to search for known priority weeds, defined as being recognised important weeds in Australia, that were also not previously known on Norfolk Island. This approach was selected to focus the survey on detection of new incursions, rather than providing a list of all known weeds on the island, which was beyond the scope of the project. Two resources were used to determine if the species were known from Norfolk Island. Firstly, existing herbarium records, downloaded from the Atlas of Living Australia on 9 March 2022. Secondly, the accepted list of indigenous and naturalised plants known to be present on the Norfolk Island group (Mills, 2010).

Materials and methods

Summer survey

The primary method for finding new weeds on Norfolk Island during the summer survey was interviews and discussions with people who had an interest in weeds or land management, followed by ground surveys in areas suggested by them to search for particular weeds. New or important weeds indicated by the interviewees were checked against the species known to be present on the island. Collections were made if they were new records, or were known from the island but did have a voucher specimen lodged at a herbarium. Plants that were sparely naturalised or of particular interest were also pressed.

Spring survey

The primary method for searching for new weeds on Norfolk Island during the spring survey was visual inspections of likely locations to find garden escapees, a very common way for weeds to naturalise in new locations. Likely locations included roadsides with areas where cars could pull of the road and parking areas at reserves, both are locations that allow easy dumping of garden waste.

Results

Seven new records of weeds or plants naturalised on Norfolk Island were made during the summer and spring surveys. Information is provided on them in the sections below, while location details for each new record are provided in Table 1.

Summer survey

Sixteen presses were made from the survey in summer 2022 (Table 1). The most important were four new records:

- Three were new records of naturalised species on Norfolk Island
  - Duckweed, *Lemna disperma*
  - Indian siris, *Albizia lebbeck*
  - Bitou bush*, Chrysanthemoides monilifera* var. *rotundata*
- One was the first herbarium press of a species previously recorded as naturalised by Mills (2010), but for which no herbarium voucher exists
  - Water cress, *Nasturtium officinale*

The remaining collections were made because:

- weed names were requested by islanders for which the identification could not be immediately determined
  - Hairy abutilon, *Abutilon grandifolium*
  - Twiggy Mullein, *Verbascum virgatum*
  - Polke-dot plant, *Hypoestes phyllostachya*
- the weed had a particularly small distribution on the island
  - Water poppy, *Hydrocleys nymphoides*

    - Marsh ludwigia, *Ludwigia palustrus*
    - Clove-strip, *Ludwigia peploides*
    - Dense waterweed, *Egeria densa*
- there was taxonomic confusion about the species of water fern present on the island.
    - Waterfern, *Azolla pinnata*
- they are known to be particularly troublesome weeds
    - Salvinia, *Salvinia molesta*
    - Water hyacinth, *Eichhornia crassipes*

Spring survey

Twenty-one presses were made from the survey in spring 2022 (Table 1). The most important were new records, or clarifications of new records:

- Five were new records of naturalised species on Norfolk Island
    - Agapanthus, *Agapanthus praecox*
    - Nelson's slime lily, *Albura nelsonii*
    - Bromeliad, *Billbergia pyramidalis*
    - Angel-wing begonia, *Begonia coccinea*
    - Ti (pink ornamental variety), *Cordyline fruticosa*
- One was the first herbarium press of a species previously recorded as naturalised by Mills (2010), but for which no herbarium voucher exists
    - Ivy-leaved Violet, *Viola hederacea*
- One was collected to resolve taxonomic confusion as there seemed to be two forms of *Potamogeton cheesmanii* present on the island.
    - Pondweed, *Potamogeton cheesemanii*

The remaining collections were made because:

- they were listed as recently naturalised (Mills 2010) and weren't widely established on the island
    - Gazania, *Gazania rigens*
    - Nasturtium, *Tropaelum majus*
- weed names were requested by islanders for which the identification could not be immediately determined
    - Maltese cockspur, *Centaurea melitensis*
    - Velvet pink, *Petrorhagia dubia*
    - Soybean, *Neontonia wightii*
    - Small broomrape, *Orobanche minor*
- the weed had a particularly small distribution on the island
    - Water poppy, *Hydrocleys nymphoides*

Table 1. Plant species collected and pressed from summer (March) and spring (November) 2022 weed survey of Norfolk Island. Species marked with an (*) refers to first herbarium presses, which have been recorded on Norfolk Island before but without voucher specimens. To add a voucher specimen is important in order to ensure that the identification is correct and/or can be confirmed in the future.

Common name	Genus	Species	Collecting number	Collecting notes	NEW Norfolk Record
Summer (March) 2022					
Water poppy	*Hydrocleys*	*nymphoides*	NI1		
Water cress	*Nasturtium*	*officinale*	NI2	First herbarium press, has been recorded on Norfolk Island but without voucher	*
Marsh ludwigia	*Ludwigia*	*palustrus*	NI3		
Clove-strip	*Ludwigia*	*peploides*	NI5		
Marsh ludwigia	*Ludwigia*	*palustrus*	NI6		
Water hyacinth	*Eichhornia*	*crassipes*	NI7		
Water cress	*Nasturtium*	*officinale*	NI8	First herbarium press, has been recorded on Norfolk Island but without voucher	*
Salvinia	*Salvinia*	*molesta*	NI9	Primary growth stage	
Salvinia	*Salvinia*	*molesta*	NI10	Tertiary growth stage	
Hairy Abutilon	*Abutilon*	*grandifolium*	NI11		
Twiggy Mullein, Green Mullein	*Verbascum*	*virgatum*	NI12		
Dense waterweed	*Egeria*	*densa*	NI13		
Waterfern	*Azolla*	*pinnata*	NI14		
Duckweed	*Lemna*	*disperma*	NI15	Not recorded on Norfolk Island before.	*
Albizia, Indian siris	*Albizia*	*lebbeck*	NI16	Not recorded on Norfolk Island before. Only known specimen on the island. Recorded as weedy in QLD and USA, native to northern Australia.	*

				No flowers or pods present (despite extensive search for pods on the ground below the tree in March and November); raised glands at base of leaf branchlets.	
Bitou bush	*Chrysanthemoides*	*Monilifera var. rotundata*	NI17	Not recorded on Norfolk Island before. Weed of National Significance.	*
Polke-dot plant; Pink speckles	*Hypoestes*	*phyllostachya*	NI20		
Spring (November) 2022					
Pondweed	*Potamogeton*	*cheesmanii*	NI21	One patch at this locality	
Pondweed	*Potamogeton*	*cheesmanii*	NI22	One patch at this locality	
Pondweed	*Potamogeton*	*?cheesmanii*	NI23	Larger and more robust than the Potamogeton cheesmanii in other waterbodies on Norfolk Island. Possibly a different species but not clear which, as taxonomy is not well resolved in Australia. Several patches at this locality.	
Agapanthus	*Agapanthus*	*praecox*	NI24	Single specimen at this locality. New record for Norfolk Island. Common in gardens but not observed natulised on the island.	*
Nelsons slime lily	*Albura*	*nelsonii*	NI25	Single specimen at this locality. New record for Norfolk Island.	*
Gazenia	*Gazenia*	*regens*	NI26	Sparely naturalised on island. Only noted here and Taylors Road, Burnt Pine.	
Maltese cockspur	*Centaurea*	*melitensis*	NI27	This patch is relatively new and has been sprayed for 2-3 years in an effort to control it. Local landholder considered it new to the island. Records indicate that it has been on the island since 1958. I also noted it in grazed pasture adjacent to Middlegate Road.	
Velvet pink	*Petrorhagia*	*dubia*	NI28		
Soybean	*Neontonia*	*wightii*	NI29	Noted by two local farmers to be relatively new to the island (very noticeable in last few years) and causing a large	

				problem, scrambling over fences, trees and ungrazed areas, including cropping areas. Locals call it serrato.	
Small broomrape	*Orobanche*	*minor*	NI30	Coastal grassland	
Ivy-leaved Violet	*Viola*	*hederacea*	NI31	Growing on roadside verge, garden nearby but seems naturlaised.	*
Nasturtium	*Tropaeolum*	*majus*	NI32	Growing on dune slope behind beach	
Bromeliad	*Billergia*	*pyramidalis*	NI33	Growing on pile of dumped soil and garden waste under thick Norfolk Pine canopy. Established and reproducing vegetatively. Several areas of dumped waste exist along this road, which leads to the rubbish tip.	*
Begonia	*Begonia*	*coccinea*	NI34	Growing under thick Norfolk Pine canopy. Several areas of dumped garden waste exist along this road, which leads to the rubbish tip.	*
Ti - pink ornamental varity.	*Cordyline*	*fruticosa*	NI35	Growing under thick Norfolk Pine canopy. Several areas of dumped garden waste exist along this road, which leads to the rubbish tip.	*
Water poppy	*Hydrocleys*	*nymphoides*	NI37	Aquatic. Growing in muddy substrate in pool of creek.	
Pondweed	*Potamogeton*	*?cheesmanii*	NI37a	Larger and more robust than the Potamogeton cheesmanii in other waterbodies on Norfolk Island. Possibly a different species but not clear which, as taxonomy is not well resolved in Australia. Aquatic. Growing in muddy substrate in pool of creek.	
Pondweed	*Potamogeton*	*cheesmanii*	NI38	Aquatic. Growing in muddy substrate in shallow water channel through wetland.	
Pondweed	*Potamogeton*	*cheesmanii*	NI39	Very abundant, covering extensive portion of the pond.	
Pondweed	*Potamogeton*	*?cheesmanii*	NI40	Abundant at this site.	
Pondweed	*Potamogeton*	*?cheesmanii*	NI41	Abundant at this site.	

Discussion

Commentary on new records for Norfolk Island

Of the seven new plant species collected from Norfolk Island as part of this project, bitou bush and agapanthus are known to be troublesome weeds in other parts of the world. Both of these species are limited to a single location on Norfolk Island, and it is prudent to attempt eradication of these naturalised populations. Of the remaining five new plant species, only Indian siris has weedy potential, although it seems that the single specimen present does not produce seed. More information on each species is provided below.

Bitou bush

The most important new weed species recorded on Norfolk Island was bitou bush (*Chrysanthemoides monilifera* subsp. *rotundata*). It is native to the coastal regions of South Africa. A patch ~30 m long by ~30 m wide was found on private property after its presence was alerted to us by the landholder. It had been known by the landholder for 4-5 years, but it had expanded rapidly since an overtopping Norfolk Pine had fallen.

Management of bitou bush has been occurring since its presence was reported at the time of the summer survey. This consisted of distributing a poster (Figure 1) and a media release, which aimed to raise awareness so that other unknown infestations may be found. Control work to remove the known infestation has also commenced. This management has been given urgency due to it being declared a Weed of National Significance in Australia, and the serious impact of bitou bush in coastal areas of New South Wales and Queensland (Winkler *et al*, 2008).

Agapanthus

African lily, Lily of the Nile, or agapanthus are all common names for the *Agapanthus praecox* subsp. *orientalis*, which was found naturalised at Point Ross Reserve (Figure 2). Although it is grown widely in gardens on Norfolk Island, it was not observed naturalised anywhere else during the surveys.

Agapanthus is native to southern Africa. Agapanthus is regarded as an environmental weed in Victoria, New South Wales, Tasmania, South Australia and Western Australia. Its dense clumping roots are capable of displacing ground cover species, and it may prevent establishment of trees and shrubs (Weeds Australia, 2016).

Natal lily

Natal lily, Nelson's lily, or Nelson's slime lily are all common names for *Albuca nelsonii* (synonym *Ornithogalum nelsonii*), which was found naturalised at Point Ross Reserve (Figure 3). It is a native of South Africa. Natal lily is sparsely naturalised in Australia, with records from a single locality in each of New South Wales and Victoria. It has a low weed risk ranking on Victoria's advisory list of environmental weeds (White *et al*, 2018). Natal lily is not known to be a troublesome weed.

Bromeliad

Bromeliad (*Billbergia pyramidalis*) was found growing alongside Ben Christian Drive, which leads to the Norfolk Island rubbish tip (Figure 4). It appeared that it had established from dumped garden waste, as piles of garden waste were evident at intervals along this roadside. Although bromeliads are grown widely in gardens on Norfolk Island, they were not observed naturalised anywhere else during the surveys. Bromeliads are not known to be troublesome weeds. *Billbergia pyramidalis* is native to south America.

Angel-wing begonia

Begonia (*Begonia coccinea*) was found growing alongside Ben Christian Drive, which leads to the Norfolk Island rubbish tip (Figure 5). Like the bromeliad, it appeared that begonia had established from dumped garden waste, as piles of garden waste were evident at intervals along this roadside. Angel-wing begonia is native to Brazil and is not known to be a troublesome weed.

Ti

A pink ornamental variety of ti or broadleaved palm lily (*Cordyline fruticosa*) was also found growing alongside Ben Christian Drive, which leads to the Norfolk Island rubbish tip (Figure 5). Like the bromeliad and begonia, it appeared to have established from dumped garden waste, as piles of garden waste were evident at intervals along this roadside. It is believed to have a very broad native tropical range, which includes northern Australia. Although it is grown widely in gardens on Norfolk Island, it was not observed naturalised anywhere else during the surveys.

Water cress

Water cress (*Nasturtium officinale* (synonym *Rorippa nasturtium-aquaticum*)) was found growing in in several watercourses on Norfolk Island, including Cockpit Creek at Cascade Reserve and the wetlands at Kingston (Figure 6). Although water cress has been recorded on the island before (Mills, 2010), no voucher specimens exist in herbaria, so a collection was made. Water cress is native to Europe and Asia but is widely naturalised globally, probably due to it being used as a salad vegetable.

Duckweed

Duckweed (*Lemna disperma*) was noted in slow moving waterbodies (
Figure 7 7). It is native to both New Zealand and Australia. Although this a new record for Norfolk Island (it is not recorded in Mills (2010), or as a herbarium voucher) it is highly likely that it has been present for a long time, possibly before European colonisation, and has simply been overlooked by previous botanists. Although duckweed plants are only very small (<8 mm), the floating plants are prolific and form dense green mats on the water surface.

Indian siris

A large mature Indian siris (*Albizia lebbeck*) tree was tentatively identified in the Hundred-Acre Reserve (Figure 8). It was growing among a Norfolk Pine forest and appears to be a solitary specimen on the island. No flowers or seed pods could be found in either survey so its identity cannot be confirmed with certainty. The lack of flowers and pods also suggests future spread is unlikely. *Albizia lebbeck* is recorded as weedy in QLD and USA, and it native to northern Australia (Weeds Australia, 2016).

Figure 1. LEFT: Poster about bitou bush prepared and distributed by Norfolk Island Regional Council. RIGHT: Bitou bush infestation on Norfolk Island, 18 March 2022.

Figure 2. Agapanthus at Point Ross Reserve, 1 November 2022.

Figure 3. Natal lily at Point Ross Reserve, 1 November 2022.

Figure 4. Bromeliads growing atop dumped garden waste, adjacent to Ben Christian Drive, 4 November 2022.

Figure 5. LEFT: Angel-wing begonia, adjacent to Ben Christian Drive, 4 November 2022. RIGHT: Ti, adjacent to Ben Christian Drive, 4 November 2022.

Figure 6. Water cress at Watermill Pond, 15 March 2022.

Figure 7. A range of aquatic plants at Watermill Pond, Kingston, 15 March 2022. Duckweed, light green in red; waterfern, most of the floating plants in the photo; salvinia, primary growth stage in blue; and water hyacinth leaf blades, note feeding scars from the biocontrol agent (*Neochetina* spp.) in orange.

Figure 8. Foliage of Indian siris (*Albizia lebbeck*), Hundred Acre Reserve, 17 March 2022.

Gaps in weed management on Norfolk Island

Although the purpose of the survey was to detect new weed species on Norfolk Island, discussions with land holders and public land managers made it clear that more needed to be done to better manage the existing, widespread weeds on the island, which are prolific and causing substantial impacts. Points that were raised to assist with this were:

1. The need for a more strategic, island-wide, weed management plan. There are weed plans in place, for example at Norfolk Island National Park and by the Norfolk Island Regional Council, indicating priority weeds and priority weed management. A process for bringing these plans together and including private

land holdings would ensure co-ordinated selection and management of priority weeds across Norfolk Island, or portions of it.

2. The need for a transparent weed prioritisation process to consider what species are the most important, what management interventions are the most useful, and how these vary by situation (i.e., different species, distribution, ecology). Such tools exist in NSW and Victoria and could be modified for use on Norfolk Island.

3. The need for more funding to undertake weed management. Norfolk Island does not have a clear regional government status, equivalent to Local Land Service areas in New South Wales or Catchment Management Authorities in Victoria, thus limiting the ability to access government weed and land management funding.

4. Clarification on the process for the release of biological control agents for weed control on Norfolk Island by the Australian Government. It is no clear if agents approved for release on the Australian mainland can be released onto Norfolk Island, or how people might go about doing this. Weeds of concern to Norfolk Islander are mistflower (*Ageratina riparia*) and madeira vine (*Anredera cordifolia*), both of which have biocontrol agents in Australia but not on Norfolk Island. In contrast, abundant scars from biocontrol agents (*Neochetina* spp.) were noted on water hyacinth leaf blades, indicating that this biocontrol agent has been released on Norfolk Island.

5. The need for more research into new biocontrol agents for Norfolk Island. A particular potential agent is the cedar tip moth (*Hypsipyla robusta*) which is native to Australia, where it attacks Australian red cedar (*Toona ciliata*), preventing commercial growth of this tree (Plant Health Australia, undated). Red cedar is considered a serious weed on Norfolk Island, the impact of which may be reduced by the cedar tip moth.

6. Access to an agronomist, or similar, that can provide land holders with weed and pest management advice. A range of weed, pest and agronomy experts have been engaged to undertake a range of projects in recent years. Better outcomes could be achieved when land holders have ongoing access to one or two experts who have knowledge from an agroecological zone similar to Norfolk Island.

7. Advice on herbicide label use and minor use permits for herbicides and pesticides applied on Norfolk Island. The Australian Pesticides and Veterinary Medicines Authority (APVMA) regulates pesticide (including herbicide) availability in Australia and use must comply with the product label. A permit can be issued by APVMA for off-label use, such as using it at a higher rate than specified on the label, applying it with different equipment to that specified on the label, or targeting a weed not listed on the label. Further, legal “off-label use” of pesticides varies between states and territories based on their regulations. It is not clear how these regulations relate to Norfolk Island.

Appendix G: References

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