Arbor Carbon *Creating Sustainable Ecosystems*

Investigation into the cause(s) of premature decline of Norfolk Island Pine

Town of Cottesloe

Report No. J20490

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REVISION SCHEDULE

Revision	Report Description	Submission Date	Author(s)
A	Norfolk Island Pine Decline	25/09/2020	Dr Harry Eslick Naviin Hardy Dr Paul Barber
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Executive Summary

ArborCarbon were engaged by the Town of Cottesloe to diagnose potential causes of decline of mature *Araucaria heterophylla* (Norfolk Island Pine) trees across the Town. Previous sampling from declining *A. heterophylla* in Cottesloe found a fungus morphologically consistent with the *Neofusicoccum* genus. Conclusive DNA identification was not undertaken.

ArborCarbon undertook visual tree and crown health assessments of a minimum of 20 trees chosen by the Town, and collected soil, root, trunk, stem and foliage samples from a subset of trees for pathology and nutrient analysis.

A range of pathogens were isolated from symptomatic trees, including but not limited to *Neofusicoccum parvum, N. australe, Phytopythium vexans, Pythium pachycaule and Pythium vanterpooli.* Additional analysis of DNA extracted from symptomatic roots and a remaining stump of a previously removed dead tree identified *Phytophthora multivora* and *P. ornamentata*. We also identified low levels of Mn, Fe and Zn in foliage, but these levels were observed in both healthy and chlorotic foliage. Cumulative rainfall recorded at the nearby Swanbourne weather station from January to May showed only 64mm in 2019, almost one-third of the average recorded during the same period since 1994. Average rainfall for the same period on Norfolk Island (origin of the species) was 537mm. The only other period since 1994 where a similar extremely low rainfall event occurred (65mm) was 2009, the same time previous sampling of declining *A. heterophylla* identified the presence of *N. parvum* associated with top-down decline of trees (Golzar & Burgess, 2011).

Our preliminary investigation suggests a prolonged period of very low rainfall from January to May, particularly during the period when the autumn flush of growth occurs (April-May) has triggered the onset of disease caused by the latent fungal pathogen *N. fusicoccum* and possibly others. This may also be exacerbated in trees through the loss of fine roots cause by putative root pathogens (i.e. Pythiaceous organisms). We make the following recommendations based on our findings:

1. DNA of *Phytophthora multivora* and *P. ornamentata* was sequenced and identified from lesioned roots of a number of trees, and stem material from a dead tree. The presence of DNA is not conclusive evidence that these organisms are living and causing the symptoms observed, but considering that they were extracted directly from the symptomatic material, and that symptoms observed are typical of *Phytophthora*, it is very possible that *Phytophthora* is playing a role in the decline observed.
2. The presence of necrotic lesions in the main stem originating from the upper crown caused by *Neofusicoccum parvum* are likely to be a cause of the decline in crown condition and death of the trees. However, the effectiveness of removal of large portions of the crown to halt the disease has not been proven and should be evaluated in an objective fashion along with other treatment options.
3. The maintenance of *A. heterophylla* in their current location will likely involve substantial effort and expense in the future, faced with a changing climate. Well designed and properly controlled management trials should be established to determine the effectiveness of a range of treatments at preventing *A. heterophylla* decline. Robust experimental design and statistical rigor is required when undertaking management trials to ensure the best outcome for the trees, ensuring council funds are not wasted on ineffective treatments. From this work we recommend the following treatments alone and in combination are worthy of further investigation:
    a. Supplemental irrigation across the late-summer autumn period,

   b. Systemic treatment with micronutrients including Mn and Zn,
   c. Systemic phosphonic acid (phosphite) and fungicide treatment,
   d. Removal of diseased crown and or diseased branches.
4. Outcomes from these trials should be used to inform a comprehensive management plan to manage the health of *A. heterophylla* throughout the Town.
5. Further work is required to identify the climatic and site conditions associated with this disease syndrome. Conditions can then be monitored and used to inform management action if the established thresholds are exceeded.
6. An objective benchmark of crown health of *A. heterophylla* throughout the town is required to monitor the future health of trees and measure response to any treatment. Remote sensing imagery provides an objective, quantitative and highly sensitive method to monitor the change in crown condition over time.
7. Remote sensing should be used to map the distribution and extent of the current disease outbreak to further examine spatial pattern of the disease outbreak, and identify possible environmental factors associated with disease expression.

Table of Contents

List of Figures

1 Introduction

ArborCarbon were engaged by the Town of Cottesloe to diagnose potential causes of decline of mature *Araucaria heterophylla* (Norfolk Island Pine) trees across the Town. Previous sampling from declining *A. heterophylla* in Cottesloe found a fungus morphologically consistent with the *Neofusicoccum* genus. Conclusive DNA identification was not undertaken.

The *Neofusicoccum* genus is a well-known group of plant pathogens, however these species are widely regarded as opportunistic pathogens. This means they are often found in association with healthy trees, and only result in disease expression in a stressed host. This study aims to conclusively identify the pathogen/s associated with declining *Araucaria heterophylla* within the Town of Cottesloe and examine the presence of other potential stressors (environmental and other pathogens) which may be involved, providing additional targets for management intervention.

1.1 Scope of Works

- Desktop analysis of images, reports and data on recent decline of up to 20 *A. heterophylla* throughout the Town
- Field assessment and sampling including ground-based and canopy level inspection and sample collection.
- Laboratory pathogen isolation and molecular identification of 5 soil/root samples and 5 aerial samples
- Laboratory pathogen isolation and molecular identification of 2 soil/root samples from stumps of previous mortalities
- Laboratory pathogen isolation and molecular identification of a further 2 samples from 20 Forrest St.
- Recommendations for future management

1.2 Additional sampling

The following analysis was conducted in addition to the quoted scope of works.

- Foliar nutrient sampling and analysis of 3 paired samples.
- An additional 1 laboratory soil and root sample
- An additional 1 laboratory sample from aerial symptoms.
- Collection and direct DNA extraction and identification from 3 root and 1 basal stem sample.

1.3 Ongoing analysis

- Broad spectrum DNA sequencing of all fungi is being conducted on several samples.

2 Background

Numerous reports of dieback in *Araucaria heterophylla* exist, going back as far as the 1970's. Benson (1980) reported on the decline of *A. heterophylla* in their native habitat of Norfolk Island. It was concluded that habitat degradation in the form of erosion, invasive species and pollution from human activity on the Island as well as adverse environmental conditions were the primary drivers of decline. Dowden and Lambert (1979) examined decline of *A. heterophylla* along the NSW coastline. They found decline was associated with high levels of salinity, exacerbated by environmental pollution of surfactants originating from marine sewer outlets.

In New-Zealand there are multiple reports of decline in *A. heterophylla* from as far back as 1998. Causes are believed to vary, including stem and root pathogens as well as herbicide damage. "Top-Down" decline matching the symptoms observed in Cottesloe has been observed throughout parts of the Bay of Plenty coast along with Tauranga and Auckland. A range of Botryosphaeriaceae pathogens were found in association with branch cankers, and often abnormally low annual rainfalls.

In NSW there are multiple reports of top-down decline dating back ~20 years, including isolations of *Neofusicoccum parvum*.

Neofusicoccum parvum [Botryosphaeriaceae] has been linked to decline in *A. heterophylla* in Perth as far back as 2009 (Golzar & Burgess, 2011). The species was frequently isolated from diseased tissue, and occasionally isolated from healthy tissue. A pathogenicity trial was conducted confirming pathogenicity towards *A. heterophylla*. This was the first scientific publication reporting decline and canker of *A. heterophylla* caused by *N. parvum* in Australia and worldwide. The authors concluded that "*High temperature and drought stress seem to be important factors predisposing Norfolk Island pine trees to N. parvum in Western Australia".*

Also, in WA, similar symptoms of decline were observed in Esperance in 2017. In response a range of treatments were applied including removal of the diseased portion of the tree crown. However, the resulting rate of survival and condition of trees receiving these treatments has not been objectively assessed.

Botryosphaeriaceae species including *N. parvum* are typically considered latent pathogens – they can exist within the host plant as endophytes, co-existing harmlessly with the plant, and disease symptoms only become apparent later in the plant's life, or if it becomes stressed (Golzar & Burgess, 2011, Sakalidis et al., 2013, Slippers and Wingfield 2007). Although, *N. parvum* is regarded as one of the most aggressive pathogens among the Botryosphaeriaceae (Slippers and Wingfield 2007).

Neofusicoccum parvum is found in association with a wide range of host species with a global distribution. However, only one record of *N. parvum* existed in Western Australia before it was found in association with declining *A. heterophylla*, suggesting it may be a recent import into Western Australia (Golzar & Burgess, 2011). ArborCarbon have since isolated and identified *N. parvum* using DNA techniques from a range of other host genera throughout Perth including *Banksia, Grevillea* and *Eucalyptus*.

Given *N. parvum* is considered a latent pathogen it is likely there are other factors triggering stress resulting in the onset of disease within trees. Possible candidates include:

- High temperature and drought stress associated with changing climate,

- Nutrient deficiencies due to changing soil and ground-water chemistry related to reducing rainfall,
- Soil-borne root pathogens.

3 Methods

ArborCarbon Managing Director (Dr Paul Barber), Senior Consultant (Dr Harry Eslick) and Consultant (Briony Williams) visited the Town of Cottesloe between 27th July 2020 and 10th September 2020. A ground-based visual tree assessment was conducted on 20 trees selected for inspection by the Town of Cottesloe (Figure 1). Details regarding the type and extent of symptoms were recorded as well as a below ground inspection of the superficial fine root system. Soil and root samples were collected from each tree and combined into 8 samples for laboratory isolation of plant pathogens.

The extent of decline was recorded by measuring the proportion of the crown length affected. The total crown length was recorded using a laser rangefinder (TruPulse) from the tree-top to the lowest primary branch. The length of 'healthy' crown was recorded from the lowest primary branch up to the typically distinct margin with the disease front, moving downwards from the top of the tree. This demarcation was defined as the point at which diseased branches and foliage were greater in proportion than healthy tissue (Figure 2).

On the 11th August 2020 five of the subject trees were selected for aerial inspection by a climbing arborist. These were trees 3, 5, 20, 24 and 25. From each tree, several primary branches and other samples of interest were removed and inspected at ground level by ArborCarbon consultants. Samples were grouped by the type of symptom observed into 7 samples which were analysed for the presence of fungal pathogens using sterile isolation techniques followed by DNA molecular analysis for the identification of pathogen species.

In addition, foliar samples were taken from paired healthy and symptomatic trees for foliar nutrient content analysis. Terminal branchlets were sampled from secondary or lower order branches in the lower canopy. Samples was taken from sunlit branches on the northern aspect. Paired samples were taken under similar conditions and positions within the tree to minimize variation between the samples other than the chlorotic symptoms.

Figure 1: Norfolk Island Pine tree locations.

Figure 2: Declining trees. Arrow indicates the margin between diseased and healthy tissue used to calculate the diseased crown ratio (42% and 19% for A and B, respectively)

4 Results and Discussion

4.1 Observations

The trees examined varied in the extent and severity of decline ranging from dead (Tree 17, Tree 11) to moderate branch-tip dieback without primary branch, or stem mortality (Tree 18). A summary of the details from each tree are provided in Table 1. Further description of the main symptoms observed is provided below.

Table 1: Norfolk Island Pine survey data

ID	Details		Photo
9	Site	Grant-Marine Playground	
	Sample ID	PAB 20-08, PAB 20-31	
	DBH (cm)	_	
	Crown Radius (m)	7	
	Height (m)	23.3	
	Diseased Crown Extent (%)	59%	
	Trunk Length (m)	3.3	
	Tip Dieback	moderate	
	Necrotic Roots	major	
	Chlorotic Foliage	N/A	
	Thinning Crown	major	
	Stem Lesion	minor	
	Branch Lesion		
	Comment	Tree has been treated with Bioprime soil drench. Branch tip dieback worse on south side. Relatively more fruits than others. Mower damage to roots.	

ID	Details		Photo
10	Site	Grant St	
	Sample ID	PAB 20-11, PAB 20-32	
	DBH (cm)	49	
	Crown Radius (m)	5	
	Height (m)	19.8	
	Diseased Crown Extent (%)	53%	
	Trunk Length (m)	2.3	
	Tip Dieback	moderate	
	Necrotic Roots	major	
	Chlorotic Foliage	moderate	
	Thinning Crown	moderate	
	Stem Lesion	minor	
	Branch Lesion	N/A	
	Comment	According to resident, severe decline over 2 months. Chlorotic decline at tips. Dieback worse on northern side of canopy. Non-irrigated weeds/lawn around tree. Tree in middle of the median strip.	
11	Site	2A Griver St	
	Sample ID	PAB 20-11	
	DBH (cm)	45	
	Crown Radius (m)	3	
	Height (m)	17.6	
	Diseased Crown Extent (%)	100%	
	Trunk Length (m)	2.4	
	Tip Dieback	N/A	
	Necrotic Roots	severe	
	Chlorotic Foliage	N/A	
	Thinning Crown	N/A	
	Stem Lesion	N/A	
	Branch Lesion	N/A	
	Comment	Soil highly organic. On front lawn, unlikely irrigated, yet maintained lawn. Some old bleeding on trunk, particularly north western side. Very few roots to sample.	

ID	Details		Photo
12	Site	Eric St	
	Sample ID	PAB 20-19	
	DBH (cm)	39	
	Crown Radius (m)	3.5	
	Height (m)	14.8	
	Diseased Crown Extent (%)	48%	
	Trunk Length (m)	2.2	
	Tip Dieback	moderate	
	Necrotic Roots	minor	
	Chlorotic Foliage	major	
	Thinning Crown	moderate	
	Stem Lesion	minor	
	Branch Lesion	N/A	
	Comment	On verge, non-irrigated, surrounded by weeds and some annual grass. Light coloured sandy soil.	
13	Site	Hammersley St	
	Sample ID	PAB 20-08	
	DBH (cm)	61	
	Crown Radius (m)	4.5	
	Height (m)	15.4	
	Diseased Crown Extent (%)	19%	
	Trunk Length (m)	1.9	
	Tip Dieback	minor	
	Necrotic Roots	minor	
	Chlorotic Foliage	minor	
	Thinning Crown	minor	
	Stem Lesion	N/A	
	Branch Lesion	N/A	
	Comment	On verge, manicured/irrigated lawn, difficult to sample. Living portion of tree generally healthy, top portion dead, stunted, with many fruits that did not reach maturity before limbs died.	

ID	Details		Photo
14	Site	Barchetta	
	Sample ID	PAB 20-08	
	DBH (cm)	42	
	Crown Radius (m)	4	
	Height (m)	14.7	
	Diseased Crown Extent (%)	78%	
	Trunk Length (m)	3.1	
	Tip Dieback	moderate	
	Necrotic Roots	N/A	
	Chlorotic Foliage	minor	
	Thinning Crown	major	
	Stem Lesion	absent	
	Branch Lesion	N/A	
	Comment	Next to beach, in sandy dune. Western side very few branches, likely affected by ocean wind. Recent clearing of vegetation and planting of small dune plants.	
15	Site	Marine Parade	
	Sample ID	PAB 20-19	
	DBH (cm)	57	
	Crown Radius (m)	5	
	Height (m)	18.3	
	Diseased Crown Extent (%)	25%	
	Trunk Length (m)	2.9	
	Tip Dieback	absent	
	Necrotic Roots	moderate	
	Chlorotic Foliage	minor	
	Thinning Crown	moderate	
	Stem Lesion	moderate	
	Branch Lesion	N/A	
	Comment	On beach front irrigated grassy area, turf right up to basal stem. Some old bleeding spots around trunk, one fresh. Dieback not from the tips but thinning throughout branches.	

ID	Details		Photo
16	Site	John St	
	Sample ID	PAB 20-19	
	DBH (cm)	102	
	Crown Radius (m)	7	
	Height (m)	28.9	
	Diseased Crown Extent (%)	11%	
	Trunk Length (m)	4.6	
	Tip Dieback	minor	
	Necrotic Roots	moderate	
	Chlorotic Foliage	absent	
	Thinning Crown	minor	
	Stem Lesion	absent	
	Branch Lesion	N/A	
	Comment	On reticulated front lawn, turf right up to base of tree. Dark green foliage, minor crown dieback in majority of tree. Major symptom appears to be unusual branching structure in top portion of tree, as if branches have died and resprouted multiple times.	
17	Site	Forest St	
	Sample ID	PAB 20-19, PAB 20-29, PAB 20-30, PAB 20-34	
	DBH (cm)	-	
	Crown Radius (m)	-	
	Height (m)	23	
	Live Crown Length (m)	-	
	Trunk Length (m)	-	
	Tip Dieback	-	
	Necrotic Roots	severe	
	Chlorotic Foliage	-	
	Thinning Crown	-	
	Stem Lesion	-	
	Branch Lesion	-	
	Comment	Completely dead. Council Verge – non-irrigated, mulched groundcover.	

ID	Details		Photo
18	Site	Napier St	
	Sample ID	PAB 20-19	
	DBH (cm)	111	
	Crown Radius (m)	9	
	Height (m)	30.2	
	Diseased Crown Extent (%)	24%	
	Trunk Length (m)	3.2	
	Tip Dieback	moderate	
	Necrotic Roots	minor	
	Chlorotic Foliage	absent	
	Thinning Crown	minor	
	Stem Lesion	minor	
	Branch Lesion	N/A	
	Comment	Front lawn likely irrigated, epicormic shoots from main stem near base. Likely historic dieback resprouting from the tips throughout the crown.	
19	Site	68 Broome St	
	Sample ID	PAB 20-20	
	DBH (cm)	69	
	Crown Radius (m)	4.5	
	Height (m)	23	
	Diseased Crown Extent (%)	22%	
	Trunk Length (m)	3.2	
	Tip Dieback	moderate	
	Necrotic Roots	moderate	
	Chlorotic Foliage	absent	
	Thinning Crown	moderate	
	Stem Lesion	absent	
	Branch Lesion	N/A	
	Comment	Patchy grass, visible limestone in soil.	

ID	Details		Photo
20	Site	50 Broome St	
	Sample ID	PAB 20-20	
	DBH (cm)	80	
	Crown Radius (m)	8	
	Height (m)	27.5	
	Diseased Crown Extent (%)	51%	
	Trunk Length (m)	4	
	Tip Dieback	major	
	Necrotic Roots	N/A	
	Chlorotic Foliage	major	
	Thinning Crown	severe	
	Stem Lesion	absent	
	Branch Lesion	N/A	
	Comment	On front verge, likely irrigated. Climbed and samples collected throughout crown. Rounds collected from branch that had suddenly died. Sapwood staining extending ~1m or more from trunk along branch. Bark discoloration with fruiting bodies present. Lesion extending along branch.	
21	Site	37 Broome St	
	Sample ID	PAB 20-20	
	DBH (cm)	82	
	Crown Radius (m)	6	
	Height (m)	22.5	
	Diseased Crown Extent (%)	31%	
	Trunk Length (m)	4.5	
	Tip Dieback	major	
	Necrotic Roots	absent	
	Chlorotic Foliage	minor	
	Thinning Crown	major	
	Stem Lesion	absent	
	Branch Lesion	N/A	
	Comment	Front verge, seasonal grass, non-irrigated.	

ID	Details		Photo
22	Site	14 Dean St	
	Sample ID	PAB 20-21	
	DBH (cm)	64	
	Crown Radius (m)	5.5	
	Height (m)	15.2	
	Diseased Crown Extent (%)	65%	
	Trunk Length (m)	3.9	
	Tip Dieback	N/A	
	Necrotic Roots	moderate	
	Chlorotic Foliage	minor	
	Thinning Crown	severe	
	Stem Lesion	minor	
	Branch Lesion	N/A	
	Comment	Previously injected with fungicide, top removed. On irrigated verge	
23	Site	3A Dean St	
	Sample ID	PAB 20-21, PAB 20-33	
	DBH (cm)	-	
	Crown Radius (m)	-	
	Height (m)	26.3	
	Diseased Crown Extent (%)	68%	
	Trunk Length (m)	4.2	
	Tip Dieback	major	
	Necrotic Roots	major	
	Chlorotic Foliage	minor	
	Thinning Crown	severe	
	Stem Lesion	absent	
	Branch Lesion	N/A	
	Comment	On irrigated front lawn.	

ID	Details		Photo
3	Site	Grant-Marine Playground	
	Sample ID	PAB 20-08	
	DBH (cm)	57	
	Crown Radius (m)	4.5	
	Height (m)	20.4	
	Diseased Crown Extent (%)	57%	
	Trunk Length (m)	3.3	
	Tip Dieback	minor	
	Necrotic Roots	major	
	Chlorotic Foliage	N/A	
	Thinning Crown	major	
	Stem Lesion	absent	
	Branch Lesion	absent	
	Comment	Lesions found on roots up to 8cm in diameter. Staining in central sapwood of branches extending a few inches from trunk. No discolouration in top portion.	
5	Site	Grant-Marine Playground	
	Sample ID	PAB 20-08, PAB 20-09	
	DBH (cm)	58	
	Crown Radius (m)	4	
	Height (m)	20.9	
	Diseased Crown Extent (%)	42%	
	Trunk Length (m)	3.1	
	Tip Dieback	minor	
	Necrotic Roots	moderate	
	Chlorotic Foliage	N/A	
	Thinning Crown	minor	
	Stem Lesion	minor	
	Branch Lesion		
	Comment	Discolouration of sapwood near trunk. Leaf dieback.	

4.2 Root Symptoms

Below ground examination of the superficial fine root system revealed a high frequency and abundance of necrotic roots. Necrotic roots were observed in 14 out of the 15 trees in which fine roots were exposed. Root lesions were also observed in the fine root system as well as extending into larger roots up to 8 cm in diameter (Figure 3). Stunted, nodular root growth was also observed on several trees (Figure 3). This often occurs as a symptom of root disease, resulting from progressive cycles of root dieback and re-growth.

Samples of soil and root material were taken from 20 trees including 2 tree stumps. These samples were aggregated into 8 samples which were tested for the presence of soil-borne pathogens via laboratory baiting techniques.

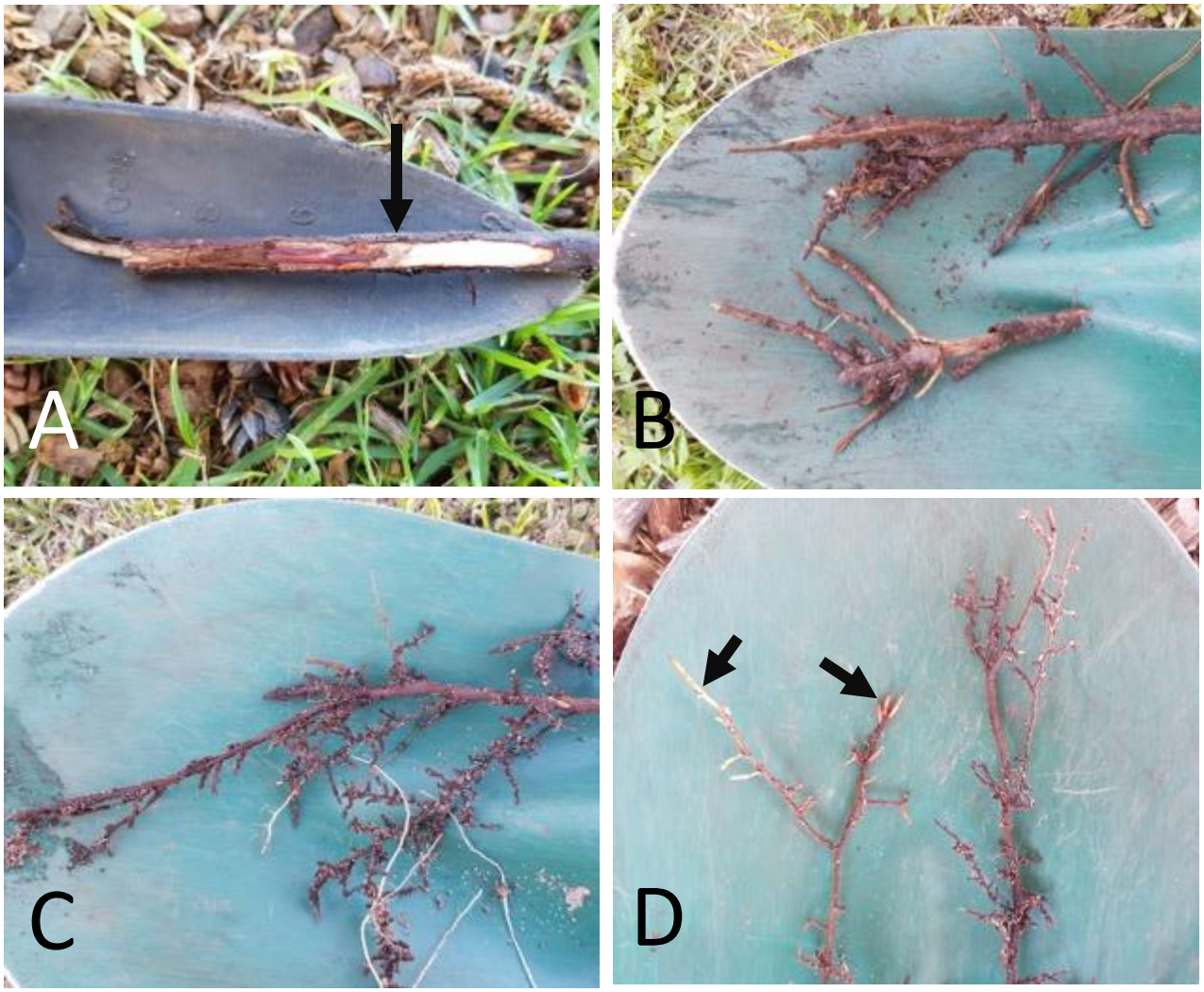

Figure 3: Necrotic roots. (A) Lesion in root exhibiting distinct margin (arrow) with healthy tissue; (B) Stunted, nodular root morphology, resulting from repeated death and re-growth, typical of soil-borne pathogen; (C) Necrotic fine root system compared with (D) healthy roots (arrows).

4.3 Foliar Necrosis and Branch Tip Dieback

Foliage on declining trees (as well as many otherwise healthy trees in the vicinity) often exhibited an irregular pattern of leaf necrosis (Figure 4A). Leaf necrosis often started as a single random leaf scale expanding to adjacent leaves. Leaf dieback also frequently occurred as a progressive tip dieback, starting at the terminal ends and working in towards the axis (Figure 4B).

In addition to leaf and branch necrosis, foliar chlorosis (yellowing) was also a common symptom occurring with diseased tissue. Several paired samples of chlorotic and healthy branches were analysed for plant nutrient content.

Samples were taken from three trees and combined to form two samples which were subjected to laboratory based fungal pathogen isolation.

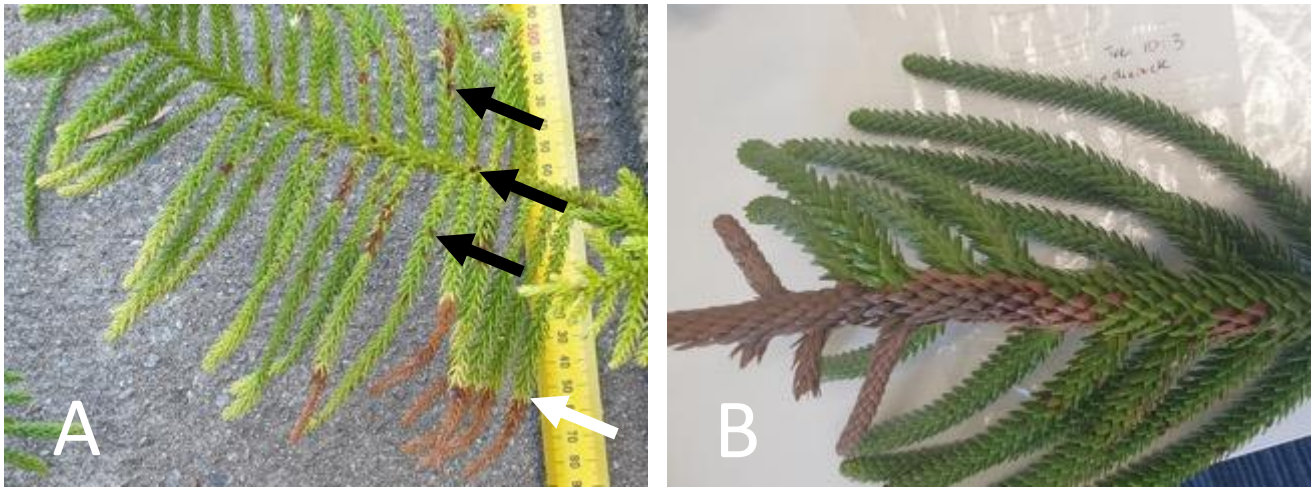

Figure 4: (A) Leaf scale necrosis (black arrows) and tip dieback (white arrow); (B) progressive tip dieback.

4.4 Crown Thinning

Crown thinning was regularly observed in trees throughout the Town of Cottesloe. It is not clear if this symptom is related to the 'top-down' decline and subsequent tree death. However, general crown thinning appears to be a precursor to top-down decline in many cases. Crown thinning was observed both originating from the terminal ends of the primary branches (Figure 5A), as well as internal lower order branches (Figure 5B).

Progressive tip-dieback and subsequent regrowth has resulted in a loss of the typical symmetrical branching structure characteristic of the species (Figure 5 B,C).

Figure 5: (A,B) Crown thinning affecting the whole crown; (C,D) Asymmetrical branching structure resulting from repeated branch-tip death (arrow) and resprouting.

4.5 Stem and Branch Lesions

Several distinct symptoms affecting the main stem or branches were observed. Blue staining was observed in branches from trees 20 and 24 (Figure 6A). This symptom is commonly caused by the presence of fungal pathogens within the vascular tissues resulting in loss of function.

Small dark lesions in the central xylem of primary branches were observed on all trees. These lesions were typically localized to a small area from the main trunk 10-20cm into the lateral branch (Figure 6B). Cross sections of the main trunk showed these lesions were contained to the branch tissue and compartmentalized by the tree preventing further spread within the trunk.

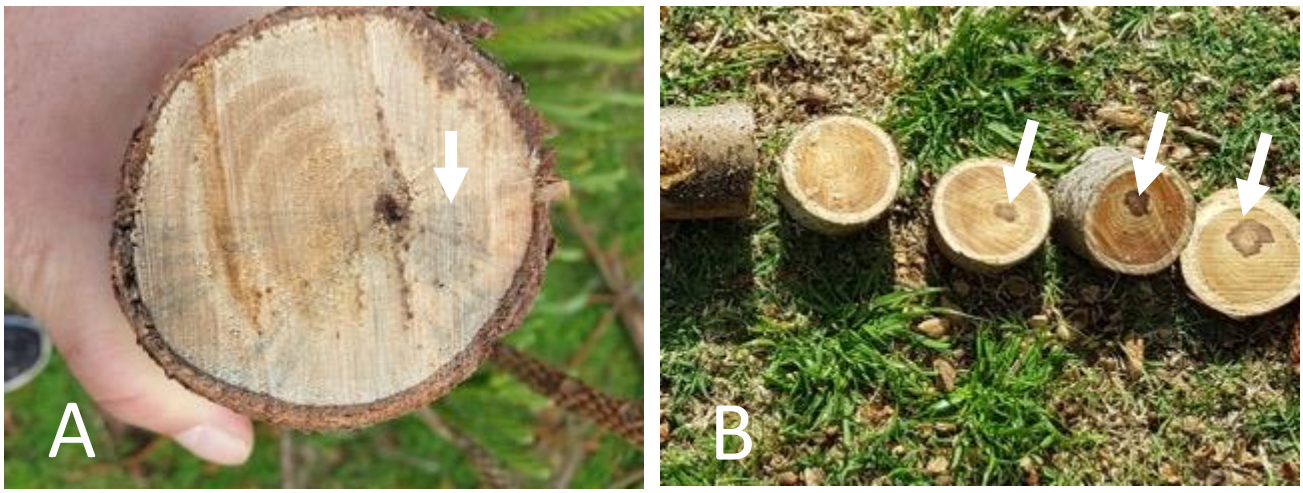

Figure 6: (A) Blue-stain lesion (arrow) in xylem tissue of primary branch; (B) Discoloured lesion in central xylem at the union with the main stem. Discs are 50-100mm thick and placed out sequentially from closest to trunk (left) to farthest (right).

Dead and dying stems typically had a soft, wet and dark phloem and cambium layer under the bark, which could be easily removed from the internal xylem tissue. Where this intersected with healthy tissue a distinct lesion could be observed (Figure 7). This lesion typically affected the underside of the branch in early stages before enclosing the full circumference resulting in branch death. The margin between healthy and diseased tissue was occasionally marked on the outer bark by small gummy exudates (Figure 7B) and cross section of the branch revealed the margin between healthy cambium tissue and diseased (Figure 7F). In diseased tissue, the bark peeled off easily by hand and fungal mycelia were observed. Microscopic examination revealed these to contain fungal pycnidia (asexual spore producing structures) (Figure 7E).

Diseased tissue within the cambial layer occasionally extended into and along the main trunk. In Tree 17 on Forrest St, this diseased tissue extended the full length of the trunk down to ground level, where it intersected with unaffected tissue (Figure 7A).

Small regions of gum exudation were observed on the main stem and multiple branches of both healthy and diseased trees. Examination below the bark often revealed these to be discrete lesions (Figure 7 C&D).

Figure 7: Stem lesions. (A) Necrotic lesion under bark on main stem; (B) Necrotic lesion on the underside of a primary branch (blue arrow) with bleeding visible at the bark surface (white arrows) at the lesion margin; (C) Bleeding branch lesions with (D) discrete necrotic patch underneath; (E) Branch lesion with peeling bark, revealing fungal mycelia and pycnidia (arrow); (F) Branch cross section showing lesion in the cambium, arrows indicate the margin with healthy tissue.

4.6 Pathogen Isolation

A range of fungal species were isolated from the different samples analysed. There were no pathogenic Phythiaceous organisms isolated from five of the eight soil and root samples analysed. This was somewhat unexpected considering the symptoms of root necrosis and lesions observed. Pathogens isolated include *Pythium pachycaule, Pythium vanterpooli* and *Phytopythium vexans.*

Pythium species are often regarded as weak pathogens of mature vegetation and are rarely regarded as a concern. Little information exists about *Pythium pachycaule*, however there is one report of it as a pathogen of Common Bean (*Phaseolus vulgaris*) seedlings in Western Australia (Li *et al.* 2014). It has also been isolated by ArborCarbon once before associated with declining coastal vegetation north of Perth. *Pythium vanterpooli* is typically associated with damping off in cereals and turf grass (Vanterpool 1938).

Phytopythium vexans (syn. *Pythium vexans*) is a known pathogen of a variety of plants. It has a wide host range, including mature trees (e.g. causing canker in rubber) and fruit trees (decline of avocado, root rot of kiwifruit). *Phytopythium vexans* has been isolated by ArborCarbon several times in the Perth region, including from declining *A. heterophylla* in the Nedlands area, as well as *Eucalyptus camaldulensis, Pinus* sp. and *Ficus rubiginosa*. There has been no research into the pathogenicity towards *A. heterophylla* , therefore it is not possible to infer that *P. vexans* is solely responsible for the dieback symptoms observed. However, considering its wide host range, it should be considered a putative pathogen until further pathogenicity testing is undertaken.

Table 2: Sample descriptions, and identification of isolated fungi, from soil and root samples. .

Sample ID	Tree ID	Isolates	Date Collected	Sample Type
PAB 20-08	3, 5, 9, 13, 14	Negative	28/07/20	Soil & root
PAB 20-09	5	Negative	28/07/20	Soil & root
PAB 20-11	10, 11	*Pythium pachycaule*	28/07/20	Soil & root
PAB 20-12	Stump, Eric St.	Negative	28/07/20	Soil & root
PAB 20-15	Stump Marine Parade	Negative	28/07/20	Soil & root
PAB 20-19	12, 15, 16, 17, 18	*Pythium vanterpooli*	28/07/20	Soil & root
PAB 20-20	19, 20, 21	Negative	28/07/20	Soil & root
PAB 20-21	22, 23	*Phytopythium vexans*	28/07/20	Soil & root

Results from the pathogen testing of stem and leaf samples are presented in Table 3. *Neofusicoccum parvum* was isolated from two samples exhibiting necrosis of the cambium and phloem tissue from Trees 3, 5 and 24. An unknown species of *Didymella* was also isolated from two samples, both exhibiting the dark staining of central xylem at the union of branches with the main stem. *Geotrichum candidum, Fusarium oxysporum, F. equiseti,* and *Neofusicoccum australe* were all isolated from one sample.

Neofusicoccum parvum belongs to the Botryosphaeriaceae family which are a diverse group of closely related and morphologically similar cryptic species known to be pathogenic fungi, causing cankers on a diverse variety of woody hosts. However, many species are also commonly known as endophytes in healthy trees, invading the tree but causing no visible symptoms of disease (Slippers & Wingfield 2007). For example, Smith et al. (1996) found *Botryosphaeria dothidea* (previously grouped with *Neofusicoccum parvum*) was common in all the *Eucalyptus* spp. tested, occurring in 93% of E. *smithii*, 77% of *E. camaldulensis*, 63% of *E. grandis* and 57% of *E. nitens* leaves tested. The change from endophytic to pathogenic phase is often related

to stress such as drought, extreme temperature fluctuations, nutrient deficiencies and mechanical injuries (Slippers & Wingfield 2007).

Neofusicoccum parvum is among the most widely distributed species of the Botryosphaeriaceae found on 90 hosts across six continents and 29 countries (Sakalidis et al. 2013). It is an economically important pathogen of several horticultural crops, notably grape vines (Massonnet et al. 2017), blueberries (Koike et al, 2014; Tennakoon et al. 2018), and plantation eucalypts (Slippers et al. 2004, Iturritxa et al. 2011), as well as natural forest stands of *Quercus ilex* (Linaldeddu et al. 2014). Symptoms typically include stem cankers, as well as leaf and branch dieback occasionally resulting in mortality.

The closely related species *Neofusicoccum australe* is a very common plant endophyte and believed to be endemic to WA (Taylor *et al.* 2009 Burgess *et al.* 2006). However, this species has been identified as an opportunistic pathogen of *Agonis flexuosa,* causing dieback symptoms in forests south of Perth (Dakin *et al.* 2010). *N. australe* was also frequently isolated from healthy tissue, leading the authors to conclude environmental stress was predisposing the tree to disease expression. It has also been identified as a canker pathogen causing dieback of grapevines in New Zealand (Amponsah *et al.* 2009).

Didymella is a genus of ascomycete fungi. There are no reports of *Didymella* species as pathogens of *A. heterophylla*, however there are reports of *Didymella* species causing disease in other plant hosts. For example, *D. fabae* causes disease in faba bean on Syria (Ozkilinic *et al.* 2015), *D. pinodes* causes blight disease of field pea (Khan *et al.* 2013), and *D. bryoniae* causes gummy stem blight and black rot on cucurbits around the world (Keinath 2011). However, no reports have been found of *Didymella* species causing heartwood discolouration, such as that observed in the current report.

Fusarium is a large genus of fungi that is widely distributed in soil and is commonly associated with plants. The majority of species are saprotrophic and do not cause disease. *Fusarium oxysporum* is a common pathogen of agricultural plants, such as muskmelon (Gordon 1986) and banana (Ploetz 2006). *Fusarium oxysporum* has been recorded as causing root rot of *Pinus wallichania* in nurseries in Kashmir (Dar *et al.* 2011). *Fusarium equiseti* has been identified as a pathogen of cucumber in Jordan Valley, causing crown rot (Aldakil *et al.* 2019). Additionally, it has been found to cause damping off disease on Aleppo pine (*Pinus halepensis*) seedlings in Algeria (Lazreg *et al.* 2014). There are no reports of *F. equiseti* causing disease in mature pines. In this instance, it is unlikely that either *Fusarium* species isolated from Norfolk Island Pines are the primary disease causing pathogens.

Mucor plumbeus and *Clonostachys rosea* are not plant pathogens.

Table 3: Sample descriptions, and identification of isolated fungi from stem and leaf samples, with references to the associated images.

Sample ID	Tree ID	Isolates	Date Collected	Sample Type
PAB 20-22	3,5,24	*Geotrichum candidum, Didymella* sp.	11/08/20	Dark staining in central core of primary branches (Figure 6B)
PAB 20-23	24	*Fusarium equiseti*	11/08/20	Stem and leaf scale necrosis (Figure 4)
PAB 20-24	3,5	*Neofusicoccum parvum*	11/08/20	Leaf scale necrosis (Figure 4)
PAB 20-25	20	*Didymella* sp.	11/08/20	Dark staining in central core of primary branches. Death of cambium (Figure 6B, Figure 7)
PAB 20-26	3	Negative	11/08/20	Main stem

PAB 20-27	24	*Fusarium oxysporum, Neofusicoccum parvum*	11/08/20	Primary branch, death of cambium, fungal pycnidia under bark. (Figure 7)
PAB 20-28	20, 24	*Neofusicoccum australe*	11/08/20	Primary branch, blue stain in xylem (Figure 6A)
PAB 20-29	17	*Neofusicoccum parvum, Mucor plumbeus*	10/09/20	Cross section of main stem, 5m from ground level
PAB 20-30	17	*Neofusicoccum luteum, Fusarium striatum*	10/09/20	Cross section of main stem, near ground level

4.7 DNA Extraction and Identification

A number of additional samples were collected for extraction and sequencing of DNA from plant tissue to investigate possible presence of *Phytophthora*. Three of the samples were fine roots collected from the top 15cm of the topsoil surrounding the tree, and one sample was from the remaining stump of a recently removed dead *A. heterophylla*. *Phytophthora multivora* DNA was sequenced from all four samples, and *Phytophthora ornamentata* from one fine root sample. *Phytophthora multivora* is a well-known pathogen of a large number of trees and diverse range of genera in the Perth region (Scott *et al*. 2009, Barber *et al*. 2013). It has been associated with dead and declining members of other Araucariaceae both in Australia and overseas. For example, it's been isolated from the rhizosphere of symptomatic Bunya pines (*Araucaria bidwillii*) in the Bunya Mountains National Park (QLD), as well as *Wollemia nobilis* (Wollemi pine) and *Araucaria cunninghamii* (Hoop Pine) in Eastern Australia, and from *Agathis australis* (Kauri) in New Zealand (Shuey *et al.* 2019, Puno *et al.* 2015).

Phytophthora ornamentata is a lesser known pathogen, but it has been reported causing decline in Mediterranean vegetation in Italy (Scanu *et al.* 2015). It has been isolated directly from lesions on the trunks of marri (*Corymbia calophylla* in Margaret River (WA) (Hardy, pers. comm.). The presence of *Phytophthora* DNA is not conclusive evidence that symptoms observed are caused by *Phytophthora*, because DNA can be present whether an organism is living or not, and can persist for years after the death of an organism. However, considering that it was extracted directly from within the roots and the stem of the trees means that it has actively colonized the host at some point. Additionally, the disease symptoms observed both above ground in the foliage (top dieback) and below ground (root lesions) are typical of *Phytophthora*. Although there were no isolations of living *P. multivora* or *P. ornamentata* from the samples during traditional baiting techniques designed to isolated *Phytophthora* species, this does not mean the organisms are not present – a number of factors, including environmental, and the current stage of disease expression, can result in the organism not being isolated. Therefore, it is important not to discount *P. multivora* and *P. ornamentata* as playing an important role in the decline observed on Norfolk Island Pines. Additional work should be considered, including more rigorous and frequent testing of the roots for *Phytophthora* in an attempt to isolate the living organism, as well as treatment of the trees with phosphite. If *Phytophthora* or *Phytopythium* are involved, the trees are likely to respond well to phosphite. Preliminary trials will be required to determine the appropriate dose.

Table 4: Results of direct DNA extraction and sequencing from fine roots and remaining stump.

Sample ID	Tree ID	DNA identified	Date Collected	Sample Type
PAB 20-31	9	*Phytophthora multivora*	14/09/20	Fine roots
PAB 20-32	10	*Phytophthora multivora, Phytophthora ornamentata*	14/09/20	Fine roots
PAB 20-33	23	*Phytophthora multivora*	14/09/20	Fine roots
PAB 20-34	17	*Phytophthora multivora*	14/09/20	Stump

4.9 Plant Nutrient Analysis

Paired samples from chlorotic and healthy leaves showed no consistent trends to support the idea that the yellowing symptom resulted from nutrient deficiency (Table 5).

Foliar nutrient standards for *Araucaria heterophylla* are not available to compare the results between symptomatic and asymptomatic trees. However, in one of the only studies available Dowden & Lambert (1979) analysed foliar nutrient content of 277 *Araucaria heterophylla* to diagnose decline of trees in NSW. When compared with the values presented here from the Town of Cottesloe, levels of Mn, Fe and Zn all appear low, with several samples outside the range observed by Dowden & Lambert. All of these micronutrient levels may be expected to reduce in availability with increasing soil pH.

Figure 8: Symptomatic (left) and Healthy (right) leaf samples submitted for nutrient analysis (PABF_20005 and PABF_20006)

Table 5: Foliar nutrient analysis results from paired healthy and symptomatic Araucaria heterophylla.

	Chlorotic			Healthy			Reference*
	A	B	C	A	B	C	
Element	PABF 20-001	PABF 20-004	PABF 20-006	PABF 20-002	PABF 20-003	PABF 20-005	
Boron (mg/kg)	31.16	37.23	14.14	33.75	26.94	35.5	13 – 23
Calcium (%)	3.42	3.52	2.17	2.66	3.4	3.71	1.38 - 1.95
Chloride (%)	0.14	0.18	0.36	0.67	0.3	0.69	0.235 - 8.8**
Copper (mg/kg)	1.88	3.64	3.92	2.41	2.28	6.87	
Iron (mg/kg)	135.19	164.02	56.06	129.58	84.49	97.68	110 – 370
Magnesium (%)	0.34	0.36	0.41	0.46	0.33	0.68	0.32 - 0.48
Manganese (mg/kg)	10.21	38.4	14.45	6.24	18.76	14.79	10 – 155
Phosphorus (%)	0.07	0.18	0.15	0.05	0.16	0.08	0.098 – 0.32
Potassium (%)	0.43	0.71	0.63	0.39	0.26	0.13	0.32- 3.35**
Sodium (%)	0.09	0.13	0.14	0.22	0.09	0.22	0.06 – 6.5**
Sulfur (%)	0.19	0.27	0.17	0.17	0.18	0.24	
Total Nitrogen (%)	0.62	0.88	0.77	0.75	0.76	0.94	
Zinc (mg/kg)	19.69	27.49	14.14	15.99	33.26	35.5	35 - 130

*Dowden and Lambert 1979

** Toxic values reported

4.10 Climate

Decline in *A. heterophylla* occurs at a time of increasing temperatures and reducing rainfall throughout south-west WA. These changing conditions are resulting in background stress to a range of trees and vegetation.

Figure 9 shows the cumulative rainfall total for the first 5 months of the year since 1994 at the Swanbourne weather station (BOM station number 9215). The data shows that in 2019 rainfall in the late summer and autumn period was 64 mm, almost one third of the average for the period (179mm). It is worthy to note that average rainfall for the same period on Norfolk Island was 537 mm.

Autumn rainfall is particularly important for tree health as it breaks an extended drought period over summer, and coincides with the annual foliage growth flush. It could be that the dramatic decline observed in the past 6 months is a delayed response to drought stress experienced in 2019. It may be coincidental, but the last period in which rainfall below 100mm from January to May was observed was 2009 (65mm), the same period in which Golzar & Burgess (2011) undertook their sampling (samples collected in 2009 & 2010).

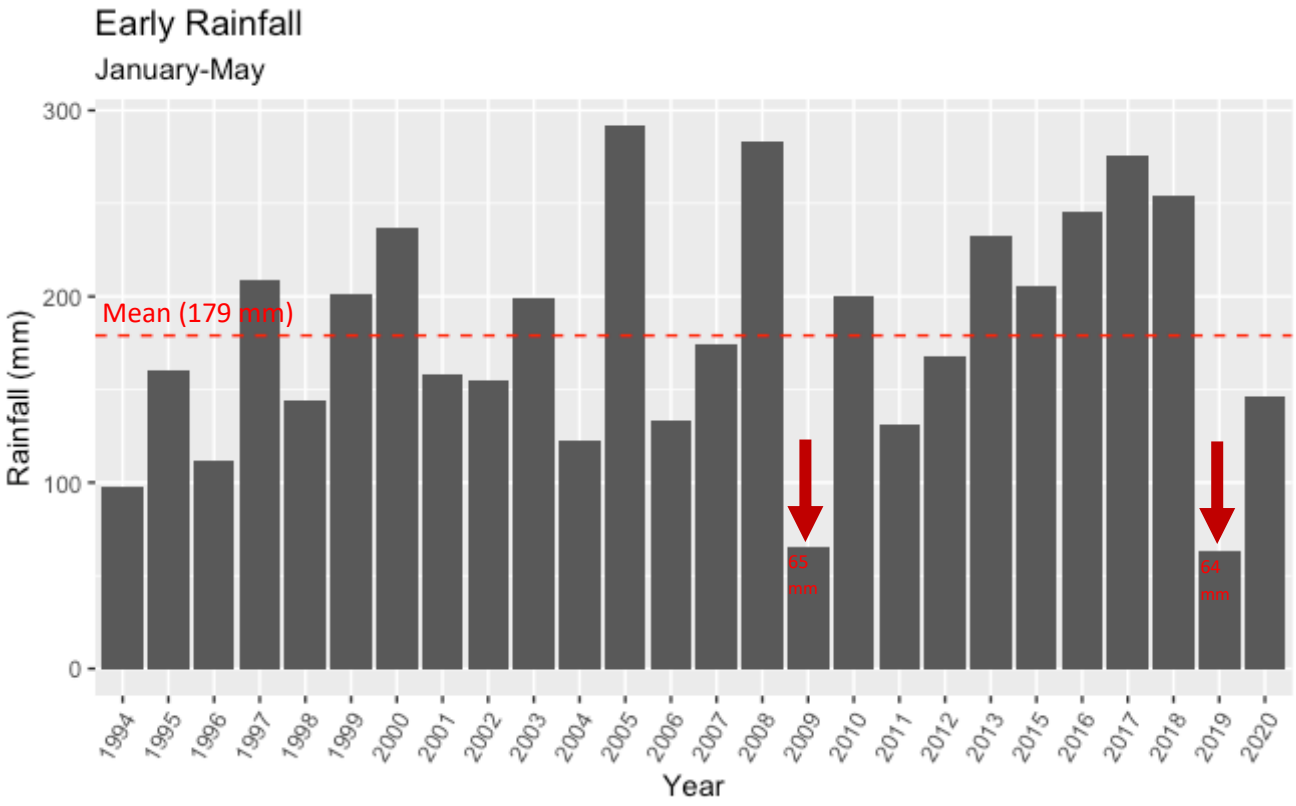

Figure 9: Cumulative rainfall, January till May, at the Swanbourne weather station (BOM station number: 9215). Arrows highlight low rainfall anomalies in 2009 and 2019, receiving 65mm and 64mm, respectively. Red dashed line shows the mean of all years. 2014 has been removed due to incomplete records.

5 Conclusions and Recommendations

The results presented here support the conclusion that *Neofusicoccum parvum* is responsible for the top-down decline resulting in tree death. The outstanding question is, given that *N. parvum* and related species are typically regarded as latent pathogens, to what extent is *N. parvum* responsible for the disease alone, and what other biotic or abiotic factors may be involved in predisposing the tree to infection.

5.1 Fungal Disease

N. parvum was isolated from stem lesions as well as individual necrotic leaves. This suggests that leaf scales may act as a point of entry for the pathogen. Under the microscope, fungal pycnidia were observed forming under the scales, suggesting that the disease may spread from an individual necrotic leaf to lower leaves and branches through gravity, wind or rain. Therefore, the pathogen may act as a leaf blight prior to advancing into the branches and stem tissue, where conspicuous decline in crown condition becomes apparent. A prolonged infection by a low level leaf blight may act as a stressor, gradually reducing the tree's resources and resulting in branch tip and crown dieback which is observed in diseased as well as healthy trees throughout the council.

The role of the other pathogens identified remains unresolved. The presence of an unknown *Didymella* species associated with discoloured lesions in central tissue of branches extending to the union of the main stem is worthy of further investigation as these lesions were observed in almost all of the branches sampled. Further work is currently underway to identify putative pathogens associated with this disease symptom.

5.2 Root Disease

The poor condition of the root system and necrotic root lesions observed on declining trees is strongly suggestive of the involvement of one or more soil-borne pathogens. Testing for *Phytophthora* and pythiaceous pathogens mostly identify suspected weak pathogens (Pythium) and one isolation of *Phytopythium vexans.* The role of *Phytopythium vexans* is unknown and deserves further investigation, as this pathogen has previously been isolated from declining *A. heterophylla* by ArborCarbon in Nedlands.

There were no *Phytophthora* species isolated during standard baiting of samples collected during this project, but this does not mean that they were not present. It is possible that isolation of pathogens using standard baiting techniques was hampered by the presence of antagonistic metabolites produced by *A. heterophylla*, which has been observed previously in other Araucariaceae (Hardy Pers. Comm.). Symptoms observed were similar to those caused by *Phytophthora*. The DNA extraction and identification results showed that *P. multivora* and *P. ornamentata* DNA was present in a number of roots and a stump sample. However, without isolating a living organism, it is impossible to draw a definitive conclusion about the role that these pathogens play in the decline observed. Considering the symptoms observed, and the presence of DNA, it is likely that at some point the pathogen has been active in the area.

5.3 Climate

It's well known that declining rainfall and increasing temperature are major stressors to trees. It was noted that Swanbourne experienced very low rainfall in 2019. This is important as it suggests that the current decline may be in response to an atypical weather event, rather than a terminal pathogen incursion. Additionally, increased stress on a tree results in it being more prone to disease caused by pathogens that would not normally cause disease expression. It also suggests that supplemental irrigation during periods of

extended summer drought may be effective in avoiding or reducing the impacts of future episodes. Key to this will be an effective system of monitoring and pre-defined management actions to be implemented when thresholds are exceeded in the future.

5.4 Nutrition

It has been hypothesized that changing soil chemistry leading to Fe and Mn deficiency is related to decline in health of a range of tree species in coastal suburbs of Perth. Grigg *et al.* 2009 demonstrated that the accumulation of saline and alkaline salts from irrigation with saline bore water resulted in increasing soil pH resulting in Fe and Mn deficiency in C*orymbia calophylla* and *Eucalyptus marginata*. It was hypothesized that decreasing rainfall led to increasing ground water salinity, increased reliance on irrigation over a longer period and reduced flushing of salts from the root zone. This together with the naturally low Mn levels in coastal soils resulted in Mn deficiency.

Results presented here do not support micronutrient deficiency as a cause of the chlorotic symptom observed in foliage frequent in declining trees. However, low levels of Mn, Fe and Zn were observed in both healthy and declining trees. Levels of these micronutrients may be expected to become deficient with increasing soil pH.

Although it has not been qualitatively compared, observation suggests that decline in *Araucaria heterophylla* occurs in both irrigated parks and verges as well as unirrigated areas at similar frequencies. However, it is likely that trees are accessing ground water reserves directly, and therefore, may still be sensitive to changes in ground water chemistry.

Further work is required to conclusively rule out changing ground water chemistry as a factor contributing to the decline. This work would ideally be carried out over the late-summer to early autumn when conditions are at their most severe. Regardless, micronutrient supplementation should be considered in future tree treatment trials.

5.5 Recommendations

1. DNA of *Phytophthora multivora* and *P. ornamentata* was sequenced and identified from lesioned roots of a number of trees, and stem material from a dead tree. The presence of DNA is not conclusive evidence that these organisms are living and causing the symptoms observed, but considering that they were extracted directly from the symptomatic material, and that symptoms observed are typical of *Phytophthora*, it is very possible that *Phytophthora* is playing a role in the decline observed.
2. The presence of necrotic lesions in the main stem originating from the upper crown caused by *Neofusicoccum parvum* are likely to be a cause of the decline in crown condition and death of the trees. However, the effectiveness of removal of large portions of the crown to halt the disease has not been proven and should be evaluated in an objective fashion along with other treatment options.
3. The maintenance of *A. heterophylla* in their current location will likely involve substantial effort and expense in the future, faced with a changing climate. Well designed and properly controlled management trials should be established to determine the effectiveness of a range of treatments at preventing *A. heterophylla* decline. Robust experimental design and statistical rigor is required when undertaking management trials to ensure the best outcome for the trees, ensuring council funds are not wasted on ineffective treatments. From this work we recommend the following treatments alone and in combination are worthy of further investigation:

a. Supplemental irrigation across the late-summer autumn period,
b. Systemic treatment with micronutrients including Mn and Zn,
c. Systemic phosphite (phosphonic acid) and fungicide treatments,
d. Removal of diseased crown and or diseased branches.

The supplemental irrigation scheme should be part of a robust trial that considers water quantities, along with frequency, season, delivery method and depth of the water penetration. These decisions will be influenced by a number of factors including water holding capacity of the surrounding soil, the area, depth and mass of roots, crown area, and site factors (e.g. irrigated/unirrigated). Similarly, the robust trial should consider the appropriate concentration of phosphite and fungicides to achieve efficacy. Phosphite can be toxic if applied in high concentrations, but these concentrations can vary widely between tree genera and species. Outcomes from these trials should be used to inform a comprehensive management plan to manage the health of the *A. heterophylla* throughout the Town.

4. Outcomes from these trials should be used to inform a comprehensive management plan to manage the health of *A. heterophylla* throughout the Town.
5. Further work is required to identify the climatic and site conditions associated with this disease syndrome. Conditions can then be monitored and used to inform management action if the established thresholds are exceeded.
6. An objective benchmark of crown health of *A. heterophylla* throughout the town is required to monitor the future health of trees and measure response to any treatment. Remote sensing imagery provides an objective, quantitative and highly sensitive method to monitor the change in crown condition over time.
7. Remote sensing should be used to map the distribution and extent of the current disease outbreak to further examine spatial pattern of the disease outbreak, and identify possible environmental factors associated with disease expression.

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