A Proteomics Study of Hormone Induction of Defence Related Proteins in Kauri (*Agathis australis*)

Thilana Nayagar

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Auckland

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Attestation of Authorship

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning."

Signed _____

Date _____

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Abstract

Kauri (*Agathis australis*) is currently threatened by a pathogen, Phytophthora Taxon agathis (PTA) against which there are few effective treatments. Hormone induction may potentially induce the plant defence system and provide some resistance against the pathogen. The objective of this thesis was to determine if treatment with the hormones, methyl jasmonate (MJ) and ethylene (ethephon, ET) induces defence related proteins.

A proteomics study was carried out to identify potential defence related proteins using SDS-PAGE and LC-MS. Numerous proteins were identified including common plant proteins such as RuBisCO as well as two potential defence related proteins, a super oxide dismutase and an acid phosphatase. This protein study was the first of its kind done in Kauri.

Acid phosphatase was selected as a target for an enzyme induction kinetics study. Kauri plants were treated with the hormones MJ and ET alone and in combination. A spectrophotometric acid phosphatase assay was carried out to quantify enzyme concentration changes with time over a period of twelve days.

The results of the enzyme assay showed a statistically significant increase in acid phosphatase activity when Kauri was treated with MJ alone and also when treated

with a combination of MJ and ET. The treatment with MJ alone caused an induction of enzyme activity of 1.2 times higher than that of the control. The treatment with MJ and ET in combination caused an induction of enzyme activity 1.8 times higher when compared to the control.

In conclusion, hormone treatment with MJ and ET has been shown to induce a plant defence related protein in Kauri which may have potential application in Phytophthora disease management. Further research will be required to determine if it is possible to prime Kauri defence related proteins by hormone treatment in order to confer PTA disease resistance.

Contents

Abbreviations

2-Mercaptoethanol: 2ME

2,6-Dichloroisonicotinic acid: INA

Activated thiol sepharose: ATS

Amino butyric acid: BABA

Benzoic acid: BA

Bovine serum albumin: BSA

Dithiothreitol: DTT

Ethephon: ET

Ethylene: ETY

Jasmonic acid: JA

Liquid chromatography-mass spectrometry: LC-MS

Min: Minute

Methyl Jasmonate: MJ

Paranitrophenyl phosphate: pNPP

Pathogenesis related proteins: PRs

Phytophthora Taxon Agathis: PTA

p-Nitrophenol: pNP

Polyvinylpolypyrrolidone: PVPP

Reactive oxygen species: ROS

Ribulose-1,5-bisphosphate carboxylase/oxygenase: RuBisCO

Salicylic acid: SA

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis: SDS-PAGE

Superoxide dismutase: SOD

Systemic acquired resistance: SAR

Tobacco mosaic virus: TMV

Trichloroacetic acid: TCA

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Chapter 1. Introduction

1.0. Introduction and Overview

Kauri dieback is a disease caused by the pathogen Phytophthora taxon Agathis (PTA). Symptoms include bleeding cankers on the tree trunk known as collar rot, and dieback of the foliage, eventually leading to the death of the tree. PTA was first identified in 2006, and while the origins of the pathogen are still unclear, most evidence suggests it is an introduced exotic species. Very few control mechanisms exist for the disease, and they rely upon early detection and proper disposal of infected plant material.

However, there is a considerable body of literature which indicates that treating plants with agents that induce the natural defence system of plants provides some protection against pathogens. Inducible defence-related proteins were first discovered in tobacco reacting to infection by *Tobacco mosaic virus* (TMV) (van Loon, 1987). Pathogenic organisms produce molecules (elicitors) that trigger defence mechanisms in the plant, such as the production of phytoalexins and defence proteins (Slusarenko et al., 2000).

Most defence-related proteins are either pathogenesis-related proteins (PRs) or the products of the systemic acquired resistance (SAR) genes (Slusarenko et al., 2000). The PRs have been shown to be induced by the signalling compounds salicylic acid (SA), jasmonic acid (JA) ethylene (ETY), benzoic acid (BA) and also certain non-protein amino acids such as beta amino butyric acid (BABA). Mutants of the biological model plant *Arabidopsis thaliana* have been developed with impaired SA, JA or ETY production. These mutants show an enhanced disease susceptibility to specific pathogens (Thomma et al., 2001), indicating that SA, JA and ETY play a role

in resistance to these pathogens. Plant susceptibility or resistance to infection seems to be dependant on the speed of which these mechanisms are activated

The induction process may be thought of as being akin to 'immunisation' in that treatment of the plants with these compounds greatly enhances the content of a variety of PR proteins, enzymes and other defensive molecules within the treated plant (Lyon et al., 1995). The increased content of defensive molecules gives the plants increased resistance compared to non-treated plants.

PRs have been shown to possess antimicrobial function through hydrolytic activities on cell walls, and contact toxicity. For example, the PR-3 family has been identified as endochitinases, which act against fungi by degrading chitin, a component of the fungal cell wall.

Several studies have demonstrated enhanced resistance in plants against *Phytophthora* species using this 'induced resistance' treatment approach. Infection of potato leaves by the late blight fungus *Phytophthora infestans* lead to a strong increase in PR-3 chitinase activity (Buchter et al., 1997). In *Piper colubrinum,* inoculation with the foot rot fungus *Phytophthora capsici,* showed a marked increase in chitinase activity in inoculated leaves, with the maximum activity after 60 h of inoculation and gradually decreasing thereafter (Varma et al., 2009). Whilst studies have been carried out on commercial crops, very little is known about the epidemiology and control of fungal pathogens in Kauri.

The research aim of the present project was to determine the extent of induction of Kauri defensive proteins after hormone treatment with regard to factors such as hormone dose-response relationships and the timing of the response.

The research undertaken in the present study involved treating Kauri seedlings (purchased from a nursery) with the resistance inducing agents methyl jasmonate and ethylene (ethephon), both individually and in combination. Changes in the levels of proteins were monitored in the leaves. Three techniques were employed to monitor the changes, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), liquid chromatography-mass spectrometry (LC-MS) , and enzyme induction kinetics assay.

1.1. *Agathis australis* (Kauri)

Agathis australis belongs to the Araucariaceae branch of the Pinophyta division (Figure 1). Pinophytes are gymnosperms and are more commonly referred to as conifers. The conifers are the largest and most diverse group of living gymnosperms (Raven et al., 1986). Conifers bear naked seeds, differentiating them from angiosperms, and are the only gymnosperms that have their seeds enclosed in a cone.

Conifers have significant ecological and economic importance. They compose an important part of the earth's terrestrial vegetation, and display considerable diversity of form, ranging from vines, creepers, and shrubs to gigantic trees. All are woody perennials, from the giant sequoias (*Sequoiadendron giganteum*) of the Sierra Nevada

of California, which reaches heights of more than 95 metres, to the natural bonsai cypresses (*Cupressus goveniana*), which only grow to 0.2 metres.

Conifers have a wide geological distribution, and are particularly prevalent in temperate climates. They can be found on nearly every part of the globe, from the Arctic Circle, to the limits of tree growth in the Southern Hemisphere. Due to their high growth capabilities, favourable wood structural properties and columnar form, conifers play a disproportionate role in meeting global demands for lumber and fibre. (Waring, 2002). Conifers provide all the world's softwood timber, the major construction wood of temperate regions, which constitutes about 45 percent of the world's annual lumber production. As a consequence, the geographic range of temperate conifers has been extended beyond the native ranges of many species. (Waring, 2002).

Coniferous leaf litter typically contains twice the ratio of carbon(C) in relation to nitrogen found in corresponding angiosperm litter. As a result, leaf litter and other detritus produced by conifers decomposes 3-4 times slower than corresponding materials produced by angiosperms. As litter decays, soils under coniferous forests maintain high C:N ratios and serve as storage sites for amounts of carbon that far exceed above-ground biomass. Coniferous forest ecosystems are therefore responsible for the sequestration of large amounts of atmospheric carbon dioxide. (Waring, 2002).

Classification of the extant conifers remains controversial, but cladistic analyses using data obtained from 28S rRNA gene sequences have identified seven monophyletic

families of conifers. (Stefanoviac et al., 1998). Figure 1 below shows the relationship of the other gymnosperms in addition to the relationship of the extant families of conifers.

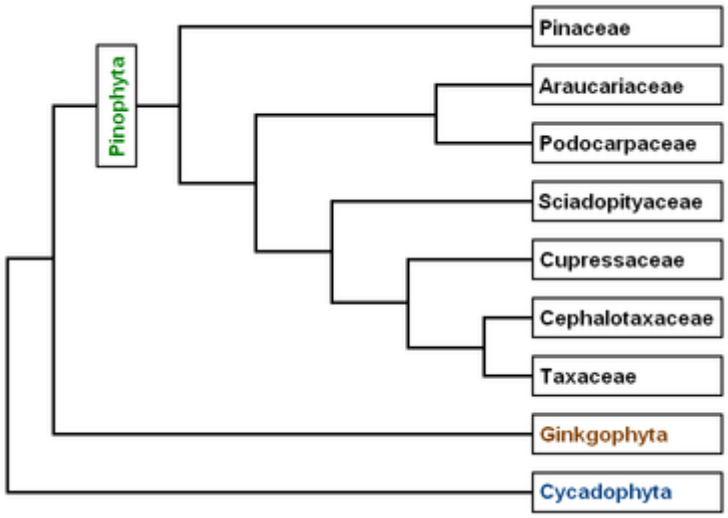


Figure 1. Phylogeny of the conifers

Araucariaceae, the family to which Kauri belongs, is one of the most primitive families of the living conifers, with a fossil record from Triassic times (Fleming, 1962). Expansion and diversification of the Araucariacea family occurred in both hemispheres in the Jurassic and Early Cretaceous and they remained a significant component of Gondwanan vegetation until the latter part of the Cenozoic (Kershaw and Wagstaff, 2001). The development of angiosperms in the Middle Cretaceous likely contributed to the demise of some Araucariaceae, but tectonic and volcanic activity, partially associated with Australia's collision with Southeast Asia, provided

new opportunities for Araucariaceae on Asia-Pacific islands. (Kershaw and Wagstaff, 2001).

Phylogenetic analysis using *rbcL* sequence work strongly indicated the Araucariacea family to be a highly coherent taxon, consisting of three well-defined genera, *Araucaria*, *Agathis* and *Wollemia* (Setoguchi et al., 1998). The first two genera have been recognized for more than a century, but *Wollemia* was recently found in New South Wales, Australia, and described in 1995 (Setoguchi et al., 1998).

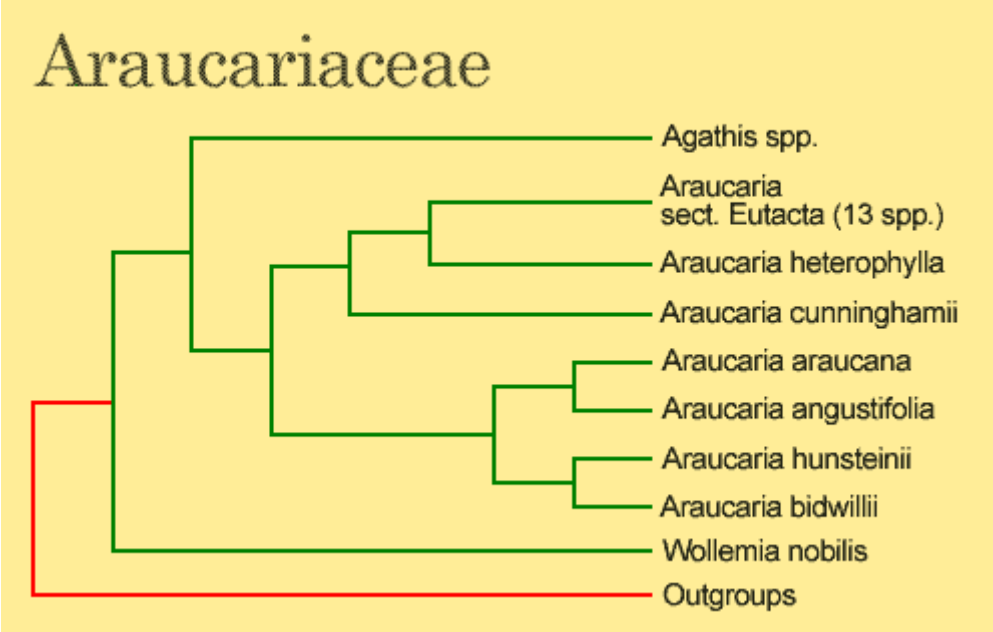
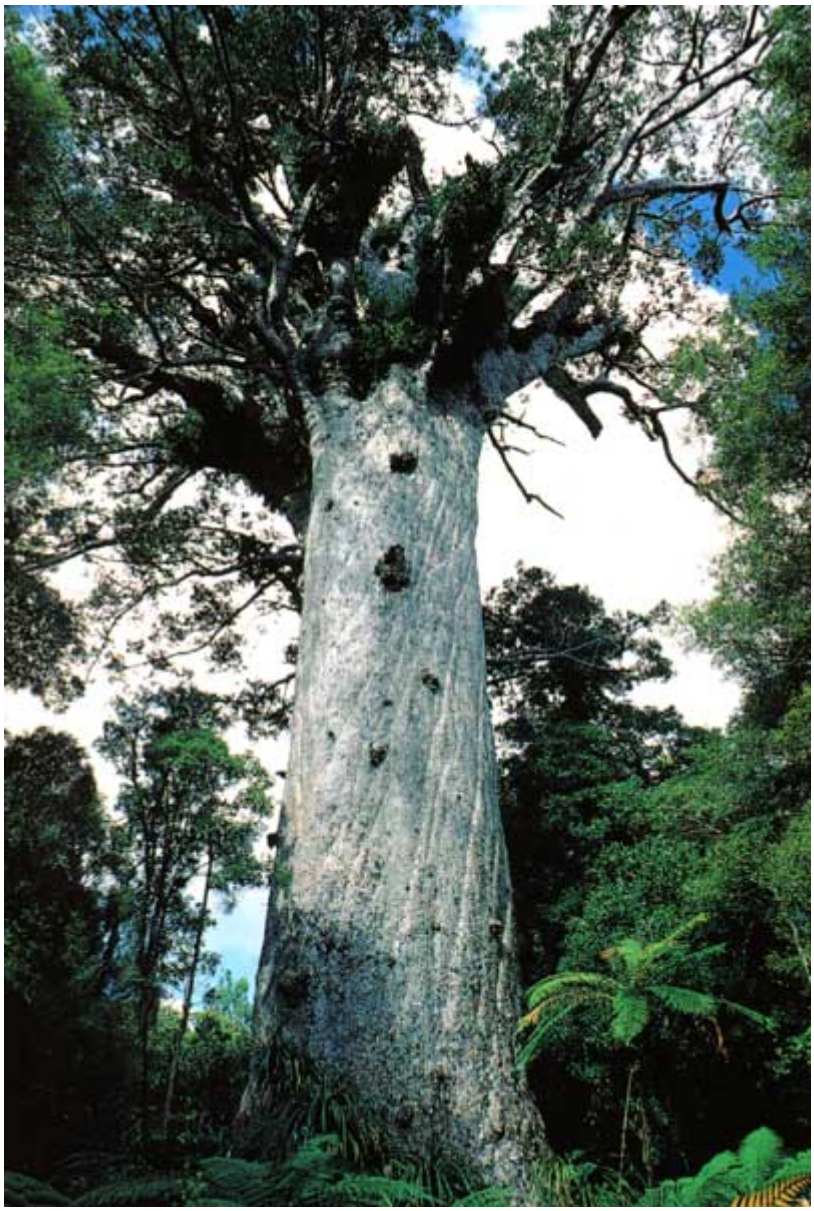


Figure 2. Phylogenetic relationships in Araucariacea (redrawn from Kershaw and Wagstaff, 2001, based on Setoguchi et al., 1998)

Macroscopic remains from *Araucaria* have been found in Middle Triassic plant beds in South Canterbury. (Fleming, 1979). Despite the molecular evidence for the sister-group relationship between *Agathis* and *Araucaria*, our current knowledge of the fossil record, only traces *Agathis* back to the Upper Cretaceous (Setoguchi et al., 1998). In contrast to the wider distribution of *Araucaria,* the fossil record for *Agathis* is limited to the Tertiary sediments of Australia and New Zealand. The oldest known fossil is a collection of leaves from the middle Cretaceous in Australia (Cantrill, 1992). Fossils of *Agathis* have also been excavated from the Eocene strata at Pakawau, north-west Nelson (Oliver and Ipcho, 1950). Resin isolated from several Lower Pliocene araucarian fossils found in the Otago region, contained agathic acid, which is characteristic of *Agathis australis* (Evans, 1937).

Agathis australis or Kauri is the only indigenous member of the Araucariaceae present in New Zealand and it is endemic (Ecroyd, 1982). It is distinguished from the other species of its genus by smaller, narrower leaves (Ecroyd, 1982). Kauri is a monoecious resin producing evergreen tree that typically reaches 30-40 metres in height. Mature trees have a columnar trunk with very little taper, and are usually free of branches due to branch abscission (Licitis-Lindbergs, 1956). Trunk diameter falls between 1-4 metres, but can on exceptional occasions reach up to 7 metres (Ecroyd, 1982). Mature trees form a crown and become globular or flat-topped after emerging above the codominant forest canopy.



Tane Mahuta, currently thought to be the largest living Kauri, in Waipoua Forest (photo Department of Conservation NZ).

Seedlings present in areas of regenerating Kauri forest have either green or reddish bronze cotyledons in approximately equal quantities (Peterson, 1963). The same variation has been observed under nursery conditions. The caretenoid pigment, rhodoxanthin, is responsible for the colouring in the red seedlings (Peterson, 1963). Greater light intensity is implicated in causing more red pigment to be deposited in the leaves. Seedlings with green cotyledons are initially more vigorous, but this advantage along with the colour variation tends to disappear with age. All mature Kauri trees have green leaves.

Young Kauri has a taproot, and long lateral roots (Ecroyd, 1982). Mature Kauri has a wide plate of spreading lateral roots, anchored with peg roots that can extend 5 metres into the ground. The feeding roots form a fine surface mat located within the litter and humus layers of the forest floor. The lateral roots usually exceed the crown in spread (Hinds & Reid,1957). Kauri has all but a few of its short roots converted to nodular structures containing mycorrhiza (Morrison & English, 1967). Mycorrhiza are a symbiotic fungi, that allow Kauri to grow on poor quality soil. Like most conifers, Kauri leaf litter is slow to decay because it includes woody components like bark and cones, as well as Kauri gum. The resulting highly acidic hummus layer contributes to the low fertility of podzolized soils by liberating acidic compounds such as phosphates in a process called leaching. It also locks in up to four times the nitrogen stored in the litter of most other forest types. The stored nitrogen is relatively inaccessible to plants, including Kauri, but the mycorrhiza present in Kauri root nodules are highly efficient nitrogen users. Mycorrhiza assist with nutrient uptake

efficiency, allowing Kauri to grow on podzolized soils, as well as soils with varying moisture content. It is an adaptation that makes it possible for slow-growing conifers like Kauri to compete successfully with faster-growing broadleaved trees.

1.1.1. Geographic distribution

Kauri is restricted to the sub-tropical forests in areas north of latitude 38° S. It occurs naturally in Auckland, Northland, the Coromandel Peninsula and on many offshore islands. The largest remaining stands of Kauri are in Northland, with Waipoua State Forest containing the largest groves and many of the notable massive trees, including Tane Mahuta (Ecroyd, 1982). Kauri can also be found on islands in the Hauraki Gulf, including Great Barrier Island, Rangitoto and Waiheke (Kirk, 1878; Atkinson, 1959; Brown, 1979).

Kauri is found from sea level to 600 metres, but it is predominantly a lowland species. Upland Kauri found on Great Barrier Island and on several parts of the Coromandel ranges are stunted in comparison to lowland Kauri, but were still extensively logged for timber (Ecroyd, 1982).

Kauri timber and gum were the first source of export revenue for New Zealand (Ecroyd, 1982). The elasticity and size of the trunk made them ideal construction material for ship spars and many cargo ships built from Kauri served the early domestic and export timber industry. Kauri timber was also used as sawn timber for building construction. In addition to timber, Kauri gum dug from Northland swamps was used in the manufacture of various products including linoleum, and varnish (Reed, 1948).

Milling reached its peak in 1905 (Simpson, 1973), and in just over 100 years, transformed Northland from forest to farmland. Although concerns about the unsupportable rate of cutting Kauri and other native forest emerged as early as 1863, the high value of Kauri timber meant that logging still continued (Ecroyd, 1982). It was not until 1985, that the growing recognition of the value of native forest, finally led to the end of all logging of live Kauri trees in state forests. By then though, Kauri were reduced from around 1 M ha, to approximately 7,500 ha (Ecroyd, 1982).

Figure 3. Geographic distribution map of Kauri, shown in red (data from Metcalf, 2002).

1.2. Phytophthora

Phytophthora is a major genus of plant pathogenic oomycetes, that cause devastating diseases which impact both agriculture and natural ecology. These diseases, include root and crown rots, foliar blights, and fruit rots, and they affect food crops, forest trees, and a variety of ornamental plants (Erwin and Ribero, 1996). The genus was first described by Heinrich Anton de Bary in 1875, when he identified *Phytophthora infestans* as the pathogen responsible for potato late blight.

Phytophthora species are relatively host specific and are pathogenic for virtually all dicotyledons, as well as for certain cereals (Erwin and Ribeiro, 1996). *Phytophthora infestans* remains a major pathogen of solanaceous crops, which include tomato and potato and can cause up to 100% yield loss (Nowicki et al., 2011). The root and stem rot pathogen *Phytophthora sojae* has also caused longstanding problems for the soybean industry (Kauffman and Gerdemann, 1958) and recently *Phytophthora ramorum*, has had devastating effects on the oak populations in North America (Rizzo et al., 2002).

Phytophthora species account for approximately $5 billion worth of crop loss annually worldwide (Stokstad, 2006), and costs for chemical management of Phytophthora diseases represent over 25% of the annual fungicide market (Schwinn et al., 1987).

Phytophthora species are oomycetes, a fungus-like eukaryote, but they are phylogenetically distinct from the true fungi (Gunderson et al., 1987). There are

several key aspects of their structural and biochemical characteristics that distinguish oomycetes from plant pathogenic fungi (Table 1). Oomycetes are diploid and lack the free haploid life stage fungi have. Oomycete cell walls are also primarily composed of cellulose, whereas fungal cell walls contain chitin as a characterising component (Bartinicki-Garcia and Wang, 1983). Oomycetes are unable to synthesize sterols, because they lack the specific enzymes required to convert sterol precursors (Tyler et al., 2006). Most traditional chemical fungicides target chitin and sterol synthesis, which means that they are inefficient against oomycetes, and to date pesticides that are adapted to specifically cure or prevent oomycete diseases do not exist (Atard et al., 2007).

The phenylamide metalaxyl, which inhibits RNA polymerase-1 has been shown to be effective against oomycetes, (Sukul and Spiteller, 2000), but resistance to metalaxyl developed rapidly and is now characteristic of pathogenic *P. infestans* and *P. capsici* populations from potato and pepper, respectively (Lee et al., 1999; Parra and Ristaino, 2001). Treatment with metalaxyl is also a preventative measure only. Metalaxyl cannot be used on plants that already have infected tissue.

Feature	Oomycete	True fungi
Neighbouring taxonomic groups	Diatoms and golden-brown algae	Animals
Hyphal architecture	Aseptate and coenocytic tubular hyphae	Either single cell or septated hyphae, with one or more nuclei per compartment
Ploidy of vegetative hyphae	Diploid, except for transient haploid nuclei in gametangia	Typically haploid or dikaryotic; often with a stable or semi-stable diploid stage following mating
Typical size of genome	50–250 Mb	10–40 Mb
Major glucans in cell walls	Cellulose (β-1,4-linked glucose), and β-1,3, and β-1,6-linked glucose polymers	Usually chitin (β-1,4-linked *N*-acetylglucosamine) and/or chitosan (β-1,4-linked glucosamine), often with other β-1,3, and β-1,6 glucans
Pigmentation	Usually unpigmented	Very common in hyphae or spores, or secreted (for example, melanin, carotenoids and others)
Toxic secondary metabolites	None described	Common (typically aromatic, heterocyclic compounds)
Mating hormones	Non-peptide, probably lipid-like	Usually small peptides or lipopeptides
Predominant asexual spore	Undesiccated, unicellular sporangia (multinucleate cells)	Desiccated single or multicellular conidia (one nucleus per cell)
Motile asexual spores	Nearly universal, biflagellated zoospore	Uncommon, only in chytrids, which are monoflagellate
Sexual spores	Oospores, formed on the termini of specialized hyphae, each containing one viable zygotic nucleus	Various types, often formed in large numbers within complex enclosures (for example, perithecia, mushroom caps and others)
Major energy reserves used by spores	Mycolaminarin and lipid, possibly polyphosphate	Glycogen and trehalose, also sugar alcohols and lipid

Table 1. Major differences between oomycete and true fungi (from Judelson and Blanco, 2005).

1.2.1. The Phytophthora infection cycle

The Phytophthora infection process is a cycle. The pathogen makes contact with the plant via motile, biflagellate zoospores (Duniway, 1983), which attach to the plant surface. Most fungal spores are wind borne but Phytophthora zoospores swim actively in water and can travel 25–35 mm in waterlogged soils (Duniway, 1983).

Phytophthora zoospores are chemotactically attracted to the roots of host and non host plants alike (Hardham, 2001). This implies that resistance or susceptibility of the plant is determined at a later stage of infection. Phenolic compounds, sugars and alcohols have all been show to be chemo-attractants for zoospores (Carlile, 1983; Morris and Ward, 1992).

Once they reach the root, the zoospores encyst and rapidly adhere to the host surface (Hardham, 2001). The adhesion process is an important part of the infection cycle as it prevents the spores from being dislodged and allows physical force to be exerted by the pathogen cell as it tries to invade the plant surface. (Hardham, 2001). The encysted zoospore then penetrates the host surface and colonisation of the plant occurs. The pathogen acquires the necessary nutrients for growth and sporulation, and the cycle begins again (Hardham, 2001).

1.2.3. Phytophthora taxon Agathis (PTA)

Phytophthora taxon Agathis (PTA) was first found in New Zealand on great Barrier Island in 1974 (Gadgil, 1974). Morphological analysis at the time mistakenly identified the pathogen as *Phytophthora heveae*. Pathogenicity tests on Kauri seedlings using both wound and soil inoculation methods, showed it was highly pathogenic (Gadgil, 1974). However, as *P. heaveae* was present in soil under healthy trees, Gadgil concluded that it was only pathogenic under specific environmental conditions and little further investigation was undertaken (Gadgil, 1974). It was not until 2006, due to the decline in health of Kauri in the Auckland region, that samples were collected and

analysed, resulting in PTA being formally identified as a new species (Beever et al., 2009). Samples that were retested from the original site on Great Barrier Island, were also confirmed positive for PTA.

PTA samples were collected from both the leading edge of a lesion margin caused by the pathogen, as well as the soil underneath trees (Beever et al., 2009). All isolates from collected samples had identical internal transcribed spacer (ITS) sequences and phylogenetic analysis indicated that PTA is more closely related to *Phytophthora katsurae* than to *P. heaveae* (Beever et al., 2009, also figure 4.). Morphological analysis however shows that PTA differs from *P. katsurae* in its rugose, as distinct from bullate, oogonia (Beever et al., 2009).

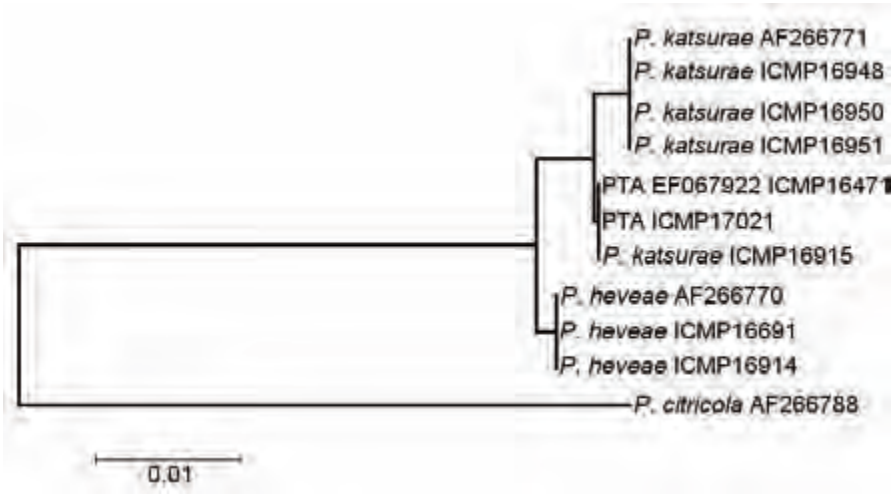


Figure 4. Phylogentic tree based on ITS sequences (from Beever et al., 2009).

Due to the ITS sequence analysis showing a close relationship with both *P. heveae* and *P. katsurae*, PTA is hypothesised to be an exotic species. (Beever et al., 2009). Studies have show that both *P. heveae* and *P. katsurae* species, are present in natural forests in Taiwan, although neither species is in obvious association with disease (Ko

et al., 2006). PTA also prefers soil temperatures of 25 ºC, which is significantly warmer than the average soil temperature in New Zealand (Beever et al., 2009).

Pathogenicity tests using small saplings and agar plug inoculation showed PTA is highly virulent to Kauri (Beever et al., 2009). In the natural ecosystem, PTA causes Kauri dieback. Symptoms include yellowing of foliage, loss of leaves and canopy thinning (Beever et al., 2009). Infected trees can also develop collar rot, which are large bleeding lesions of resin that can eventually gird the entire trunk and lead to tree mortality. (Beever et al., 2009). Trees of a wide size range and age are affected in the natural ecosystem (Beever et al., 2009). Quantitative plot analysis of two separate sites in the Waitakere ranges showed that 30% of Kauri to be either dead or have unusual gummosis (Beever et al., 2009). The photographs below show the before and effect of PTA on Kauri foliage.

The selective mortality of Kauri caused by PTA may lead to changes in forest composition, with forest dominance likely to shift towards unaffected podocarp species such as *Dacrydium cupressinum* (rimu) (Beever et al., 2009).

A healthy Kauri

PTA infected Kauri crown

The before and after effect of PTA on Kauri foliage (Pictures courtesy of Auckland Regional Council).

In the Auckland region, the presence of PTA has now been confirmed in the Waitakere Ranges Regional Park, Great Barrier Island and Rodney districts (see Figure 5 for map data). In Northland, it has also been identified at Waipoua Forest and Trounson Kauri Park (Beever et al., 2009).

PTA is a soil-borne species that spreads predominantly through soil and soil water movement. (Beever et al., 2009). It can also spread via plant to plant transmission through underground root contact, and human and animal vectors, including feral pigs (Beever et al., 2009).

As the native range of PTA is unclear (Beever et al., 2009), information on long distance dispersal methods is lacking. Human vectors are likely to be responsible via the movement of contaminated soil from footwear and equipment. Stations utilising TriGene, a disinfectant, have been installed along public walking tracks in Auckland to assist trampers with removal of potentially PTA spore infested soil from their footwear and equipment (Waipara et al., 2010).

In October 2008, MAF Biosecurity New Zealand declared PTA an unwanted organism under the Biosecurity Act.

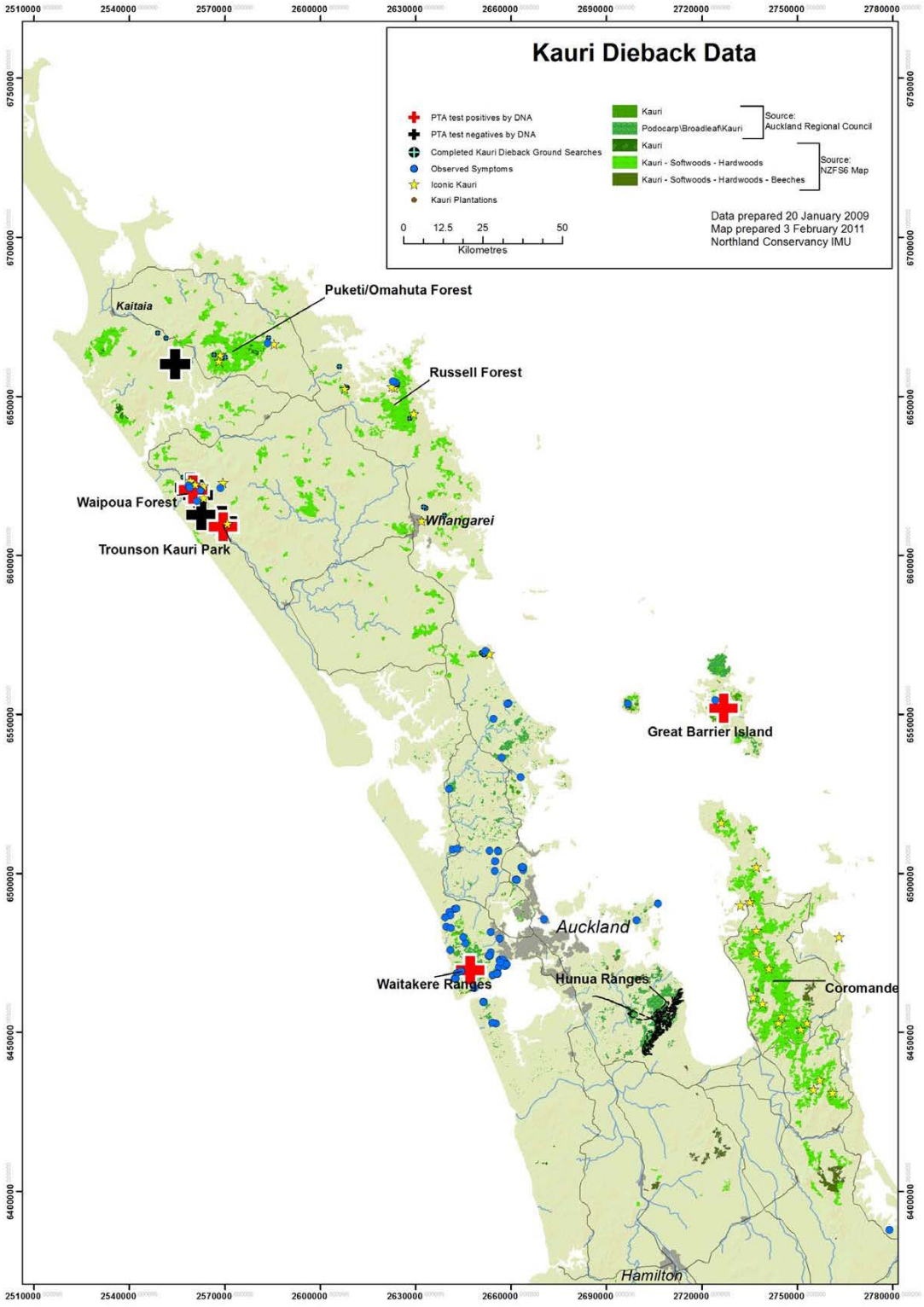


Figure 5. Map of areas affected by Kauri dieback (Beever et al., 2009).

1.3. Plant defence

Plants represent the most abundant and important group of autotrophic organisms on the planet. Despite the large numbers of bacteria and fungi that are involved in the breakdown of dead plant material, very few microbiological organisms have acquired the ability to colonise living plants. Extensive studies have shown that plants have developed a large arsenal of defence responses when attacked by pathogens. The hypersensitive reaction to a pathogen is one of the most efficient defence mechanisms in nature and leads to the induction of numerous plant genes encoding defence proteins. These proteins include structural proteins that are involved in the reinforcement of cell walls, the production of phytoalexins, and the synthesis of pathogenesis-related proteins (Cutt and Klessig, 1992; Kombrick and Somssick, 1995).

The term pathogenesis-related (PR) proteins was coined in 1980 to define a group of plant peptides that are produced in response to pathogens or pathological related situations (Cutt and Klessig, 1992). PR proteins were first discovered in tobacco cultivars (*Nicotiana tobaccum*) reacting to infection by *Tobacco mosaic virus* (TMV) (van Loon, 1987). The PR proteins were originally identified as extra bands in polyacrylamide gels and were characterised as acidic, low molecular weight proteins, soluble at low pH and relatively resistant to proteases (Stintzi et al., 1993). These physicochemical properties mean that PR proteins are resistant to acidic pH and

proteolytic cleavage and thus can survive in harsh cellular environments like the vacuolar compartment or intercellular spaces (Stintzi et al., 1993).

Since the discovery of the first PRs in tobacco, similar proteins have been isolated from a large variety of other plant species, and PR proteins are now assumed to be ubiquitous in the plant kingdom (Van Loon, 1987). In the absence of known biological function, the 10 major tobacco PR proteins were first classified into 5 major groups based first on serological properties, and later on sequence data (Cutt and Klessig, 1992, Stintzi et al., 1993). The recognized PRs currently comprise 17 families of induced proteins as shown in Table 2 (van Loon et al., 2006).

Family	Type member	Properties	Gene symbols
PR-1	Tobacco PR-1a	Unknown	*Ypr1*
PR-2	Tobacco PR-2	β-1,3-glucanase	*Ypr2*, [*Gns2* ('*Glb*')]
PR-3	Tobacco P, Q	Chitinase type I, II, IV, V, VI, VII	*Ypr3*, *Chia*
PR-4	Tobacco 'R'	Chitinase type I, II	*Ypr4*, *Chid*
PR-5	Tobacco S	Thaumatin-like	*Ypr5*
PR-6	Tomato Inhibitor I	Proteinase-inhibitor	*Ypr6*, *Pis* ('*Pin*')
PR-7	Tomato P_{69}	Endoproteinase	*Ypr7*
PR-8	Cucumber chitinase	Chitinase type III	*Ypr8*, *Chib*
PR-9	Tobacco "lignin-forming peroxidase"	Peroxidase	*Ypr9*, *Prx*
PR-10	Parsley "PR1"	Ribonuclease-like	*Ypr10*
PR-11	Tobacco "class V" chitinase	Chitinase, type I	*Ypr11*, *Chic*
PR-12	Radish Rs-AFP3	Defensin	*Ypr12*
PR-13	Arabidopsis THI2.1	Thionin	*Ypr13*, *Thi*
PR-14	Barley LTP4	Lipid-transfer protein	*Ypr14*, *Ltp*
PR-15	Barley OxOa (germin)	Oxalate oxidase	*Ypr15*
PR-16	Barley OxOLP	Oxalate-oxidase-like	*Ypr16*
PR-17	Tobacco PRp27	Unknown	*Ypr17*

Table 2. Table of PR protein familes (fom Van Loon et al., 2006).

PR proteins have been implicated in pathogen resistance response, specifically in the acquired resistance that is induced in the hypersensitive response (HR) in various plant species **(**Pietersen and Van Loon, 2004). The term hypersensitive response was first used to describe rapid host cell death in resistant wheat plants upon infection by rust fungi (Stakman, 1915). Hypersensitivity is a rapid defence reaction induced in incompatible host by a plant pathogen, which results in the death of a limited number of host cells (Stakman, 1915). When plants are infected with a pathogen that triggers HR necrosis, they acquire systemically enhanced resistance to subsequent infections from various pathogens. This type of enhanced resistance is referred to as systemic acquired resistance (SAR). SAR is associated with the induction of PR proteins in locations that are distant from the original inoculation site (Taylor et al., 1993).

In tobacco, SAR activation results in a significant reduction of disease symptoms caused by the oomycete *Phytophthora parasifica*, as well as various fungi, bacteria and viruses, including *Cercospora nicotianae*, *Peronospora tabacina*, TMV and *Pseudomonas syringae* (Vernooij et al., 1994).

Group PR-I contains the first discovered PRs of 15–17 kDa molecular mass, whose biological activity is still unknown, but some members have been shown recently to be induced by oomycete activity (Van Loon et al., 2006). Potato for example accumulates PR-1 in the vicinity of the successfully colonized leaf area in response to infection by *Phytophthora infestans* (Hoegen et al., 2002).

Group PR-3 consists of various chitinases-lysozymes that belong to three distinct classes. Chitinases are found in the leaves of many plants, but have no known function in growth and development. Chitin and β-1, 3-glucanase are major structural polysaccharides components of the fungal cell. Chitinases either alone or in combination with 1,3-ß-glucanases, have been shown to hydrolyze the 1,4 linkages between N-acetylglucosamine residues of chitin, weakening the fungal cell wall and exposing the fungi to osmotic pressure. The enzyme is also linked with the thinning of the growing hyphal tips of fungi (Van Loon et al., 2006). A combination of chitinase and -1,3-glucanase is known to be more effective than each enzyme alone against many fungi (Van Loon et al., 2006).

Studies on tomato infected with *Fusarium oxysporum* revealed that chitinase accumulated earlier in the non infective interaction than in the pathogenic one (Benhamou et al., 1990). Chitinase deposition was found to be largely correlated with pathogen distribution, with chitinase accumulating in areas where host walls were in close contact with fungal cells. The substantial amount of chitinase found at the fungal cell surface supports the view of an antifungal activity (Benhamou et al., 1990). In the *C. fulvum*-tomato host-pathogen interaction, resistance against the fungus correlates with the early induction of transcription of the chitinase gene and accumulation of this protein in inoculated tomato leaves (Wubbem et al., 1996). Upregulation of the chitinase gene was observed most abundantly in resistant tomato genotypes (Wubbem et al., 1996).

1.3.1. PR protein induction and signalling compounds

Plants can activate separate defence pathways depending on the type of pathogen encountered (Garcia-Brugger et al., 2006) . Upon infection with various types of pathogens, defence-related genes are activated in coordination by specific signalling compounds or hormones, of which the three most important are salicylic acid (SA), jasmonic acid (JA) and ethylene (ETY) (Van Loon et al., 2006). Different pathogens trigger different combinations of signalling compounds and although different plant species react to infection by activation of similar defensive mechanisms, their regulation may differ in important details. For example, basal resistance against the fungus *Botrytis cinerea* is regulated by SA in tomato but by JA and ET in tobacco (Achuo et al., 2004, Geraats et al., 2006). Such differences may be the basis of specificity in plant-pathogen interactions, as only a small number of potential pathogens are able to infect any given plant species (van Loon et al., 2006).

Crosstalk between defence pathways mediated by salicylates, jasmonates, ethylene, and pathogen infection has been proposed, due to the fact that upon hypersensitive necrosis, the levels of SA, JA and ETY production have all been shown to be strongly enhanced. (Seo et al., 2001). In *Arabidopsis*, in addition to SA-inducible defence-related genes, such as PR-1, other JA and ETY-inducible genes such as PR-3 basic

chitinase are also activated (Thomma et al., 2001). ETY is also able to sensitize plant tissue to respond to SA, as evidenced by a lowering of the concentration of SA that is required for PR-1 expression when Arabidopsis is exposed to ETY (Lawton et al., 1994). On the other hand, Niki et al., (1998) showed that the expression of all acidic PR1 genes in tobacco that were induced by SA, had their induction in response to SA inhibited by JA. They also showed that basic PR genes that were induced in response to wounding and JA, were actually inhibited by SA. Induction and inhibition in response to both hormones occurred in a dose responsive manner (Niki et al., 1998). A combination of ethylene and methyl jasmonate (MJ) has also been shown to cause a synergistic induction of PR-1 and PR-5 protein accumulation in tobacco seedlings (Xu et al., 1994).

High throughput microarray data analysis of *Arabidopsis* by Schenk et al. (2000), revealed a surprising level of coordinated defence responses between SA, MJ and ETY. The largest number of genes co-induced was found after treatments with SA and MJ (Schenk et al., 2000), In addition, 50% of the genes induced by ethylene treatment were also induced by MJ treatment (Schenk et al., 2000). These results indicate the existence of a substantial network of regulatory interactions and coordination occurring during plant defence among the different defence signaling pathways (Schenk et al., 2000). The nature and extent of the cross-talk between the three defence-regulating hormones depend on the timing and magnitude of their increases, which, in turn can be modulated by the action of the attacking pathogen (van Loon et al., 2006).

The objective of the present research was to study the induction of defensive proteins in Kauri after treatment with the hormones methyl-jasmonate and ethylene (applied as ethephon) with the long term objective of potentially developing a hormone based induced resistance 'immunisation type' treatment against *Phytophthora*. Such immunisation type treatments hold promise as an alternative or supplemental treatment to the use of conventional chemical treatments which directly target the pathogen (Lyon et al., 1995).

Chapter 2. Kauri Proteomic Study

2.0. Introduction

Kauri seedlings were treated with the plant hormones methyl jasmonate (MJ) and ethephon (ET) in an attempt to induce the synthesis of pathogenesis related proteins. Previous studies have shown that PR-1 is the most abundant of the inducible PR proteins and accounts for 1-2% of the total leaf protein content. PR-1 proteins are selectively soluble in acidic buffers and have low molecular weights (14-16 kDa). PR-3 protein (chitinase) is also a low molecular weight acid soluble protein. A low pH extraction buffer can be used to therefore enrich for these particular proteins.

2.1. Kauri protein extraction using a low pH citrate phosphate buffer (McIlvaine's buffer)

McIlvaine's buffer is a citrate phosphate buffer system that can be volumetrically set for specific pH over a wide range (2.2 to 8) (McIlvaine, 1921).

In this protocol, 2g of Kauri leaf tissue was homogenised in a mortar and pestle with liquid nitrogen and 4mL of pH 2.8 citrate phosphate buffer. Due to it's volatile nature, 3µL of 2-mercaptoethanol (2ME), a reducing agent was only added to the citrate phosphate buffer, immediately prior to homogenisation. The buffer and the homogenised leaf tissue were transferred to a 50mL centrifuge tube and centrifuged at 25,000*g* for 15min at 4ºC. The supernatant was collected and a second centrifuge step at 13,000*g* for 2mins at 4ºC was carried out.

The supernatant was collected and subjected to a Bradford assay (Wang and Fan, 2000) in order to determine protein concentration. The extraction was repeated again with 1.5g of Kauri leaf tissue and 3mL of citrate phosphate buffer.

Bradford assay results are shown in table 3.

Table 3. Bradford assay results for pH 2.8 citrate phosphate buffer

Treatment		Absorbance 410nm	
Weight		2g	1.5g
Blank		0	0
Protein Standards:	25µg	0.083	0.121
	50µg	0.307	0.255
	75µg	0.431	0.412
	100µg	0.599	0.608
Kauri Protein		0.365	0.391
Kauri Protein (50% dilution)		0.262	0.281

The Bradford assay on both samples showed an initial protein yield in the 50-75µg/100µL range, which is sufficient for SDS-PAGE, so it was decided to proceed with a hormone treatment.

2.2. Methyl jasmonate and ethephon hormone application

A solution compromising of 23mg methyl jasmonate (MJ), 50mg Tween, 200µL 99% ethanol and 25mL water was made and sprayed onto a Kauri seedling in a fume hood.

A second solution containing 6.7mg of ethephon (ET) dissolved in 10mL of water was poured directly onto the soil of the pot the seedling was planted in.

Leaf tissue was harvested on day 2 and day 6, along with root tissue on day 6. No root tissue was removed prior to day 6 as it would have damaged the plant significantly. All plant material was homogenised into a powder using liquid nitrogen directly after collection and stored at -20ºC. Leaf and root tissue was also harvested from an untreated plant. Quantities are shown in Table 4.

Table 4. Quantity of leaf and root tissue harvested after MJ and ET treatment

	Raw weight (g)	Powdered weight (g)
Day 2 leaf tissue	3.04	2.26
Day 6 leaf tissue	7.5	6.38
Day 6 root tissue	7.12	5.97
Day 0 untreated leaf tissue	5.22	4.55
Day 0 untreated root tissue	7.81	6.53

Protein extraction was carried out using 1.5g of plant tissue and 3mL of citrate phosphate buffer as described in the method above. The Bradford assay results of the protein extraction are shown in Table 5.

Table 5. Bradford assay results for MJ and ET treated Kauri tissue using pH 2.8 citrate phosphate buffer

Treatment		Absorbance 410nm
Blank		0
Protein standards:	25µg	0.09
	50µg	0.238
	75µg	0.416
	100µg	0.613
Day 2 leaf tissue		0.362
Day 6 leaf tissue		0.405
Day 6 root tissue		0.104
Day 0 untreated leaf tissue		0.315
Day 0 untreated root tissue		0.04

The leaf tissue protein yield was once again in the 50µg-75µg/100µL range. A Centricon centrifugal filter unit was used to further concentrate the protein, where 1mL each of the day 6 treated leaf tissue and the day 0 untreated leaf tissue were placed in a 2 mL Centricon tube. The Centricon tube was centrifuged for 1hr at 5000*g* at 4ºC. It was then inverted and centrifuged at 700*g* for 2min. The final eluted volume after centrifugation was 250µL. The Bradford assay for the concentrated protein extract is shown in Table 6.

Table 6. Bradford assay result comparing concentrated untreated kauri tissue with MJ and ET treated kauri tissue

Treatment		Absorbance 410nm
Blank		0
Protein standards	25µg	0.085
	50µg	0.129
	75µg	0.358
	100µg	0.544
Day 0 untreated leaf tissue		0.277
Day 6 leaf tissue		0.414

2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The ingredients for SDS-PAGE sample buffer are listed in Table 7. A commercial precast gel and running buffer were used.

Table 7. Sample buffer for SDS-PAGE

SDS	4mL
Tris-HCL pH 6.8	2.5mL
Glycerol	2mL
Bromophenol Blue	1mL
2-Mercaptoethanol	500μL

The protein extract and the sample buffer were mixed in a 1:1 ratio, with 25μL of each being used, for a total volume of 50μL. The gel was run for 20min at 150V, washed three times in 200mL of deionised water, and stained with Coomassie Brilliant Blue for 5min. Destaining was carried out in a methanol/acetic acid solution for 1hr, after which the gel was washed 3x with 200mL deionised water. No bands were visible, so the gel was left to destain overnight in a methanol/acetic acid solution.

Overnight destaining did not yield any visible bands, so the silver nitrate staining method was attempted as per Mortez et al., (2001), but this method was not successful either.

2.4. Discussion

Protein extraction methods remain a challenge for the accurate analysis of proteins due to the presence of contaminants that affect the performance of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Natarajan et al, 2005). These contaminants include salts, detergents, denaturants and organic solvents. Additionally plants contain many compounds which can potentially interfere with the SDS-PAGE process.

Kauri leaves are tough and leathery in texture and contain waxes, phenols and tannins. Leaves accumulate these substances in order to lower the palatability of leaf tissue (Feeny, 1976). Ionic contaminants such as lipids, detergents, and phenolic compounds interfere with isoelectric focusing of proteins resulting in poor gel resolution and reproducibility. Plant-specific compounds such as tannins, lignins, and chlorophyll can also cause disruption of electrophoresis.

Because of differences between the chemical properties of proteins, different extraction methods may favour proteins from different materials. Unfortunately there is no single protocol that has proved successful in extracting protein from all types of plant material, so several have to be tried in order to determine exactly which protocol would yield a better performance using SDS-PAGE. The most versatile method to selectively separate proteins from contaminants like phenolics consists of protein precipitation by trichloroacetic acid (TCA)/acetone followed by resolubilisation in electrophoresis sample buffer.

Phenol extraction of proteins is an alternative method to classical TCA/acetone extraction. It allows for efficient protein recovery and removes non protein components in plant tissues rich in polysaccharides, lipids, and phenolic compounds. (Hurkman and Tanaka, 1986).

Multiple Bradford assays confirmed the presence of protein in the pH 2.8 citrate phosphate buffer extracts, yet no protein was visualised on the SDS-PAGE gel. The Bradford assay is a dye binding, direct, non-enzymatic spectroscopic method, where the dye binding takes place via basic amino acids. High or low pH may affect binding by changing the conformation of proteins. It is therefore possible that the low pH of the citrate phosphate extraction buffer was interacting with the bovine serum albumen (BSA) standard and causing incorrect readings. Further work was needed to determine if the buffer pH is affecting the Bradford assay.

2.5. Bradford assay and citrate phosphate buffer interaction

A lower ionic strength pH 2.8 citrate phosphate buffer was made by dissolving 3.53g of citric acid and 0.91g of disodium hydrogen phosphate in 195mL of water. Both this lower ionic strength buffer and the original pH 2.8 buffer were then separately added to BSA at a 1:1 ratio, with 50µL of each being added. Results are shown in Table 8.

Table 8. Bradford assay results for normal and low ionic strength pH 2.8 citrate phosphate buffer

Treatment	Absorbance 410nm
Blank	0
50 µL BSA +50 µL water	0.218
50 µL BSA +50 µL water	0.235
50 µL BSA +50 µL water	0.239
50 µL BSA +50 µL water	0.273
50 µL BSA +50 µL low ionic strength buffer	0.289
50 µL BSA +50 µL low ionic strength buffer	0.317
50 µL BSA +50 µL low ionic strength buffer	0.308
50 µL BSA +50 µL low ionic strength buffer	0.233
50 µL BSA +50 µL standard ionic strength buffer	0.267
50 µL BSA +50 µL standard ionic strength buffer	0.253
50 µL BSA +50 µL standard ionic strength buffer	0.263
50 µL BSA +50 µL standard ionic strength buffer	0.267

The results show that neither of the citric phosphate buffers are affecting the ability of Coomassie Blue to bind to BSA, so the buffer itself was not causing any discrepancy in protein readings.

2.6. Lowry Assay

The Lowry assay (Lowry, 1951) is another general use protein assay. The Lowry method is very sensitive, but it is a two step procedure that requires a minimum of 40 minutes incubation time (Stoscheck, 1990). The Lowry assay was carried out as per protocol using 100µL each of day 6 raw and concentrated Kauri leaf protein extract (Lowry, 1951). Colour changes were observed upon addition of NaOH, with the day 6 raw extract turning yellow and the Centricon centrifugal filter unit concentrated day 6 extract turning yellow-brown.

Further colour changes were observed after the heated incubation stage, with both extracts turning a dark red. Addition of the Folin reagent caused the colour to shift to a dark blue, with the concentrated Kauri protein extract, a darker, almost black shade of blue. The pH 2.8 citrate phosphate buffer with no protein present also turned yellow at this step. The assay gave an off the scale reading on the spectrophotometer at both 750nm and 550nm wavelengths, possibly indicating that there is a very high degree of phenolic contamination in the samples.

2.7. Phenol Extraction

For recalcitrant tissues, the phenol-based extraction method has the potential to generate samples of higher purity than TCA-acetone, as compounds such as phenols and other water-soluble contaminants are separated from the proteins that are solubilised in the phenolic layer (Hurkman and Tanaka, 1986).

An extraction buffer was made up as per protocol (Carpenter et al., 2005) and contained 50 mM Tris-HCL pH 8.5, 5 mM EDTA, 100 mM KCL, DTT 1% w/v, and sucrose 30% w/v. Kauri leaf tissue was homogenised using liquid nitrogen and 150mg of powder was added to 500µL of extraction buffer. An equal volume of Tris-HCL buffered phenol, pH 8.0, was then added, and the sample was shaken on a platform shaker on ice for 30 min. The extraction was centrifuged at 5000g and the upper phenol layer removed and re-extracted twice with an equal volume of extraction buffer. The final volume of phenol recovered was typically one-third the starting

volume of phenol. To precipitate the proteins, the final phenol phase was added to 5 volumes of 0.1 M ammonium acetate dissolved in methanol. The proteins were precipitated at –20°C for at least 12 h. After precipitation, the pellets were washed twice in ice-cold methanol, twice in ice-cold acetone, and air-dried. Pellets were stored at –80°C until used.

2.8. Trichloroacetic acid (TCA)/acetone extraction method

The TCA-acetone protocol is based on protein denaturation and precipitation under acidic/hydrophobic conditions, which help to concentrate proteins and remove contaminants (Wang et al., 2008). To date, this is the most widely used protocol for protein extraction from plant tissues for proteomic analysis (Jorrín et al., 2007).

Kauri leaf tissue was homogenised in liquid nitrogen using a mortar and pestle, and 6.6g of powder was transferred to a 50mL centrifuge tube. A 30mL volume of ice cold acetone was added and the suspension was centrifuged at 15000*g* for 30min (Karenlampi, 1994). The pellet was dissolved in 15mL of 50 mM citrate buffer and polyvinylpolypyrrolidone (PVPP), which precipitates tannins and binds phenolics, was added at 1:10 of the powdered leaf weight. The suspension was stirred for 20min on ice and centrifuged at 12000*g* for 30min. A Bradford assay was carried out on the supernatant in order to confirm protein content (shown in Table 2.8).

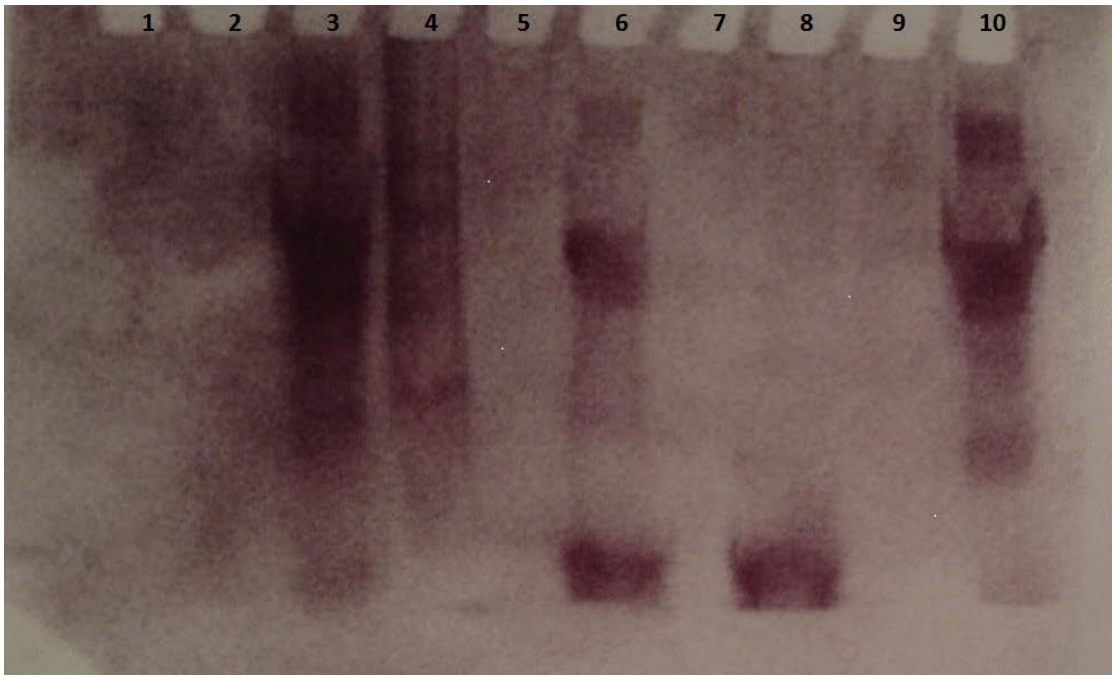
Table 9. Bradford assay results for simple TCA/Acetone protocol

Treatment		Absorbance 410nm
Blank		0
Protein standards:	25µg	0.89
	50µg	0.156
	75µg	0.285
	100µg	0.365
Kauri leaf protein extract		0.262

Trichloroacetic acid (TCA) was added to the supernatant, to give a final concentration of 10% and kept on ice for 30min. Centrifugation was carried out at 4000*g* for 10min and the pellet resuspended in 1.5mL of 10% TCA. The pellet was very gummy and would not solubilise, so a further 5mL of TCA was added and the sample left overnight at 4ºC. The solution was centrifuged at 4000*g* for 10min and the supernatant was discarded. The pellet was washed with 3mL of acetone and left on ice for 10min. The sample was centrifuged at 4000*g* for 10min and the supernatant discarded. An acetone wash was carried out 3x, before the pellet was resuspended in 1.5mL SDS-PAGE running buffer.

SDS-PAGE was carried out using 25µL of TCA/acetone protein extract. Lysosyme and BSA on their own and combined with each other were also run on the gel in order to confirm that protein separation was occurring. Bromophenol blue was also run on its own. The gel was run at 100V for 25min and at 85V for a further 20min. The gel was visualised by the using the standard Coomassie Blue staining method (Sambrook et al., 1982). The gel is shown in Figure 6.

Figure 6. SDS-PAGE gel of simple TCA/Acetone extraction method



Key

Lane 1	Empty
Lane 2	Bromophenol
Lane 3	BSA
Lane 4	Protein standard
Lane 5	Phenol protein extract
Lane 6	BSA/Lysozyme
Lane 7	TCA/Acetone protein extract
Lane 8	Lysozyme
Lane 9	Bromophenol
Lane 10	BSA

No protein bands were observed in the Kauri bands with either the simple TCA/Acetone method or the phenol extraction method.

2.9. TCA/acetone extraction using UKS resolubilisation

Due to the gummy pellet and difficulty with getting the protein back into solution, a modified TCA/acetone method, which ressuspends the protein pellet in a urea- K_2CO_3 -sodium dodecyl sulfate (UKS) solution was attempted next.

The solutions required are listed below as per the protocol (Mechin et al., 2007).

Precipitation solution freshly prepared: 10% TCA, 0.07% 2ME (v/v) in cold acetone.

Rinsing solution: 0.07% 2ME (v/v) in cold acetone.

UKS solubilisation solution: 9.5 M urea, 5% DTT, 1.25% SDS, 6% Triton X-100, 5 mM K_2CO_3. SDS was prepared as a 10% (w/v) stock solution and K_2CO_3 prepared as a 2.8% (w/v) stock solution.

Untreated individual Kauri leaves were ground into a fine powder in liquid nitrogen using a mortar and pestle. The powder from one leaf was transferred to a previously weighed Eppendorf tube and covered with 1.8mL of TCA-2ME precipitation solution. The solution was mixed and stored overnight at -20C for to denature and precipitate

the proteins. Centrifugation of the sample was then carried out at 4ºC for 10min at 10,000*g*.

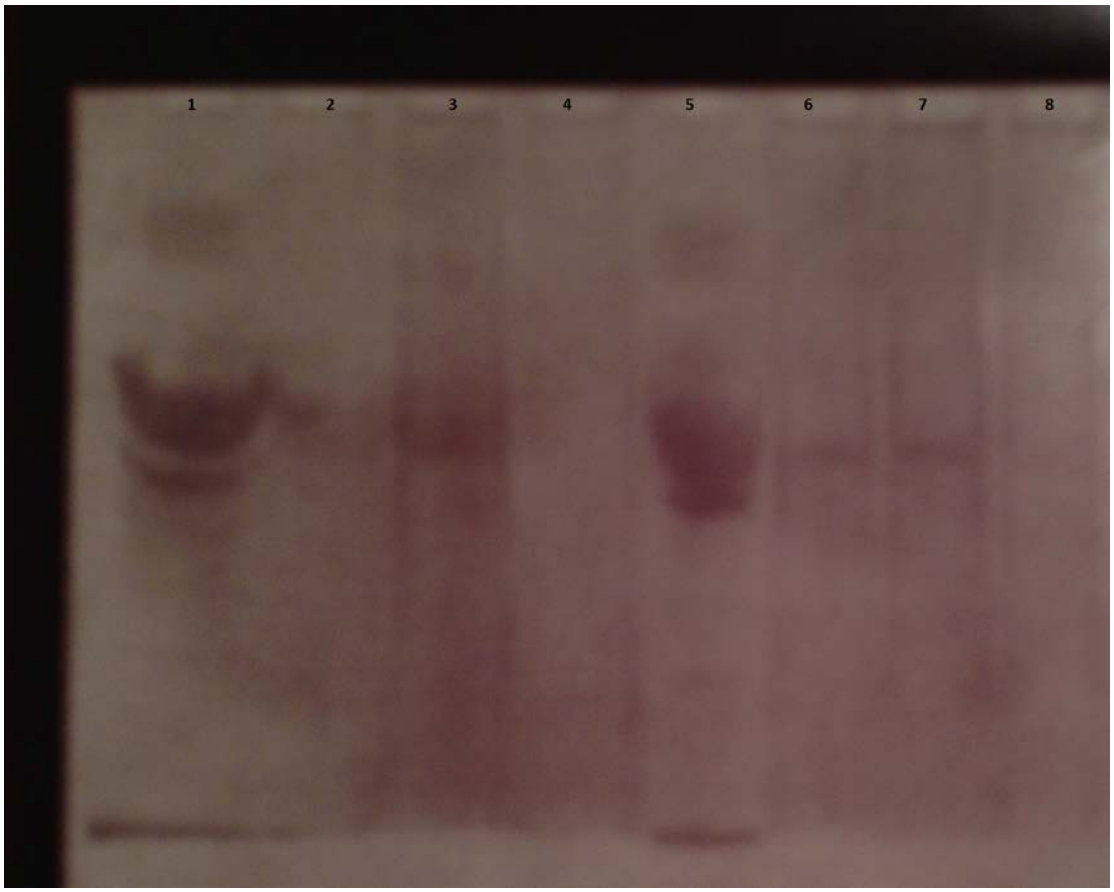
The supernatant was discarded and the pellet resuspended in 1.8mL of cold rinsing solution to eliminate the acidity from TCA that would otherwise interfere with protein recovery. The sample was centrifuged again for 15min at 10,000*g* at 4ºC. The supernatant was discarded and the rinsing step was repeated twice more. After the third rinse, the pellet was dried for 1h in order to eliminate the acetone.

The pellet was weighed and resolubilised in UKS solution at a ratio of 60µl/mg of dry leaf tissue. Resolubilisation was achieved by vortexing the pellet for 1min. The sample was centrifuged at room temperature for 15min at 10,000*g* and the supernatant was collected. The centrifugation step was repeated (15min, 10,000*g*) and the sample stored at -80º C until needed. All but 100µL of the extraction was concentrated using a Centricon centrifugal filter unit as described previously.

The method was also scaled up by a factor of 10 and 0.9g of untreated powdered leaf tissue was used and extracted as per above protocol.

SDS-PAGE was carried out using 25µL of TCA/acetone protein extract. The 10x scaled up extract was diluted and only 15µL of protein extract added to 35µL of the running buffer. The gel was run at 100V for 40min and visualised as per standard Coomassie Blue staining protocol. The gel is shown in Figure 7.

Figure 7. SDS-PAGE gel of TCA/Acetone UKS resolubilisation extraction method



Key

Lane 1	BSA/Lysozyme
Lane 2	TCA/Acetone UKS resolubilisation extract
Lane 3	TCA Acetone UKS resolubilisation extract centricon concentrated
Lane 4	Simple TCA/Acetone extract
Lane 5	BSA/Lysoszyme
Lane 6	10x scaled up TCA/Acetone UKS resolubilisation extract
Lane 7	10x scaled up TCA/Acetone UKS resolubilisation extract diluted
Lane 8	10x scaled up TCA/Acetone UKS resolubilisation extract room temp

Protein bands were visible at approximately 60 KDa using the BSA peptide as a marker, which corresponds with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the most abundant plant protein.

This extraction method was successful in terms of achieving protein gel visualisation, due to the UKS solubilisation solution being more efficient at resuspending the protein pellet, than the standard TCA/acetone protocol.

2.10. Discussion of further selective protein extraction methods

Coomassie staining of SDS-PAGE has so far only yielded one clear visible band on the gel, which likely corresponds with the 60 KDa beta subunit of RuBisCO, the most abundant plant protein. PR proteins are generally smaller than RuBisCO, and are usually found in the 14 kDa-30 kDa range.

Although it has become feasible to rapidly identify proteins from crude cell extracts using mass spectrometry after two-dimensional electrophoretic separation, it can be difficult to isolate low-abundance proteins of interest in the presence of a large excess of relatively abundant proteins. Therefore, for effective proteome analysis it becomes critical to enrich the sample to be analysed for the proteins of interest, in this case the PR proteins.

Hormone induction should increase the relative abundance of PR proteins in the plant itself, but there are also several methods that can be used to increase extraction efficiency. For example the pathogenesis related class of proteins contain many disulphide bridges which can be reduced to thiols and used as an enrichment target.

Protein thiols have been shown to contribute to antioxidant defence (Dat et al., 1998). Plants are constantly exposed to one or more conditions that generate reactive oxygen species (ROS) that serve as second messengers in signal transduction (Jaleel et al., 2009). Factors that influence the formation of ROS include a number of environmental stresses such as light, heat and drought conditions. Oxidative stress has been shown to induce plant defence by increasing salicylic acid (SA) production in heat stressed mustard (Dat et al., 1998). Selective oxidation of cysteines is important in signal transduction even in sub-stress scenarios, but cysteine is the second rarest residue in proteins and it can be difficult to target low-abundance thiol (-SH)-containing proteins in proteomic separations. However activated thiol sepharose (ATS) allows for the covalent selection of -SH-containing proteins, which can then be recovered by reduction with dithiothreitol (DTT) as an eluting agent (Hu et al., 2010). In the present study this method was attempted to enrich the PR proteins from the Kauri extract.

Pathogenesis related protein 3 (PR3) is a chitinase that hydrolyses the β-1,4 linkages of N-acetyl-D-glucosamine polymers of chitin, a major component of fungal cell walls (Bartnicki-Garcia, 1969). Hyunh et al., (1992) have developed a protocol that has successfully described the purification of two basic chitinases from maize seed by

selectively binding the PR3 protein to chitin. This offered a second method for enrichment.

The next section describes the attempt to selectively extract pathogenesis proteins using activated thiol sepharose and chitin following hormone treatment.

2.11. Second application of methyl jasmonate and ethephon

A solution of methyl jasmonate and ethephon as described in section 2.2, was applied to a Kauri seedling. Leaf tissue was harvested prior to hormone application and stored at -80ºC. On the sixth day after treatment, 7.3g of leaf tissue was harvested, with 5.1g stored at -80 ºC and 2.2g powdered using liquid nitrogen.

The TCA/acetone UKS resolubilisation method was carried out with 0.13g of day 0 leaf tissue and 0.11g day 6 leaf tissue.

2.12. Activated thiol sepharose (ATS) purification

The protocol was followed as per Hu et al., (2010), with 200 µL each of day 0 and day 6 protein extract. The binding buffer consisted of 0.1M Tris-HCL, 0.5M NaCl and 1mM EDTA. The elution buffer contained 25mM DTT and 20mM N-ethyl maleimide (NEM). A Bradford assay was carried out to determine protein yield and the results are show in Table 10.

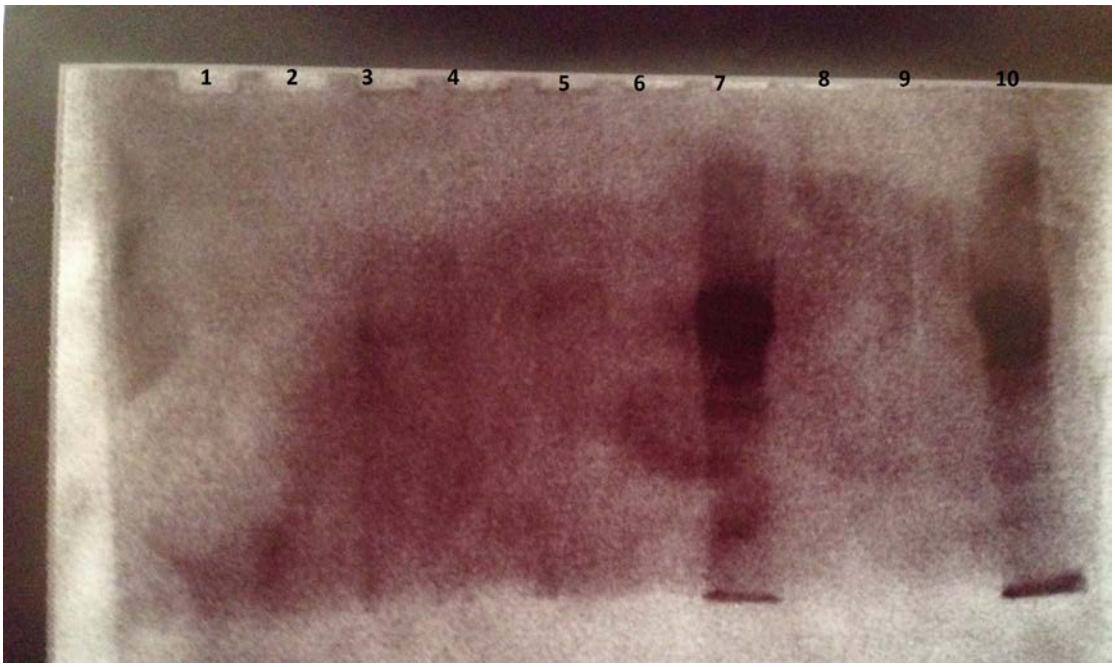
Table 10. Bradford assay results after Thiol Sepharose purification

Treatment		Absorbance 410nm
Blank		0
Protein Standards:	25µg	0.263
	50µg	0.462
	75µg	0.56
	100µg	0.661
Day 0 Kauri protein extract after NEM treatment		0.244
Day 6 Kauri protein extract after NEM treatment		0.246

SDS-PAGE was carried out using 25µL of the thiol sepharose purified Day 0 and Day 6 protein extract. The gel was run at 100V for 40min and is shown in Figure 8 after Coomassie blue staining.

The RuBisCO band was still visible in the raw protein extract, but no bands were visible after thiol sepharose treatment. The gel also had much more background staining. Three more thiol purification treatments of the raw Kauri protein extracts were carried out, including protein concentration via Centricon, but no visible bands were detected in the SDS-PAGE gels.

Figure 8. SDS-PAGE gel of kauri protein extracts after purification with thiol sepharose



Key

Lane 1	Blank
Lane 2	Blank
Lane 3	Day 6 MJ and ethephon treated raw protein extract
Lane 4	Day 0 MJ and ethephon treated raw protein extract
Lane 5	Day 6 MJ and ethephon treated raw protein extract room temp
Lane 6	Day 0 MJ and ethephon treated raw protein extract room temp
Lane 7	BSA/Lysozyme
Lane 8	NEM Day 0
Lane 9	NEM Day 6
Lane 10	BSA Lysozme

2.13. Chitinase extraction

Chitinase extraction was carried out as per the protocol described by Hyunh et al., (1992). The protocol is pH sensitive so raw Kauri protein extract was adjusted to pH 8.0 before chitinase extraction was attempted. A 1.5mL volume of day 0 and day 6 MJ and ethephon treated Kauri protein extract was added to 0.1g of chitin. A Centricon column was used to concentrate and elute the final protein product.

SDS-PAGE was carried out to visualise the protein composition of the chitinase extraction. The gel was run at 100V for 40min and stained with Coomassie. No bands were visible on the gel lanes corresponding to the chitinase extraction.

2.14. Discussion

Two methods were attempted in order to try and select for enrichment of PR proteins. SDS-PAGE was unable to confirm the presence of any protein using thiol purification or chitinase extraction. Due to time constraints, extraction methods for specific selection for PR proteins were abandoned. The TCA/Acetone UKS resolubilisation method was used for all further protein extractions.

2.15. Repetition of MJ and ethephon treatments and extraction via TCA/acetone UKS resolubilisation

Several more treatments of Kauri seedlings with MJ and ethephon were carried out with leaf tissue subjected to TCA/Acetone UKS resolubilisation extraction. SDS-PAGE was used to confirm the purity of the extract. Bands from the cleanest gels (see Figure 9 for example) were then excised using a scalpel and sent for Liquid chromatography–mass spectrometry (LC-MS) at the University of Auckland Proteomics Facility. The raw protein extract was concentrated via Centricon and also sent for LC-MS analysis.

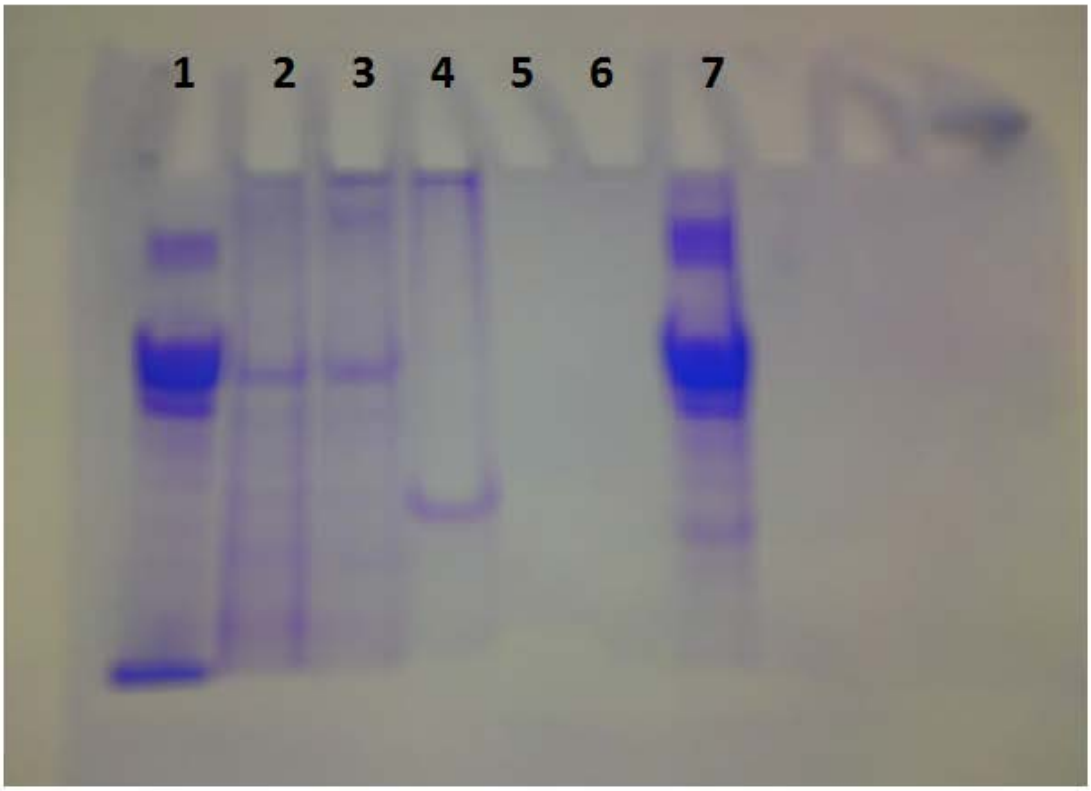


Figure 9. SDS-PAGE gel of MJ and ET treated Kauri protein extract

Lane 1 BSA/Lysozyme

Lane 2 Day 6 MJ and ethephon treated Kauri protein extract

Lane 3 Day 0 MJ and ethephon treated Kauri protein extract

Lane 4 Protein Marker

Lane 5 Blank

Lane 6 Blank

Lane 7 BSA/Lysozyme

2.16. Liquid chromatography–mass spectrometry (LC-MS)

LC-MS was carried out at Auckland University Proteomics Facility and the results subjected to a BLAST type search with the MASCOT search engine against the NCBI Conifers subset in order to identify protein homologues. Several protein sequences were identified including an oxygen-evolving enhancer protein 3-2, Cu-Zn superoxide dismutase, a cytochrome b559 protein, with an actual Agathis match and an acid phosphatase. Table 11 shows an example of the strongest protein matches and their sequences, after a MASCOT search against green plants. Table 12 shows protein sequences for superoxide dismutase and acid phosphatase MASCOT search results against the conifers. The full LC-MS data is available in Appendix 1.

Table 11. Example of LC-MS results of Kauri protein extract

Names	Sequence
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	TVTAMDVVYALKR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	TVTAMDVVYALKR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	TVTAMDVVYALKR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	DNIQGITKPAIR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	IFLENVIR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	DNIQGITKPAIR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	TVTAMDVVYALKR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	DNIQGITKPAIR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	ISGLIYEETR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	TVTAMDVVYALK
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	ISGLIYEETR
histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]	ISGLIYEETR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	KAWPYVQNDLR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	LFDTLDNLDYAAR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	YYAETVSALNDVISK
unknown [Picea sitchensis]; unknown [Picea sitchensis]	LFDTLDNLDYAAR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	LFDTLDNLDYAAR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	LFDTLDNLDYAAR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	YYAETVSALNDVISK
RecName: Full=Histone H2AX; putative histone H2B [Pinus pinaster]	AGLQFPVGR
RecName: Full=Histone H2AX; putative histone H2B [Pinus pinaster]	AGLQFPVGR
cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]	FDSLEQLDEFTK
cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]	QEVPLVTGR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	ISVLNDALR
unknown [Picea sitchensis]; unknown [Picea sitchensis]	LITPSILSDR
Cu-Zn superoxide dismutase [Larix gmelinii var. gmelinii]	GGHELSLSTGNAGGR
Putative acid phosphatase PS18	SLSIMEPIR

Table 12. Protein sequence of Superoxide Dismutase and Acid Phosphatase in Kauri

Accession	Names	Protein Sequence
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	AVVVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	VVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	AVVVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	AVVVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	AVVVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	HAGDLGNVTVGTDGTVEFSITDSQIPLSGPHSIVGRAVVVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	HAGDLGNVTVGTDGTVEFSITDSQIPLSGPHSIVGRAVVVHADPDDLGKGGHELSK
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]	LACGVVGLQG
gi|109892853	Putative acid phosphatase PS18	SLSIMEPIR
gi|109892853	Putative acid phosphatase PS18	SLSIMEPIR
gi|109892853	Putative acid phosphatase PS18	SLSIMEPIR

2.17. Discussion

The data results in Appendix 1 are the first reported proteomic analysis of Kauri. Although many protein sequences were identified, few were the sort after defence related proteins. However two of the identified proteins, superoxide dismutase and acid phosphatase have been shown to be implicated in plant stress response and defence (protein sequences shown in Table 12).

Superoxide dismutase (SOD) is the first line of defence in plants against toxic radical oxygen species (ROS). It is a metal-containing enzyme that catalyzes the dismutation of ROS to oxygen and hydrogen peroxide, thus eliminating free radicals (Bowler et al., 1992). The enzyme has been found in all aerobic organisms, where it plays a major role in the defence against ROS, which are generated as byproducts of both biological oxidations and environmental stress (Bowler et al., 1992).

Acid phosphatases are ubiquitous enzymes found in plants that catalyse the dephosphorylation of a wide variety of substrates by hydrolysis of phosphate esters (Duff et al., 1994). These enzymes are believed to have roles in energy transfer and metabolic regulation in plant cells (Duff et al., 1994).

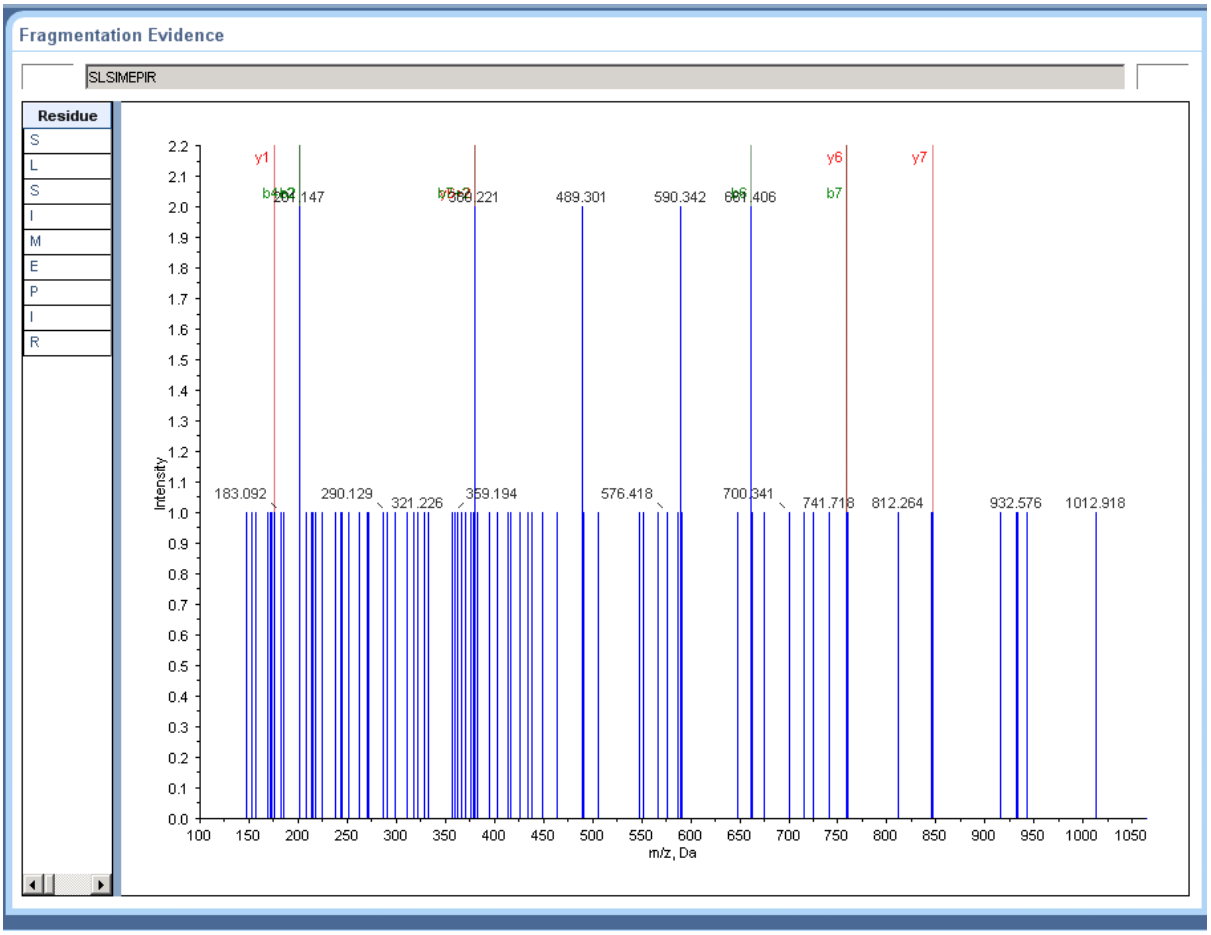
More importantly for this study, acid phosphatases have also been shown to be associated with disease resistance in plant systems. For example, transcript of a putative acid phosphatase accumulates in barley in response to chemical compounds which activate systemic resistance to pathogens (Beber et al., 2000). Acid phosphatase activity has also been shown to increase in tobacco following inoculation with bacteria, which induced a hypersensitive response in the plant (Kenton et al., 1999). Acid phosphatases have multiple functions related to phosphate metabolism and a large pool of potential substrates (Duff et al., 1994), therefore, the putative acid phosphatase identified via LC-MS could have any one of a variety of roles related to disease resistance.

Furthermore a proteomic study (Smith et al., 2006) in which a comparison of needles from Blister Rust-resistant and susceptible *Pinus strobus* seedlings revealed up-regulation of putative disease resistance proteins, one of which, an acid phosphatase had a perfect match with an acid phosphatase sequence in the current study.

The disease resistance up-regulated acid phosphatase peptide from the *Pinus strobus* study had the amino acid sequence SLSIMEPIR (Smith et al., 2006) as did an acid phosphatase peptide from the current study as shown in the mass spectrum below in Figure 10.

The mass spectrum shown has a confidence score of 67, but the quality of the mass spectrum was only relatively mediocre. However given that this was the only disease resistance protein identified in the proteomic analysis in the present study that was also shown to be pathogen induced in a previous study (Smith et al., 2006), in a related species (*Pinus strobus*), it was decided to further investigate the putative Kauri acid phosphatase protein using an enzyme assay.

Figure 10. Mass Spectrum of putative acid phosphatase peptide sequence SLSIMEPIR



An enzymatic assay was selected as the proteomic methods used up to now were strongly dependent on the quality of the protein extractions, which had so far proved to be troublesome. In addition an enzyme assay for acid phosphatase would also be able to measure the degree of induction under various hormone treatments quantitatively.

Chapter 3. Enzyme Induction assay of Acid Phosphatase

3.0. Acid Phosphatase assay

Acid phosphatase activity is measured by the rate of conversion of the chromogenic substrate paranitrophenyl phosphate (pNPP) into p-nitrophenol (pNP). The pNPP phosphatase activity is assayed in a reaction mixture containing 50mM pNPP and a protein phosphatase buffer supplemented with additional components when required. The reaction is initiated by addition of enzyme and quenched by addition of 1 ml of 1M NaOH. The amount of product, pNP, is determined by reading the absorbance at 410 nm and using a molar extinction coefficient of $18mM^{-1}$ cm^{-1} (Mackintosh, 1993). One unit of the protein phosphatase activity is defined as the amount of enzyme that hydrolyzes 1 nanomole of pNPP in one minute at 30°C in a total reaction volume of 50µL under standard reaction conditions.

Several factors affect the rate at which enzymatic reactions proceed, temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators. Optimal parameters for temperature and time needed to be determined, as well as the extraction buffer itself modified to extract phenolic compounds that may inhibit the assay.

Sodium acetate (100 mM) was used as both a lysis and extraction buffer. In order to extract the enzyme from Kauri seedlings in a biologically active form, all procedures were performed at 4°C. Leaves were ground in a mortar with liquid nitrogen until they turned into a fine powder. The powder was transferred to a test tube and weighed, before the addition of cooled sodium acetate buffer at a ratio of 1:5 w/v. Phenolic compounds are known to cause interference with enzyme assay measurements so 1:10 w/w Polyvinylpyrrolidone (PVP) and Polyvinylpolypyrrolidone (PVPP) , were added to the buffer to bind the phenolics. An ultra-turrex homogeniser was used to further break up the leaf tissue. The mixture was centrifuged at 12,000*g* for 20min and the supernatant collected for the acid phosphatase enzyme kinetics measurements.

The assay itself consisted of adding 50µL of Kauri protein extract to 2mL of pNPP substrate. A control was also setup, which consisted of 200µL Kauri protein extract added to 2mL sodium acetate buffer only, with no substrate. The initial assay was carried out at 37ºC in a water bath for 10min. The reaction was stopped with the addition of 1mL NaOH (1M). Enzyme activity was determined by using a spectrophotometer to measure the absorbance increase at 410nm. Results are shown in Table 13.

Table 13. Acid phosphatase assay results after the addition of PVP and PVPP

	Absorbance 410nm
Blank	0
Control	0.234
Kauri protein extract	0.23
Kauri protein extract	0.322
Kauri protein extract	0.276
Kauri protein extract + PVP	0.415
Kauri protein extract + PVP	0.511
Kauri protein extract + PVP	0.586
Kauri protein extract + PVP +PVPP	0.299
Kauri protein extract + PVP +PVPP	0.318
Kauri protein extract + PVP +PVPP	0.34

The results showed that the addition of PVP improved the acid phosphatase reaction, so the decision was made to use it in all further assays.

Another series of assays were carried out where the reaction was stopped at 5 min intervals over the course of a 30 min period in order to determine the optimal incubation time. The results are shown below in Table 14.

Table 14. Acid phosphatase assay results over a 30min time period

	Time (min)	Absorbance 410nm
Blank	0	0
Blank	15	0
Blank	30	0
Control	0	0.092
Control	15	0.123
Control	30	0.115
Kauri protein extract	0	0.143
Kauri protein extract	5	0.219
Kauri protein extract	10	0.25
Kauri protein extract	15	0.265
Kauri protein extract	20	0.274
Kauri protein extract	25	0.361
Kauri protein extract	30	0.4

A 30min incubation period was used going forward as it gave the highest enzyme activity when compared with the control absorbance reading.

The temperature of the incubation period was the final parameter tested and the results shown in Table 15.

The pNPP substrate became unstable at temperatures greater than 60 ºC, as evidenced by the greater than 0 absorbance value in the blank. A 30ºC temperature was selected as the optimum temperature going forward.

Table 15. Acid phosphatase assay results showing enzyme activity at different temperatures

	Temperature ºC	Absorbance 410nm
Blank	0	0
Kauri protein extract	0	0.245
Kauri protein extract	0	0.254
Kauri protein extract	0	0.244
Kauri protein extract	0	0.264
Blank	20	0
Kauri protein extract	20	0.395
Kauri protein extract	20	0.347
Kauri protein extract	20	0.332
Kauri protein extract	20	0.327
Blank	40	0
Kauri protein extract	40	0.498
Kauri protein extract	40	0.579
Kauri protein extract	40	0.623
Kauri protein extract	40	0.632
Blank	60	0.876
Kauri protein extract	60	1.819
Kauri protein extract	60	1.88
Kauri protein extract	60	1.981
Kauri protein extract	60	1.971
Blank	80	>3
Kauri protein extract	80	>3
Kauri protein extract	80	>3
Kauri protein extract	80	>3
Kauri protein extract	80	>3
Blank	100	>3
Kauri protein extract	100	>3
Kauri protein extract	100	>3
Kauri protein extract	100	>3
Kauri protein extract	100	>3

3.1.1. Discussion

When NaOH was added to the Kauri protein control, it turned yellow and showed an absorbance reading at 410nm. Changing the absorbance to 401nm lowered this background interference, but it was still present. The control readings of just the Kauri protein itself will need to be subtracted from the substrate readings, in order to adjust for the background interference.

3.2. Hormone treatment with methyl jasmonate and ethephon

A Kauri seedling was treated with a mixture containing 0.23g methyl jasmonate (MJ), 0.5g tween, 200µL 99% ethanol and 25mL of water. The soil was also doused with 6.6mg of ethephon (ET) dissolved in 10mL of water. Leaves were collected on Day 0, 3, 6, 9 and 12 and placed at -80 ºC. A control plant without any hormone treatment was also harvested at the same three day intervals.

Five leaves from each day were individually subjected to the acid phosphatase enzyme assay as described in section 3.1. The assay was done four times for each individual Kauri leaf. The hormone treatments were then repeated on two more Kauri seedlings as well as leaves harvested from another untreated control plant.

The data was pooled and subjected to statistical analysis via a Two Tailed t-test. A p-value of .05 or less rejects the null hypothesis and accepts that the result is statistically significant.

The t-test values of the raw absorbance differences comparing the leaves from the treated plants against day 0 treated are shown in table 16.

Table 16. T-test p value comparing MJ & ET treated Kauri against day 0 treated

Day	p value
Day 3	0.3
Day 6	0.03
Day 9	0.01
Day 12	0.05

Day 6 ,9 and 12 were significantly different from day 0.

Table 17 shows the t-test of the absorbance values of the hormone treated plant compared with the untreated control plant. Day 6, 9 and 12 once again showed that they are statistically different with $p < 0.05$.

Table 17. T-test p value comparing MJ & ET treated kauri against the untreated Control

Day	p value
Day 0	0.7
Day 3	0.6
Day 6	0.003
Day 9	0.03
Day 12	0.007

The following equation was used to calculate enzyme activity:

Equation 1. Acid phosphatase enzyme activity calculation

$$\text{Ap activity } (\mu\text{mol/min/mL}) = \Delta\text{Abs}/30\text{min} \times 2\text{mL}/0.05\text{mL} \times 18\ \text{mM}^{-1}\ \text{cm}^{-1}$$

The results are shown in Figures 11 and 12. There is a 1.8x difference between enzyme activity on day 0 and day 6 of the MJ and ET treated plants. Day 12 also shows a 1.7x difference. The control untreated plant phosphatase activity remained relatively unchanged over the course of the 12 days (Figure 12), but the MJ and ET treated plants showed increased enzyme activity, particularly on day 6 and day 12.

Figure 11. Comparison of the amount of phosphatase activity between MJ & ET treated plants vs untreated Control

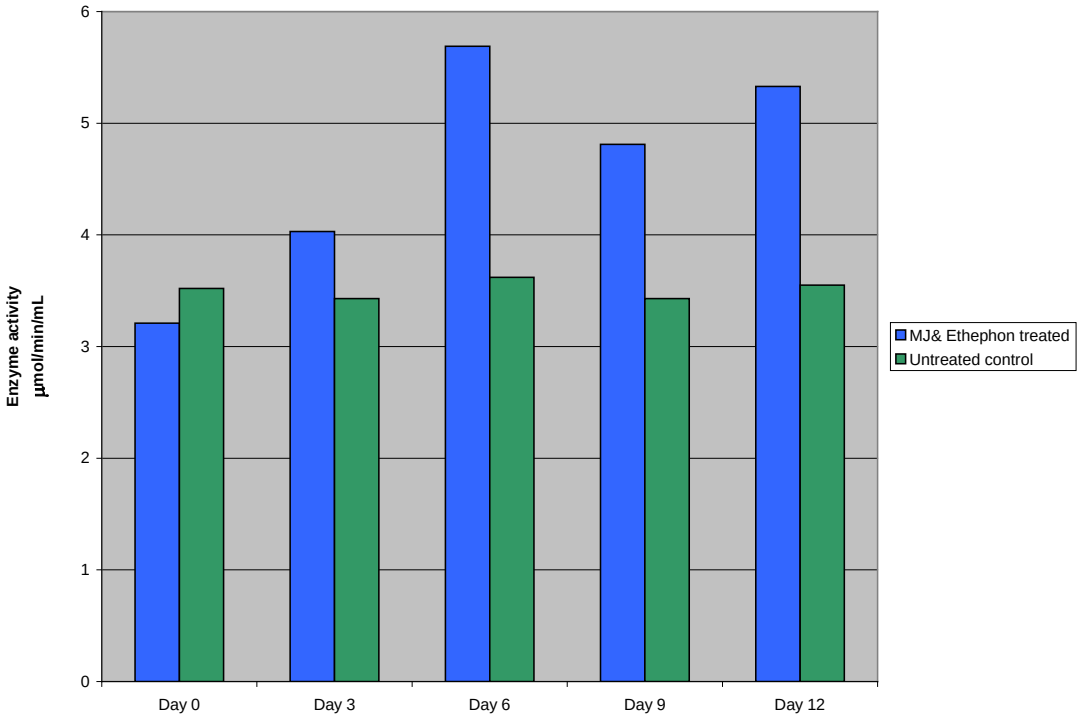
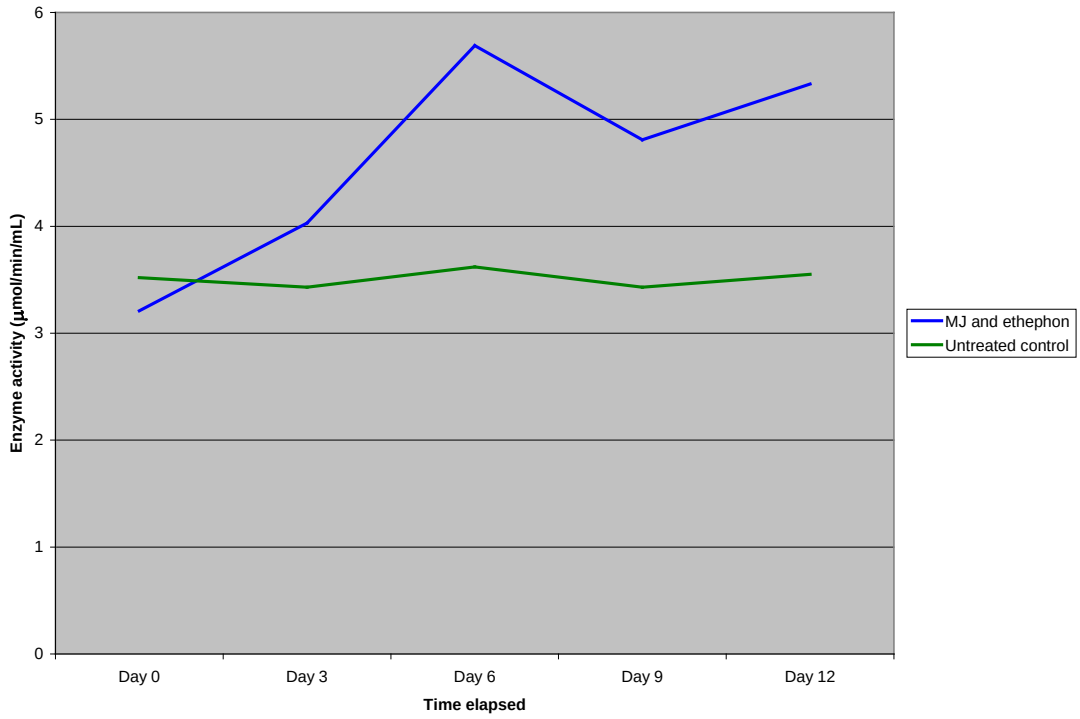


Figure 12. Phosphatase activity after MH and ET treatment over time



3.3. Hormone treatment with methyl jasmonate single and double dose

Kauri seedlings were treated with either a single dose of MJ (0.23g methyl jasmonate, 0.5g tween, 200μL 99% ethanol and 25mL of water) or a double dose consisting of (0.5g methyl jasmonate, 0.5g tween, 200μL 99% ethanol and 25mL of water). Leaves were harvested at day 0, 3, 6, 9 and 12 and assayed as per section 3.1.

The t-test results comparing single, double and control untreated plants are shown in Table 18. Table 19 shows the t-test results using each plant's day 0 leaves as its own control.

There was a large variation with the results. When compared against the untreated control only day 6 and day 12 on the double dose MJ treated plant showed statistically significant differences. Both the single and double dose MJ treated plants showed statistically significant differences on day 9, when the day 0 from the actual plant itself was used as a negative control.

Table 18. T-test showing the comparison between single and double dose MJ treated Kauri and the untreated Control

	Single dose p value	Double dose p value
Day 0	0.2	0.8
Day 3	0.1	0.2
Day 6	0.05	0.04
Day 9	0.2	0.07
Day 12	0.7	0.01

Table 19. T-test results showing p values for MJ single and double treated plants using each plant as its own control

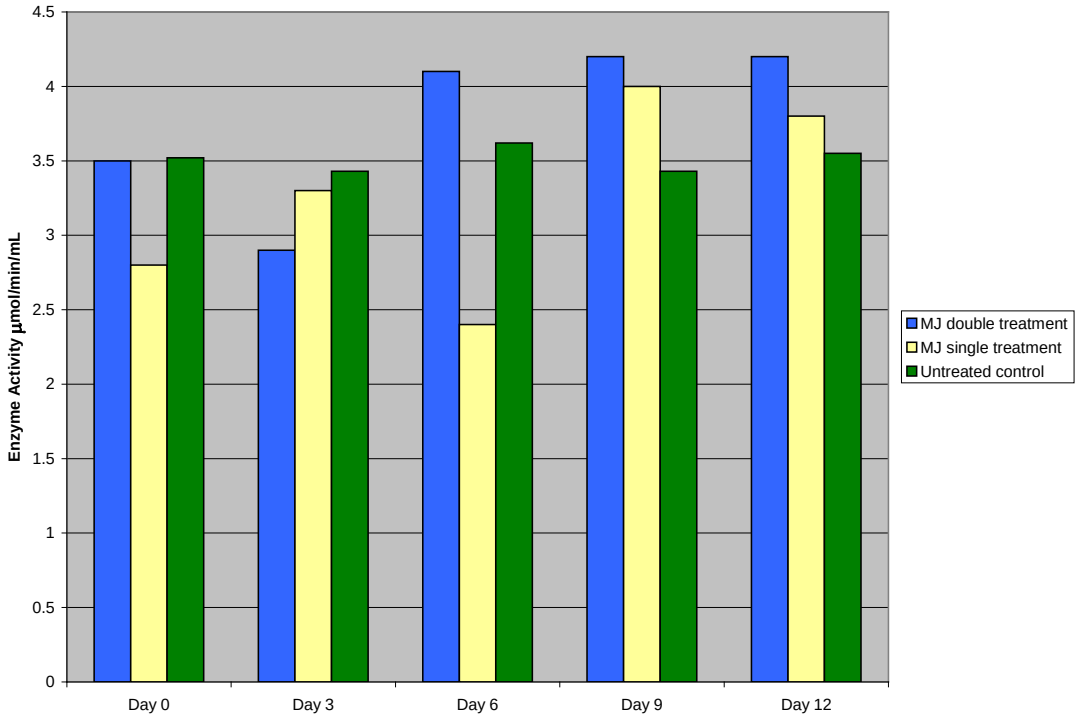
	Single dose p value	Double dose p value
Day 3	0.2	0.07
Day 6	0.3	0.09
Day 9	0.04	0.05
Day 12	0.1	0.02

The double dosage also appeared to have a detrimental effect on the plant itself, with leaves turning brown and eventually dying (shown in the photograph below).

Browning effect on Kauri leaves of a high dose of methyl jasmonate

Enzyme activity was calculated as previously described in section 3.2 and the results are shown in Figure 13.

Figure 13. Enzyme activity of MJ double and single dose treated plants and the untreated Control



There is approximately a 1.2x difference between day 0 and day 12 enzyme activity in the double dose MJ treated plant.

3.4. Hormone treatment with a salicylic acid analogue, INA

The synthesis of PR proteins have been shown to be induced by treatment with SA and also by the application of functional analogues of SA, such as 2,6-dichloroisonicotinic acid (INA) (Metraux et al., 1991).

Two different dosages of 2,6-dichloroisonicotinic acid (INA) were applied to Kauri seedlings. One dose consisted of 0.0078g of INA dissolved in 200 μL 99% ethanol and then added to 400 mL distilled water. The second dose consisted of .0832g INA dissolved in 600 μL of 99% ethanol and added to 100 mL distilled water. Leaves were collected on day 0, 3, 6, 9 and 12 and the phosphatase assay carried out as per section 3.1.

The results showed no statistically significant differences with the INA treated Kauri seedlings.

3.5. Discussion

Although there is considerable variation observed between not only different plants, but individual leaves from the same plant, data analysis does show that there was a

statistical significant difference between methyl jasmonate and ethephon treated seedlings over the course of the treatment. The combination of the two hormones was the most effective in terms of acid phosphatase induction, with a 1.8x difference observed with the combination of hormones as opposed to a 1.2x difference with just methyl jasmonate on its own.

The level of PR proteins in plants are strongly dependent on growing conditions, so factors like temperature, light cycle and humidity need to be properly regulated. The Kauri seedlings used in this work were grown in a general lab environment over the course of the year, so were exposed to different environmental and seasonal variables. Some of the variability in the results will be due to not growing the seedlings in a controlled environment, but the variation observed in individual leaves from the same plant indicates that there are also other factors involved. The position of the leaves on the stem may play a role and create differences in response. Poiatti et al., (2009) have shown that on inoculation with bacteria, the basal leaves of potato (*Solanum tuberosum)* showed higher peroxidase activity and lower levels of total phenolic compounds and flavonoids, compared to the apical leaves.

A leaf disc treatment may also be a more effective method in measuring enzyme activity as it is easier to standardise the application of the treatment to an individual leaf, as opposed to spraying the entire plant.

Chapter 4. Conclusion

4.0. Conclusion

Kauri leaf tissue proved to be rather recalcitrant in terms of protein extraction and purification, and several methods were tried before a sufficiently useful extract was achieved. LC-MS analysis of the kauri protein extract resulted in the first known protein sequences for Kauri. The LC-MS analysis lead to the identification of a defense related protein, a putative acid phosphatase, that was selected as a target for an enzyme induction assay study.

Treatment of Kauri seedlings with methyl jasmonate and ethephon showed a statistically significant induction of acid phosphatase. Due to time constraints it was not possible to test the induced trees for resistance against the pathogen Phytophthora taxon agathis (PTA). However a number of plant acid phosphatases have been implicated in resistance to pathogens and nematodes (Heil and Bostock, 2002). They appear to be induced by different regulatory pathways, where barley acid phosphatase was shown to be induced by the salicylic acid mimic, 2,6-dichloroisonicotinic acid and JA, while potato acid phosphatase responded only to intact bacterial organisms (Heil and Bostock, 2002).

Further work needs to be done in terms of treating Kauri seedlings with MJ and ET (or other combinations of hormones) and then challenging the plants with PTA. It would definitely be of value to determine if artificially inducing the kauri defence system prior to PTA infection has any effect on PTA disease progression. It is important to note though, that any form of induced resistance can only be of selective

advantage if the eliciting attack has a predictive value and can be used as a cue to indicate future attack by a given enemy (Karban et al., 1999). The current consensus is that PTA is an exotic species to New Zealand (Beever et al., 2009), so the host-pathogen interaction between Kauri and PTA would be something new to both plant and pathogen. Given that to date, no Kauri has been found to be resistant to PTA, there is a possibility that the pathogen is bypassing the Kauri defence system entirely. It will therefore be of interest to see whether priming Kauri with the application of MJ and ET prior to inoculation with PTA shows any difference in terms of disease progression and pathogenicity.

It is important to note that several differences have also been reported between chemically and biologically induced resistance (Schweizer et al.,1997). Studies using external application of signalling compounds like MJ or ET may therefore suffer from physiologically unrealistic concentrations and spatial distributions of the resistance inducers. There are also likely to be mechanisms regulating the interplay among resistance pathways in response to natural induction that are bypassed when resistance is induced chemically (Heil and Bostock, 2002).

It is clear that defence related protein induction is a complex series of interactions between the host plant, the environment and the attacking pathogen. Experiments that thoroughly explore signalling conflicts and synergies in plant pathogen interactions will be essential to fully understanding the potential of inducible resistance strategies in pathogenic resistance management. There is also such a wide variation in response between individual plants, that high throughput microarray analysis will be a must in terms of gaining consistent results. Any future work will need to use a large sample

population, that was unfortunately beyond the scope and time constraints of this work.

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Appendix

Appendix 1.1 LC-MS MASCOT analysis of Kauri Protein compared against Green Plants

Unused	Total	%Cov	%Cov(95)	Accession	Name
7.27	7.27	58.25	33.98	gi|62642127	histone 4 [Picea abies]
0	7.27	58.25	33.98	gi|383162100	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162098	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162096	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162094	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162092	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162090	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162088	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162086	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162084	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162082	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162080	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162078	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162076	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162074	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162072	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162070	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|383162068	hypothetical protein 0_18315_01 [Pinus taeda]
0	7.27	58.25	33.98	gi|361067525	hypothetical protein 0_18315_01 [Pinus radiata]
0	7.27	58.25	33.98	gi|224285053	unknown [Picea sitchensis]
0	7.27	58.25	33.98	gi|116793524	unknown [Picea sitchensis]
0	7.27	58.25	33.98	gi|116788052	unknown [Picea sitchensis]
0	7.27	58.25	33.98	gi|116782704	unknown [Picea sitchensis]
0	7.27	58.25	33.98	gi|116778467	unknown [Picea sitchensis]
0	4.76	48.54	24.27	gi|62642125	histone 4 [Picea abies]
2.26	2.26	19.75	11.52	gi|224285725	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|224285419	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|224285087	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|224284923	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|224284653	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|224284078	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|148909289	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|116793677	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|116793667	unknown [Picea sitchensis]
0	2.26	21.92	12.79	gi|116793613	unknown [Picea sitchensis]
0	2.26	19.75	11.52	gi|116785681	unknown [Picea sitchensis]
2	2	40.54	40.540001	gi|76150398	Cu-Zn superoxide dismutase [Larix gmelinii var. gmelinii]

0	2	57.69	57.690001	gi|66841108	Cu/Zn superoxide dismutase [Larix sibirica]
0	2	57.69	57.690001	gi|66841106	Cu/Zn superoxide dismutase [Larix gmelinii]
1.89	1.89	6.923	6.9229998	gi|294463748	unknown [Picea sitchensis]
0	1.89	6.923	6.9229998	gi|294461482	unknown [Picea sitchensis]
0	1.89	6.923	6.9229998	gi|116784403	unknown [Picea sitchensis]
0	1.89	6.923	6.9229998	gi|116781396	unknown [Picea sitchensis]
1.66	1.66	39.81	9.2589997	gi|224286704	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|224286580	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|224284082	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|148906786	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|148906644	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|116793648	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|116790134	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|116788763	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|116779909	unknown [Picea sitchensis]
0	1.66	39.81	9.2589997	gi|116779097	unknown [Picea sitchensis]
0	1.66	9.259	9.2589997	gi|224286268	unknown [Picea sitchensis]
0	1.66	9.259	9.2589997	gi|116789769	unknown [Picea sitchensis]
0	1.66	9.259	9.2589997	gi|116785734	unknown [Picea sitchensis]
1.51	1.51	41.33	12	gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]
0	1.51	37.35	10.84	gi|354991441	psbE (chloroplast) [Agathis australis]
0	1.51	37.35	10.84	gi|347977320	cytochrome b559 alpha chain [Agathis dammara]
0	1.51	41.33	12	gi|33327799	cytochrome b-559 alpha subunit [Agathis australis]
0	1.51	41.33	12	gi|154001321	cytochrome b-559 alpha subunit, partial (chloroplast) [Agathis robusta]
0	1.51	41.33	12	gi|154001316	cytochrome b-559 alpha subunit, partial (chloroplast) [Wollemia nobilis]
0	1.51	28	12	gi|154001326	cytochrome b-559 alpha subunit, partial (chloroplast) [Araucaria cunninghamii]
0	1.28	40.26	11.69	gi|56236639	cytochrome b-559 alpha subunit [Juniperus communis]
0	1.28	40.26	11.69	gi|56236634	cytochrome b-559 alpha subunit [Torreya californica]
0	1.28	41.33	12	gi|56236624	cytochrome b-559 alpha subunit [Saxegothaea conspicua]
0	1.28	35.63	10.34	gi|354991439	psbE (chloroplast) [Halocarpus kirkii]
0	1.28	36.47	10.59	gi|352951105	cytochrome b559 alpha chain (chloroplast) [Taiwania cryptomerioides]
0	1.28	36.47	10.59	gi|347977435	cytochrome b559 alpha chain [Taiwania cryptomerioides]
0	1.28	40.26	11.69	gi|33327907	cytochrome b-559 alpha subunit [Thuja plicata]
0	1.28	41.33	12	gi|33327859	cytochrome b-559 alpha subunit [Phyllocladus alpinus]
0	1.28	40.26	11.69	gi|33327825	cytochrome b-559 alpha subunit [Cunninghamia lanceolata]
0	1.28	40.26	11.69	gi|33318703	cytochrome b-559 alpha subunit [Metasequoia glyptostroboides]
0	1.28	36.9	10.71	gi|239794368	photosystem II cytochrome b559 alpha subunit [Cryptomeria japonica]
0	1.28	36.9	10.71	gi|239794286	photosystem II cytochrome b559 alpha subunit [Cryptomeria japonica]
0	1.28	36.9	10.71	gi|172072898	photosystem II protein V [Cryptomeria japonica]
0	1.28	36.9	10.71	gi|171854917	photosystem II cytochrome b559 alpha subunit [Cryptomeria japonica]
0	1.28	40.26	11.69	gi|138277505	cytochrome b-559 alpha subunit [Taxus brevifolia]
0	1.28	40.79	11.84	gi|138277502	cytochrome b-559 alpha subunit [Taxodium distichum]
0	1.28	34.94	10.84	gi|353351849	cytochrome b559 alpha chain (chloroplast) [Picea morrisonicola]
0	1.28	22.35	10.59	gi|352950974	cytochrome b559 alpha chain (chloroplast) [Cephalotaxus wilsoniana]
0	1.28	22.35	10.59	gi|350529168	cytochrome b559 alpha chain [Cephalotaxus wilsoniana]
0	1.28	34.94	10.84	gi|347977512	cytochrome b559 alpha chain [Picea morrisonicola]

0	1.28	24.68	11.69	gi|33327912	cytochrome b-559 alpha subunit [Widdringtonia cedarbergensis]
0	1.28	24.68	11.69	gi|33327815	cytochrome b-559 alpha subunit [Cephalotaxus harringtonia]
0	1.28	38.67	12	gi|33318678	cytochrome b-559 alpha subunit, partial (chloroplast) [Cedrus deodara]
0	1.28	34.94	10.84	gi|309322351	cytochrome b559 alpha chain [Cedrus deodara]
0	1.28	34.94	10.84	gi|307683633	cytochrome b559 alpha chain [Picea morrisonicola]
0	1.28	34.94	10.84	gi|307683277	cytochrome b559 alpha chain [Cedrus deodara]
0	1.28	20.48	10.84	gi|7524642	photosystem II protein V [Pinus thunbergii]
0	1.28	22.67	12	gi|56236619	cytochrome b-559 alpha subunit [Pseudotsuga menziesii]
0	1.28	22.67	12	gi|56236614	cytochrome b-559 alpha subunit [Abies lasiocarpa]
0	1.28	20.48	10.84	gi|357001915	photosystem II protein V [Pinus clausa]
0	1.28	20.48	10.84	gi|357001842	photosystem II protein V [Pinus arizonica var. cooperi]
0	1.28	20.48	10.84	gi|357001769	photosystem II protein V [Pinus coulteri]
0	1.28	20.48	10.84	gi|357001696	photosystem II protein V [Pinus cubensis]
0	1.28	20.48	10.84	gi|357001623	photosystem II protein V [Pinus culminicola]
0	1.28	20.48	10.84	gi|357001554	photosystem II protein V [Pinus fenzeliana var. dabeshanensis]
0	1.28	20.48	10.84	gi|357001482	photosystem II protein V [Pinus dalatensis]
0	1.28	20.48	10.84	gi|357001409	photosystem II protein V [Pinus densiflora]
0	1.28	20.48	10.84	gi|357001336	photosystem II protein V [Pinus densata]
0	1.28	20.48	10.84	gi|357001263	photosystem II protein V [Pinus devoniana]
0	1.28	20.48	10.84	gi|357001190	photosystem II protein V [Pinus discolor]
0	1.28	20.48	10.84	gi|357001117	photosystem II protein V [Pinus hartwegii]
0	1.28	20.48	10.84	gi|357001042	photosystem II protein V [Pinus douglasiana]
0	1.28	20.48	10.84	gi|357000968	photosystem II protein V [Pinus echinata]
0	1.28	20.48	10.84	gi|357000895	photosystem II protein V [Pinus edulis]
0	1.28	20.48	10.84	gi|357000822	photosystem II protein V [Pinus elliottii]
0	1.28	20.48	10.84	gi|357000749	photosystem II protein V [Pinus engelmannii]
0	1.28	20.48	10.84	gi|357000676	photosystem II protein V [Pinus fragilissima]
0	1.28	20.48	10.84	gi|357000603	photosystem II protein V [Pinus glabra]
0	1.28	20.48	10.84	gi|357000530	photosystem II protein V [Pinus greggii]
0	1.28	20.48	10.84	gi|357000458	photosystem II protein V [Pinus halepensis]
0	1.28	20.48	10.84	gi|357000385	photosystem II protein V [Pinus hartwegii]
0	1.28	20.48	10.84	gi|357000313	photosystem II protein V [Pinus heldreichii]
0	1.28	20.48	10.84	gi|357000240	photosystem II protein V [Pinus hwangshanensis]
0	1.28	20.48	10.84	gi|357000167	photosystem II protein V [Pinus jeffreyi]
0	1.28	20.48	10.84	gi|357000094	photosystem II protein V (chloroplast) [Pinus johannis]
0	1.28	20.48	10.84	gi|357000021	photosystem II protein V [Pinus kesiya]
0	1.28	20.48	10.84	gi|356999948	photosystem II protein V [Pinus latteri]
0	1.28	20.48	10.84	gi|356999875	photosystem II protein V [Pinus pringlei]
0	1.28	20.48	10.84	gi|356999802	photosystem II protein V [Pinus lawsonii]
0	1.28	20.48	10.84	gi|356999729	photosystem II protein V [Pinus leiophylla]
0	1.28	20.48	10.84	gi|356999656	photosystem II protein V [Pinus lumholtzii]
0	1.28	20.48	10.84	gi|356999583	photosystem II protein V [Pinus massoniana]
0	1.28	20.48	10.84	gi|356999510	photosystem II protein V [Pinus maximartinezii]
0	1.28	20.48	10.84	gi|356999437	photosystem II protein V [Pinus montezumae]
0	1.28	20.48	10.84	gi|356999365	photosystem II protein V [Pinus morrisonicola]
0	1.28	20.48	10.84	gi|356999292	photosystem II protein V [Pinus mugo]

0	1.28	20.48	10.84	gi|356999220	photosystem II protein V [Pinus muricata]
0	1.28	20.48	10.84	gi|356999147	photosystem II protein V [Pinus nigra]
0	1.28	20.48	10.84	gi|356999074	photosystem II protein V [Pinus pseudostrobus var. apulcensis]
0	1.28	20.48	10.84	gi|356999001	photosystem II protein V [Pinus occidentalis]
0	1.28	20.48	10.84	gi|356998928	photosystem II protein V [Pinus palustris]
0	1.28	20.48	10.84	gi|356998855	photosystem II protein V [Pinus patula]
0	1.28	20.48	10.84	gi|356998782	photosystem II protein V [Pinus pinceana]
0	1.28	20.48	10.84	gi|356998709	photosystem II protein V [Pinus pinea]
0	1.28	20.48	10.84	gi|356998636	photosystem II protein V [Pinus ponderosa var. benthamiana]
0	1.28	20.48	10.84	gi|356998563	photosystem II protein V [Pinus ponderosa var. scopulorum]
0	1.28	20.48	10.84	gi|356998491	photosystem II protein V [Pseudotsuga menziesii var. menziesii]
0	1.28	20.48	10.84	gi|356998418	photosystem II protein V [Pinus pseudostrobus]
0	1.28	20.48	10.84	gi|356998349	photosystem II protein V [Pinus pumila]
0	1.28	20.48	10.84	gi|356998276	photosystem II protein V [Pinus pungens]
0	1.28	20.48	10.84	gi|356998203	photosystem II protein V [Pinus quadrifolia]
0	1.28	20.48	10.84	gi|356998130	photosystem II protein V [Pinus radiata]
0	1.28	20.48	10.84	gi|356998057	photosystem II protein V [Pinus remota]
0	1.28	20.48	10.84	gi|356997984	photosystem II protein V [Pinus rigida]
0	1.28	20.48	10.84	gi|356997911	photosystem II protein V [Pinus roxburghii]
0	1.28	20.48	10.84	gi|356997839	photosystem II protein V [Pinus sabiniana]
0	1.28	20.48	10.84	gi|356997766	photosystem II protein V [Pinus serotina]
0	1.28	20.48	10.84	gi|356997694	photosystem II protein V [Pinus strobiformis]
0	1.28	20.48	10.84	gi|356997621	photosystem II protein V [Pinus sylvestris]
0	1.28	20.48	10.84	gi|356997548	photosystem II protein V [Pinus taiwanensis]
0	1.28	20.48	10.84	gi|356997475	photosystem II protein V [Pinus tropicalis]
0	1.28	20.48	10.84	gi|356997403	photosystem II protein V [Pinus virginiana]
0	1.28	20.48	10.84	gi|356997331	photosystem II protein V [Pinus wallichiana]
0	1.28	20.48	10.84	gi|356997259	photosystem II protein V [Pinus kwangtungensis]
0	1.28	20.48	10.84	gi|356997186	photosystem II protein V [Pinus yecorensis]
0	1.28	20.48	10.84	gi|356997116	photosystem II protein V [Pinus yunnanensis]
0	1.28	20.48	10.84	gi|356997044	photosystem II protein V [Pinus amamiana]
0	1.28	20.48	10.84	gi|356996971	photosystem II protein V [Pinus arizonica]
0	1.28	20.48	10.84	gi|356996899	photosystem II protein V [Pinus brutia]
0	1.28	20.48	10.84	gi|356996827	photosystem II protein V [Pinus bungeana]
0	1.28	20.48	10.84	gi|356996754	photosystem II protein V [Pinus caribaea]
0	1.28	20.48	10.84	gi|356996681	photosystem II protein V [Pinus cembroides]
0	1.28	20.48	10.84	gi|356996608	photosystem II protein V [Pinus chiapensis]
0	1.28	20.48	10.84	gi|352950949	cytochrome b559 alpha chain (chloroplast) [Larix decidua]
0	1.28	20.48	10.84	gi|351653793	cytochrome b559 alpha chain (chloroplast) [Pseudotsuga sinensis var. wilsoniana]
0	1.28	20.48	10.84	gi|347977627	cytochrome b559 alpha chain [Pseudotsuga sinensis var. wilsoniana]
0	1.28	20.48	10.84	gi|347977604	cytochrome b559 alpha chain [Larix decidua]
0	1.28	20.48	10.84	gi|324986509	photosystem II protein V [Pinus nelsonii]
0	1.28	20.48	10.84	gi|324986438	photosystem II protein V [Pinus monophylla]
0	1.28	20.48	10.84	gi|324986366	photosystem II protein V [Pinus lambertiana]
0	1.28	20.48	10.84	gi|323522697	photosystem II protein V [Pinus nelsonii]
0	1.28	20.48	10.84	gi|323522533	photosystem II protein V [Pinus monophylla]

0	1.28	20.48	10.84	gi|323514215	photosystem II protein V [Pinus lambertiana]
0	1.28	20.48	10.84	gi|31340255	RecName: Full=Cytochrome b559 subunit alpha; AltName: Full=PSII reaction center subunit V
0	1.28	20.48	10.84	gi|309322390	cytochrome b559 alpha chain [Cathaya argyrophylla]
0	1.28	20.48	10.84	gi|307683539	cytochrome b559 alpha chain [Larix decidua]
0	1.28	20.48	10.84	gi|307683445	cytochrome b559 alpha chain [Pseudotsuga sinensis var. wilsoniana]
0	1.28	20.48	10.84	gi|307683316	cytochrome b559 alpha chain [Cathaya argyrophylla]
0	1.28	20.48	10.84	gi|29565605	photosystem II protein V [Pinus koraiensis]
0	1.28	20.48	10.84	gi|29469704	PSII cytochrome b559 alpha chain [Pinus koraiensis]
0	1.28	20.48	10.84	gi|257042594	photosystem II protein V [Pinus monticola]
0	1.28	20.48	10.84	gi|237688667	photosystem II protein V [Pinus krempfii]
0	1.28	20.48	10.84	gi|237688599	photosystem II protein V [Pinus gerardiana]
0	1.28	20.48	10.84	gi|237688467	photosystem II protein V [Picea sitchensis]
0	1.28	20.48	10.84	gi|228017387	photosystem II subunit V [Larix occidentalis]
0	1.28	20.48	10.84	gi|228017321	photosystem II subunit V [Pinus lambertiana]
0	1.28	20.48	10.84	gi|228016698	photosystem II subunit V [Pinus aristata]
0	1.28	20.48	10.84	gi|228016572	photosystem II subunit V [Abies firma]
0	1.28	20.48	10.84	gi|228016452	photosystem II subunit V [Pinus torreyana subsp. insularis]
0	1.28	20.48	10.84	gi|228016385	photosystem II subunit V [Pinus thunbergii]
0	1.28	20.48	10.84	gi|228016314	photosystem II subunit V [Pinus taeda]
0	1.28	20.48	10.84	gi|228016190	photosystem II subunit V [Pinus squamata]
0	1.28	20.48	10.84	gi|228015932	photosystem II subunit V [Pinus ponderosa]
0	1.28	20.48	10.84	gi|226951125	photosystem II protein V [Pinus krempfii]
0	1.28	20.48	10.84	gi|226876060	photosystem II protein V [Pinus gerardiana]
0	1.28	20.48	10.84	gi|226875296	photosystem II protein V [Picea sitchensis]
0	1.28	20.48	10.84	gi|222084101	cytochrome b559 alpha chain [Keteleeria davidiana]
0	1.28	20.48	10.84	gi|220983653	cytochrome b559 alpha chain [Keteleeria davidiana]
0	1.28	20.48	10.84	gi|1262636	PSII cytochrome b559 subunit [Pinus thunbergii]
0	1.28	20.48	10.84	gi|1172678	RecName: Full=Cytochrome b559 subunit alpha; AltName: Full=PSII reaction center subunit V
0	1.28	10.84	10.84	gi|257042679	photosystem II protein V [Pinus monticola]
0	1.28	10.84	10.84	gi|257042564	photosystem II protein V [Pinus monticola]
0	1.28	10.84	10.84	gi|228017707	photosystem II subunit V [Pinus pinaster]
0	1.28	10.84	10.84	gi|228017514	photosystem II subunit V [Pinus monticola]
0	1.28	10.84	10.84	gi|228017015	photosystem II subunit V [Pinus canariensis]
0	1.28	10.84	10.84	gi|228016951	photosystem II subunit V [Pinus banksiana]
0	1.28	10.84	10.84	gi|228016887	photosystem II subunit V [Pinus ayacahuite]
0	1.28	10.84	10.84	gi|228016631	photosystem II subunit V [Pinus albicaulis]
0	1.28	10.84	10.84	gi|228016516	photosystem II subunit V [Pinus torreyana subsp. torreyana]
0	1.28	10.84	10.84	gi|228016069	photosystem II subunit V [Pinus rzedowskii]
0	1.28	10.84	10.84	gi|228016001	photosystem II subunit V [Pinus resinosa]
1.2	1.2	27.54	6.5219998	gi|462234	RecName: Full=Histone H2AX
0	1.2	27.54	6.5219998	gi|30024112	putative histone H2B [Pinus pinaster]
0	1.2	27.54	6.5219998	gi|297871	histone H2A [Picea abies]
0	1.2	30.65	7.2580002	gi|215809515	putative histone [Pinus sylvestris]
0	1.2	30.65	7.2580002	gi|215809513	putative histone [Pinus sylvestris]
0	1.2	30.65	7.2580002	gi|215809511	putative histone [Pinus sylvestris]

0	1.2	30.65	7.2580002	gi|215809509	putative histone [Pinus sylvestris]
0	1.2	25.19	6.8700001	gi|148909493	unknown [Picea sitchensis]
0	1.2	25.19	6.8700001	gi|116786377	unknown [Picea sitchensis]
0	1.2	27.54	6.5219998	gi|116779069	unknown [Picea sitchensis]
0	1.14	31.51	6.1640002	gi|116791114	unknown [Picea sitchensis]
0	1.14	42.45	6.4750001	gi|30024110	putative histone H2A [Pinus pinaster]
0	1.14	28.78	6.4750001	gi|257741326	unnamed protein product [Pinus taeda]
0	1.14	28.78	6.4750001	gi|257707962	unnamed protein product [Pinus taeda]
0	1.14	28.78	6.4750001	gi|2317760	H2A homolog [Pinus taeda]
0	1.14	28.78	6.4750001	gi|219926373	unnamed protein product [Pinus taeda]
0	1.14	28.78	6.4750001	gi|219728576	unnamed protein product [Pinus taeda]
0	1.14	25	6.25	gi|148908587	unknown [Picea sitchensis]
0	1.14	24.82	6.3830003	gi|116783667	unknown [Picea sitchensis]
0	1.14	22.76	6.2070001	gi|116781203	unknown [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018579	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018577	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018575	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018573	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018571	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018569	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018567	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018565	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018563	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018561	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018559	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018557	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018555	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018553	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018551	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018549	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018547	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018545	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018543	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018541	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018539	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018537	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018535	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018533	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018531	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018529	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018527	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018525	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018523	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018521	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018519	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018517	histone H2A-like protein, partial [Picea sitchensis]

0	1.14	24.24	6.8180002	gi|306018515	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018513	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018511	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018509	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018507	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018505	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018503	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018501	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018499	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	24.24	6.8180002	gi|306018497	histone H2A-like protein, partial [Picea sitchensis]
0	1.14	13.19	6.25	gi|294464123	unknown [Picea sitchensis]
0	1.14	13.29	6.2940001	gi|294461845	unknown [Picea sitchensis]
0	1.14	22.86	6.4290002	gi|294461162	unknown [Picea sitchensis]
0	1.14	15	6.4290002	gi|224286646	unknown [Picea sitchensis]
0	1.14	13.19	6.25	gi|148905752	unknown [Picea sitchensis]
0	1.14	19.73	6.1220001	gi|116794149	unknown [Picea sitchensis]
0	1.14	16.31	6.3830003	gi|116793703	unknown [Picea sitchensis]
0	1.14	22.86	6.4290002	gi|116790854	unknown [Picea sitchensis]
0	1.14	22.86	6.4290002	gi|116778705	unknown [Picea sitchensis]
0	1.14	12.68	12.68	gi|383128836	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128835	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128834	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128833	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128832	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128831	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128830	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128829	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128828	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128827	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128826	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128825	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|383128824	hypothetical protein CL1360Contig1_05, partial [Pinus taeda]
0	1.14	12.68	12.68	gi|361068817	hypothetical protein CL1360Contig1_05, partial [Pinus lambertiana]
0	1.14	12.68	12.68	gi|361068815	hypothetical protein CL1360Contig1_05, partial [Pinus radiata]
0	1.14	6.122	6.1220001	gi|148907948	unknown [Picea sitchensis]
0	1.14	6.338	6.3380003	gi|116794370	unknown [Picea sitchensis]
0.55	0.55	41.06	0	gi|116780952	unknown [Picea sitchensis]
0	0.55	23.11	0	gi|116785854	unknown [Picea sitchensis]
0	0.55	31.63	0	gi|16798638	Cu-Zn-superoxide dismutase precursor [Pinus pinaster]
0	0.55	31.21	0	gi|20697	CuZn superoxide dismutase [Pinus sylvestris]
0	0.55	31.21	0	gi|134685	RecName: Full=Superoxide dismutase [Cu-Zn], chloroplastic
0.43	0.43	18	0	gi|210162074	putative dehydrin [Cupressus sempervirens]
0.25	0.25	11.56	0	gi|294461824	unknown [Picea sitchensis]
0.23	0.25	100	0	gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
0.11	0.11	44.44	0	gi|56236662	photosystem II subunit H [Araucaria bidwillii]
0	0.11	21.33	0	gi|354991429	psbH (chloroplast) [Agathis australis]

0	0.11	21.33	0	gi|347977324	photosystem II 10 kDa phosphoprotein [Agathis dammara]
0	0.11	21.33	0	gi|239794346	photosystem II phosphoprotein [Cryptomeria japonica]
0	0.11	21.33	0	gi|239794264	photosystem II phosphoprotein [Cryptomeria japonica]
0	0.11	21.33	0	gi|172072876	photosystem II protein H [Cryptomeria japonica]
0	0.11	21.33	0	gi|171854895	photosystem II phosphoprotein [Cryptomeria japonica]
0.1	0.11	100	0	gi|109892853	Putative acid phosphatase PS18
0.06	0.06	1.931	0	gi|116781547	unknown [Picea sitchensis]
0.05	0.05	5.488	0	gi|116791650	unknown [Picea sitchensis]
0	0.05	5.556	0	gi|116788927	unknown [Picea sitchensis]

Appendix 1.2 LC-MS MASCOT analysis of Kauri Protein compared against the Conifer Family Protein Sequence data.

Accessions	Names
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]

gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|354991381	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Agathis australis]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]

gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|21913294	fructose-1,6-diphosphate aldolase [Metasequoia glyptostroboides]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|354991561;	atpA (chloroplast) [Agathis australis]; ATP synthase CF1 alpha chain [Agathis dammara]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|116787106	unknown [Picea sitchensis]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|7524738	photosystem I subunit VII [Pinus thunbergii]; photosytem I subunit VII [Pinus clausa]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]

gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|347977268;	ATP synthase CF1 beta chain [Agathis dammara]; ATP synthase beta subunit [Agathis robusta]
gi|224284407	unknown [Picea sitchensis]
gi|224284407	unknown [Picea sitchensis]
gi|224284407	unknown [Picea sitchensis]
gi|224284407	unknown [Picea sitchensis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|56236629	cytochrome b-559 alpha subunit [Araucaria bidwillii]; psbE (chloroplast) [Agathis australis]
gi|224284458;	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284458;	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284458;	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284458;	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284458;	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]
gi|296514048	atpE (chloroplast) [Agathis australis]; ATP synthase CF1 epsilon chain [Agathis dammara]
gi|296514048	atpE (chloroplast) [Agathis australis]; ATP synthase CF1 epsilon chain [Agathis dammara]
gi|296514048	atpE (chloroplast) [Agathis australis]; ATP synthase CF1 epsilon chain [Agathis dammara]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148908967	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116785854	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116785854	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116785854	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116785854	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116785854	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116785854	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]

gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787538	unknown [Picea sitchensis]
gi|116787582	unknown [Picea sitchensis]
gi|116787582	unknown [Picea sitchensis]
gi|116787582	unknown [Picea sitchensis]
gi|116787582	unknown [Picea sitchensis]
gi|116787582	unknown [Picea sitchensis]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|311698155	glyoxysomal malate dehydrogenase [Sequoia sempervirens]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
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gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284112	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
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gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285725	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
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gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
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gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116791650	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|20695	CuZn superoxide dismutase [Pinus sylvestris]; RecName: Full=Superoxide dismutase [Cu-Zn]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|62642127	histone 4 [Picea abies]; hypothetical protein 0_18315_01 [Pinus taeda]
gi|224286258	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224286258	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284992	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224284992	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148907083	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116787373	unknown [Picea sitchensis]
gi|116787373	unknown [Picea sitchensis]
gi|116787373	unknown [Picea sitchensis]
gi|116787373	unknown [Picea sitchensis]
gi|116787373	unknown [Picea sitchensis]
gi|294463383	unknown [Picea sitchensis]
gi|7620557	F1 ATPase alpha subunit [Metasequoia glyptostroboides]
gi|7620557	F1 ATPase alpha subunit [Metasequoia glyptostroboides]
gi|7620557	F1 ATPase alpha subunit [Metasequoia glyptostroboides]
gi|7620557	F1 ATPase alpha subunit [Metasequoia glyptostroboides]
gi|90704785	putative glycine-rich RNA binding protein [Cryptomeria japonica]
gi|90704785	putative glycine-rich RNA binding protein [Cryptomeria japonica]
gi|90704785	putative glycine-rich RNA binding protein [Cryptomeria japonica]
gi|90704785	putative glycine-rich RNA binding protein [Cryptomeria japonica]
gi|90704785	putative glycine-rich RNA binding protein [Cryptomeria japonica]
gi|90704785	putative glycine-rich RNA binding protein [Cryptomeria japonica]
gi|238636853	cyclophilin [Chamaecyparis obtusa]; putative cyclophilin [Pinus taeda]
gi|238636853	cyclophilin [Chamaecyparis obtusa]; putative cyclophilin [Pinus taeda]
gi|238636853	cyclophilin [Chamaecyparis obtusa]; putative cyclophilin [Pinus taeda]

gi|238636853	cyclophilin [Chamaecyparis obtusa]; putative cyclophilin [Pinus taeda]
gi|238636853	cyclophilin [Chamaecyparis obtusa]; putative cyclophilin [Pinus taeda]
gi|238636853	cyclophilin [Chamaecyparis obtusa]; putative cyclophilin [Pinus taeda]
gi|294460278	unknown [Picea sitchensis]
gi|294460278	unknown [Picea sitchensis]
gi|294460278	unknown [Picea sitchensis]
gi|294460278	unknown [Picea sitchensis]
gi|294460278	unknown [Picea sitchensis]
gi|294460278	unknown [Picea sitchensis]
gi|294460278	unknown [Picea sitchensis]
gi|224285693	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285693	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116792150	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116792150	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|116792150	unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|73919919	RecName: Full=Histone H3.3; histone 3 [Picea abies]; histone 3 [Picea abies]; histone 3 [Picea abies]
gi|73919919	RecName: Full=Histone H3.3; histone 3 [Picea abies]; histone 3 [Picea abies]; histone 3 [Picea abies]
gi|73919919	RecName: Full=Histone H3.3; histone 3 [Picea abies]; histone 3 [Picea abies]; histone 3 [Picea abies]
gi|209778903	unknown [Cupressus sempervirens]
gi|209778903	unknown [Cupressus sempervirens]
gi|209778903	unknown [Cupressus sempervirens]
gi|209778903	unknown [Cupressus sempervirens]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|148909668	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285421	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285421	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285421	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
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gi|224285421	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285421	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|4138395	glutamine synthetase [Pinus sylvestris]; glutamate--ammonia ligase [Pinus sylvestris]
gi|4138395	glutamine synthetase [Pinus sylvestris]; glutamate--ammonia ligase [Pinus sylvestris]
gi|4138395	glutamine synthetase [Pinus sylvestris]; glutamate--ammonia ligase [Pinus sylvestris]
gi|4138395	glutamine synthetase [Pinus sylvestris]; glutamate--ammonia ligase [Pinus sylvestris]
gi|87132971	beta-actin [Abies alba]; actin [Picea abies]; actin [Picea rubens]; actin [Pinus taeda]; actin 2 [Picea abies]
gi|296514444	unnamed protein product [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224286816	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224286816	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224286816	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|54399504	S-adenosyl methionine synthetase 1 [Pinus taeda]; S-adenosyl methionine synthetase 1 [Pinus taeda]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]

gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
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gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
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gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
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gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
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gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|21360794	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Pseudotaxus chienii]
gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]
gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]
gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]

gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]
gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]
gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]
gi|168812226	putative ribulose-1,5-bisphosphate carboxylase/oxygenase activase alpha 2 [Cupressus sempervirens]
gi|62642100	dehydrin 2 [Picea abies]; dehydrin 2 [Picea obovata]; dehydrin-like protein [Picea abies]
gi|62642100	dehydrin 2 [Picea abies]; dehydrin 2 [Picea obovata]; dehydrin-like protein [Picea abies]
gi|62642100	dehydrin 2 [Picea abies]; dehydrin 2 [Picea obovata]; dehydrin-like protein [Picea abies]
gi|224285637	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285637	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|224285637	unknown [Picea sitchensis]; unknown [Picea sitchensis]; unknown [Picea sitchensis]
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|109892850	RecName: Full=Putative cytochrome c oxidase subunit II PS17
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]

gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
gi|386802266	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Cephalotaxus harringtonia]
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