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**Plant Functional Traits Associated with  
Frost Susceptibility**

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A Dissertation  
submitted in partial fulfilment  
of the requirements for the Degree of  
Bachelor of Science (Honours)

at  
Lincoln University  
by  
Georgia May Stevenson

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Lincoln University  
2015

Abstract of a dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Science (Honours)

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Frost Susceptibility**

by

Georgia May Stevenson

Frost can affect the productivity and distribution of plants, as it can cause plant cell rupture and xylem cavitation, which may limit the water transport, growth, and survival of plants. Climate change is expected to increase the effect of frost on plants, making them more susceptible to frost events. Therefore it is necessary to determine the frost susceptibility of species, which may be done using a plant functional trait approach. Plant functional traits are any attribute of a plant which can influence its establishment, survival and fitness. Frost tolerant species are expected to possess traits that reflect a conservative life history strategy aimed at stress tolerance, such as small and thick leaves, low specific leaf area, high leaf dry matter content, high leaf vein density and vein length per unit area, and high wood density. We sampled twenty-three plant species in Australia and twenty-five plant species in New Zealand, and compared their functional traits to existing species-specific frost susceptibility datasets. The traits most likely to influence frost susceptibility appear to be leaf size traits, leaf venation traits, and wood density, which is most likely due to the important effects these traits have on plant hydraulics, which is known to be significantly impacted by frost. Higher leaf vein density and vein length per unit area provide greater leaf hydraulics and better water use efficiency, which helps plants survive the water stress conditions that can be caused by frost. Higher wood density is related to having narrow xylem vessels, which are more resistant to freeze-thaw induced xylem cavitation and embolism, compared to wider xylem vessels. This study has provided globally-new information about which plant functional traits may be associated with frost susceptibility in plant species, and demonstrates for the first time the promise of leaf venation traits as a means of predicting and understanding response of plants to frost.

**Keywords:** Frost, climate change, functional traits, leaf area, leaf width, leaf length, specific leaf area, leaf dry matter content, leaf thickness, vein density, vein length per unit area, Australia, New Zealand, easy traits.

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# Chapter 1

## Introduction

### 1.1 Frost

Frost is an important environmental factor which can limit the productivity and influence the distribution of plants (Sakai and Larcher 1987). For example, severe frost damage has been observed to reduce the productivity of woody plants for years after the damage occurs, by limiting new growth in subsequent seasons, resulting in lower leaf area developing, which reduces the overall productivity of the tree (Larcher 1981). An example of the influence of frost on plant distribution was observed by Davidson and Reid (1985) who found that the distribution of six *Eucalyptus* species was closely related to the minimum temperature at the site where the species were growing.

Frost occurs when temperatures fall below 0°C, and it affects vegetation because freezing of water in the plant tissues occurs at temperatures below 0°C (Sakai and Larcher 1987). One of the most important effects caused by frost is water stress (Ansley et al. 1992). The low temperatures associated with frost can cause water transport within the plant to be reduced or completely stopped (Ansley et al. 1992), this is caused by xylem conduits becoming embolised after freeze-thaw events, which affects water transport to the leaves (Hacke and Sperry 2001). When the water freezes, gases dissolved in the water are pushed out to form bubbles surrounded by ice. Once the xylem sap thaws, these bubbles can either dissolve, or cause xylem cavitation, where the xylem conduit is filled with the gas (Sakai and Larcher 1987, Choat et al. 2011). Xylem cavitation causes the xylem conduit to become non-functional, which can limit the plant's water transport, growth, and survival (Willson and Jackson 2006).

There are two main types of frost: radiation frost and advection frost. Radiation frosts are caused by the loss of infrared radiation at night (Trought et al. 1999). On clear nights, the surface of the earth radiates heat upwards into the atmosphere and receives infrared radiation from the sky (Trought et al. 1999). The ground radiates more because it is usually warmer than the sky, resulting in cooling on the ground surface, due to the net loss of radiation (Trought et al. 1999). Therefore, greater exposure to the cold night time skies increases the susceptibility of leaves to radiation frost, a net loss of radiant energy can lead to frost formation on leaves even when temperatures remain well above freezing (Jordan and Smith 1995). Advection frosts are the result of large below 0°C air masses moving into an area, which causes tiny ice spikes to form over surfaces, such as the leaves and branches of trees (Trought et al. 1999). Advection frosts differ from radiation frosts as they can occur



at any time, day or night (Trought et al. 1999). The effects frost have on plants are expected to increase with future climate change (Bannister 2007).

## 1.2 Frost and climate change

Anthropogenic climate change will affect ecosystems due to changes in mean conditions, such as increases in both maximum and minimum temperatures (Barker et al. 2005, Woldendorp et al. 2008), changes in water availability, and increases in atmospheric CO<sub>2</sub>; these changes in abiotic conditions are likely to affect plant productivity (IPCC 2014). Climate change is also expected to increase the frequency and severity of climatic extremes, such as affecting the occurrence, severity, and distribution of frost (Woldendorp et al. 2008, IPCC 2014). Climate change is predicted to increase the effect of frost on plants, by causing them to become more susceptible to frost events (Bannister 2007).

Loss of frost resistance in plants is expected with climate change. Cold hardening in plants is activated by exposure to low temperatures; it induces a state of minimum growth in plants, and brings about a level of freezing tolerance which helps the plant survive through the winter (Woldendorp et al. 2008). The reversal of this cold-hardening is triggered by warming in spring (Harrison et al. 2010). These triggers are important, as plants adapted to cold winters seldom thrive or reproduce without them (Harrison et al. 2010).

Climate change is predicted to make plants even more susceptible to late frost events. Although climate change is expected to increase the number of frost-free days, it is also predicted to cause mild temperature spells in winter and spring, which are expected to accelerate the phenology and growth onset of plants, which will make vegetation even more prone to late spring frost events (Woldendorp et al. 2008, St. Clair et al. 2009). Changes in the timing and duration of growing seasons, due to climate warming, can increase the vulnerability of plants to freezing damage caused by early or late season frosts (Woldendorp et al. 2008). Early leaf flushing in response to late winter warming, followed by extremely low temperatures, can cause extensive forest defoliation (St. Clair et al. 2009).

Higher CO<sub>2</sub> concentrations are associated with climate change. This may make plants more susceptible to frost, as plants grown under elevated CO<sub>2</sub> levels have been found to have lower freezing tolerance (Barker et al. 2005). Freezing tolerance appears to be influenced by CO<sub>2</sub> in two main ways: elevated CO<sub>2</sub> increases the temperature at which ice nucleation occurs in plant tissues (Lutze et al. 1998). For example, elevated CO<sub>2</sub> levels were found to promote spring frost damage in field grown seedlings of *Eucalyptus pauciflora*, which is one of the most frost tolerant species of *Eucalyptus* (Lutze et al. 1998). Woldendorp et al. (2008) also found that ice nucleation temperatures

increase rapidly with increasing CO<sub>2</sub> up to around 350 ppm, and continue to increase at a slower rate at high CO<sub>2</sub> levels in numerous species (Woldendorp et al. 2008). It is already believed that plants growing under the current atmospheric CO<sub>2</sub> levels may be exposed to greater freezing stress today, than before the industrial revolution (Woldendorp et al. 2008). In order to understand how plants will respond to changes in frost regimes due to climate change, it is necessary to determine the frost resistance or susceptibility of plant species. This can be measured in a number of ways.

### **1.3 Frost resistance and susceptibility in plants**

One of the most common ways to determine frost resistance involves exposing a plant or a part of a plant to a range of freezing temperatures, then determining a critical lethal temperature (LT) which causes a particular level of frost damage to that plant, usually 50 percent (LT<sub>50</sub>) (Bannister 2007). The degree of frost damage on the plant is usually estimated subjectively by visual assessment; frost damaged leaves may become discoloured (black or brown), or translucent (Bannister and Lord 2006). This can also be done by digitally scanning the frost damaged plant or plant parts, which is a more quantitative measure and may be more accurate than visual estimates (Bannister 2007).

Another way frost resistance can be determined is by using electrical conductivity to measure the electrolyte leakage from frost damaged plants (Scowcroft et al. 2000, Bannister and Polwart 2001). This can be standardised between plants by using uniform amounts of plant tissue, or by relating the conductivity of a control or treated sample to the conductivity of the same sample after it has been completely killed by heat or freezing (Bannister and Polwart 2001).

The percentage foliage retention of plant species after a frost event can also be estimated to determine frost resistance (Harwood 1980, Scowcroft et al. 2000, Curran et al. 2010). At extremely low temperatures, or during a sensitive phase of development of a plant, frost can cause leaf death leading to partial or complete canopy defoliation, depending on how susceptible the plant species is (St. Clair et al. 2009).

While these methods are useful to determine the frost susceptibility of plant species, they may time-consuming making it difficult to measure enough plant species to be able to predict the effect of future frosts on plant communities. A more efficient way to estimate the frost susceptibility of plant species could be via a plant functional trait approach, by determining which traits are associated with frost resistance or susceptibility.

### **1.4 Plant functional traits**

Plant functional traits are any attribute of a plant which can influence its establishment, survival and fitness (Reich et al. 2003, Pérez-Harguindeguy et al. 2013). They are important because they are

linked to the ecological strategy of a plant; that is, the way a plant species secures both carbon profit during vegetative growth, and gene transfer to the next generation (Westoby et al. 2002). A widely used example of a plant ecological strategy is the leaf economics spectrum (Wright et al. 2004). One end of the spectrum describes species that are long-lived, have small and thick leaves, and have low return on carbon and nutrient investment, the other end of the spectrum describes species which are the opposite; short lived, have large and thin leaves, and have high return on carbon and nutrient investment (Wright et al. 2004). Differences in the way plants acquire, process, and invest resources can have a major influence on the functioning of ecosystems and species composition in the community (Diaz et al. 2004). Environmental filters, such as abiotic conditions, can exclude species which do not have suitable physiological characteristics (defined by functional traits), from entering and remaining in a community, consequently shaping community structure (Venn et al. 2011). Therefore plant functional traits can help us understand the local community structure, biodiversity, and ecosystem function (Read et al. 2014), as well as enabling us to predict how plant species and communities may respond to global environmental changes.

Plant traits have often been separated into easy and hard traits. Hard traits are those most directly linked to a certain plant function, but are often difficult to measure and may require experimental manipulation or measurement over a long time period (Gibson 2015). Easy traits are less directly related to a given plant function, but are easier to measure. Logistical constraints can make hard traits too difficult to determine for large numbers of species and individuals, therefore easy traits can be measured instead (Harrison et al. 2010). Links between easy and hard traits have been found in various environmental conditions (Hodgson et al. 1999, Lavorel and Garnier 2002), which suggest that easy traits are promising way to connect plant traits to important plant and ecosystem processes (Diaz et al. 2004). For example, specific leaf area may be measured to understand a plant's strategy, as it has been observed to be highly correlated with a relative growth rate, photosynthesis rate, and leaf longevity (Weiher et al. 1999). In this study we will focus on measuring easy leaf traits which may help to predict how plants will respond to frost.

## **1.5 Traits associated with frost resistance**

### **1.5.1 Leaf size traits: leaf area, leaf length, and leaf width**

Leaf area has important consequences for the leaf energy and water balance (Cornelissen et al. 2003). Leaf area variation among plant species has been associated with climatic variation, where cold stress, among other abiotic stresses, has been found to select for relatively small leaves (Cornelissen et al. 2003). Small leaves may be an adaptation of the plant used to limit the effects of extreme temperatures, such as those caused by radiation frost (Harrison et al. 2010, Jordan and

Smith 1995). Leaves with large area have also been found to be colder than smaller leaves (Leuning and Cremer 1988).

Leaf length and width, along with overall leaf size, are related to resource capture and use efficiency in plants (Forgiarini et al. 2015). Longer and wider leaves are expected to increase the resource capture efficiency, therefore increasing the relative growth rate of the plant (Forgiarini et al. 2015). Stress tolerant plants are not expected to have high growth rates, and are instead expected to use their resources on stress tolerant traits. Therefore we expect that stress susceptible plant species will have wider and longer leaves, and stress tolerant species will have narrower and shorter leaves. These relationships have previously been found by Jordan and Smith (1995), who observed both leaf length and width to decrease with sky infrared radiation, which is related to frost. Leaf length has also been found to be sensitive to moderate water stress (Deblonde and Ledent 2001). Since frost causes a type of water stress, the response of leaf length to drought conditions may indicate how length, and possibly other leaf traits leaf, may respond to frost.

### **1.5.2 Leaf toughness traits: specific leaf area, leaf dry matter content, and leaf thickness**

Specific leaf area (SLA) is the one-sided area of a fresh leaf, divided by its oven dry mass (Pérez-Harguindeguy et al. 2013) and is known to be affected by environmental stress (Carevic et al. 2015). Low SLA has been associated with stress tolerant traits, such as high investments in leaf defences and leaf longevity (Poorter et al. 2009). For example, plant species growing in low temperature habitats generally have low SLA (Atkin et al. 2006, Poorter et al. 2009), which is thought to be due to cell expansion being limited at lower temperatures, leading to a larger number of smaller cells per unit area, and therefore more cell wall material per unit leaf volume and more cell layers (Poorter et al. 2009). Ball et al. (2002) showed that a low SLA, particularly having more cell layers, reduced the incidence and severity of freezing stress, by slowing down the rate of freezing. Frost has also directly been shown to affect SLA. Carevic et al. (2015) evaluated the variation in physiological parameters, including SLA, in two populations of the plant species *Prosopis burkartii*, during frost-prone winter periods. They found that at the within population level variations in SLA indicated an increase in the non-structural biomass during the months with the most frost days. Increased biomass likely helped the plants to survive the months with increased number of frost days (Carevic et al. 2015). Therefore, we would expect frost resistant species to have low values of SLA (Scheepens et al. 2010). SLA has also been observed to decrease under water stress, and frost is similar to drought as it can also reduce or inhibit water transport within the plant (Ansley et al. 1992), suggesting that in our study frost susceptible species will have high SLA compared to frost resistant species.

Leaves with high leaf dry matter content (LDMC) tend to be relatively tough and are assumed to be more resistant to physical hazards compared to leaves with low LDMC (Cornelissen et al. 2003). For example, Prasil et al. (2001) found leaf dry matter content substantially increased when barley plants (*Hordeum vulgare* L.) were exposed to freezing temperatures. LDMC has also been found to increase under water stress (Ansley et al. 1992). Therefore, we expect that frost susceptible species will have low LDMC compared to frost resistant species.

Leaf thickness is another leaf toughness trait, which plays an important role in the physical strength of leaves (Pérez-Harguindeguy et al. 2013). Increased cell wall rigidity, which occurs with increased leaf thickness, is thought to protect cells against intracellular freezing (Stefanowska et al. 1999). Therefore, when plants are grown in cold temperatures, changes in the leaf structure and cell wall thickness occur. Alpine species also provide evidence of this, as they often have high resource input into structural traits, including thick leaves, as a response to the stressful conditions which occur in alpine areas, including cold temperatures (Soudzilovskaia et al. 2013). It has been suggested that changes in leaf thickness due to cold are related to modification in the size and expansion patterns of mesophyll cells, and to a thickening of the cell walls (Mediavilla et al. 2012). The increase of cell wall thickness is a characteristic of leaves growing in cold climates (Kubacka-Zebalska and Kacperska 1999). Thicker cell walls have also been suggested to cause a change in the expansion of mesophyll cells from a longitudinal to perpendicular direction, which would contribute to the decrease in leaf area that is observed in cold climates (Stefanowska et al. 1999).

Hence, it would be expected that frost resistance plant species would possess leaf traits that reflect a conservative life history strategy aimed at stress tolerance, meaning that they would have lower specific leaf area, smaller leaves, and thicker leaves than frost susceptible species (Cornelissen et al. 2003).

### **1.5.3 Leaf venation traits: vein length per unit area, and vein density**

Leaf veins are extremely important in the functioning of plants, as they form the transport network for water, nutrients, and carbon (Brodribb et al. 2007). Two important leaf venation traits are: vein length per unit area (VLA) and vein density. VLA refers to the vein length per unit area for the total vein system, whereas vein density only refers to the vein length per unit area for the minor veins. Since these two measurements are similar, they are expected to have a corresponding relationship with frost.

Vein length per unit area (VLA) is a key feature of the leaf vein system (Sack and Scoffoni 2013). It is mainly determined by minor vein length per unit area, which accounts for over eighty percent of the total vein length. A high VLA can be beneficial for the leaf as it related to an increase in efficiency of

important leaf processes, such as: leaf hydraulic conductance ( $K_{\text{leaf}}$ ), stomatal density, stomatal conductance, and gas exchange (Brodribb et al. 2007, Sack and Scoffoni 2013). A high VLA may also help plants tolerate abiotic stresses, as it may provide tolerance to vein blockage and fine scale vein damage (Sack and Scoffoni 2013).

Vein density has been found to generally increase due to factors which raise plant transpiration rates and reduce water availability, such as increasing temperature, soil dryness, and decreasing air moisture (Uhl and Mosbrugger 1999). It is assumed that one of the most important factors influencing venation density is water availability (Uhl and Mosbrugger 1999). Some field studies show that venation density has been observed to be higher in plants from dry habitats, than moderate moisture habitats (Herbig and Kull 1992). Experimental studies also support this relationship. For example, the vein density of several herb species was found to increase with increasing soil and surrounding air dryness (Uhl and Mosbrugger 1999). This relationship suggests that having a higher vein density may make plants more tolerant to water stress, as a higher vein density represents more xylem flow pathways in parallel per leaf area and shorter pathways for water movement outside the xylem (Sack and Frole 2006, Brodribb et al. 2007, McKown et al. 2010). Therefore, we expect that leaves with lower vein densities will be more susceptible to frost, and leaves with higher vein densities will be more frost resistant.

As yet there has been no research done on the direct relationships between leaf venation traits and frost susceptibility. However, what we do know, such as the increase in efficiency of leaf processes, potential tolerance to vein blockage provided by higher VLA, and the relationship between water availability and vein density, leads us to predict that frost susceptible species will have lower VLA and vein density compared to frost tolerant species.

#### **1.5.4 Wood density**

Wood density is important in mechanical support, defence, architecture, hydraulics, carbon gain, and the growth potential of plants (Fan et al. 2012). Low wood density has been related to fast growth, in contrast to high wood density which is thought to limit growth but increase stress tolerance, through increasing a plants ability to handle limited resources, resistance to natural enemies, hydraulic safety (Meinzer et al. 2010), and mechanical strength (Jacobsen et al. 2007). There have been no studies on the direct relationship between frost and wood density, but for the same reasons as discussed above with venation traits, water stress may indicate how wood density is influenced by frost. Fu et al. (2012) observed wood density to be significantly correlated with leaf water stress tolerance. Other studies have also showed that species with higher wood density generally have a lower leaf water potential (Ackerly et al. 1992, Bucci et al. 2004). Therefore, we would expect frost susceptible species to have a lower wood density, compared to frost resistant species.

## 1.6 Aim and predictions

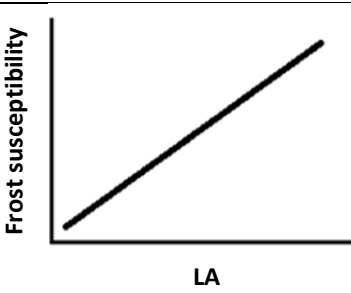
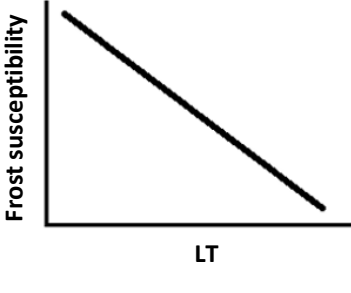
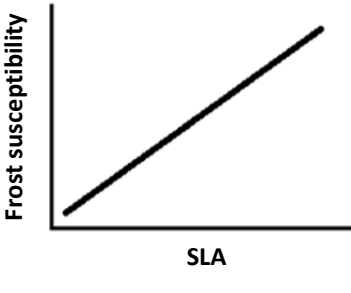
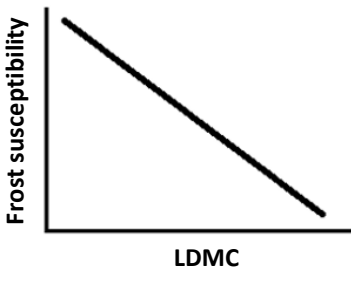
The main aim of this study was to determine which easily measured leaf functional traits are associated with frost susceptibility in plants. To achieve this, leaf trait measurements were taken from plant species in Australia and New Zealand. Trait measurements from Australian species were compared to percentage foliage retention values obtained from Curran et al. (2010), and the New Zealand species trait measurements were compared with  $LT_{50}$  and frost damage values obtained from Bannister (2003). New Zealand is an ideal place to conduct this research because there is an abundance of frost damage and  $LT_{50}$  data in the literature.

Frost susceptible species are expected to have (Table 1.1):

- Large leaf area, long and wide leaves
- Thin leaves with high specific leaf area and low leaf dry matter content
- Low vein density and low vein length per unit area
- Low wood density

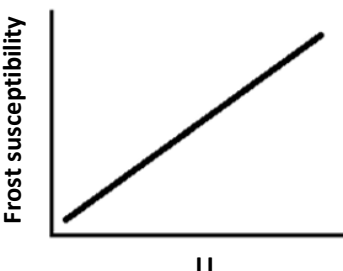
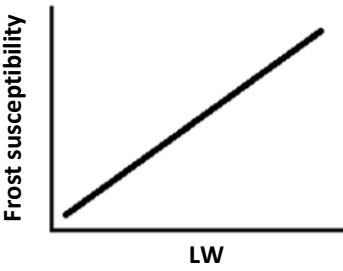
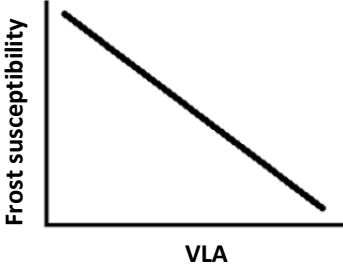
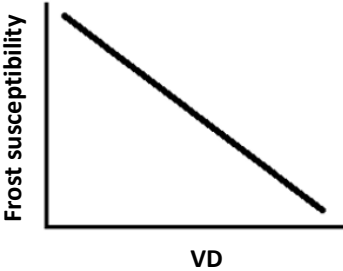
Easily measured leaf traits may provide an efficient way to determine foliar frost resistance and can be easily quantified for most of the world's plant species. This research will enable us to find out which leaf functional traits correlate with frost resistance. This information can then be used to predict which plant species are likely to be at risk of frost damage with future climate change and to decide which species should be used in restoration projects around the world, particularly in frost-prone ecosystems.

**Table 1.1: Leaf traits included in this study, their relation to frost susceptibility and their predicted relationships with frost susceptibility (i.e. higher frost damage, less negative LT50).**

Leaf trait	Description	Relation to frost susceptibility	Prediction
<b>Leaf area (LA)</b>	LA is the one sided projected surface area of a leaf, expressed in mm <sup>2</sup> (Cornelissen et al. 2003).	Cold stress has been found to select for small LA (Cornelissen et al. 2003). This may be an adaptation for limiting the effects of extreme temperatures, such as those cause by radiation frost (Harrison et al. 2010, Jordan and Smith 1995). Leaves with large LA have also been found to be colder than smaller leaves (Leuning and Cremer 1988).	 <p>Frost susceptible species will have large leaves.</p>
<b>Leaf thickness (LT)</b>	LT is the thickness of the leaf lamina and is one of the key components of SLA and LDMC, expressed in μm or mm (Perez et al. 2013).	LT plays an important role in the physical strength of leaves (Perez-Harguindeguy et al. 2013). Alpine species have been found to have high resource input into structural traits, including thick leaves (Soudzilovskaia et al. 2013).	 <p>Frost susceptible species will have thin leaves.</p>
<b>Specific leaf area (SLA)</b>	SLA is the one sided area of a fresh leaf divided by its oven dry mass, expressed in mm <sup>2</sup> mg <sup>-1</sup> (Perez Harguindeguy et al. 2013)	Leaves with low SLA have been found to be more tolerant of abiotic stress, including cold temperatures (Poorter et al. 2009). SLA has been found to decrease under drought conditions (Ansley et al. 1992). Frost is similar to drought, as low temperatures can cause water transport within the plant to be reduced or completely interrupted (Ansley et al. 1992).	 <p>Frost susceptible species will have high SLA.</p>
<b>Leaf dry matter content (LDMC)</b>	LDMC is the oven-dry mass of a leaf divided by its water-saturated fresh mass, expressed in mg g <sup>-1</sup> (Cornelissen et al. 2003).	Leaves with high LDMC tend to be tough and may be more resistant to physical hazards compared to leaves with low LDMC (Cornelissen et al. 2003). LDMC has also been found to increase under water stress (Ansley et al. 1992).	 <p>Frost susceptible species will have low LDMC.</p>



**Table 1.1 cont: Leaf traits used in this study, their relation to frost susceptibility and their predicted relationships with frost susceptibility (i.e. higher frost damage, less negative LT50).**

Leaf trait	Description	Relation to frost susceptibility	Prediction
<b>Leaf length (LL)</b>	LL is the longest distance measured from the tip of the leaf to the end of the petiole, where it would have attached to the branch/tree (Lee et al. 2012).	Shorter LL has been found to significantly decrease with increasing sky infrared radiation, which is linked to frost development on plants (Jordan and Smith 2009). Shorter LL has also been observed under water stress (Deblonde and Ledent 2001). Leaf size traits, including LL, are related to resource capture and use efficiency, and are expected to increase the relative growth rate of plants, which is not related to stress tolerance in plants (Forgiarini et al. 2015).	 <p>Frost susceptible species will have long leaves.</p>
<b>Leaf width (LW)</b>	LW is the distance in mm across the widest part of the leaf (Lee et al. 2012).	Wider leaves are expected to reach critical lethal temperatures faster and experience leaf death earlier than narrower leaves (Tozer et al. 2015). LW has been found to decrease significantly with increasing sky infrared radiation, which is linked to frost development on plants (Jordan and Smith 2009).	 <p>Frost susceptible species will have wide leaves.</p>
<b>Vein length per unit area (VLA)</b>	VLA is the vein length per unit area of the total vein system, expressed in mm mm <sup>-2</sup> (Sack and Scoffoni 2013).	High VLA can provide tolerance of fine-scale damage to the leaf or tolerance to vein blockage and may confer benefits for biomechanical support and protection (Sack and Scoffoni 2013).	 <p>Frost susceptible species will have low VLA.</p>
<b>Density of minor veins (VD)</b>	VD is the length of minor veins per unit leaf area, expressed in mm mm <sup>-2</sup> (Scoffoni et al. 2011).	Factors which reduce water availability, such as frost, tend to increase VD (Uhl & Mosbrugger 1999). Higher VD represents more xylem flow pathways in parallel per leaf area and shorter pathways for water movement outside the xylem (Sack and Frole, 2006; Brodribb et al., 2007; McKown et al., 2010).	 <p>Frost susceptible species will have low VD.</p>

## Chapter 2

### Methods

#### 2.1 Study sites

This study was conducted in 2015 in two locations: a restoration planting alongside the Barron River, which is 7.5 km east of Atherton in north Queensland, Australia (17°16'8"S, 145°32'20"E) (Figure 2.1), and Lincoln University campus, Lincoln, Christchurch, New Zealand (43. °38'41" S, 172°.28'8" E). The environment of the Australian site is described by Curran et al. (2010). It is located at approximately 700 m.a.s.l and receives 1379 mm rainfall per year, the warmest month is December, with a maximum temperature of 29°C, and the coldest is July with a minimum temperature of 10°C (Curran et al. 2010). A weather station nearby the site (Kairi) from 1965 to 2011 recorded a mean of 0.7 frost days per year (BOM 2015a) and, from 1994 to 2008 Atherton recorded a mean of 1.7 frost days per year (BOM 2007b).



**Figure 2.1: Australian site, showing the restoration planting and the Barron River.**

The New Zealand site has a temperate climate with an overall moderate rainfall, which in Christchurch between 1981 and 2010 was an average of 618.2 mm per year (NIWA 2013). January is the warmest month with a mean daily maximum air temperature of 22.7°C and July is the coldest month with a mean minimum daily temperature of 1.9°C (NIWA 2013). In winter, the night time

temperature commonly falls below 0°C and there are on average 99 days of ground frost per year (NIWA 2013).

## **2.2 Study species and frost resistance values**

The Australian plant species used in this study were taken from Curran et al. (2010) who examined the impact of a severe frost on rainforest saplings. In 2007, severe frosts caused considerable crop losses and seedling mortality of a riparian rainforest restoration planting. During this severe frost the temperatures were not recorded at the study site, but the study site is known to often have substantially lower temperatures and more frosts than Atherton, where four frost days with temperatures as low as -0.6°C were reported (BOM 2007; Curran et al. 2010). After this severe frost Curran et al. (2010) determined the frost resistance of species by visually estimating the proportion of foliage retention to the nearest 5% of the full canopy, for forty-five species. Our study assessed twenty-three of these species (Table 2.1).

The species used in the New Zealand dataset were taken from Bannister (2003). An exceptional period of frost, causing extensive damage to vegetation, occurred in the first two weeks of July in 1996 in Southland and South Otago (Bannister 2003). During this period, the lowest temperature recorded was -15.3°C in Tapanui. Bannister (2003) used this severe frost period to check measures of frost hardiness and experimentally-determined measures of frost resistance against field damage. In this study he used two types of frost susceptibility measures: frost damage and LT<sub>50</sub>. The LT<sub>50</sub> values were experimentally determined and were obtained from published and unpublished sources. During winter the year after the severe frost, Bannister (2003) also determined frost resistance for forty species which were not able to be found in any literature sources. Frost damage was determined using field observations of damage, which were then translated into a five point scale from 0, which is undamaged, to 4, which is apparently dead. The field observations were then allocated to USDA (United States Department of Agriculture) climatic zones. The USDA hardiness ratings are based on the coldest zone in which plants are able to survive and in this study were obtained from the RHS Encyclopaedia of Gardening (Huxley et al. 1992). For our study, we used twenty-five species (Table 2.2) from the Bannister (2003) dataset which had values for both the LT<sub>50</sub> frost measurement and the frost damage measurement.

## **2.3 Additional data**

Additional data from other sources were also included in this study. Wood density measurements were added for all twenty-three species in the Australian dataset. Eight additional species were also added to the Australian dataset; all eight had wood density values and four of the eight also had leaf length and leaf width values (Table 2.1). Wood density, leaf length, and leaf width values for the

Australian species were from unpublished reports, Eickhoff (2007) and Rubin (2007). Phenology, whether the species is evergreen or deciduous, was also added for each species in the Australian dataset. Wood density values were added for eighteen out of the twenty-five species in the New Zealand dataset. These wood density values were obtained from Richardson et al. (2013), and Beets and Oliver (2008).

## **2.4 Sampling**

We sampled twenty-three species from the Australian site (Table 2.1) and twenty-five species from the New Zealand site (Table 2.2). Five individuals of each species were sampled. Ideal leaves selected for sampling were relatively young, fully expanded and hardened leaves from adult plants that did not show any damage from pathogens or herbivores (Pérez-Harguindeguy et al. 2013). We collected whole twig sections approximately 10 cm in length, from parts of the tree which were most exposed to direct sunlight. The samples were put in bags while collection was occurring and were then refrigerated until measurements were made. Measurements were made as soon as possible after collection. Before measurement of the plant traits, plant samples were cut into small twig sections and then rehydrated by placing the cut end into water at room temperature for twenty-four hours.

**Table 2.1: Species list for the Australian dataset, including the family, species name, authority, species code, and the foliage retention for each species. ✕ = additional species added with only wood density values, ✕ ✕ = additional species added with wood density, leaf length and leaf width values.**

Australian dataset				
Family	Species	Authority	Code	% foliage retention
Rutaceae	<i>Zanthoxylum veneficum</i>	Bailey, F.M.	ZANven	0.33
Euphorbiaceae	<i>Aleurites rockinghamensis</i>	(Baill.) P.I.Forst.	ALERoc	0.82
Combretaceae	<i>Terminalia sericocarpa</i>	Decne.	TERser	1.98
Meliaceae	<i>Dysoxylum mollissimum</i> <i>ssp. molle</i> ✕	(Miq.) Mabb.	DYSmol	5.77
Myrtaceae	<i>Syzygium cormiflorum</i>	(F.Muell.) B.Hyland	SYZcor	7.56
Moraceae	<i>Ficus hispida</i> var. <i>hispida</i>	L.f.	FICHis	12.38
Sapindaceae	<i>Diploglottis diphylostegia</i> ✕	Hook.f.	DIPdip	14.17
Moraceae	<i>Ficus septica</i> var. <i>septica</i>	Burm.f.	FICsep	19.67
Meliaceae	<i>Melia azedarach</i> ✕ ✕	L.	MELaze	20.29
Rutaceae	<i>Melicope elleryana</i>	Ferdinand von Mueller	MELell	22.5
Euphorbiaceae	<i>Mallotus mollissimus</i>	Airy Shaw, H.K.	MALmol	26.1
Malvaceae	<i>Brachychiton acerifolius</i>	(A.Cunn. ex G.Don) Macarthur & C. Moore	BRAace	28.4
Apocynaceae	<i>Alstonia scholaris</i>	L. R. Br.	ALSsch	30.75
Boraginaceae	<i>Cordia dichotoma</i>	G.Forst.	CORdic	33.25
Rutaceae	<i>Acronychia acidula</i>	F. Muell.	ACRaci	33.8
Rutaceae	<i>Melicope rubra</i>	Lauterb. & K.Schum.	MELrub	41.67
Sapindaceae	<i>Castanospira alphanthii</i>	Mueller, F.J.H. von	CASalp	46.5
Meliaceae	<i>Toona ciliata</i> ✕ ✕	M. Roem	TOOcil	47.86
Elaeocarpaceae	<i>Elaeocarpus grandis</i>	Blume	ELAgra	48.57
Fabaceae	<i>Pararchidendron</i> <i>pruinatum</i>	(Benth.) I.C.Nielsen	PARpru	50
Lauraceae	<i>Cryptocarya triplinervis</i> var. <i>riparia</i> ✕ ✕	R.Br.	CRYtri	52.56
Fabaceae	<i>Castanospermum australe</i>	A.Cunn & C.Fraser ex Hook.	CASaus	55.58
Elaeocarpaceae	<i>Elaeocarpus coorangooloo</i>	J.F.Bailey & C.T.White	ELAcoo	60
Sapindaceae	<i>Guioa acutifolia</i> ✕	Ludwig Radlkofer	GUIacu	63
Lauraceae	<i>Neolitsea dealbata</i>	(R.Br.) Merr.	NEOLit	67.4
Moraceae	<i>Ficus fraseri</i>	Miq.	FICfra	69.43
Rutaceae	<i>Flindersia schottiana</i>	F.Muell.	FLIsch	72.5
Rutaceae	<i>Sarcomelicope simplicifolia</i> <i>ssp. simplicifolia</i> ✕	(Endl.) T.G.Hartley	SARsim	79.38
Myrtaceae	<i>Syzygium australe</i> ✕ ✕	(H.L.Wendl. ex Link) B.Hyland	AYZaus	79.62
Myrtaceae	<i>Melaleuca viminalis</i>	(Sol. ex Gaertn.) Byrnes	MELvim	84.37
Euphorbiaceae	<i>Mallotus philippensis</i>	(Lam.) Muell.Arg.	MALphi	87.73

**Table 2.2: Species list for the New Zealand dataset, including the family, species name, authority, species code, and the two frost variables LT<sub>50</sub> and frost damage by site. ✧ = Species introduced into New Zealand.**

New Zealand dataset					
Family	Species	Authority	Code	LT <sub>50</sub>	Frost damage
Sapindaceae	<i>Dodonaea viscosa</i>	Jacq.	DODvis	-3	4
Violaceae	<i>Melicytus ramiflorus</i>	J.R. & G. Forster	MELram	-4.9	4
Elaeocarpaceae	<i>Aristotelia serrata</i>	(J.R.Forst. & G.Forst.)	ARIserr	-5.4	3.7
Winteraceae	<i>Pseudowintera colorata</i>	(Raoul) Dandy	PSEcol	-6.3	3
Fabaceae	<i>Acacia melanoxylon</i> ✧	R.Br.	ACAmel	-6.3	4
Rousseaceae	<i>Carpodetus serratus</i>	J.R.Forst	CARserr	-6.7	4
Fabaceae	<i>Acacia baileyana</i> ✧	F.Muell.	ACAbai	-6.7	3.5
Asteraceae	<i>Olearia paniculata</i>	(J.R.Forst. & G.Forst.) Druce	OLEpan	-7	4
Malvaceae	<i>Hoheria angustifolia</i>	Raoul	HOHang	-7.5	2
Araliaceae	<i>Pseudopanax crassifolius</i>	(Sol. ex A.Cunn.) C.Koch	PSEcra	-7.5	3
Araliaceae	<i>Pseudopanax arboreus</i>	(L.f.) Philipson	PSEarb	-7.9	3.8
Myrtaceae	<i>Eucalyptus viminalis</i> ✧	Labill.	EUCvim	-8	4
Nothofagaceae	<i>Fuscopora fusca</i>	(Hook.f.) Heenan & Smissen	FUSfus	-8	1.3
Myrtaceae	<i>Kunzea ericoides</i>	(A.Rich.) Joy Thomps.	KUNeri	-8	4
Griselinaceae	<i>Griselinia littoralis</i>	Raoul	GRlitt	-8.6	3.3
Plantaginaceae	<i>Hebe salicifolia</i>	(G. Forst.) Pennell	HEBsal	-9	3.3
Pittosporaceae	<i>Pittosporum eugenioides</i>	A.Cunn.	PITeug	-9	3.3
Pittosporaceae	<i>Pittosporum tenuifolium</i>	Gaertn.	PITten	-9.5	2.6
Asteraceae	<i>Olearia ilicifolia</i>	Hook.f.	OLEill	-9.6	4
Scrophulariaceae	<i>Hebe topiaria</i>	L.B.Moore	HEBtop	-10.8	0
Nothofagaceae	<i>Lophozonia menziesii</i>	(Hook.f.) Heenan & Smissen	LOPmen	-11.7	1.3
Nothofagaceae	<i>Fuscopora cliffortioides</i>	(Hook.f.) Heenan & Smissen	FUScli	-13	0.5
Ericaceae	<i>Arbutus unedo</i> ✧	L.	ARBune	-14.9	0
Asteraceae	<i>Olearia macrodonta</i>	Baker	OLEmac		4
Asteraceae	<i>Olearia traversii</i>	(F. Muell.) Hook.f.	OLEtra		4

## 2.5 Measurement of traits

For each species five individuals were sampled and two leaves were measured from each sampled individual (ten leaves per species in total). The mean from the two leaves provided a trait value for each individual, which was then used as a single replicate. Leaf thickness, leaf area, specific leaf area, leaf width, leaf length, and leaf dry matter content were measured for each sample collected. The petiole of each leaf was included in the leaf trait measurements, except leaf thickness. Leaf thickness was measured with a micrometer (Insize Metric Digital Outside Micrometer series 3108) to the nearest 0.01mm. Three leaf thickness measurements per leaf were taken at a position as standard as

possible, within the lamina between the margin and the midrib, and were then averaged to provide one leaf thickness measurement per leaf (Pérez Harguindeguy et al. 2013). Leaf area was determined by taking a photograph of each individual leaf pressed flat with a piece of clear Perspex positioned next to a ruler. Then the leaf area was measured using the computer program ImageJ (National Institutes of Health, Bethesda, Maryland, USA). To determine the specific leaf area and the leaf dry matter content, leaf samples were dried in an oven for 48 hours at 80°C. Each individual sample was weighed. The SLA was calculated by dividing the leaf area by the dry mass. The leaf dry matter content was calculated by dividing the dry mass by the rehydrated weight for each leaf. Leaf width and leaf length were also measured using ImageJ. This was done using the straight line or segmented line drawing tool, by drawing to the tip of the leaf to the end of the petiole, along the midrib, to determine leaf length, and drawing across the leaf at the widest point to determine leaf width.

## 2.6 Leaf venation

For the New Zealand dataset two leaf venation traits were also measured for twelve species (Figure 2.2): vein density and vein length per unit area. To measure the leaf venation traits, leaves were first chemically cleared using methods adapted from Scoffoni and Sack (2013) and Berlyn and Miksche (1976). The leaf vein clearing was done on fresh leaves, as soon as possible after collection. Only twelve out of twenty-five species were able to be successfully cleared (Figure 2.2), as some leaves were too small to work with, too thin and delicate, or had a thick cuticle and were not able to be stained easily. Clearing was originally attempted on three leaves per species. However, out of the twelve species which were able to be cleared, three leaves were successfully able to be cleared for some species, whereas for other species only one or two were able to be cleared. The leaf clearing method used in this study is described below but was modified slightly for each individual species, based on their leaf thickness, leaf pigmentation, and size, as recommended by Scoffoni and Sack (2013).

The chemical leaf clearing method used in this study (adapted from Berlyn and Miksche 1976, Scoffoni and Sack 2013) was as follows:

1. Leaves were first immersed in 70% or 100% ethanol depending on the species, 70% for thin and light pigmented species and 100% for thicker and darker species. This step is used to remove the chlorophyll from the leaf. The leaves were left in the ethanol for up to one week, and were monitored throughout this time to decide when to remove them from the ethanol.
2. The leaves were then soaked in a 5% NaOH aqueous solution for a few hours to a few days. Thicker leaves were placed in the solution for a longer time than thinner leaves. The NaOH

solution was changed throughout the time the leaves were soaking in it, due to the leached leaf contents changing the colour of the solution.

3. The leaves were then rinsed with water and soaked in 50% bleach for 2-30 minutes until the colour was eliminated from the leaf.
4. The leaves were then rinsed with water again and brought into alcohol using an ethanol dilution series of 30%, 50%, 70%, and 100%, putting the leaves in these ethanol solutions for five minutes each.
5. Next the leaves were stained. After the 100% ethanol stage the leaves were covered with 1% safranin stain for about 1-30 minutes depending on the leaf thickness, then taken out and rinsed in 100% ethanol. They were then covered in 1% fast green stain for a few seconds, after which they were rinsed with 100% ethanol.
6. The leaves are then brought back into water in a reverse dilution series of ethanol, 100%, 70%, 50%, 30%, then into water.
7. The leaves were then placed into a 30% ethanol solution for storage, and scanned as soon as possible after clearing.

The chemically cleared leaves were then analysed to determine leaf venation traits using Leafgui (Price et al. 2011).

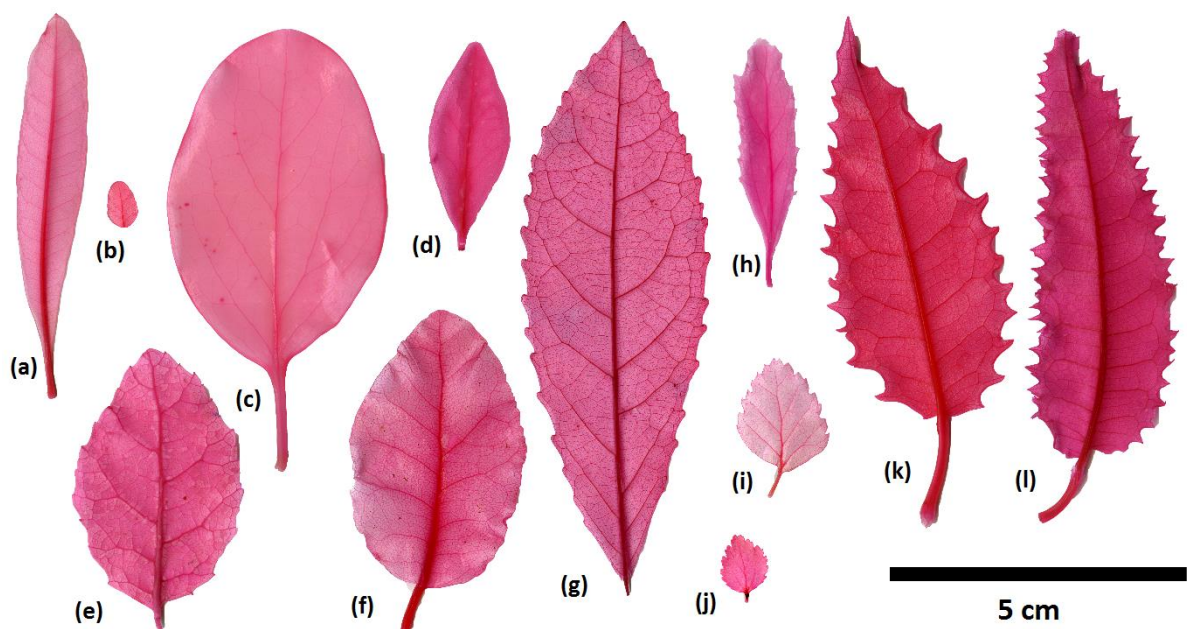


Figure 2.2: Chemically cleared and stained leaves from each of the twelve species, (a) *Dodonaea viscosa*, (b) *Fuscopora cliffortioides*, (c) *Griselina littoralis*, (d) *Olearia traversii*, (e) *Carpodetus serratus*, (f) *Olearia paniculata*, (g) *Melicytus ramiflorus*, (h) *Hoheria angustifolia*, (i) *Fuscopora fusca*, (j) *Lophozonia menziesii*, (k) *Olearia macrodonta*, (l) *Olearia ilicifolia*.



## 2.7 Statistical analysis

Pearson's correlation coefficient was used to assess intercorrelations among trait and frost variables for both the New Zealand and Australian datasets, and to select appropriate variables for subsequent regression analysis. Univariate and multivariate linear regression models were then used to model the relationships between the frost variable (percentage foliage retention,  $LT_{50}$ , frost damage by site) and the plant traits.

For the Australian dataset the relationships between percentage foliage retention and the leaf traits were modelled. Both the multivariate model and the univariate models included twenty-three species. The multivariate model included five plant traits: rehydrated leaf thickness, specific leaf area, leaf area, leaf dry matter content, and wood density. The univariate models were done on seven plant traits: rehydrated leaf thickness, specific leaf area, leaf area, leaf width, leaf length, leaf dry matter content, and wood density.

For the New Zealand dataset there were two frost variables:  $LT_{50}$  and frost damage. The multivariate and univariate models of the relationships between the frost damage variable and the plant traits included all twenty-five species. Whereas, the multivariate and univariate models of the relationships between the  $LT_{50}$  variable and the plant traits included only twenty-three species, as two species did not have  $LT_{50}$  values available for them. The multivariate models for both frost variables included four plant traits: rehydrated leaf thickness, specific leaf area, leaf area, and leaf dry matter content. The univariate models for both frost variables included eight plant traits: rehydrated leaf thickness, specific leaf area, leaf area, leaf width, leaf length, and leaf dry matter content. Univariate models were also constructed on a reduced New Zealand dataset for three traits: vein density (eleven species for  $LT_{50}$  and twelve species for frost damage), vein length per unit area (eleven species for  $LT_{50}$  and twelve species for frost damage), and wood density (eighteen species for both frost variables).

The software program R version 3.2.0, implemented in RStudio version 0.98.1103 was used for all the statistical analyses of this study.

## Chapter 3

### Results

#### 3.1 Australian dataset

Pearson's correlation coefficient values obtained for the Australian dataset showed that rehydrated weight, dry weight, leaf area, leaf width and leaf length were all highly correlated with each other, and fresh leaf thickness and rehydrated leaf thickness were highly correlated with each other (Table 3.1).

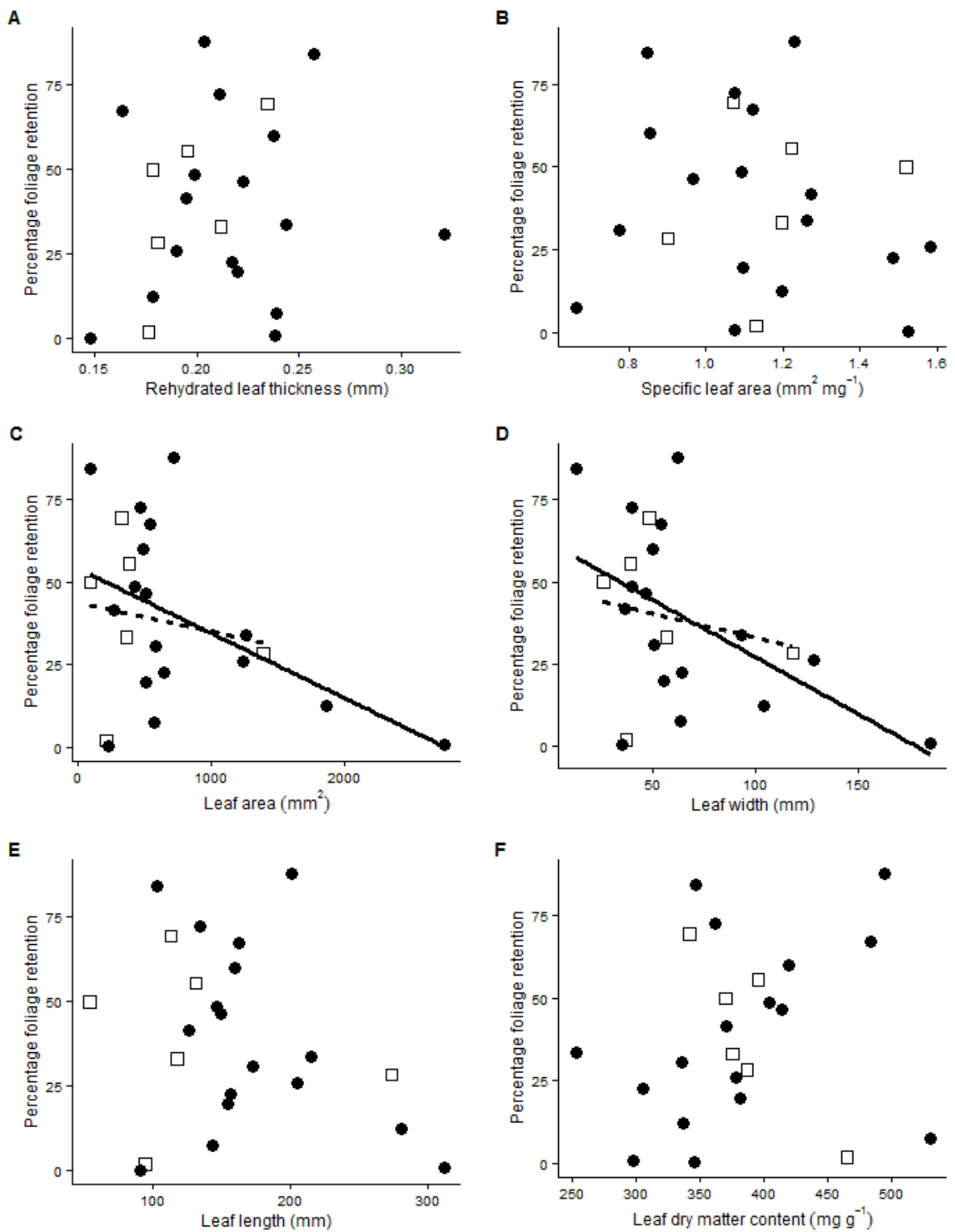
**Table 3.1: Pearson's correlation coefficient values for all plant traits and the percentage foliage retention (frost) for the Australian dataset, which contained twenty-three species. FLT = fresh leaf thickness, RLT = rehydrated leaf thickness, RW = rehydrated weight, DW = dry weight, LA = leaf area, LW = leaf width, LL = leaf length, SLA = specific leaf area, LDMC = leaf dry matter content, WD = wood density, Frost = percentage foliage retention.**

Variable	FLT	RLT	RW	DW	LA	LW	LL	SLA	LDMC	WD	Frost
FLT	1.00	0.88	0.15	0.12	0.03	0.02	0.06	-0.58	-0.26	0.02	-0.13
RLT	0.88	1.00	0.19	0.15	0.06	0.02	0.11	-0.59	-0.26	0.04	0.13
RW	0.15	0.19	1.00	0.98	0.97	0.91	0.89	-0.13	-0.41	-0.47	-0.43
DW	0.12	0.15	0.98	1.00	0.97	0.92	0.93	-0.21	-0.25	-0.45	-0.42
LA	0.03	0.06	0.97	0.97	1.00	0.96	0.94	0.00	-0.35	-0.44	-0.41
LW	0.02	0.02	0.91	0.92	0.96	1.00	0.89	0.07	-0.28	-0.37	-0.46
LL	0.06	0.11	0.89	0.93	0.94	0.89	1.00	-0.15	-0.22	-0.43	-0.27
SLA	-0.58	-0.59	-0.13	-0.21	0.00	0.07	-0.15	1.00	-0.32	-0.04	-0.14
LDMC	-0.26	-0.26	-0.41	-0.25	-0.35	-0.28	-0.22	-0.32	1.00	0.24	0.18
WD	0.02	0.04	-0.47	-0.45	-0.44	-0.37	-0.43	-0.04	0.24	1.00	0.33
Frost	-0.13	0.13	-0.43	-0.42	-0.41	-0.46	-0.27	-0.14	0.18	0.33	1.00

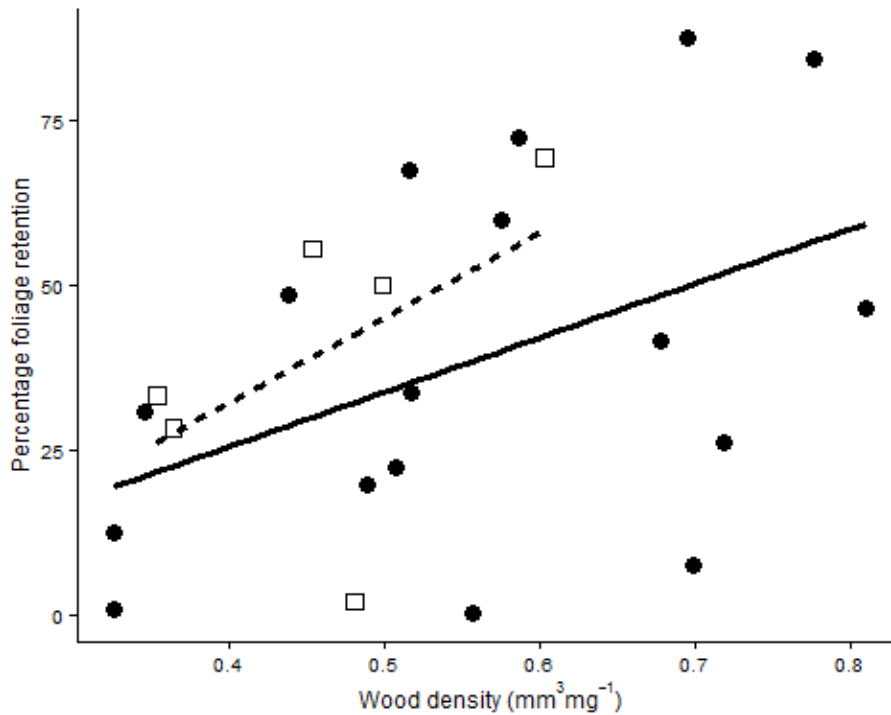
The multivariate model for the Australian dataset showed no significant relationships between the percentage foliage retention and any of the plant functional traits (Table 3.2). The univariate models done on traits separately showed no significant relationships between percentage foliage retention and the functional leaf traits: rehydrated leaf thickness, specific leaf area, leaf length, leaf dry matter content, but showed significant negative relationships between percentage foliage retention and leaf width and leaf area (Figure 3.1), and a significant positive relationship between percentage foliage retention and wood density (Table 3.2, Figure 3.2)

**Table 3.2: The results from the multivariate model and univariate models showing the relationships between percentage foliage retention and the plant functional traits for twenty-three Australian plant species. Multivariate model:  $R^2$  is -0.00, 17 degrees of freedom. Leaf length and leaf width were not included in the multivariate model due to collinearity with rehydrated weight, fresh weight, and leaf area. Univariate models all have 21 degrees of freedom, \* =  $P < 0.05$ .**

Variable	Multivariate model	Univariate models	
	Regression coefficient (S.E.)	Regression coefficient (S.E.)	$R^2$
Rehydrated leaf thickness	3.39 (9.00)	95.47 (154.62)	-0.03
Specific leaf area	-1.25 (9.17)	-15.57 (23.54)	-0.03
Leaf area	-8.86 (6.62)	-0.02 (0.01) *	0.13
Leaf width		-0.31 (0.13) *	0.17
Leaf length		-0.11 (0.09)	0.03
Leaf dry matter content	1.02 (8.20)	0.07 (0.09)	-0.02
Wood density	8.19 (6.37)	77.927 (36.24) *	0.14



**Figure 3.1:** The relationships between percentage foliage retention and the leaf functional traits: A) rehydrated leaf thickness, B) specific leaf area, C) leaf area, D) leaf width, E) leaf length, and F) leaf dry matter content. Black circles represent the seventeen plant species in the Australian dataset that are evergreen, and the white squares represent six plant species in the Australian dataset which are deciduous. The significant relationships are indicated by a solid line for evergreen species and a dashed line for deciduous species.

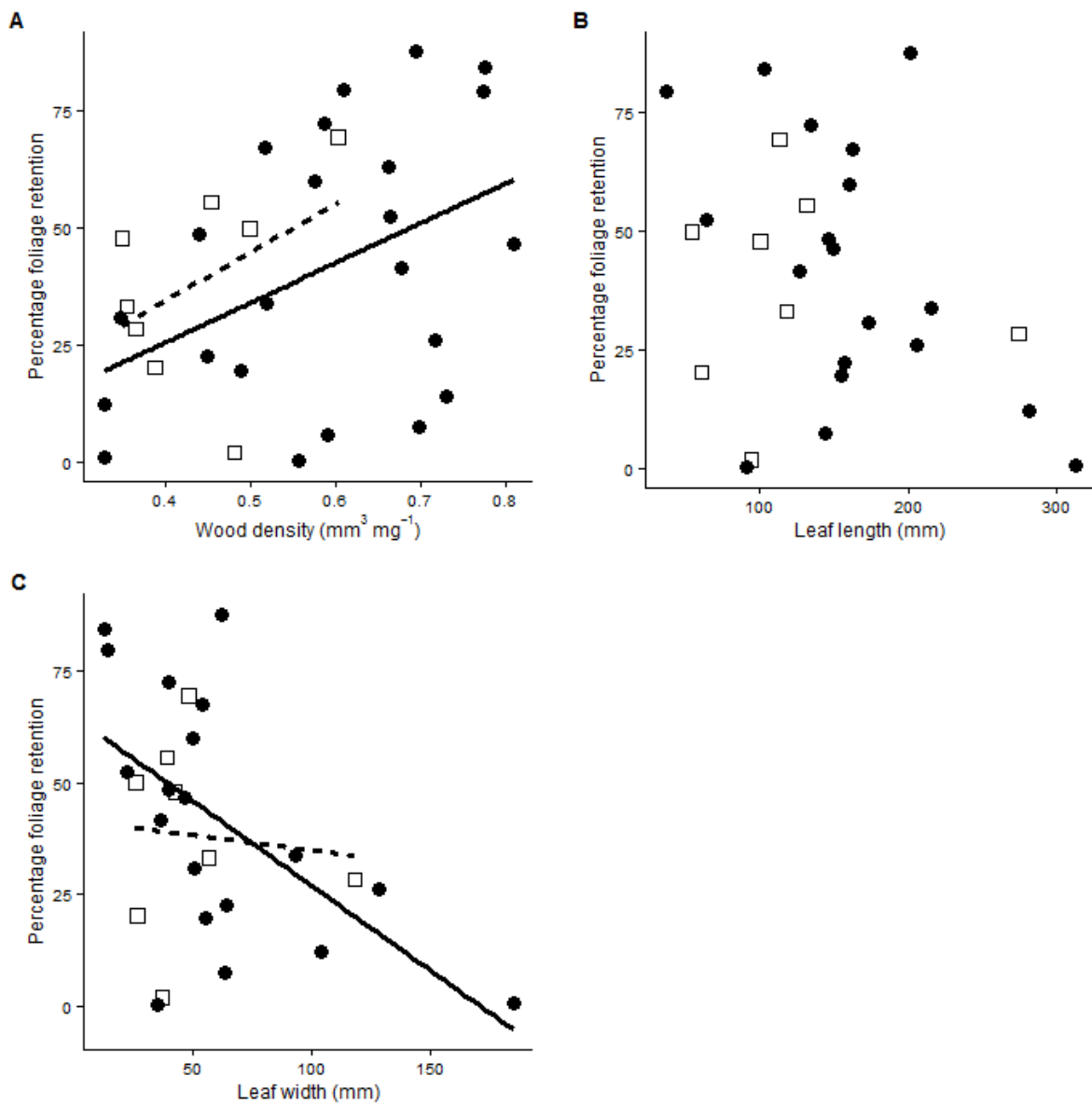


**Figure 3.2:** The relationship between percentage foliage retention and wood density for the Australian dataset. Black circles represent the seventeen plant species in the Australian dataset that are evergreen, and the white squares represent the six plant species in the Australian dataset which are deciduous. The significant relationship is indicated by a solid line for evergreen species and a dashed line for deciduous species.

When eight additional plant species were added to the wood density data, a significant positive relationship was observed between percentage foliage retention and wood density (Table 3.3). When four additional plant species were added to both the leaf length and leaf width data, there was still no relationship observed between percentage foliage retention and leaf length (Table 3.3), but a significant negative relationship was observed between percentage foliage retention and leaf width (Table 3.3, Figure 3.3).

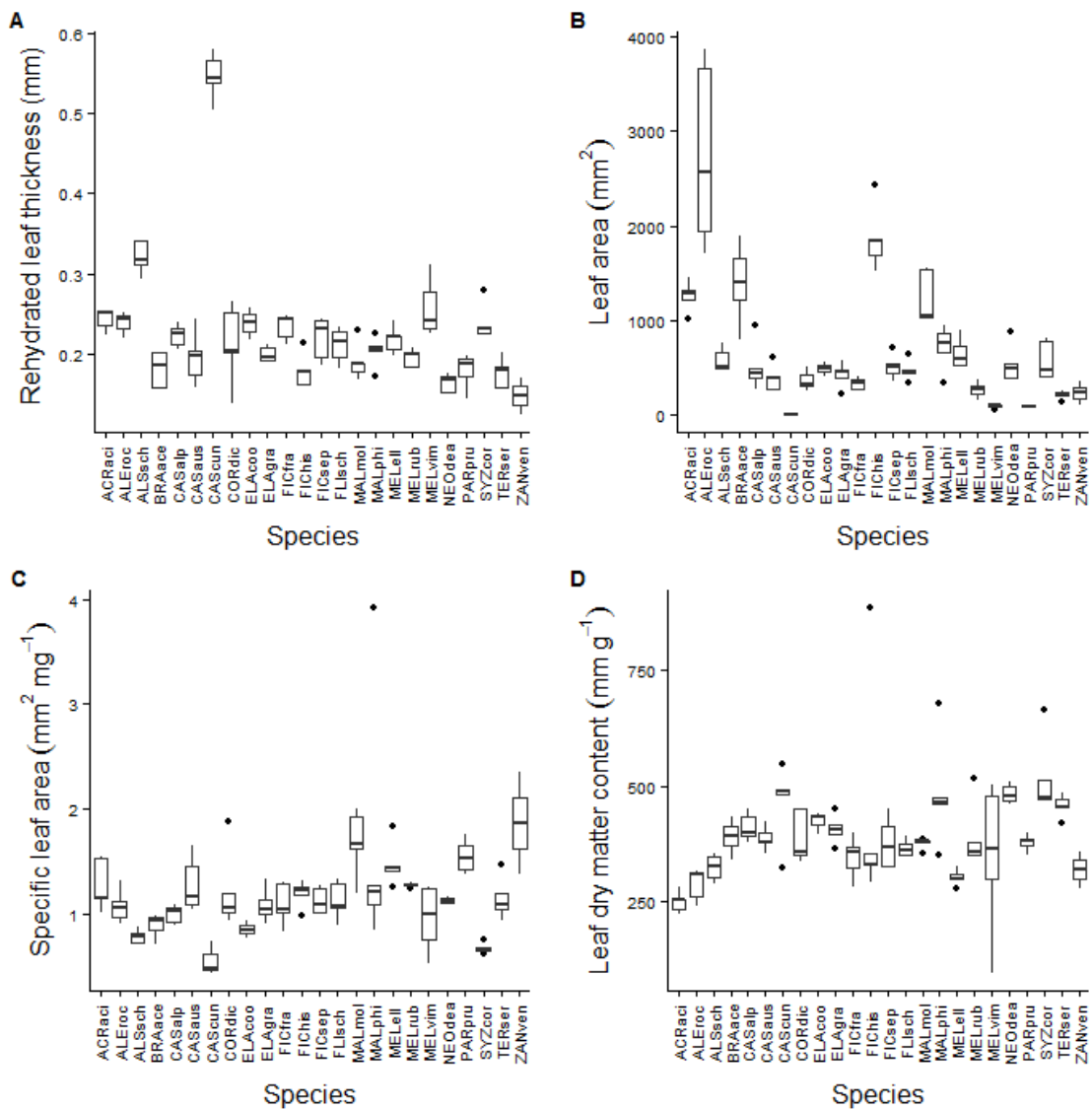
**Table 3.3:** The results from the univariate models showing the relationships between percentage foliage retention and the leaf functional traits: wood density, leaf length, and leaf width, when eight additional species (total of thirty-one species) have been added to the wood density data, and four species have been added to both the leaf length and leaf width data (total of twenty-seven species), \* = P < 0.05.

Variable	Regression coefficient (S.E.)	d.f.	R <sup>2</sup>
Wood density	73.24 (30.87) *	29	0.13
Leaf length	-0.12 (0.07)	25	0.06
Leaf width	-0.32 (0.12) *	25	0.19



**Figure 3.3: The relationships between percentage foliage retention and the leaf functional traits: A) wood density, B) leaf length, and C) leaf width. When an additional eight species have been added to the dataset for wood density (thirty-one species total), and an additional four species added to the dataset for both leaf area and leaf length (twenty-seven species total). Black circles represent the plant species in the Australian dataset that are evergreen, and the white squares represent plant species in the Australian dataset which are deciduous. The significant relationships are indicated by a solid line for evergreen species and a dashed line for deciduous species.**

The within species variability for leaf thickness, leaf area, specific leaf area, and leaf dry matter content was inconsistent between species for the Australian dataset (Figure 3.4). Some species exhibited very little variation, with all five individuals having very similar trait values, whereas others are highly variable with a wide range of trait values. For example, the species with the least variable leaf thickness is *Elaeocarpus grandis* with a leaf thickness range of 0.19 to 0.21, and the species with the most variable leaf thickness is *Cordia dichotoma* with a leaf thickness range of 0.137 to 0.255 (Figure 3.4).



**Figure 3.4: Within species variability from five individuals for each of the twenty-three original species from the Australian dataset, for four leaf traits: A) rehydrated leaf thickness, B) leaf area, C) specific leaf area, and D) leaf dry matter content. Boxplot explanation: the central line is the median, the top and bottom of the box are the interquartile ranges, the whiskers of the plot are 1.5 times the interquartile range, and the black dots represent the outliers.**

### 3.2 New Zealand dataset

Pearson’s correlation coefficient values obtained for the twenty-five New Zealand plant species showed that rehydrated weight, dry weight, leaf area, leaf width and leaf length were all highly correlated with each other, and fresh leaf thickness and rehydrated leaf thickness were highly correlated with each other. SLA was also moderately correlated with fresh leaf thickness and rehydrated leaf thickness (Table 3.4 A). The same correlations among traits were found when wood density was added to the dataset for eighteen species, and wood density was not found to be significantly correlated with any of the other traits (Table 3.4 B). These correlations among traits were also found when the leaf venation traits were added to the dataset for twelve species, and vein length per unit area and vein density were found to be highly correlated with each other (Table 3.4 C).

**Table 3.4: Pearson's correlation coefficient values for leaf functional traits and the frost variables: LT<sub>50</sub> (FR) and frost damage by site (FD) for the New Zealand dataset, which contained twenty-five species. FLT = fresh leaf thickness, RLT = rehydrated leaf thickness, RW = rehydrated weight, DW = dry weight, LA = leaf area, LW = leaf width, LL = leaf length, SLA = specific leaf area, LDMC = leaf dry matter content, WD = wood density, VLA = vein length per unit area, VD = vein density, FR = LT<sub>50</sub> value, FD = frost damage by site. (A) includes all twenty-five species from the New Zealand dataset, (B) only included the eighteen species with wood density values available, and (C) only included the twelve species with VLA and vein density values available.**

**(A)**

Variable	FLT	RLT	RW	DW	LA	LW	LL	SLA	LDMC	FR	FD
FLT	1.00	0.94	0.52	0.52	0.35	0.25	0.39	-0.68	-0.14	-0.32	-0.13
RLT	0.94	1.00	0.54	0.52	0.37	0.36	0.45	-0.71	-0.28	-0.31	-0.11
RW	0.52	0.54	1.00	0.99	0.96	0.83	0.83	-0.42	-0.29	0.14	0.34
DW	0.52	0.52	0.99	1.00	0.95	0.79	0.84	-0.44	-0.22	0.12	0.33
LA	0.35	0.37	0.96	0.95	1.00	0.90	0.87	-0.34	-0.32	0.24	0.40
LW	0.25	0.36	0.83	0.79	0.90	1.00	0.72	-0.32	-0.43	0.22	0.32
LL	0.39	0.45	0.83	0.84	0.87	0.72	1.00	-0.38	-0.36	0.27	0.45
SLA	-0.68	-0.71	-0.42	-0.44	-0.34	-0.32	-0.38	1.00	-0.24	0.39	0.17
LDMC	-0.14	-0.28	-0.29	-0.22	-0.32	-0.43	-0.36	-0.24	1.00	-0.28	-0.21
FR	-0.32	-0.31	0.14	0.12	0.24	0.22	0.27	0.39	-0.28	1.00	0.76
FD	-0.13	-0.11	0.34	0.33	0.40	0.32	0.45	0.17	-0.21	0.76	1.00

**(B)**

Variable	FLT	RLT	RW	DW	LA	LW	LL	SLA	LDMC	WD	FR	FD
FLT	1.00	0.92	0.50	0.51	0.26	0.06	0.30	-0.76	-0.03	-0.03	-0.24	-0.03
RLT	0.92	1.00	0.50	0.47	0.25	0.16	0.33	-0.70	-0.27	-0.01	-0.23	-0.01
RW	0.50	0.50	1.00	0.99	0.95	0.80	0.81	-0.37	-0.42	-0.28	0.20	0.38
DW	0.51	0.47	0.99	1.00	0.94	0.75	0.82	-0.41	-0.34	-0.23	0.18	0.36
LA	0.26	0.25	0.95	0.94	1.00	0.89	0.84	-0.12	-0.47	-0.37	0.33	0.46
LW	0.06	0.16	0.80	0.75	0.89	1.00	0.63	0.11	-0.63	-0.49	0.32	0.34
LL	0.30	0.33	0.81	0.82	0.84	0.63	1.00	-0.18	-0.51	-0.13	0.37	0.52
SLA	-0.76	-0.70	-0.37	-0.41	-0.12	0.11	-0.18	1.00	-0.36	-0.25	0.56	0.28
LDMC	-0.03	-0.27	-0.42	-0.34	-0.47	-0.63	-0.51	-0.36	1.00	0.37	-0.41	-0.48
WD	-0.03	-0.01	-0.28	-0.23	-0.37	-0.49	-0.13	-0.25	0.37	1.00	-0.05	-0.08
FR	-0.24	-0.23	0.20	0.18	0.33	0.32	0.37	0.56	-0.41	-0.05	1.00	0.78
FD	-0.03	-0.01	0.38	0.36	0.46	0.34	0.52	0.28	-0.48	-0.08	0.78	1.00

**(C)**

Variable	FLT	RLT	RW	DW	LA	LW	LL	SLA	LDMC	VLA	VD	FR	FD
FLT	1.00	0.87	0.51	0.48	0.31	0.31	0.15	-0.67	-0.09	-0.31	-0.70	-0.18	0.29
RLT	0.87	1.00	0.75	0.72	0.54	0.60	0.39	-0.50	-0.44	-0.56	-0.77	-0.10	0.41
RW	0.51	0.75	1.00	0.99	0.94	0.94	0.76	-0.21	-0.62	-0.61	-0.72	0.15	0.49
DW	0.48	0.72	0.99	1.00	0.96	0.93	0.84	-0.22	-0.62	-0.66	-0.76	0.21	0.58
LA	0.31	0.54	0.94	0.96	1.00	0.93	0.90	-0.05	-0.63	-0.64	-0.70	0.34	0.59
LW	0.31	0.60	0.94	0.93	0.93	1.00	0.75	-0.08	-0.71	-0.69	-0.65	0.31	0.56
LL	0.15	0.39	0.76	0.84	0.90	0.75	1.00	0.07	-0.62	-0.71	-0.70	0.52	0.75
SLA	-0.67	-0.50	-0.21	-0.22	-0.05	-0.08	0.07	1.00	-0.40	-0.13	0.29	0.50	-0.03
LDMC	-0.09	-0.44	-0.62	-0.62	-0.63	-0.71	-0.62	-0.40	1.00	0.80	0.52	-0.48	-0.62
VLA	-0.31	-0.56	-0.61	-0.66	-0.64	-0.69	-0.71	-0.13	0.80	1.00	0.80	-0.71	-0.85
VD	-0.70	-0.77	-0.72	-0.76	-0.70	-0.65	-0.70	0.29	0.52	0.80	1.00	-0.43	-0.81
FR	-0.18	-0.10	0.15	0.21	0.34	0.31	0.52	0.50	-0.48	-0.71	-0.43	1.00	0.70
FD	0.29	0.41	0.49	0.58	0.59	0.56	0.75	-0.03	-0.62	-0.85	-0.81	0.70	1.00



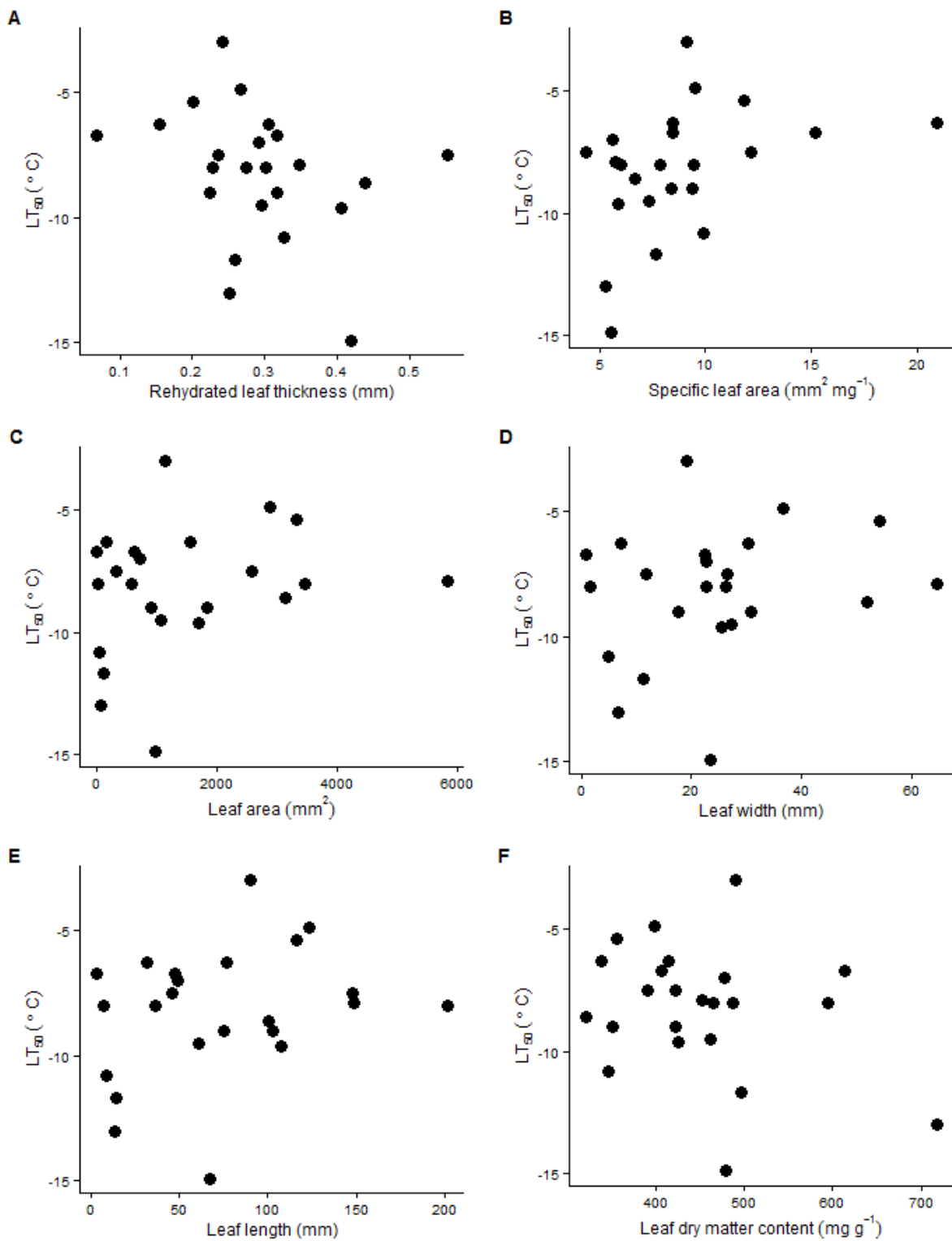
The multivariate models for the New Zealand dataset showed no significant relationships between either of the frost variables: LT<sub>50</sub> (Table 3.5), frost damage by site (Table 3.6), and any of the plant functional traits. The univariate models for the plant functional traits and the LT<sub>50</sub> frost variable showed a significant negative relationship between LT<sub>50</sub> and vein length per unit area, but no significant relationships were observed between LT<sub>50</sub> and any of the other leaf functional traits (Table 3.5, Figure 3.5). The univariate models for the leaf functional traits and frost damage showed significant negative relationships between frost damage and vein length per unit area and vein density, and a significant positive relationship between frost damage and leaf length, but no significant relationships were observed between frost damage and any of the other leaf traits (Table 3.6, Figure 3.6).

**Table 3.5: The results from the multivariate model and univariate models showing the relationships between LT<sub>50</sub> and the plant functional traits for the New Zealand dataset. Multivariate model: R<sup>2</sup> = 0.20 degrees of freedom. Leaf length and leaf width were not included in the multivariate model due to collinearity with rehydrated weight, fresh weight, and leaf area. Wood density, vein length per unit area, and vein density were not included in the multivariate model due to missing data for some of the species. There were twenty-three species in the New Zealand dataset with LT<sub>50</sub> values, eighteen of these species had wood density data, and eleven of these species had vein length per unit area and vein density data. \* = P < 0.05.**

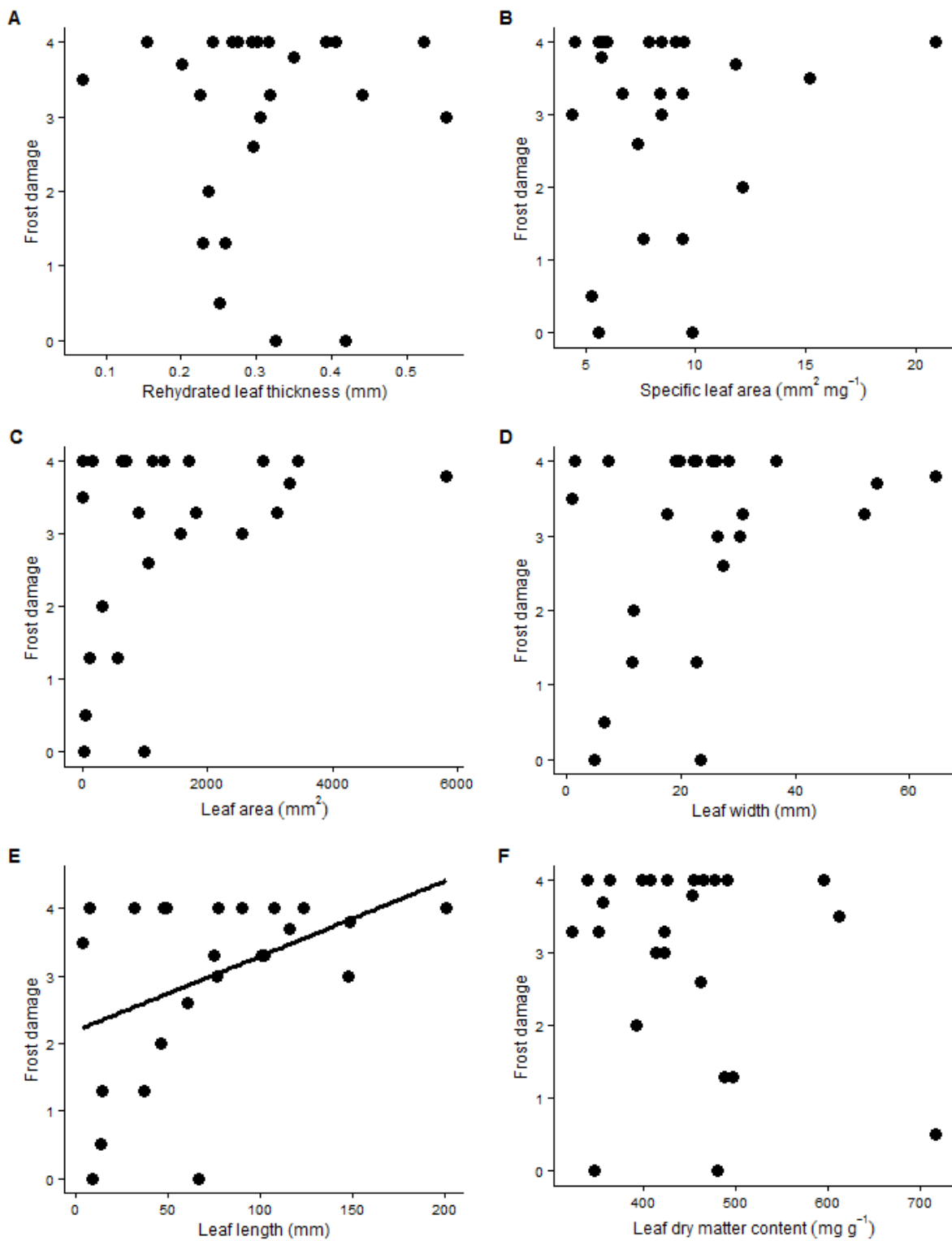
Variable	Multivariate model	Univariate models		
	Regression coefficient (S.E.)	Regression coefficient (S.E)	R <sup>2</sup>	d.f.
Rehydrated leaf thickness	-0.97 (1.04)	-8.19 (5.46)	0.05	21
Specific leaf area	0.61 (1.01)	0.28 (0.14)	0.11	21
Leaf area	0.98 (0.58)	0.00 (0.00)	0.01	21
Leaf width		0.03 (0.03)	0.00	21
Leaf length		0.01 (0.01)	0.03	21
Leaf dry matter content	-0.50 (0.74)	-0.01 (0.01)	0.03	21
Wood density		-1.35 (6.40)	-0.06	16
Vein length per unit area		-9786.98 (3240.51) *	0.45	9
Vein density		-275900 (193500)	0.09	9

**Table 3.6: The results from the multivariate model and univariate models showing the relationships between frost damage and the plant functional traits for the New Zealand dataset. Multivariate model: R squared is 0.02, 20 degrees of freedom. Leaf length and leaf width were not included in the multivariate model due to collinearity with rehydrated weight, fresh weight, and leaf area. Wood density, vein length per unit area, and vein density were not included in the multivariate model due to missing data for some of the species. There were twenty-five species in the New Zealand dataset with frost damage values, eighteen of these species had wood density data, and twelve of these species had vein length per unit area and vein density data. \* = P <0.05, \*\* = P <0.01, \*\*\* = P<0.001.**

Variable	Multivariate model	Univariate models		
	Regression coefficient (S.E.)	Regression coefficient (S.E)	R <sup>2</sup>	d.f.
Rehydrated leaf thickness	-0.03 (0.54)	-0.02 (2.59)	-0.04	21
Specific leaf area	0.29 (0.56)	0.03 (0.08)	-0.03	21
Leaf area	0.54 (0.31)	0.00 (0.00)	0.10	21
Leaf width		0.03 (0.02)	0.06	21
Leaf length		0.01 (0.01) *	0.14	21
Leaf dry matter content	-0.08 (0.41)	-0.00 (0.00)	0.012	21
Wood density		-0.91 (2.92)	-0.06	16
Vein length per unit area		-5599.41 (1067.31) ***	0.71	10
Vein density		-244000 (53280) **	0.64	10

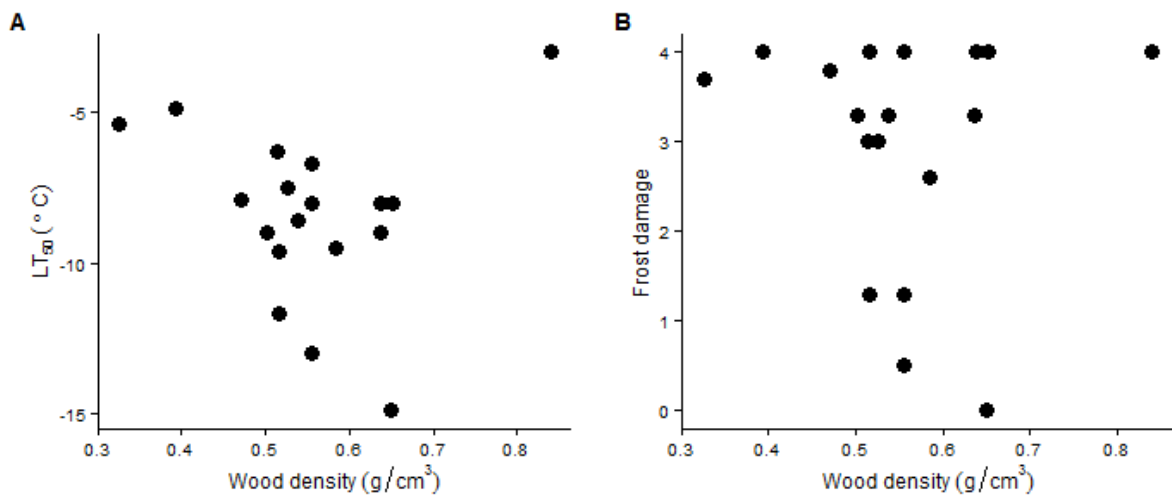


**Figure 3.5: The New Zealand dataset relationships for twenty-three species, between  $LT_{50}$  and the leaf functional traits: A) rehydrated leaf thickness, B) specific leaf area, C) leaf area, D) leaf width, E) leaf length, and F) leaf dry matter content.**



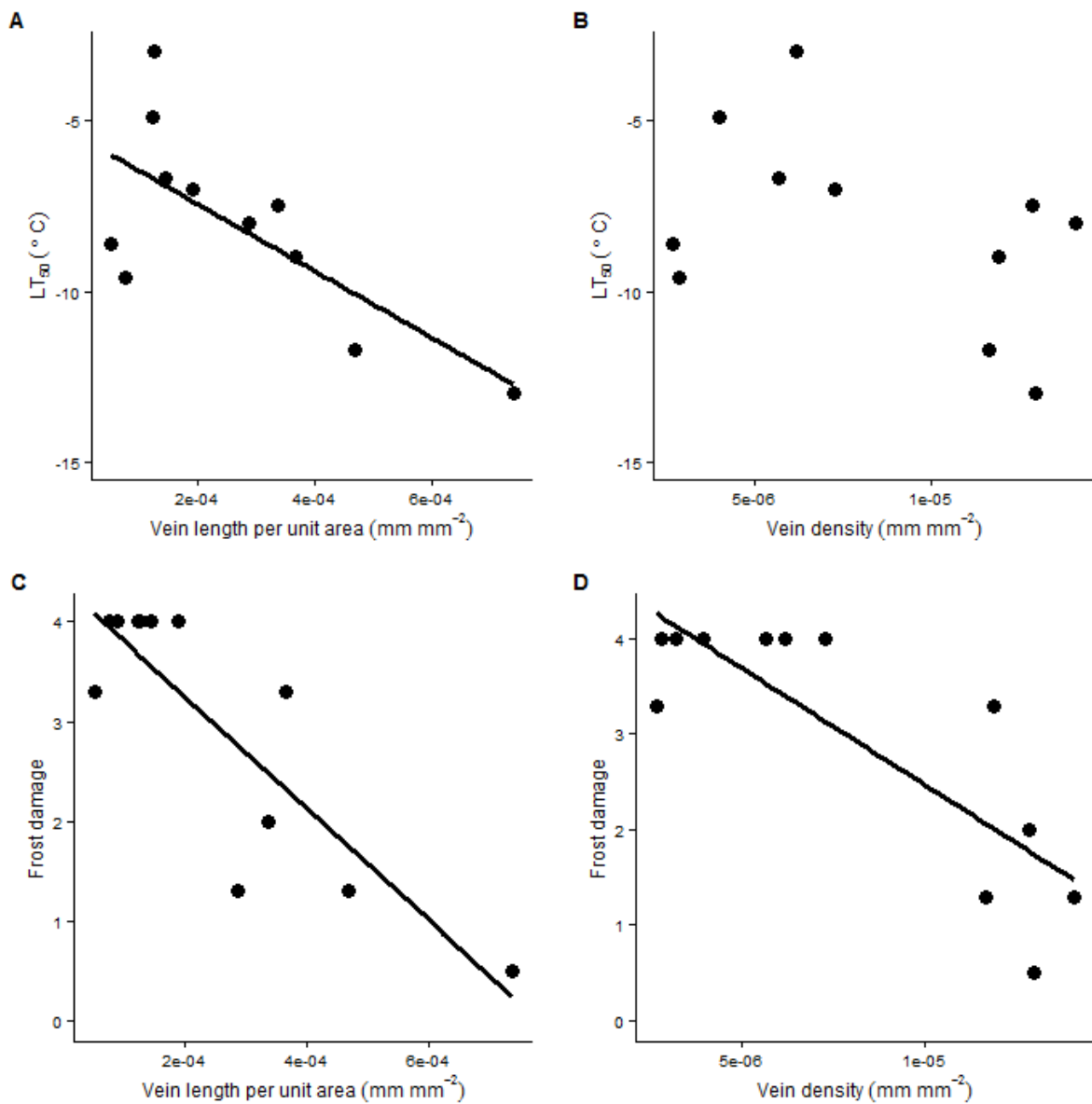
**Figure 3.6: The New Zealand dataset relationships for twenty-five species, between frost damage and the leaf functional traits: A) rehydrated leaf thickness, B) specific leaf area, C) leaf area, D) leaf width, E) leaf length, and F) leaf dry matter content.**

When wood density was added to the New Zealand dataset for eighteen of the species, no significant relationship was found between wood density and either of the frost variables,  $LT_{50}$  (Table 3.5, Figure 3.7) or frost damage (Table 3.6, Figure 3.7).



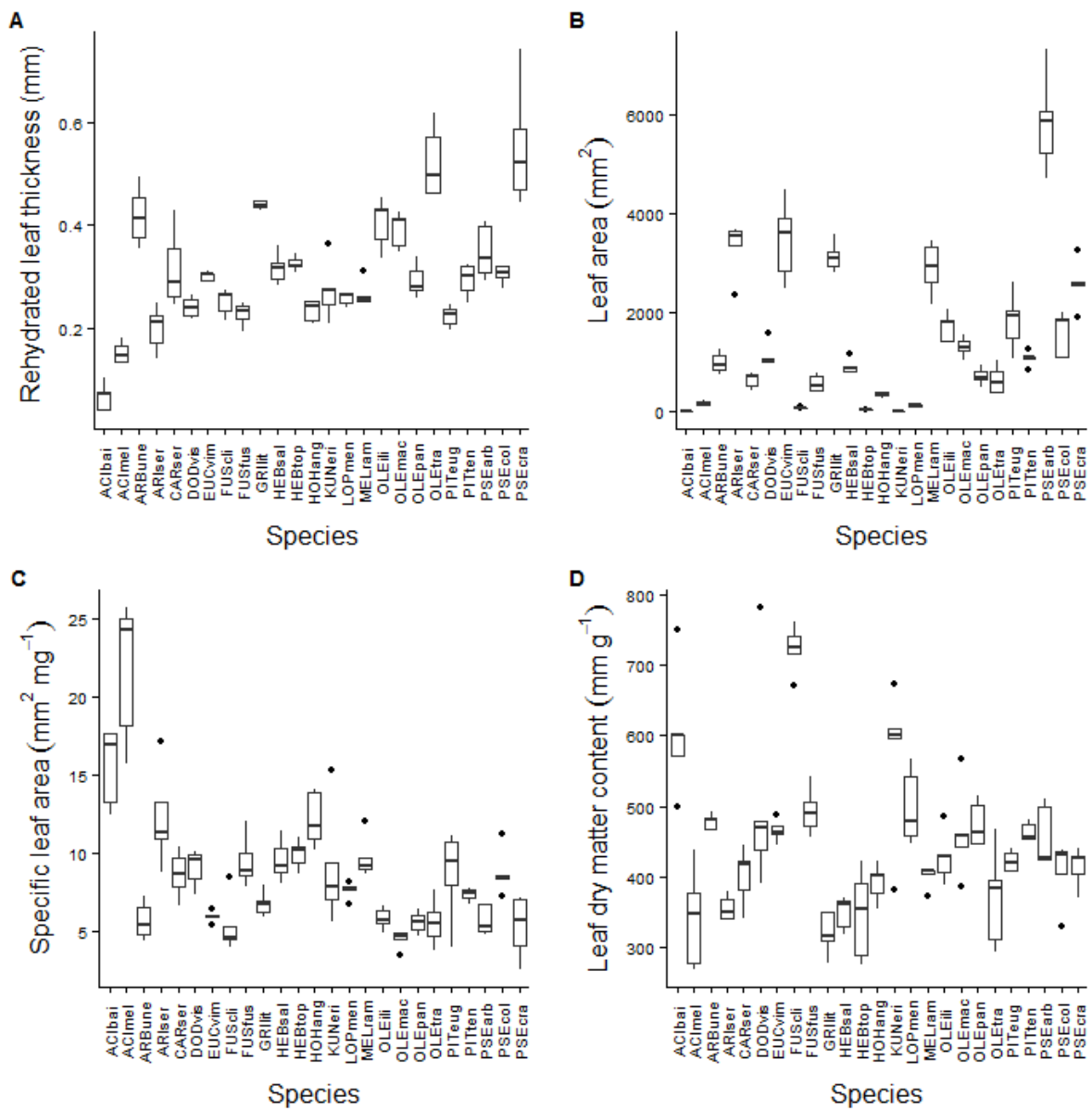
**Figure 3.7: The New Zealand dataset relationships for eighteen species between wood density and frost variables: A) LT<sub>50</sub> and B) frost damage.**

There was a significant negative relationship observed between LT<sub>50</sub> and vein length per unit area (Table 3.5, Figure 3.8), but no relationship between frost resistance and vein density. Vein length per unit area and vein density both had significant negative relationships with frost damage (Table 3.6, Figure 3.8).



**Figure 3.8: The New Zealand dataset relationships between the frost variables:  $LT_{50}$  (eleven species) and frost damage (twelve species) and the leaf venation traits: vein length per unit area and vein density. Significant negative relationships are shown with a simple linear regression trendline.**

The within-species variability for leaf thickness, leaf area, specific leaf area, and leaf dry matter content was inconsistent between species for the New Zealand dataset. Figure 3.9 shows that values for the leaf traits leaf thickness, leaf area, specific leaf area, and leaf dry matter content were inconsistent among species. Some species exhibited very little variation, with all five individuals having very similar trait values, whereas others were highly variable with a wide range of trait values. For example, the species with the least variable leaf thickness is *Griselinia littoralis* with a leaf thickness range of 0.43 to 0.45, and the species with the most variable leaf thickness was *Pseudopanax crassifolius* with a leaf thickness range of 0.446 to 0.74 (Figure 3.9).



**Figure 3.9: Within-species variability for five individuals of each of the twenty-five species in the New Zealand dataset, for four leaf traits: A) rehydrated leaf thickness, B) leaf area, C) specific leaf area, and D) leaf dry matter content. Boxplot explanation: the central line is the median, the top and bottom of the box are the interquartile ranges, the whiskers of the plot are 1.5 times the interquartile range, and the black dots represent the outliers.**

## Chapter 4

### Discussion

This study provides new information about which leaf traits and leaf venation traits may affect the frost susceptibility of plant species. Overall, the traits which are most likely to be correlated with frost resistance appear to be leaf size traits, leaf venation traits, and possibly wood density (Table 4.1). However, none of the significant relationships we observed were consistent across the three frost variables we used (percentage foliage retention,  $LT_{50}$ , and frost damage). We also found inconsistency between the multivariate and univariate models; none of the multivariate models showed significant results, whereas some of the univariate models showed significant results for the same traits (Table 4.1). These inconsistencies found between datasets and models are likely due to the low sample size of only twenty-three species in the Australian dataset and twenty-five species in the New Zealand dataset, and the high species variability, due to only five individuals of each species being measured (Figure 3.4, Figure 3.9). Therefore, the significant relationships may have been more consistent if more species, and individuals per species, had been sampled.



**Table 4.1: Summary of results, including the initial predictions from hypotheses and the model results for both the Australian and New Zealand datasets. ✓ = results agree with the prediction, - = Plant trait not included in model, ✗ = plant trait showed no relationship with frost resistance. Multi = multivariate model results, Uni = univariate model results, Uni add = results from univariate model when additional data were added to the Australian dataset, LT<sub>50</sub> = models using LT<sub>50</sub> as frost variable, FD = models using frost damage as frost variable.**

Plant trait	Prediction	Australian dataset			New Zealand dataset			
		Multi	Uni	Uni add	Multi LT <sub>50</sub>	Uni LT <sub>50</sub>	Multi FD	Uni FD
Leaf area	Species with a smaller LA will be more frost resistant	✗	✓	-	✗	✗	✗	✗
Leaf thickness	Species with thicker leaves will be more frost resistant	✗	✗	-	✗	✗	✗	✗
Specific leaf area	Species with a lower SLA will be more frost resistant	✗	✗	-	✗	✗	✗	✗
Leaf dry matter content	Species with a higher LDMC will be more frost resistant	✗	✗	-	✗	✗	✗	✗
Leaf length	Species with shorter leaves will be more frost resistant	-	✗	✗	-	✗	-	✓
Leaf width	Species with narrower leaves will be more frost resistant	-	✓	✓	-	✗	-	✗
Vein length per unit area	Species with a higher VLA will be more frost resistant	-	-	-	-	✓	-	✓
Vein density	Species with a higher VD will be more frost resistant	-	-	-	-	✗	-	✓
Wood density	Species with a higher WD will be more frost resistant	✗	✓	✓	-	✗	-	✗

#### 4.1 Leaf size traits: leaf area, leaf length, and leaf width

Leaf area was expected to have a negative relationship with frost, whereby frost-susceptible species would have larger leaves compared to frost resistant species. We observed this pattern in the Australian dataset, but not in the New Zealand dataset. Frost susceptible species were expected to have larger leaves, because higher temperatures have been related to larger leaves (Ackerly et al. 1992), whereas, small leaves are thought to help plants survive extremes of cold, such as those caused by radiation frost (Jordan and Smith 1995). For example, Jordan and Smith (1995) observed leaf size to decrease significantly with increasing frost exposure (as inferred by sky infrared radiation), which suggests that frost may be an important influence on leaf size. Other studies have found no relationship between frost resistance and leaf area. For example, Warrington and Southward (1995) observed no correlation between leaf area and the frost tolerance of *Hebe* species and cultivars, when assessed in both summer and winter. They suggested this result may be due to some *Hebe* species being bred to be more frost tolerant, whereas other *Hebe* species may be bred for different desirable traits associated with horticulture (Warrington and Southward 1995). Unlike Warrington and Southward (1995), who just focussed on the *Hebe* genus, our study looked at the variability of leaf traits within a range of species. This suggests that our study is likely to show more variability in traits among species, therefore the low sample size in our study may be why the relationship between leaf area and frost was not consistent among the frost variables.

Frost susceptible species were predicted to have longer and wider leaves than frost resistant species. Leaf length was observed to have a significant relationship with frost damage in the New Zealand dataset, and leaf width was observed to have a significant relationship with percentage foliage retention in the Australian dataset. Jordan and Smith (1995) looked at the relationships between likely frost exposure and leaf length and width. They found the expected relationships, where both leaf length and leaf width in *Erigeron peregrinus* and *Taraxacum officinale* decreased with increasing exposure of plants to frost (Jordan and Smith 1995). They also observed that individuals with leaves sheltered from radiative losses, due to growing near saplings or fallen trees, averaged wider and longer leaves, compared to individuals whose leaves were not sheltered from infrared radiation. This may be why the expected relationships between frost and leaf length and width were not consistent between the different frost variables and between the two datasets in our study. However, in this study the outer canopy (sun-leaves) were sampled where possible, which should account for some of the environmental variability which may affect the frost susceptibility of plants (Pérez-Harguindeguy et al. 2013).

## **4.2 Leaf toughness traits: leaf thickness, specific leaf area, and leaf dry matter content**

Frost susceptible species were expected to have thinner leaves compared to frost resistant species, but no relationships between leaf thickness and any of the frost variables were observed. There are several studies which have observed leaf thickness to increase with a decrease in temperature. Mediavilla et al. (2012) analysed the adaptations in the leaves of three evergreen species and their responses to the intensity of winter harshness. They found that leaf thickness showed a pronounced response to the increasing harshness of winter climatic variables, with minimum temperatures and number of frost days best accounting for leaf thickness variation observed at different winter harshness intensities (Mediavilla et al. 2012). Gorsuch et al. (2010) observed that leaves grown in cold temperatures had considerably greater thickness than their warm grown counterparts. They also observed that extended exposure of warm grown leaves to cold temperatures had little effect on their thickness (Gorsuch et al. 2010). These findings from Gorsuch et al. (2010) suggest that the environmental conditions which occur during the main leaf expansion period, are most important in determining leaf thickness. This may be why we found no relationship between the frost variables and leaf thickness in our study. The temperature at the time of leaf expansion may not have been cold enough to cause an increase in the leaf thickness of the plants which we sampled, especially in the Australian species, because severely low temperatures are rare at the site they were sampled from.

Specific leaf area was expected to increase with frost susceptibility. However, this relationship was not observed in either the Australian or New Zealand dataset. SLA is thought to be an indicator of important plant characteristics such as growth rate, leaf longevity, and stress tolerance, and is known to be highly variable depending on environmental conditions (Weiher et al. 1999). SLA has been found to have a significant relationship with temperature, irradiance, and water availability (Poorter et al. 2009), and species with low SLA generally occur in stressful environments, which is why frost susceptible species were expected to have higher SLA values (Knight et al. 2012). Hekneby et al. (2006) observed that species with higher frost resistance have lower SLA values, and suggested that freezing tolerance of plants was related to vegetative growth modifications developed during the cold acclimation process. The link between SLA and stress tolerance can also be observed on altitudinal gradients, as among species SLA generally decreases with an increase in altitude (Poorter et al. 2009). This is thought to be a response to the environment becoming more stressful with increasing altitude, due to declining water availability and temperature (Poorter et al. 2009). Other studies have also found that SLA is generally reduced under water stress (Li et al. 2000). Knight et al. (2012) proposed that reduced SLA is a trait in plant lineages which have evolved into thermally stressful environments with low water availability. They suggest that low SLA is a trait associated

with high temperature stress, due to low SLA leaves being better able to recover photosynthetic electron transport (which is decreased during high temperature stress) after high temperature stresses better than species with higher SLA (Knight et al. 2012). This study indicates that having a low SLA may be more related to high temperature stress rather than low temperature stress, hence why we saw no relationship between frost resistant and SLA. Adler et al. (2014) suggested that SLA may be a poor indicator of life history strategy due to its plasticity, and because SLA values can be variable and context dependent. Values of SLA may change as new leaves develop, allowing the plants to continually adapt to the environment (Sims and Pearcy 1992). Therefore, SLA may be better related to forms of plant stress other than frost, such as high temperature stress, or may not be a good indicator of plant strategy as it can change depending on the plant's environment. These may be reasons why we did not observe a relationship between frost susceptibility and SLA.

Frost susceptible species were expected to have a lower leaf dry matter content compared to frost resistant species, but no relationships between frost and LDMC were observed in this study. Other studies have found LDMC to increase in plants growing in cold temperature habitats. Gorsuch et al (2010) observed an increase in LDMC in pre-existing leaves of warm grown plants when they are first exposed to cold temperatures (Gorsuch et al. 2010). Dry matter accumulation which occurs when plants are first exposed to cold temperatures usually consists of large amounts of carbohydrates, which peaks after a few days, then starts to decrease as the leaves become cold acclimated (Ristic and Ashworth 1993). This indicates that leaves which are already cold acclimated may not have a high LDMC as we would expect, this may indicate why we found no relationship between LDMC and frost susceptibility in our study. Whole plant dry matter has been found to decrease with a decrease in temperature to suboptimal conditions (Sysoeva et al. 1999). Sysoeva et al. (1999) observed a two hour temperature drop to cause a significant reduction (18-20%) in plant dry matter, which was also accompanied by an increase in cold resistance (Sysoeva et al. 1999). This was unexpected, as a high plant dry matter content is considered to be a stress tolerant trait (Sysoeva et al. 1999). However, it is also known that the resistance to survive unfavourable conditions is often accomplished at the cost of other traits such as growth rate, reproductive rate, and biomass production (Larcher 1995). This suggests that in Sysoeva et al. (1999) the increase in cold resistance with the temperature drop may have occurred at a cost to dry matter production, and indicates that other plant characteristics, rather than plant dry matter, may be acting in increasing plant cold resistance. Sysoeva et al. (1999) referred to whole plant dry matter, whereas our study focused on specifically leaf dry matter, but the same argument may be able to be applied to our study, suggesting that other plant traits may be responsible for cold and frost resistance. This may be a possible reason as to why we saw no relationships between the frost variables and leaf dry matter content.

Our results suggest that leaf size and toughness traits are not generally useful in explaining frost resistance in plants. We saw some significant relationships between the leaf size traits: leaf area, leaf length, and leaf width, but they were not consistent between both datasets and the different frost variables. The significant relationships between the leaf size traits may be due to leaf size being related to leaf venation (Roth-Nebelsick et al. 2001, Sack and Scoffoni 2013). Larger leaves generally have larger petioles and major veins, which contain greater and larger xylem and phloem conduits (Sack and Scoffoni 2013). Vein length per unit area is generally related negatively and linearly to leaf size. This is due to the major veins arising early in leaf development, which then become more spaced apart during leaf expansion (Brodribb et al. 2002).

### **4.3 Leaf venation traits: vein density and vein length per unit area**

Frost susceptible species were expected to have low vein density and vein length per unit area compared to frost resistant species. Leaf hydraulics are a major determinant of the productivity of plants and their responses to environmental stresses (Brodribb et al. 2010). The hydraulic function of a leaf's venation network is linked to the plant's capacity for photosynthesis; the venation network supplies water to the photosynthetic tissues, which prevents them drying out during photosynthetic CO<sub>2</sub> exchange with the atmosphere (Sack and Frole 2006, Brodribb et al. 2007).

We observed the expected relationship between frost susceptibility and vein length per unit area (VLA), where frost susceptible species had lower VLA compared to frost resistant species. VLA plays an important role in gas exchange and plant growth, and is a major influence on a plant's photosynthetic rate, and hydraulic and stomatal conductance (Niinemets and Sack 2006, Hao et al. 2010). Currently VLA is known to be influenced by water availability. For example, a decrease in annual precipitation generally causes an increase in VLA. In evergreen shrubs and trees, VLA and rainfall have been found to have a strong negative correlation. Li et al. (2015) found that as both mean annual precipitation and temperature increased, the VLA of jujube (*Ziziphus jujube*) leaves decreased, which suggests that VLA can be used to indicate adaptation of plants to the local climate and habitat (Li et al. 2015). Having a high VLA during water stress conditions, such as those caused by frost, is believed to be a benefit to plants, as it can increase leaf xylem hydraulics, which corresponds to a larger number of flow pathways, increasing total permeability for water flow out of the veins (Roth-Nebelsick et al. 2001). A higher VLA may also be used as a protective mechanism for leaves as it can provide redundant pathways around damaged parts of the leaf (Roth-Nebelsick et al. 2001). Therefore the physical damage caused by frost, such as cell rupture or freeze-thaw cavitation of leaf xylem, may be able to be managed by the plant if it has a high VLA.

Frost susceptible species were expected to have lower vein densities compared to frost resistant species. We observed the expected relationship between frost damage and vein density, but no

relationship between  $LT_{50}$  and vein density. There is currently no literature which has tested the direct relationship between vein density and frost susceptibility. However, variation in vein density has been linked to other climatic variables, such as temperature, precipitation, and water availability (Sack and Scoffoni 2013), with water availability assumed to be the most important factor influencing vein density (Uhl and Mosbrugger 1999). This is due to vein density being a major determinant of leaf hydraulic supply in plants, as high water transport within a leaf requires the leaf to have a high density of veins (Brodribb et al. 2007). Evidence of this comes from species in dry sites being observed to generally have high vein densities (Sack and Scoffoni 2013). Higher vein densities provide greater leaf hydraulic conductance and carbon isotope discrimination, which indicates better water use efficiency, which is essential in dry environments (Uhl and Mosbrugger 1999). As discussed previously, water availability is related to frost damage because frost can reduce water transport within the plant (Ansley et al. 1992). For instance, Dunbar-Co et al. (2009) observed total vein density to have a significant negative relationship with mean annual precipitation. Higher vein density contributes to a higher maximum leaf hydraulic conductance (Sack and Frole 2006, Brodribb et al. 2007).

This study is the first time the relationship between leaf venation traits and frost susceptibility has been demonstrated. Our results suggest that leaf venation traits do play a key role in the frost susceptibility of species, due to the part they play in leaf hydraulics. However, this may be also be related to overall plant hydraulics, which in this study, is also represented by wood density.

#### **4.4 Wood density**

Wood density was expected to be higher in frost resistant species. We observed this expected relationship between percentage foliage retention and wood density in the original Australian dataset and when eight additional species were added to the dataset. However, we did not find this relationship in the New Zealand dataset.

Wood density is considered to be one of the main determinants of life history variation in woody plants (Fearnside 1997, Swenson and Enquist 2007), due to the correlation of wood density with transport capacity and stem water storage, which both have an important impact on leaf and whole-plant performance. Plants with high wood density are thought to be more frost resistant because dense wood is related to having narrow xylem vessels, which are more resistant to freeze-thaw induced xylem cavitation and embolism, compared to wider xylem vessels (Davis et al. 1999). Many studies have found correlations between wood density and climatic variables and environmental gradients. Fu et al. (2012) found wood density to be significantly correlated with leaf water stress tolerance. Other studies have shown that species with high wood density generally have more negative minimum leaf water potentials (Ackerly et al. 1992, Bucci et al. 2004). This correlation

between wood density and water stress has also been observed in species from dry habitats. For example, Ishida et al. (2008) found that wood density has a negative relationship with water-potential at turgor loss point. They suggested that wood density is an important and easily measured trait which can be used as an indicator of water-stress tolerance and leaf photosynthetic capability. Variation in wood density is often a good predictor of variation in other characteristics related to stem water storage capacity, xylem water transport efficiency, regulation of leaf water status, and avoidance of loss of turgor (Meinzer et al. 2003). Consequently, it seems likely that wood density is associated with frost susceptibility in plants, due to its impact on plant hydraulics, which is known to be affected by frost. This hypothesis was not supported by the results from the New Zealand dataset; however, was supported by the results from the Australian dataset. The wood density results from the Australian dataset are probably more reliable as a significant relationship was observed in the original dataset and also when an additional eight species were added.

#### **4.5 Intraspecific variability**

This study mainly focused on trait differences among species. However, functional traits also vary at the individual level. We observed that the degree of intraspecific trait variability differed among species, with some species having a wide range of trait values and other species having a very narrow range of trait values (Figure 6, Figure 11). This individual variation is also known to influence the interactions among organisms and their environments, and therefore also plays a role in the composition and functioning of plant communities (Bolnick et al. 2003). Plants have often been found to have wide intraspecific variation in their functional traits, due to inherited genetic variation and phenotypic plasticity. This variation can influence the response of individual plants to the environmental conditions and interactions with other organisms (Fridley et al. 2007, Fridley and Grime 2015). Siefert et al. (2015) found intraspecific trait variability to contribute substantially to the total trait variation within and among plant communities, with intraspecific trait variability accounting for 25% of the total plant community trait variation, and interspecific variability accounted for the remaining 75%. They also observed that intraspecific trait values tended to be relatively variable for specific leaf area and leaf dry matter content (Siefert et al. 2015), which also appeared to be true for the species used in this study, particularly for the New Zealand species. Other studies have shown that leaf size traits generally have low intraspecific variability, due to their limited plasticity (Rozendaal et al. 2006). These previous studies suggest that intraspecific trait variation may play an important role in the overall plant species life strategy and that plant traits may vary considerably with different environments and conditions. Therefore, the intraspecific variability observed in this study also needs to be considered along with the interspecific variability, as it may have had a considerable influence on the overall frost susceptibility of the species, especially because

only five individuals from each species were measured, which, for some species, may have been too few for the traits we measured.

## **4.6 Limitations**

### **4.6.1 Number of species and sample bias**

We observed significant relationships between some leaf size traits, venation traits and wood density; however, these results were not consistent between the two datasets. These inconsistencies could be due to the small number of species we measured in this study. The New Zealand dataset only included twenty-five species and the Australian dataset only included twenty-three species. If more species were measured, the relationships (or lack of) between plant traits and frost susceptibility may become more apparent.

The species measured in this study were also not necessarily chosen randomly, as some species were excluded due to difficulty with identifying them. These species were often ones with small leaves (hence being difficult to identify), which may have created a size bias in the dataset, with the easily identifiable ones being larger or more conspicuous, and therefore creating a dataset that was not representative of the whole plant community. This problem was also encountered with the leaf venation traits, where larger and thinner leaves tending to be more easily processed for leaf vein analysis. These biases may influence our ability to detect trait relationships with frost susceptibility because only a limited portion of the occupied trait space was considered. For example, if more small-leaved species were included, these species may have reduced the high observed variability in frost resistance at the small-leaved end of this relationship.

### **4.6.2 Leaf venation**

To obtain the leaf venation trait measurements, three main steps were involved: leaf clearing, leaf staining, and vein analysis. There were limitations which occurred at each of these steps.

The leaf clearing was very time consuming, where the leaves of some species would take over a week to clear. We based our leaf clearing methods on Scoffoni and Sack (2013); however, this method was found to be ineffective and was subsequently modified using methods from other sources, such as Berlyn and Miksche (1976), and from experimentation, in order to clear leaves effectively. During the leaf clearing process it was also difficult to know how long to leave in and when to take the different species out of the various chemical solutions, resulting in some leaves (e.g. thin or small) becoming damaged, and therefore were not able to be used. Leaves of the same species also reacted differently to the length of time in the chemical solutions, which meant that a method for each species was not able to be developed and used as a baseline, it was just guessing and



experimentation with every individual leaf. Thick leaves generally were not easily or able to be cleared, such as lancewood (*Pseudopanax crassifolius*), which has very thick and leathery leaves. The colour was also not able to be removed from some leaves, of usually highly pigmented species, which meant these species were not able to be stained and analysed. Therefore, it was only certain species which were able to be cleared, such as those with moderate leaf thickness and low pigmentation.

Staining the cleared leaves also had limitations. Again, it was difficult to know how long to cover each species in the stains, which was not always dependent on the size or thickness of the leaf. Some leaves, usually small and thin ones, were stained very quickly (1-2 seconds) and would often become over-stained. Whereas, others could be leaf in the stain for hours or days and not become stained enough, and therefore, were not able to be used.

Problems also occurred with analysis of the venation, which were mostly due to the low quality of the images. If the leaves were not cleared and stained evenly across the leaf surface, they were generally not able to be analysed. Problems were also encountered when the leaves were digitally scanned, as the light from the scanner reflected off the leaf surface of some leaves, which meant they were not able to be analysed.

These leaf venation limitations meant that overall leaf venation traits were only measured for twelve out of the twenty-five species in the New Zealand dataset. The species which were able to be cleared successfully were also mostly species with thinner and less pigmented leaves due to the problems associated with the leaf clearing and staining. This means that the species with venation trait values used in this study may not be representative of the whole range of plant strategies. The species with thicker and larger leaves were often not able to be cleared, and these species are the ones that may be more frost resistant.

### **4.6.3 Further research**

This study showed that the frost susceptibility of plant species is most likely associated with the functional traits related to plant hydraulics: leaf venation and wood density. No other research to date has been conducted on the relationship between these traits and frost susceptibility. In this study, the venation traits were only able to be measured for twelve out of the twenty-five species in the New Zealand dataset; however, the results still showed a significant relationship, suggesting these may be important traits indicating frost susceptibility. Consequently, venation traits should be measured on more species. Wood density was found to have a significant relationship with percentage foliage retention in the Australian dataset, but not in the New Zealand dataset, which suggests that wood density may be a trait associated with frost susceptibility, therefore further research should also be done on wood density.

## Chapter 5

### Conclusion

This dissertation has provided new information about which plant functional traits may be associated with the frost susceptibility of plants. Frost is an important environmental factor which can limit the productivity and distribution of plants, and climate change is predicted to increase the effect of frost on plants. Therefore, it is necessary to determine the frost resistance or susceptibility of plant species. There are currently a number of different ways to determine plant frost susceptibility, however, these are often difficult and costly to use, therefore, easily measured plant traits may be used instead. Our results show that leaf size traits, leaf venation traits, and wood density appear to be most associated with frost susceptibility in plants. Leaf size traits are most likely associated with frost susceptibility due to their association with leaf venation traits, and in turn leaf venation and wood density are most likely associated with frost susceptibility due to their impacts on plant hydraulics. This study makes an important contribution to the understanding of which traits are associated with frost resistance in plant species. This study also provides completely new information as it is the first time that relationships between frost and leaf venation traits, and frost and wood density, have been demonstrated. Therefore, it is highly recommended that future studies focus on leaf venation traits and wood density, to understand how plants will respond to future frost regimes.

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