SEED DORMANCY AND GERMINATION OF A PANEL OF NEW ZEALAND PLANT SPECIES:

Carex trifida, Coprosma robusta, Cyperus ustulatus, Hebe stricta, Muehlenbeckia australis, Myrsine australis, Phormium tenax and Sophora prostrata

A thesis presented in partial fulfilment of the requirements for the Degree of Master of Science at Massey University, Palmerston North, New Zealand.

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2004

Abstract

Literature was reviewed on the germination and possible uses for revegetation of the New Zealand indigenous species selected. Seeds of *Carex trifida*, *Coprosma robusta*, *Cyperus ustulatus*, *Hebe stricta*, *Leptospermum scoparium*, *Muehlenbeckia australis*, *Myrsine australis*, *Phormium tenax*, *Phormium* 'Yellow Wave' and *Sophora prostrata* were assessed for germination rates, percentage germination, dormancy and the effects that temperature has on germination. Seeds of *Carex*, *Cyperus* and Myrsine showed no germination in light or dark at 20°C. In contrast, 12 weeks of low temperature stratification resulted in a high percentage of seed germinating for *Carex* and *Cyperus*. There was no germination of *Myrsine* despite high viability in the initial germination experiment and the stratification experiment. Removal of the endocarp and a period of stratification increased germination percentage of *Myrsine* to 91%. Germination was low for Muehlenbeckia in the light at 20°C, but 4 weeks of low temperature stratification increased germination rate. After 2 years, 80% of *Coprosma* seeds germinated but germination rate increased after subjecting the seed to 8 weeks or more of stratification. No seeds of *Coprosma* or *Muehlenbeckia* germinated in the dark. Rapid germination of *Hebe* seeds was obtained, with 100% of the seed germinating in the light while only 7% germinated in the dark. *Leptospermum* had rapid germination, with 100% germinating in the light, while only 3% germinated in the dark. A low percentage of *Phormium* seed germinated in both the light and dark in the first month and no further germination was observed. In contrast, 8 weeks or more of low temperature stratification resulted in almost complete germination. There was rapid germination of *Sophora* seeds with 100% of the seed germinating in the light and dark. *Carex* seed had a limited temperature range at which it germinated (22°C to 26°C), while *Cyperus* had a wider range (18°C to 32°C) but did not germinate at low temperatures (6°C to 14°C). The optimum germination range for *Cyperus* was 24°C to 30°C. *Hebe* did not germinate at high temperatures (30°C to 32°C) but successfully germinated at all other temperatures with the optimum germination range being 6°C to 24°C. *Leptospermum* did not germinate at 6°C but had maximum germination at most other temperatures. *Muehlenbeckia* and *Phormium* germinated at all temperatures tested (6°C to 32°C) with the most seed germinating at 20°C for *Muehlenbeckia* and between 14°C to 22°C for *Phormium*. *Sophora* did not germinate at the low temperatures (6°C to 10°C). The germination rate increased with temperature for *Cyperus*, *Hebe*, *Leptospermum*, *Muehlenbeckia*, and *Phormium*. Generally, for *Carex* and *Sophora* as temperature increased germination rate slowed. It appeared that light is required for *Hebe* and *Leptospermum* to germinate. *Sophora* required scarification but not light. *Coprosma* and *Muehlenbeckia* required light and a period of chilling to increase the rate of germination. A small

percentage of the *Phormium* population is not dormant but a period of chilling increased the germination percentage for that portion of the population that is dormant. *Carex* and *Cyperus* required a period of chilling in order to break dormancy. *Myrsine* required removal of endocarp and a period of chilling to germinate. A list of cleaning descriptions and the equipment that was used for each species studied is reported. Preliminary results of a hydroseeding trail using the species studied were also reported.

Acknowledgements

I would like to express my sincere gratitude and appreciation to the following people and organisations:

Firstly, thank you to Associate Professor David Fountain, my chief supervisor, for his guidance, supervision and help throughout the duration of the research and editing of this thesis. Thankyou also to Dr John Clemens my second supervisor, for advice given throughout the research, his help with the hydroseeding trial, and detailed editing of this thesis.

I would like to acknowledge help and assistance from those at the Centre for Plant Reproduction and Seed Technology, especially Craig McGill and Robert Southward. Thanks also goes to Ruth Morrison for seed testing of the grasses used in the hydroseeding trial.

Thank you to Raymond Bennett and Doug Hopcroft of HortResearch Institute for their assistance with the histology and light microscopy.

I am very grateful to The Foundation for Research, Science & Technology for funding the research. Thank you also, to Robert Coulsen of Rural Supply Technologies Ltd. for his involvement and ideas with regards to this research.

Finally, a special thanks to my daughters, Melissa, Jessica and Louise, who spent hours helping to collect seeds and to my husband Craig for his support and encouragement as well as his help with the computer technology.

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1

INTRODUCTION

In New Zealand there is an increasing awareness of the importance of using indigenous vegetation for re-vegetation work. Native vegetation can play an effective role in the stabilisation and restoration of eroded land, can reduce or halt soil erosion on roadside reserves and cuttings, and can enhance local bio-diversity. Some planting practices in New Zealand are unsuitable because of the planting of unsuitable indigenous or exotic species that may be difficult to trim from the roadside or give shading or excessive growth. Criteria for selection of suitable indigenous species for re-vegetation depend on the climatic and the biological and physical characteristics of the site. It is also necessary to assess the severity of current erosion, or the likelihood and type of potential erosion. It is important, when selecting indigenous plants, to preserve the ecological attributes of the area. When making the indigenous species selection it is important to consider the natural distribution of the species as this gives some indication of their site and climate requirements. Criteria for selection should also consider whether the species are wind, drought, frost or waterlogging tolerant, depending on the climatic conditions of the site.

An understanding of the germination requirements of native seeds is also vital to develop successful land restoration procedures. This applies whether the re-vegetation strategy involves direct seeding or is secondary following establishment of whole plants or cuttings, which themselves seed into the re-vegetation area. Germination requirements of a number of New Zealand species have been described in an ecological context (e.g., Burrows 1995; 1996a, b, c), but there is a general paucity of dormancy and germination data for the New Zealand flora (Fountain & Outred 1991; Bannister & Jameson 1994). A technology currently being investigated, in order to more efficiently establish New Zealand's indigenous species on roadside cuttings, is hydroseeding (Appendix A). Here, collected (and sometimes pre-treated) seed is mixed with fertilisers (usually slow release fertilisers), mulch, adhesives and other additives, and pumped hydraulically onto the site. In order for this technology to be successful it is essential to understand the germination and dormancy requirements of the seeds being used.

Seeds of certain New Zealand species germinate poorly. This may be due to poor viability but frequently it is due to seed dormancy. This can include embryo dormancy due to immature

embryos, e.g. in *Arthropodium cirratum* (Conner & Conner 1988a), seed coat impermeability to water, e.g. *Chordospartium stevensonii* (Conner & Conner 1988b), inhibitors in the fruit, e.g. *Solanum laciniatum* (Burrows 1996a), and *Melicytus ramiflorus* (Partridge & Wilson 1990), or requirements for specific factors such as light, e.g. *Leptospermum scoparium* (Herron et al. 2000) or chilling e.g. *Hoheria glabrata* (Haase 1987). Seeds of many temperate species require a period of chilling (stratification). *Phormium tenax*, *Coprosma repens* and *Myrsine australis* belong to this group (unpublished Conservation New Zealand pamphlet, cited in Fountain & Outred 1991). Metcalf (1995) recommended that *Carex* spp., *Coprosma*, *Muehlenbeckia australis*, *Myrsine australis* and *Phormium tenax* be subjected to a period of stratification before sowing. Some seed may have a double dormancy. Seed of matagouri (*Discaria toumatou*) was shown to have a double dormancy with both testal (impermeability to water) and embryo dormancy (requiring chilling) (Keogh & Bannister 1994). Seeds of many other species (e.g. *Hebe* spp.) are not dormant and germinate readily once provided with moisture at room temperature.

The species investigated

The following criteria were used for the selection of indigenous plants used in this research:

- low stature (result: high visibility, low maintenance)
- root systems with high tensile strength (result: soil holding)
- deep rooting (result: high soil penetration, drought resistance)
- mat-forming (result: water retention, soil holding, drought resistance)
- rapid establishment (result: rapid stabilisation suitability for poor soils resulting in adequate growth and development in nutrient poor substrate)
- low soil shear potential (result: low aerial mass loading on steep slopes, high soil mass in roots)
- persistence (result: natural sustained cover by in situ reproduction via seeds).

Carex trifida Cav is a clumping sedge, which spreads rapidly and can be deep rooted and persistent (Metcalf 1998). *Carex trifida* was used here as representative of the genus for its potential usefulness in revegetation applications and for its natural distribution in relatively hostile environments.

Coprosma robusta Raoul suits poor soils and is deep rooted. It can spread readily by seed (Cave & Paddison 1999). It is a pioneering shrub (Langer et al. 1999) suitable for

establishment of many new plantings. *Cyperus ustulatus* A.Rich is a tall sedge which is easily grown in a variety of soils and situations, as long as conditions are not too dry. As it is mainly a coastal plant, it could be used in revegetation projects in coastal areas (Metcalf 1998). It is a useful plant for revegetation in wet areas as it can be grown in wet ground (Cave & Paddison 1999).

Hebe stricta (Benth.) L.B.Moore is a widespread, early colonising species that has a very fibrous root system.

Leptospermum scoparium J.R. et G.Forst is a good pioneer shrub (Langer et al. 1999) and mature *L. scoparium* stands provide reinforcement to the soil and inhibit the development of shallow landslides (Watson & O'Loughlin 1985).

Muehlenbeckia australis (Forst.f.) Meissn. could act as a good weed ground cover (Cave & Paddison 1999). These tangling vine-like shrubs are often seen on road batters from natural spread.

Myrsine australis (A.Rich.) Allan is a suckering indigenous shrub with good rooting and persistence. It is tolerant of shade (Williams & Buxton 1989).

Phormium tenax J.R. et G.Forst. has been found to be a good nurse plant (Reay & Norton 1999). *Phormium* is potentially useful for high water absorbing, deep rooting qualities.

Sophora prostrata Buchan. is a low-growing kowhai with nitrogen fixing ability and is deep-rooted (Salmon 1996).

The objectives of this research were:

- To report on the germination responses, under controlled conditions, of seeds from a group of indigenous New Zealand plants selected for potential revegetation use.
- To examine the effects of seed moisture content in relationship to storage.
- To report on the effect that temperature has on the germination response for the selected species.

2

LITERATURE REVIEW

2.1 Overview of Dormancy

2.1.1 Background

A seed has all the genetic information that is needed to reproduce a fully formed plant and thus initiate the seedling cycle of the next generation. Apart from a few exceptions, seeds result from sexual reproduction and arise from a mature fertilised ovule. They consist of an embryo, protective covering (seed coat or testa) and storage tissue. When the seed is fully formed, the embryo generally consists of one or two cotyledons (there can be many e.g. *Pinus*), a radicle (the embryonic root), an embryonic axis, and a more or less developed plumule. The axis of the embryo incorporates the embryonic root, the hypocotyl to which the cotyledons are attached, and the shoot apex with the first true leaves (plumule). Generally, the cotyledons of dicotyledons contain stored nutritional reserves of the seed. In most monocotyledons, nutritional reserve storage tissue is the endosperm, and for gymnosperms, the storage tissue is haploid gametophyte tissue (Bewley & Black 1994). In some species the ovary wall and other organs also contribute to the outer protective layers.

Germination has been defined physiologically (i.e. not agronomically) by Bewley (1997) as beginning with water uptake by the seed (imbibition) and ending with the elongation of the embryonic axis and penetration by the radicle of the structures surrounding the embryo. The result is often called visible germination (Bewley 1997). The process can be divided into three successive phases (Hartmann et al. 1997). Within each phase there are independent and some dependent chemical and biochemical processes.

Phase I is the imbibition phase where there is a rapid water uptake (there is an increase in seed fresh weight due to accelerating water uptake), reorganisation and repair of membranes (subcellular changes), detoxification of free radicals, formation of polyribosomes, DNA repair, reactivating of existing metabolic systems and the commencing of new ones. Phase II, which is known as the lag phase (little water uptake), is where there is a change to germinative metabolism, e.g. enzymes and macromolecules are synthesised (formation of more complex substances from simpler ones), food reserve mobilisation begins and growth

metabolism is initiated. The major metabolic events take place in phase II in preparation for radicle emergence from non-dormant seeds. Dormant seeds are also metabolically active at this time (Bewley & Black 1994). They have the processes initiated but do not culminate in germination. Phase III can be classed as the germination phase, which consists of cell elongation, cell division and consequently embryo growth and visible germination. The fresh weight of the seed begins to increase again as water uptake drives the emergence of the radicle. Two discrete phases of DNA synthesis occur in the radicle cells after imbibition. The first takes place soon after imbibition and involves the repair of DNA damaged during maturation, drying and re-hydration, as well as the synthesis of mitochondrial DNA. DNA synthesis associated with post-germinative cell division accounts for the second phase (Bewley 1997).

(i) Water Potential

The process of water uptake relies on the water potential of the cells in the seed and embryo and also on the surrounding environment of the seed. Water potential is described as-

$$\text{Water potential } (\psi_{cell}) = \text{Matric potential } (\psi_m) + \text{Osmotic potential } (\psi_\pi) + \text{Pressure potential } (\psi_p)$$

Matric potential is the major force responsible for water uptake during imbibition (phase I). Because most seeds are dry (dry weight <15 percent moisture), they have a very low water potential and so water uptake during imbibition is usually rapid due to the matric forces. Matric forces are due to the state of hydration of dry components of the seed that include cell walls and macromolecules like starch and proteins (matrices). Imbibition is a physical process related to matric forces and occurs in dry seeds with water-permeable seed coats whether they are alive or dead, dormant or non-dormant (Hartmann et al. 1997). Within the imbibition phase of germination there are two stages: 1) the rapid uptake of water (previously discussed), and 2) a slower wetting stage. The seed does not wet uniformly but has what is known as a "wetting front". This develops as the outer portions of the seed hydrates while the inner tissues are still dry. There is a transformation of a dehydrated or "dry" seed with a resting embryo that has a barely detectable metabolism (the sum of all chemical processes occurring within a living cell or organism) into one that has a vigorous metabolism culminating in growth (Bewley & Black 1994). The term "dry" does not necessarily mean that water is absent. Some water is present in the "dry" seed and the metabolic reactions that proceed in the mature seed will depend on its water content.

Another characteristic of seeds during imbibition is that they are "leaky" (solute leakage). As seeds take up water there can be a rapid leakage of solutes such as inorganic and organic ions, proteins, sugars and amino acids (Simon 1984). "Leakiness" is probably due to the inability of cellular membranes to function normally until they assume their normal lipid bi-layer configuration that was lost during desiccation drying (Bewley & Black 1994). Seeds that leak excessively tend to be more susceptible to attack by insects and microorganisms. In some seed, the enclosing structures, e.g. testa and pericarp, not only restrict uptake of water but can also act as a barrier to the efflux of solutes. It has been shown that the testa plays an important protective role in *Phaseolus vulgaris* (common bean), soybean and pea (Bewley & Black 1994) in such a way. There can be cell wall leakage and cell membrane leakage.

Seeds can be physically damaged during imbibition (soaking injury) due to an inrush of water (or in some instances, the influx of water is too slow). Large seeded crops, such as soybean, can be damaged if the inrush of water is too great (Hartmann et al. 1997). Henner (1986) discovered that some subtropical and tropical species, such as corn and limabean, are sensitive to chilling injury if they rehydrate with cold water so the water temperature is also important when imbibition takes place for some species.

During phase II and phase III water begins to be drawn into the seed due to osmosis so there is an osmotic potential (the greater the concentration of solutes in the cell the lower the osmotic water potential). Osmotic potential is a measure of the osmotically active solutes in a cell including molecules like organic or amino acids, sugars, and inorganic ions. Thus, the concentration of solutes in the cell influences water uptake. Osmotic potential is expressed as a negative value (Hartmann et al. 1997). Because the osmotic potential is more negative than the outside solution, more water can move into the cell.

During phase II and III there is an opposing force, expressed as a positive value, which is the pressure potential. The pressure potential is the turgor force due to water in the cell pressing against the cell wall. Osmotic and pressure potential determine water uptake during imbibition.

The soil has its own water potential (ψ_{soil}). The difference in water potential between seed and soil determines the rate of flow of water to the seed. Initially, the difference in ψ between the dry seed and moist soil is usually very large because of a high ψ_m. As the seed moisture content increases during imbibition and the matrices become hydrated, the water potential of

the seed increases (i.e. becomes less negative) and the rate of water transfer from the soil to seed decreases with time.

(ii) Respiration-Oxygen Consumption

There are three respiratory pathways assumed to be active in the imbibed seed. These are glycolysis, the pentose phosphate pathway, and the Kreb Cycle (Bewley & Black 1994). Generally, during these processes energy is produced. Respiration, or the complete oxidation of glucose, is the chief source of energy in most cells. In the first phase in the breakdown of glucose (in glycolysis) in which the six-carbon glucose molecule is split into two three-carbon molecules of pyruvate, two molecules of ATP and two of NADH are formed. The three-carbon pyruvate molecules are broken down within the mitochondrion to two-carbon acetyl groups, which enter the Krebs cycle as Acetyl-CoA. According to Hartmann et al. (1997), within hours of imbibition, both respiration and ATP synthesis increase substantially and this is usually during the lag phase of germination (phase II). Bewley & Black (1994) state that mitochondrial oxidative phosphorylation is the major source of ATP from the start of imbibition which is in phase I. In the Krebs cycle, each acetyl group is oxidised in a series of reactions to yield two additional molecules of carbon dioxide, one molecule of ATP, and four molecules of reduced electron carriers (three NADH and one $FADH_2$) (Raven et al. 1999). With two turns of the cycle, the carbon atoms derived from the glucose molecule are completely oxidised. The final stage of respiration is the electron transport chain, which takes place in the inner membrane of the mitochondrion, where more energy is released and thus ATP is formed from ADP and phosphate (oxidative phosphorylation is accomplished). In the course of aerobic respiration (oxygen is present), 36 molecules of ATP are generated.

Respiration by mature "dry" seeds is extremely low when compared to developing or germinating seed (Bewley & Black 1994).

Respiration involves several phases-

Phase I – Due to activation and hydration of mitochondrial enzymes (in the Kreb Cycle and electron transport chain), there is an initial sharp increase in O_2 consumption. Respiration in this phase increases linearly with time.

Phase II – O_2 uptake increase slowly. Usually, hydration of the seed parts is complete and all pre-existing enzymes are activated. Between phases II and III in the embryo the radicle penetrates the surrounding structures and so germination is complete.

Phase III – Due to increase in newly synthesised mitochondria in the embryo and storage tissues there is a second respiratory burst. There could also be an increased O_2 supply through the punctured testa or other enclosing structures.

Phase IV – There is a decrease in O_2 consumption due to senescence following depletion of the stored reserves (this phase only occurs in the storage tissues and not the embryo).

The lengths of phases I-IV vary from species to species due to such differences as seed-coat permeability to oxygen, or different metabolic rates (Bewley & Black 1994). Other factors expected to influence the length of phases I – IV includes size of reserve, tissues and seed size.

Seeds can experience conditions of temporary anaerobiosis during lag phase II of respiration (Bewley & Black 1994). Anaerobic respiration takes place in the absence or shortage of oxygen. Aquatic species, such as rice (*Oryza sativa*), can germinate in a totally O_2-free environment (although root growth is inhibited) (Bewley & Black 1994). With anaerobic respiration, pyruvate produced by glycolysis may be converted to either lactic acid (as in many bacteria, fungi and animals) or to ethanol and CO_2 (in yeast and most plant cells) (Raven et al. 1999). Bewley & Black (1994) state that ethanol and lactic acid accumulate within the seed but are found in different proportions in different species. Generally, the energy for glucose breakdown is limited to the net gain of 2 ATP molecules produced during glycolysis.

(iii) The First Physical Signs of Germination

The first visible evidence of germination is emergence of the radicle (Bewley 1997; Hartmann et al. 1997). This is initially the result of cell enlargement rather than cell division (Gordon 1973). The initiation of radicle elongation is due to a combination of factors – 1) the osmotic potential of the cells in the radicle becomes more negative due to metabolism of storage reserves; 2) cell walls in the radicle become more flexible (this results from relaxation of bonding system within the wall structure) to allow for cell expansion; and 3) cells in the seed

tissues surrounding the radicle weaken to allow cell expansion in the radicle (Ni & Bradford 1993; Bewley & Black 1994).

Generally, cell wall loosening in the radicle is determined by the physical properties of the cell wall and the counter-pressure exerted by the seed tissues covering the radicle. A combination of increasing osmotic potential (more negative) and/or change in the pressure potential can result in cell enlargement and initiate radicle elongation. This is termed growth potential (Baskin & Baskin 1971). In some seeds there appears to be insufficient growth potential (radicle does not elongate enough to push out of testa) generated from these processes to allow physical germination. Such seeds are termed dormant. Other causes of dormancy also exist and are discussed below.

(iv) Seedling Growth

Seedling growth commences when germination finishes. The seedling plant begins cell division at the two ends of the embryo axis. The initiation of cell division in the growing points appears to be independent of the initiation of cell elongation (Berlyn 1972). The growing point of the root (radicle) emerges from the base of the embryo axis. The plumule, which is the growing point of the shoot, is at the upper end of the embryo axis, above the cotyledons. Once growth begins from the embryo axis, fresh-weight and dry-weight of the new seedling increases while the storage tissue weight decreases due to storage reserve utilisation. Respiration rate (O_2 uptake) increases steadily as the seedling grows. The storage tissues of the seed cease to be involved in metabolic activities except with plants where cotyledons emerge from the ground and become active in photosynthesis. As new roots explore the germination medium, water absorption increases resulting in an increase in fresh-weight of the seedling.

Seed technologists are more interested in the seedling growth or emergence from the soil than emergence of the radicle (which is an indication of the end of germination) because they are monitoring the establishment of a vigorous plant of agronomic value. If the seed germinated but failed to emerge from the soil then it has no agronomic value.

(v) Storage Reserve Utilisation

Until the seedling emerges in the light and begins to photosynthesise the embryo in the seed is dependent upon reserves that were manufactured during seed development. The major

reserves are proteins, carbohydrates (starch), and lipids (oils). Storage proteins, that are stored in protein bodies, are catabolised into amino acids, which are used by the embryo for new protein synthesis. Starch that is stored in seeds is broken down by enzymes into sugars and used by the growing embryo. There have been extensive studies on the catabolism of starch found in cereal grains, such as barley, wheat and corn (Halmer 1985; Fincher 1989). Lipids are stored in oil bodies. The oil is biochemically processed resulting in the production of sucrose, which is used by the developing embryo.

2.1.2 Dormancy and Quiescence

To have a successful reproductive strategy, it is advantageous for species to possess mechanisms to regulate the time and place of seed germination. By delaying germination until favourable environmental conditions are present for seedling growth, and by spreading germination over time and space, the species' chance of survival is increased (Foley & Fennimore 1998). A critical factor in the survival of the resulting seedlings is the timing of germination (Fenner 1992). While the seed is still on the parent plant, delayed germination, or dormancy, is advantageous when it acts as a barrier to germination in the last stages of seed development (Bryant 1985). Dormant seeds may have achieved all of the metabolic steps required to complete germination, but for some known or unknown reason, the embryonic axis (usually the radicle) fails to elongate (Bewley 1997)

The term 'dormancy' has been a subject for much debate over the years but it has been defined by some as "when a viable (alive) and intact seed fails to germinate when subjected to suitable environmental conditions" (Richards & Beardsell 1987; Bewley & Black 1994; Bewley 1997; Foley & Fennimore 1998). Favourable conditions are classed as sufficient moisture, good oxygenation and a suitable temperature (usually within a range of around 10 °C to 30 °C) (Salisbury & Ross 1992). The favourable conditions would normally favour germination within a specific time period (Hilhorst 1995). Some authors (Bewley & Black 1994) include favourable light quality and quantity in addition to the above factors. Simpson (1990) listed three criteria for classifying dormancy-

- The apparent origin of the control of dormancy, e.g. genetic, structural or environmental;
- The depth of the dormancy, e.g. shallow, deep or relative;
- The timing of the dormancy, e.g. primary, secondary.

Most seeds are thought to go through an inactive stage, which is known as a quiescent stage. A seed is said to be quiescent when it is in a non-dormant state, but unable to germinate because the external conditions normally required for growth are not present (Salisbury & Ross 1992). Quiescence is distinct from dormancy where specific priming treatments are required to initiate embryo growth (Fountain & Outred 1991). Lack of dormancy is indicated by rapid germination of the seed. Hartmann et al. (1997) states that in most cases, seeds may only be quiescent when they are separated from the plant.

2.1.3 Primary (Innate) and Secondary (Induced) Dormancy

It has been proposed that there are two types of dormancy: primary (or innate dormancy) and secondary (induced) dormancy (Karssen 1982; Hilhorst 1995). This classification of the different types of dormancy, according to Hilhorst & Toorop (1997), is based entirely on timing. They stated that primary dormancy is associated with development (established while the seed is developing on the parent plant), and the intensity of the effects (relative dormancy) may depend on environmental stimuli experienced by the parent plant during the ripening. Secondary dormancy occurs essentially after dispersal and is basic to seasonal dormancy cycling in soil seed banks. Hartmann et al. (1997) states that primary dormancy is where seeds will not germinate even when the environmental conditions (water, temperature, and aeration) are suitable for germination. Primary dormancy may have evolved to aid the survival of the species (Koller 1972; Thompson 1973) by delaying germination until a favourable time in an annual seasonal cycle. Release from primary dormancy may be in response to a specific environmental factor (for example, a period of low temperature). Secondary dormancy is a further survival mechanism that can be induced under unfavourable environmental conditions (Hartmann et al. 1997), such as low oxygen and/or high carbon dioxide levels, water stress, unfavourable temperature or illumination. There is a further time delay, resulting in germination not taking place. Chilling, light or treatment with germination-stimulating hormones such as gibberellic acid and/or cytokinin can induce release from secondary dormancy. Foley & Fennimore (1998) state that freshly harvested dormant seeds are in a state of primary dormancy. They also state that non-dormant (quiescent) or partially after-ripened seeds that encounter unfavourable germination conditions are often induced into a state of secondary dormancy.

There are two common ways by which primary dormancy is imposed: embryo dormancy, where control of dormancy resides within the embryo itself, and coat-imposed dormancy enforced by restrictive seed coverings (Bewley & Black 1982).

2.1.4 Embryo Dormancy

Seeds that require light or low temperature before germination is initiated are thought to exhibit embryo dormancy. Seeds of many species require an environmentally imposed stimulus to initiate germination. Some seed contain undeveloped embryo that require time to develop such as seed of the Apiaceae, Ericaceae and Gentianaceae families. Seed with immature embryo require a period of after-ripening.

(i) Light

One environmental trigger is light. Light can act as a prerequisite to release seeds from their dormant state but it can also induce a dormant state (Pons 1992). In the case of New Zealand indigenous plants, Wardle (1970) found that *Metrosideros umbellata* requires light for germination and Bannister (1990) found that *Pernettya macrostigma* (snowberry) also requires light. Other New Zealand species that require light to germinate (as cited in Bannister and Jameson, 1994) are *Acaena* spp. (biddibiddi) (Conner 1987), *Celmisia* spp. (Scott 1975), *Gaultheria antipoda* (snowberry) (Bannister 1990) and *Hebe elliptica* (Simpson 1976).

Habitats can influence light requirements. Shaded habitats where light is enriched in green and far-red wavelengths, drive the light quality-sensing pigment phytochrome from an "activating" state, which promotes germination. Herron et al. (2000) discovered that 90% of *Leptospermum scoparium* (manuka) seed germinated after 100 minutes of exposure to red light. This was nullified by immediate exposure to far-red light, implying phytochrome was the pigment involved. This experiment indicated that light may release seeds from their dormant state, but could also prevent germination. *Arthropodium cirratum* (rock lily) is inhibited by high irradiance (Conner & Conner 1988a).

(ii) Temperature

Another environmental stimulus that can trigger germination is subjecting the dormant seed to a period of low temperature (stratification). Stratification is a method of handling seeds in which the imbibed seeds are subjected to a period of chilling to after-ripen the embryo. The usual stratification temperature is 0 to 10 °C (Hartmann et al. 1997). High proportions of species from temperate and cooler climates are released from their dormancy after seed experience a period of cold temperatures in an imbibed state (Bewley & Black 1994). Probert (1992) stated that the requirement of chilling acts as a mechanism to ensure germination

would occur in the spring. Minchin (1983) recommends pre-chilling for a number of common ornamentals and vegetable including *Aquilegia* ("granny's bonnets"), some *Impatiens* ("busy lizzy"), and *Apium (*celery). Depending upon the species, the period of chilling required varies widely. Usually 1-4 months of moist, cool stratification is required (Metcalf 1995). Metcalf recommends 2-3 months of cool-moist stratification at 5 °C to successfully germinate *Muehlenbeckia australis* (Bush pohuehue) and *Myrsine australis* (Mapou, Red matipo) but only 1 month for *Carex* spp.

It is thought that the possible mechanisms for dormancy release during stratification are changes in membrane fluidity at chilling temperatures and differential enzyme activity (Bewley & Black 1994). Lewak & Rudnicki (1977) found that a protease and lipase enzyme increased during chilling stratification. They found that there is a decrease in storage lipids and an increase in sugars and amino acids. These increase the osmotically active solutes, increase the growth potential seen in embryos following stratification and thus release the seed from dormancy.

(iii) After-ripening

Another dormancy mechanism that fits into the embryo dormancy category is "after-ripening". After-ripening is generally used to describe the process by which seeds gradually lose their dormancy in dry conditions. It is also sometimes used when referring to the release of dormancy of imbibed seeds kept at low temperatures (Simpson & Webb 1980) and to seed with immature embryos benefiting from warm-moist storage (Metcalf 1995). Some seeds may only germinate after a period of dry or moist storage (Bannister & Jameson 1994). Conner & Conner (1988a) found that rock lily (*Arthropodium cirratum*) required up to six months of dry storage.

2.1.5 Coat-imposed Dormancy

Another category by which dormancy can be classed is "coat-imposed" dormancy. This is where the seed is unable to germinate as it is inhibited by a factor from outside the embryo. This can be a function of the seed coat and also, where present, the pericarp, endosperm and other covering tissues (Bewley & Black 1994).

(i) Mechanical Dormancy

There are a number of ways the enclosing tissues can constrain embryo growth. One such way is by some structure, which interferes with gas exchange. Some species such as white mustard have mucilaginous layers inside and outside the seed coats that develop under high moisture conditions. These impede oxygen uptake (Hartmann et al. 1997). Another example is where the hard woody testa of *Canarium schweinfurthii* seeds restricts oxygen uptake (Nwosu 2000).

Endosperm can constrain the embryo, as found in tomato and lettuce seeds (Welbaum et al. 1998). The breaking of dormancy in tomato seeds is due to a weakening in the endosperm surrounding the radicle (Ni & Bradford 1993). Studies have revealed that the tissues enclosing the embryo in the tomato seed influence germination by mechanically restraining the expansion of the embryo (Karssen et al. 1989). Endo-ß-mannanase may be the enzyme involved in the cell wall degradation leading to endosperm weakening and subsequent radicle protrusion (Nascimento et al. 2001).

Hard seed coats can physically restrain the embryos from emerging. Embryos isolated from seeds that exhibit coat enhanced dormancy are not in fact dormant (Bewley 1997). This is common in drupes, which exhibit a hardened endocarp that surrounds the seed, e.g. stone fruit (Nikolaeva 1977). Conner & Conner (1988a) found that scarification (physical seed coat disruption) over the point of radicle emergence in *Arthropodium cirratum* seeds is required for germination.

Hard seed coats can prevent the uptake of water. This is common in the Fabaceae/Leguminosae family, where the testa is extremely hard and thick. Conner and Conner (1988b) found seeds of the weeping broom (tree broom, *Chordospartium stevensonii*), which is endemic to New Zealand, has a water-impermeable testa and so it needs disruption to alleviate dormancy. White clover becomes impervious to water uptake because of vertically oriented macrosclereid cells that are covered by a layer of cuticle (Hartmann et al. 1997). In the majority of cases, it is the testa that is responsible for this dormancy mechanism (Bewley & Black 1994). Other New Zealand genera exhibiting this type of delay mechanism are *Clianthusa, Notospartium, Carmichaelia* and *Sophora* (Metcalf 1995).

In nature, the seed coat is broken down over time by the action of micro-organisms in the soil during warm periods, or by passage through the digestive tracts of birds and mammals (Hartmann et al. 1997) or by physical abrasion induced by abiotic influences such as wind, or water flow over sand and rock. For example, germination in *Coprosma* is greatly increased by abrasion, which occurs as the seeds pass through the bird's crop. When birds (e.g. blackbirds (*Turdus merula)*, thrushes (*T. philomelos*) and silvereyes (*Zosterops lateralis*)) eat the *Coprosma* fruit the fleshy part is digested but the seeds within are thick-coated and so withstand the passage through the gut. However, the seed endocarp is sufficiently damaged to enable the seed to germinate after it has left the bird. Seed that is subjected to temperature extremes (freeze/thaw), as found with *Astragalus mongholicus* (a herbaceous plant found in northern China), can be released from seed coat imposed dormancy (Shibata et al. 1995). Physical abrasion by soil particles, and with some species, fire, (i.e. *Iliamna corei*, Baskin & Baskin 1997) can also break down seed coats.

In laboratories, to break hard-seediness, seeds may be mechanically scarified by piercing, chipping, filing or sand papering (Stilinovic & Grbic 1988; Duval & NeSmith 1999; Rehman & Park 2000), by soaking in hot water (Baskin & Baskin 1998; Archana et al. 1999), applying gibberelin (Rehman & Park 2000) or being scarified by placing in sulphuric acid (Metcalf 1995, Rehman et al. 1999).

(ii) Chemical Dormancy

Germination inhibitors are chemicals that accumulate in fruit and seed-covering tissues during development and are still present after harvest of the fruits and seeds (Evenari 1949 - cited in Hartmann et al. 1997). At least 20 inhibitory compounds have been identified (Bewley & Black 1982). Partridge & Wilson (1990) found the presence of a germination inhibitor in *Melicytus ramiflorus* (mahoe) seeds. Horrell et al. (1990) discovered that the fleshy layer of fruits of *Pseudopanax crassifolius* (lancewood, horoeka) and *P. ferox* (toothed lancewood) reduces germination percentage and they suggested that the same inhibitory substance delays germination, especially so in *P. crassifolius.* Inhibitors have been found in the seeds from Polygonaceae and Chenopodiaceae (*Atriplex*) families. It is common to find inhibitors in fleshy fruits and juices of citrus, stone fruits, grapes, tomatoes, apples, pears and cucurbits (Hartmann et al. 1997). Seeds from the families of Lamiaceae (*Lavendula*), Cruciferae (mustard), Violaceae (violet), and Linaceae (flax) have a thin seed coat with a mucilaginous inner layer that contains inhibitors (Atwater 1980). It has been found, to overcome this type of

dormancy mechanism, that germination can sometimes be improved if the seeds are subjected to prolonged leaching with water, removal of the seed coverings or both (Nikolaeva 1977; du Toit et al. 1979).

2.2 Selected Species and their Seeds

The following section details descriptions of the species selected for this work and reviews existing literature on seed storage and germination.

2.2.1 *Carex trifida*

Plate 2.1: *Carex trifida* seed heads. (Sourced from Metcalf 1998)

2.2.1.1 Species Description

Carex trifida Cav. (mutton-bird sedge, tataki, tataka) belongs to the Cyperaceae family, which consists of 115 genera and about 3,600 species (Mabberly 1987-cited in Hong et al. 1998a). Carex is a large genus of more than 1000 species of grass-like plants, which are commonly known as sedges (Metcalf 1998; Cave & Paddison 1999). About 70 species are found in New Zealand. *Carex trifida* is distinguished from other native species by its large size (growing up to 90 cm), stout habit, numerous large flower-spikes (6-9 spikes) and its wide (6-15 mm), medium to deep green leaves which are bluish-green on the back (Moore & Edgar 1970, Metcalf 1998; Cave & Paddison 1999). *Carex trifida* forms very robust, dense clumps (Moore & Edgar 1970).

Sedges can be easily recognised by their flowers as the sedge flower head is made up of cone-like spikelets (Edgar 1975). *Carex* is a monomorphic genus where the sexes are on separate spikes (Godley 1979). *Carex trifida* have characteristic sturdy, brown spikes (3.5 to 11 cm long and 5 mm to 1.5 cm in diameter) that are carried on stiff flowering stems (15 to 90 cm tall) (Metcalf 1998; Salmon 1999; Cave & Paddison 1999). The upper 2 to 4 spikes have dark brown male flowers, sometimes intermingled with a few female flowers, while the lower spikes have shorter light-coloured female flowers (Moore & Edgar 1970; Metcalf 1998;

Salmon 1999). Sedge flowers are wind pollinated, therefore, do not have showy petals. When in flower, *Carex* is very easily recognised because a utricle, or small bag, through the tip of which the stigmas protrude surrounds each female flower. A utricle is a modified glume and the sedge flower is borne in the axil of the single glume. In contrast, grass flowers are enclosed by two glumes, called the lemma and the palea (Edgar 1975). Sedge flowers have no tepals.

Sedge fruits are nutlets, single seeds enclosed by a hard fruit wall. These are also known as achenes (Hong et al. 1998a). In *Carex* an extra thin but hard cover (the utricle) encloses the fruit. *Carex trifida* have 2 mm long, trigonous nutlets, which are oblong-obovoid and dark brown (Moore & Edgar 1970).

Carex originates from the Greek word *keiro* which means 'to cut', alluding to the sharp cutting edges of the leaves. The common name of *C. trifida* originates from Stewart Island, where it is fairly common. The islanders generally refer to it as 'tataki grass' (Metcalf 1998).

Carex trifida is normally found on coastal cliffs and rock outcrops or boggy shorelines (Metcalf 1998). It is naturally distributed in localised areas of the South Island from Stephens Island to Banks Peninsula and scattered localities south of North Otago. *Carex trifida* is also found on southern offshore islands including Stewart, Chatham, Snares, Auckland, Campbell and Macquarie Islands (Metcalf 1998; Cave & Paddison 1999). It also occurs in Chile and the Falkland Islands (Metcalf 1998).

On Cook' s third voyage to New Zealand a young gardener called David Nelson collected seeds and *Carex* was one of the genera mentioned. These seeds were grown in many places throughout the United Kingdom and Europe and planted in private and botanical gardens (Cooper & Cambie 1991). Many *Carex* are available commercially for horticultural use. They are grown for their foliage colour and texture (Cave & Paddison 1999). *Carex* spp. makes good ground cover and is often planted in quite large groups, particularly in pedestrian areas (Crowe 1997b). *Carex trifida* is best grown in a moist soil in sun or semi-shade (Cave & Paddison 1999). *Carex trifida* is propagated from seed or by division of clumps (Bryant 1997). *Carex* divide easily and, as with grasses, best results are achieved by shortening the foliage by half. According to Metcalf (1995) it is best to divide the plants in spring, although some species can be divided at other times of the year (Bryant 1994). Bryant (1994) states

that, depending on the species, it can be 2-6 months for the new plant to mature and start flowering.

2.2.1.2 Seed Storage

Metcalf (1995) reports that *Carex* seed stores well. According to Hong et al. (1998a) seed storage behaviour appears to be orthodox, that is, tolerates drying to a moisture content of 5 to 15% without losing viability.

2.2.1.3 Seed Germination

Metcalf (1995) suggests that *Carex* be sown when ripe. Germination of some species is hastened and improved if the seed is cool-moist stratified for 1 month before sowing. Depending on the species germination can be erratic taking from 1-7 months. A commercial packet of *C. trifida* seed (New Zealand Tree Seeds) recommends sowing seed direct from the packet and keeping moist until germinated. Metcalf (1995) also recommends that *Carex* be subjected to cool conditions when germinating the seed.

2.2.2 *Coprosma robusta*

Plate 2.2: *Coprosma robusta* planted as part of a revegetation project at Massey University (Turitea Campus)

2.2.2.1 Species description

Coprosma robusta Raoul (karamu) belongs to one of the largest plant families: Rubiaceae. This family consists of 630 genera with about 10,400 species (Mabberley 1987 – cited in Hong et al. 1998b) of shrubs, trees, lianes and herbs (Hong et al. 1998b). *Coprosma robusta* can grow as a shrub or tree up to 6 m high with spreading stout, hairless branches and branchlets and smooth trunk. Leaves are leathery and dullish dark green on the upper surface and paler underneath (Allan 1961; Salmon 1996) and are up to 12 cm long and 5 cm wide. A useful diagnostic feature for identifying *Coprosma* species is the presence of small pits (domatia) situated between the midvein and one or more of the lateral veins of the undersurface of the leaf (Greensill 1902; Salmon 1996).

Coprosma robusta is dioecious. Male and female flowers arise on separate plants (Salmon 1996; Cave & Paddison 1999). Both the male and female flowers are in separate clusters (Allan 1961). The female flower is normally erect, consisting of a cup-like structure from which protrude two long slender styles. For most of their length they are stigmatic. The male flowers are often pendulous, consisting of a similar cup-like structure to the female flowers from which long anthers hang on long, thread-like filaments (Allan 1961; Salmon 1996). In *C. robusta* there is usually no sign of androecium (the stamens of a flowering plant collectively) in the female or gynoecium (the carpels of the flowering collectively) in the

male (Godley 1979). Wild & Zotov (1930-cited in Godley 1979) have recorded departures from this condition in 10 species. Separation of the sexes in *Coprosma* is not always as clear-cut, as this literature state, as hermaphrodite flowers with anthers, stigmas and functional ovary have been observed (Taylor 1975a). Hermaphrodite flowers (with both male and female sexual characteristics) were existent early in the flowering season in many male plants but disappeared as the season advanced. Female plants tended to stay consistent. *Coprosma robusta* begins flowering in September and continues into November (Salmon 1999). Sometimes a single plant may produce separate female and male flowers on the same branchlet, or may bear female flowers early in the season and male flowers later on (Taylor 1975a).

Coprosma robusta is recognisable by its fruit of bright orange-red drupes. The drupes are oblong or narrow-ovoid in shape, 7 to 10 mm long and 4 to 5 mm wide (Allan 1961; Metcalf 2000). Drupes contain 2, 1-seeded pyrenes. The pyrenes are oblong to elliptic, 2.3 to 3.5 mm long and 2.5 to 3.5 mm wide (Webb and Simpson 2001). The thousand seed weight = 6 g (Hong et al. 1998b). Birds such as blackbirds (*Turdus merula*), thrushes and white-eyes disperse the brightly coloured fruits. The fleshy parts of *C. robusta* fruits are digested but the hard seed is rejected, which drops to the ground, usually some distance from the parent plant (Dobson 1975; Taylor 1975a). Williams & Karl (1996) have observed bellbirds eating *C. robusta* in a forest remnant, Nelson, New Zealand.

In 1773 J. R. and G. Forster devised a scientific name for hupiro (*C. foetidissma*) which translates as "extremely-vile-dung-smell', as hupiro has an unforgettable smell when bruised or cut. The generic name *Coprosma* (dung-smell) went on to being applied to the hundred or so other species later discovered growing in New Zealand and other parts of the Pacific (Taylor 1975a).

Karamu is the common name among the Maori for any of the large leafed *Coprosmas*, the name implies sturdy tree (MacDonald 1973). Maori considered *C. robusta* to be a sacred tree. The tohungas held branches of it when intoning a karakia (a prayer) over a sick person (MacDonald 1973). The pre-European Maori created crude girdles and aprons from the branches and wore them when performing sacred duties. They were not allowed to wear any other clothing (Metcalf 2000). Maori children ate the berries. They are sweet and juicy with a slightly bitter aftertaste (Crowe 1997a). The Maori boiled the leaves and drank the liquid to aid kidney troubles, the young shoots were boiled and liquid drunk for bladder stoppage and

inflammation, and the bark was boiled and the water taken in small amounts for stomach ache and to stop vomiting (MacDonald 1973). Roasting and grinding the seeds of *C. robusta* and *C. repens* has produced coffee. J. C. Crawford presented a sample of *Coprosma* coffee to the Wellington Philosophical Society recommending that it be wholesale manufactured (Crawford 1877). The small size of the seeds has discouraged anyone taking up the recommendation (Crowe 1997a). In 1870, according to the notes *Transactions of the New Zealand Institute*, T. H. Potts and W. Gray discussed the cultivation and uses of some 150 species of native trees and shrubs and recommended that *C. robusta* (as well as many other New Zealand species) be used for screen hedging (Cooper & Cambie 1991).

Coprosma robusta is very widely spread, from the Three King Islands, throughout the North and South Islands and on the Chatham Islands (Salmon 1996; Cave & Paddison 1999), from sea level to 1200 m (Pollock 1986; Metcalf 2000). It does not occur in the extreme south of the South Island (Taylor 1975). It is common in lowland and mountain forests, coastal and lowland scrubland, and swamp and rock associations (Metcalf 2000). *Coprosma robusta* is seen to best advantage growing in open places at the forest margin sand beside roads or streams (Taylor 1975a).

There are a large number of *Coprosma* cultivars available. Many are grown for their coloured and shiny foliage (Cave & Paddison 1999). *Coprosma robusta* is grown in many gardens in most lowland districts but not in great numbers (Pollock 1986). If planting it in the garden it needs a certain amount of pruning as it is fast growing and pruning it will produce a more compact shape (Cave & Paddison 1999; Metcalf 2000). *Coproma robusta* is tolerant of a wide range of conditions so is commonly grown as coastal hedging and shelter planting (Bryant 1997) although it has low salt wind tolerance (New Zealand Ecological Restoration Network [NZERN] 2002). It is wind hardy, but does not thrive where it is exposed to persistent and strong winds. Early growth is fast so it provides good shelter for other plants especially on barren sites. Tolerates shade or full sun equally well and grows well in most soils except very dry soils (Pollock 1986). Most *Coprosma* spp. can be propagated by seed or cuttings but to keep the cultivars true they need to be propagated by cuttings only (Cave & Paddison 1999). There are some varieties of *C. robusta* available, e.g. *Coprosma robusta* 'Williamsii Variegata' and *C. robusta* 'Gordon's Gold', which was discovered in Taihape growing among some wild *Coprosma* plants on a hillside.

Coprosma robusta can be susceptible to some fungi causing shoot die back, stem cankers and anthracnose. *Glomerella cingulata* was found to be the primary pathogen causing these symptoms on *C. robusta* grown in Auckland. Other fungi caused symptoms to a lesser degree (Forbes & Pearson 1987). A new cercosporoid leaf spot disease, caused by the pathogen *Pseudocercospora coprosmae*, has been reported (Braun et al. 2003).

Coprosma robusta is a primary colonising species. It is often among the pioneers where roadside cuttings are being healed and where shrubland is reverting to forest (Taylor 1975a; Pollock 1986). *Coprosma* can be used to advantage as a nurse cover and be part of the natural process of restoring the forests (Porteous 1993; Crowe 1997b). It also has a mid-successional role (NZERN 2002). For example, on sites where gorse is dominant *Coprosma* can be planted underneath it, as it is shade tolerant, and it will overtop the gorse and eventually replace it (Porteous 1993). In this example of succession, gorse acts as the nurse plant. *Coprosma robusta* roots are very dense and fibrous so it makes an excellent soil stabiliser especially for slope stabilisation (NZERN 2002). It is suitable for revegetation on bare infertile soils or subsoil's and can be planted to provide low-tier shelter under taller shelter (Pollock 1986). Pioneer shrubs *C. robusta* and *Leptospermum scoparium* were successfully established after sowing seed on the opencast coal mine sites of Westland. This was a technique that was investigated by Langer et al. (1999) to establish native forest. Some forest remnants, especially in high-risk areas, such as areas adjacent to roadsides or areas of rank grasses, may need a firebreak established. *Coprosma robusta* is relatively fire-resistant (Porteous 1993; Crowe 1997b) and can be used to establish a buffer between a forest remnant and a potential source of fires (Porteous 1993). It is often seen growing, along with *Hebe stricta* and *Phormium tenax* (fire resistant plants), between road verges and plantations of *Pinus radiata*. *Coprosma robusta* has a medium water-logging tolerance so it can be used on stream banks (NZERN 2002).

2.2.2.2 Seed Storage

Metcalf (1995) reports that *Coprosma* seed does not keep particularly well in dry storage. Bergin & Van Dorsser (1988) recommend moist, cool storage at 2°- 4°C. According to Hong et al. (1998b) this species does not show recalcitrant seed storage behaviour. A commercial packet of *C. robusta* seed (New Zealand Tree Seeds) states there are approximately 80 viable seed per gram.

2.2.2.3 Seed Germination

Metcalf (1995) reported that untreated *C. robusta* seed sown in March, as soon as it was collected, did not germinate until August and seed sown in April took 4 months to germinate. The optimum germination temperatures for quick and even germination of *Coprosma* are 15-22°C and at these temperatures it should take 10-28 days to germinate (Bryant 1994). He recommends lightly covering the seed with a thin layer of soil when germinating the seed.

2.2.2.4 Seed Germination Experiments

Germination of *C. robusta* seed is spread out in time, as discovered by Burrows (1995) who collected seeds from Ahuriri Summit, western Banks Peninsula in February 1989 and Riccarton Bush, Christchurch in September 1989 and then performed some germination experiments. Seeds were germinated fresh and the first set of seeds began germinating 18 days later and completed germinating by 68 days with 90% success. The second set of seeds began germinating after 34 days and completed germinating by 52 days with 82% success. The remaining seeds were dead. Seeds that were germinated in the dark were a lot slower than in the standard test resulting in only 60 % of the seed germinating. Burrows (1995) recommend further experimentation in controlled conditions to unravel the specific causes of the spread of germination for *C. robusta.*

2.2.2.5 Other Seed Germination Experiments

It appears that stratification seems to have some benefits in germinating *C. robusta.* Metcalf (1995) states that a 2-3 month period of cool-moist stratification is recommended, but the benefits of stratification have not been proven. For example, stratified seed sown in September took 1 month for the first seedling to appear, but germination of most seedlings was not finished until over 2 months later. This still shows that seed germination is spread over a period. In contrast, Bergin & Van Dorsser (1988), who recommend moist, cool storage, stated that germination was even. They stored the seed at 2-4°C for a period of 6 months thus subjecting the seeds to a period of stratification. A commercial packet of *C. robusta* seed (New Zealand Tree Seeds) recommends that the seed be kept moist in refrigerator at 2-5°C for 28 days before sowing.

Coprosma robusta left to germinate with the fleshy fruit intact germinated at a slower rate than the standard treatment but a high percentage of the seed germinates (72%) (Burrows 1995). Burrows (1995) tested what influence the fleshy fruit had on the germination of lettuce

seed and concluded that *C. robusta* had an inhibitory effect on the rate of germination and root and shoot growth of the new seedlings. The exudate may have an allelopathic role rather than an auto-inhibitor (inhibiting to its own species) role as Burrows (1993) noticed that some fruit of *C. robusta* still attached to the parent plant germinated viviparously. Metcalf (1995) stated that leaching does not make any difference on the germination rate. Herron (1999) leached *C. robusta* seed for 30 minutes and discovered that leaching appeared to increase germination but the final germination percentage and rates of germination were not significantly different to the control.

Herron (1999) demonstrated that mechanical scarification (chipping the exocarp) appeared to increase germination but the final germination percentage and rates of germination were not significantly different to the control. Acid scarification, using various concentrations of sulfuric acid, resulted in none of the *C. robusta* seeds germinating and this treatment damaged the embryos.

2.2.3 *Cyperus ustulatus*

Plate 2.3: *Cyperus ustulatus* with seed heads. (Sourced from Metcalf 1998)

2.2.3.1 Species Description

Cyperus ustulatus A. Rich. (giant umbrella sedge, toetoe upokotangata, sea rush, coastal cutty grass) belongs to the Cyperaceae family, which consists of 115 genera and about 3,600 species (Mabberly 1987-cited in Hong et al. 1998a). The genus *Cyperus* consists of about 600 species of sedges but *C. ustulatus* is the only species native to New Zealand (Cave & Paddison 1999). It is very vigorous, growing into large clump- or tussock-forming plants 60 cm-1 m tall. The deep olive-green, very sharp-edged leaves are 60-120 cm long by 1-1.5 cm wide (Moore & Edgar 1970; Metcalf 1998; Cave & Paddison 1999).

Clusters of long, leafy bracts (2.5 to 7 cm long) are on the top of 1.2 m flowering stems. Atop of the leafy bract are clusters of dark brown or yellow brown, shining spikelets 8 to 13 mm long (Moore & Edgar 1970; Metcalf 1998). Within each spikelet there are 5 to 20 glumes with the 3 lowermost and 1 to 2 uppermost glumes being 'empty'. The rest of the glumes are fertile (Moore & Edgar 1970). The flowering stems are distinctly three-angled.

The nutlet is 1.5 to 2 mm long and 0.5mm wide, linear-oblong and brown. The seed is about half the length of the glume that encloses it.

Cyperus comes from the old Greek name *kypeiros* that means sedge. It is also known as umbrella sedge because their inflorescences sit atop a cluster of leafy bracts, which gives them an umbrella-like appearance (Metcalf 1998).

Cyperus ustulatus was of great value to the Maori as it was one of the salt marsh plants used for the outer thatch of houses (Cooper & Cambie 1991; Metcalf 1998). In 1873, Thomas Kirk recommended that *C. ustulatus* be used to stabilise sand further inland from advancing sand dunes that were burying fertile coastal farms (Cooper & Cambie 1991).

Cyperus ustulatus is a coastal sedge commonly found growing in sand hollows, moist sand flats or coastal swamps (Edgar 1975), and lowland areas, mainly in damp ground near rivers (Metcalf 1998). It is wide-spread along the coast throughout the North Island, and in the South Island as far south as Motukarara and the Rakaia River mouth on the east, and Fiordland on the west. It is on the Chatham Islands and Kermadec Islands (Metcalf 1998). In 1788 the *Lady Penrhyn*, an escort for the first convoy of convict ships to Botany Bay, stopped at Macauley Island in the southern Kermadecs and were disappointed to see such an inhospitable, waterless island, largely covered with *C. ustulatus* and ngaio (*Myoporum laetum*) (Sykes 1975).

Cyperus ustulatus is not in cultivation as it is considered to be too large and vigorous for most garden situations (Cave & Paddison 1999). If placed in the garden then it should be in a large area. It is highly tolerant of waterlogging and tolerant of salt wind and persistent wind (NWERN 2002). Useful planting in large wet areas or next to streams (Metcalf 1998). *Cyperus ustulatus* is a primary colonising plant. It is useful for sand dune restoration (NZERN 2002).

The smut fungi *Bauerago abstrusa* is found on *C. ustulatus* (Vanky 1999).

A commercial packet of *C. ustulatus* seed (New Zealand Tree Seeds) states that viable seed per gram is approximately >100.

2.2.3.2 Seed Germination Experiments

No germination experiments have been performed on *C. ustulatus* but other species have been. The literature reveals that some other species appear to have dormancy mechanisms and

require some form of pre-treatment to break dormancy. For example, germination percentages of *C. sanguinolentus* were increased by pectinase (polygalacturonase) treatment (Shim et al. 1998). *Cyperus serotinus* require strong light for germination (Ku et al. 1996). Seeds of some species forms part of soil seed banks and remain viable. When exhumed and subjected to light treatments the seeds germinate. For example, 91-100% of *C. erythrorhizos* and *C. flavicomus* (annuals found on the mudflats at Lake Barkley, USA) seed germinated in the light after being buried for 25 months and less than 8% of the exhumed seed germinated in the dark (Baskin et al. 1993).

2.2.3.3 Seed Storage

A commercial packet of *C. ustulatus* seed (New Zealand Tree Seeds) recommends that the seed be kept moist in refrigerator at 2-5°C for 28 days before sowing.

2.2.4 *Hebe stricta*

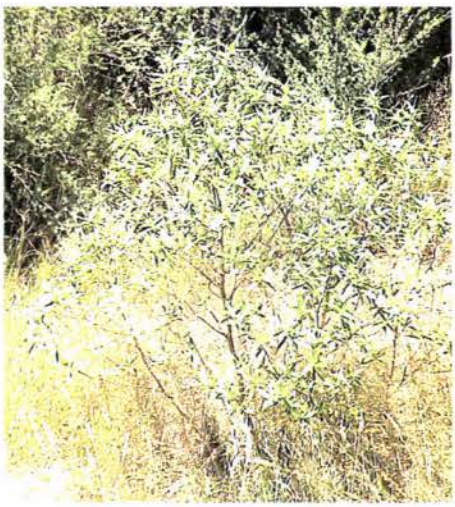

Plate 2.4: *Hebe stricta* planted as part of a revegetation project at Massey University (Turitea Campus).

2.2.4.1 Species Description

Hebe stricta (Benth.) L. B. Moore (koromiko, koromuka, willow koromiko) belongs to the foxglove family Scrophulariaceae, which consists of 292 genera according to Brummitt (1992 – cited in Hong et al. 1998b). *Hebe* is a genus with around 100 species found mainly in New Zealand. The 79 species found in New Zealand are shrubs or small trees (Salmon 1996). *Hebe stricta* is an open branching shrub 1.5 m to 4 m tall. The long narrow leaves (5 to 12.5 cm long and 1.6 to 2.5 cm wide) are deep green and slightly glossy (Allan 1961; Cave & Paddison 1999; Metcalf, 2000). *Hebe stricta* was formerly included with *H. salicifolia* (koromiko) but it is separated, as the latter leaf bud does not have a sinus. The presence or absence of a sinus provides a means of distinction (Metcalf 2000). Leaves are arranged in opposite pairs that stand in four rows when looked at from above; they are simple, never compound, and generally lack obvious veins, except the midrib. Leaves of seedlings often differ from those of adults, especially in having toothed margins (Moore 1975). Chromosome number of *H. stricta* is n=20 (Kristensen 1990).

Hebe stricta is a gynodioecious shrub (Delph & Lively 1992). The flowers of *Hebe* are produced in specialised axillary racemes (Metcalf 2000). *Hebe stricta* has inflorescences, usually much exceeding the leaves, 7.5 to 15 cm long and about 2.5 cm in diameter (Allan 1961). The flowers are white, or sometimes pale bluish, fairly closely placed (Metcalf 2000). The flower consists of four petals, one of them slightly apart from the other three, joined into a tube to which the two stamens are attached. The ovary is seated within the tube and has a single style. There is hermaphroditic (male) and male-sterile morphs with the flowers of the

male morphs have significantly larger anthers that exceed a greater distance out of the floral tube (Delph & Lively 1992). *Hebe stricta* has a long flowering period from November to March (Salmon 1999) but personal observations have found that they flower longer than this period. Godley (1979) has observed a plant in the Christchurch Botanic Garden for two seasons flowering from April to December with most inflorescences present in October. It is long flowering because pairs of inflorescences are produced one after another, throughout the flowering period, in the axils of the new leaves at the continuously growing tips of branches. The arrangement in inflorescences of *Hebe* indicates that they are likely to attract pollinators rather than being wind pollinated (Kristensen 1990). Van Eaton (1983) recommends that *H. stricta* be planted to provide pollen for bees to overcome the spring deficit faced by many hives in Southland/South Otago. Bees use *Hebe* as a pollen and nectar source. Delph & Lively (1992) observed that the solitary bee *Lasioglossum sordidum* visit *H. Stricta* more than other species of insects. Male *L. sordidum* forage on flowers throughout the day (foraging exclusively for nectar). The male bees prefer flowers on the male plants, which produce 4 times the nectar as the male-sterile plants. The female bees forage on the male plants only in the morning and not in the afternoon (forage for nectar and/or pollen). Hover flies also preferred flowers on the male plants. Although visits by pollinators to the female morph were fewer, they seemed sufficient to ensure maximal fruit set.

The fruit consists of capsules that are less than 4 mm long. The fruit dries as it ripens and splits along the septum or dividing wall into two halves, each with numerous seeds on the central wall (Moore 1975; Metcalf 2000). Seeds of most *Hebe* species are small, flattened, smooth and extremely light (Simpson 1976). The seed are buff-brown and are 0.8 to 0.9 (-1.0) mm in length. The seed is ripe from December onwards.

Koromiko was well regarded by the Maori for its astringent qualities (an agent producing contractions of organic tissues or arrest of a discharge). The young leaf tips were used to cure diarrhoea and dysentery (MacDonald 1973; Cooper & Cambie 1991). When doctors were few in rural areas, and chemists practised only in distant towns, country people had their own cures for ailments. Koromiko was a well-tried cure for diarrhoea. A sprig of koromiko was found and a good leaf bud was chosen. The two outer leaves were peeled off and the inside was chewed slowly. The bitter leaves had an action that was prompt and effective (Moore 1975). During World War II the young leaves were sent to the Maori troops in the Middle East who were suffering from dysentery. So good was the cure that the pakeha solders also accepted the leaves (MacDonald 1973; Cooper & Cambie 1991). The leaves were steeped in

water and the Maori people drank the water to cure kidney and bladder trouble. Steeping the young leaves in hot water produced an infusion, which was given to expectant mothers to bring on easy and rapid childbirth. Leaves were also used to cure ulcers and boils, bruising, venereal disease, skin disease and used as an effective mouthwash (MacDonald 1973).

The medicinal uses of koromiko have been long known by the Maori people. Koromiko has been recognised in British medicine, being listed as remedy for diarrhoea in the 1895 Extra Pharmacopoeia, London. The value of the plant has been traced to its anti-peristaltic action. The active principle is thought to be phenolic glycosides (Cooper & Cambie 1991). Kellam et al. (1992, 1993) has isolated forsthiaside and mevalonolactone glucoside derivatives and two flavone glycosides.

Hebe stricta is found throughout the North Island but especially common in lowland and hill country from about the Manawatu district northwards. It is found mainly at the forest margin (Kristensen 1990). *Hebe stricta* var. *stricta* has not been recorded as being naturally found in the South Island but several specimens from Marlborough Sounds and Nelson suggest that it may be present (Allen 1961).

There are many cultivated *Hebes* and many have been in cultivation for along time. They were originally known as *Veronica.* In 1839, live *Hebe* plants were shipped back to be grown at Kew gardens. By the 1890s there were about forty New Zealand species of *Hebe* at the Royal Botanic Gardens in Edinburgh. The number of New Zealand species of trees and shrubs in Kew Gardens had risen to about 140 by 1934, of which *Hebe* was one of them. There were in fact seventy kinds of *Hebe* growing (Cooper & Cambie 1991). They are cultivated in many countries including England, Scotland and Denmark (Cooper and Cambie 1991). There are five named varieties of *H. stricta*, var. *stricta* being the most common and widespread (Pollock 1986). It is commonly cultivated in many parts of the country but not in great numbers. Koromiko is fast growing to about 2 m and is a useful filler when large areas are to be planted. Pruning is important to maintain a good shape (Cave & Paddison 1999). It prefers well-drained soils with good moisture but will establish in on moderately heavy clays (Pollock 1986). *Hebe stricta* can be grown in sand (Crowe 1997b). It can be grown in full sunlight but can tolerate some shade (Crowe 1997b). Roots are shallow but have a good network of very fibrous roots.

Hebe species and cultivars are susceptible to several pests. Some sapsucker insects induce "big bud" galls on koromiko. Insects use these outgrowths for food and shelter (Dugdale 1975). Downy mildew of *Hebe* (*Peronospora grisea*) distorts the leaves, which after turning yellow, later turns black, resulting in death of the leaves. Septoria leaf spot (Septoria *exotica*) causes purplish blotches on the leaves and is very damaging. Veronica gallery fly (*Agromyza flavocentralis*) larvae tunnel into the leaves forming long narrow galleries. The attacks of this insect can seriously affect the young growth and consequently the flowering of *Hebes* (Metcalf 2000).

Hebe stricta can be used as a fast-growing nurse plant for revegetation, providing early cover for subsequent establishment of forest species (Pollock 1986; Crowe 1997b). It is best used on moist sites but they can be exposed (Porteous 1993) as *H. stricta* is very tolerant of wind (NZERN 2002). *Hebe stricta* can be grown in a hedgerow to create a wind break and is excellent to use in areas that have high possum numbers, as *H. stricta* is possum hardy (Crowe 1997b). It is very suitable for planting on earthworks and slip sites (Pollock 1986). *Hebe stricta* is relatively fire-resistant (Porteous 1993; Crowe 1997b) and can be used to establish a buffer between a forest remnant and a potential source of fire (Porteous 1993). It can be used for slope stabilisation (NZERN 2002).

2.2.4.2 Seed Storage

According to Hong et al. (1998b) the seed storage behaviour in all species examined in the Scrophulariaceae family is orthodox. There appeared to be no intermediate or recalcitrant seed storage behaviour reported. Metcalf (1995) reports that *Hebe* does store well, but as a general rule, seed should be sown when fresh. There appears to be no difference between sowing the seed immediately it has ripened, after 4 months of dry storage or after being-moist stratified. Seeds of *H. stricta* var. *stricta* stored in paper bags at room temperature remained 100% viable for 10 months and then showed a gradual loss of viability. At 24 months there was complete loss of viability (Simpson 1976). After 11 years under long-term storage conditions (-18°C in airtight containers at a seed moisture content of 5 ± 1%), *Hebe brachhysiphon* had 81.3% germination (Hong et al. 1998b). A commercial packet of *H. stricta* seed (New Zealand Tree Seeds) states that viable seed per gram is approximately >500.

2.2.4.3 Seed Germination

Metcalf (1995) reports that germination percentages are normally very good. All hebes will grow easily from seed (Bryant 1994). Bryant (1994) suggests lightly covering the seed with a thin layer of soil and that the tray is placed in light shade until the seeds germinate. Metcalf (1995) recommends that a suitable fungicide be used when germinating *Hebe* seed due to the seedlings being susceptible to downy mildew and damping off which can completely destroy a batch of seedlings within a day or two. The optimum germination temperatures for quick and even germination of *Hebe* are 13-24°C and at these temperatures it should take 14-28 days to germinate (Bryant 1994). A commercial packet of *H. stricta* seed (New Zealand Tree Seeds) recommends that the seed be kept moist in a refrigerator at 2-5°C for 28 days before sowing.

2.2.4.4 Seed Germination Experiments

All varieties of *H. stricta* readily germinate at 25°C in light (Simpson 1976). Comparisons were made between germination of *H. stricta* var. *atkinsonii* seed in the light and dark, resulting in a significant difference. Germination percentages of 86% and 4%, respectively, were obtained. Simpson concluded that light is necessary for germination of *Hebe* species that respond to a temperature of 25°C but this requirement can be overcome for some species if they are exposed to low temperature. Miller & Henzell (2000) subjected eight invasive weeds (commonly invade New Zealand native forests) and 4 native plants (*Hebe stricta* included) to two different light regimes, either total darkness (zero light for 24 h/day) or a 16 h light and 8 h dark cycle. His conclusion was that light did not significantly increase germination of *Hebe stricta,* as there was no significant difference between the germination (%) of seeds in the light (7.0 ± 2.6) and seeds germinated in the dark (5.0 ± 2.2).

2.2.5 *Leptospermum scoparium*

Plate 2.5: *Leptospermum scoparium* planted as part of a revegetation project at Mt Biggs, Sandon Road.

2.2.5.1 Species Description

Leptospermum scoparium J. R. et G. Forst. (manuka, red tea tree, kahikatoa) belongs to the large Myrtaceae family and the sub-family Leptospermoideae. According to Turnbull & Doran (1987) there are over 3000 species in about 155 genera. Mabberley (1987 – cited in Hong et al. 1998b) states there are 120 genera and 3,850 species. *Leptospermum scoparium* grows either as a shrub of varying form and habit or as a tree up to 8 m high with spreading ascending branches. Manuka has bark that is brown, thin and stringy which peels in strips, and leaves that are 4-12 mm long and 1-4 mm wide (Allan 1961; Poole & Adams 1994; Salmon 1996). The leaves have aromatic glands and pungent tips (Salmon 1996), which when crushed smell like myrtle (Molloy 1997).

The flowers usually arise singly in the axils of the leaves and are about 12-15 mm in diameter (Molloy 1975; Salmon 1996). They are distributed rather sparsely along the branchlets (Molloy 1975). Usually *L. scoparium* has white flowers, consisting of five petals (Allan 1961: Turnbull & Doran 1987), but flower colour ranges from white to pink (Dawson 1997) in the wild. There is an extremely rare red-flowered mutant plant, best known as *L. 'Nichollsii'* (Metcalf 1987) and double-flowered plants that have been discovered in the wild (Dawson 1997). Cultivated varieties include deep red to purple- coloured flowers, both single and double forms. *Leptospermum scoparium* usually begins flowering in September and continues into February (Salmon 1999) but Burrell (1965) has observed stands of *L. scoparium,* found in Otago, flowering at other times of the year. *Leptospermum scoparium* produce two kinds of

flowers (andromonoecy)-male flowers that have functional stamens and a non-functional pistil and hermaphrodite flowers where both the stamens and pistil are functional (Primack & Lloyd 1980). The flowers produce ample pollen and nectar and are visited by various flies, moths, native bees and nectar-feeding gecko (Molloy 1975; Godley 1979). Van Eaton (1983) recommends that *L. scoparium* be planted to provide pollen for bees to overcome the spring deficit faced by many hives in Southland/South Otago. Bees use manuka as a nectar source rather than a pollen source but it is still worth while planting in order to provide some pollen to the bees.

Leptospermoideae fruits are predominantly capsular and have a dry, often woody fruit wall (pericarp) (Hong et al. 1998b). The woody fruit of *L. scoparium* consists of a woody capsule with a five-valved structure (Allan 1961; Molloy 1975; Salmon 1996). When ripe the capsule splits open and numerous brown, linear seeds (many often sterile) are ejected through slit-like openings (Allan 1961; Molloy 1974). Seeds are (1.6-) 2.00 to 3.00 mm long (Webb & Simpson 2001). Thousand seed weight = 1.5 g (Hong et al. 1998b). Wind is probably the important agent of seed dispersal in the Leptospermoideae (Burrell 1965; Turnbull & Doran 1987). Due to their smallness Manuka seed is easily blown long distances by the wind (Dobson 1975). Whole capsules are readily entangled or water-borne so that man, stock, and rivers also carry seed (Burrell 1965; Porteous 1993). The very small seeds take at least one year to mature (Salmon 1996) but seeds removed from current seasons capsules have been known to germinate (Burrell 1965). The capsules produce from 200 to 500 seeds (Silvester 1962, cited in Grant 1966). Unopened capsules from at least three seasons can remain on the trees and the seed remains viable (Burrell 1965; Mohan et al. 1984a).

In New Zealand *L. scoparium* is highly polymorphic (Dawson 1997). Manuka has evolved many forms adapted to different habitats, and some of these are genetically determined and permanent (Molloy 1975). Yin et al. (1984) studied the variation in plant form, flowering, leaf size and shape of *L. scoparium* populations throughout New Zealand. They found there is significant correlation with geographic and climatic factors-latitude, distance from coast, annual and winter temperatures. The variation between the populations had a significant genetic component, as well as a plastic basis, thus indicating that forms of *L. scoparium* adapt to different habitats.

Leptospermum comes from the Greek *leptos* (fine or slender) and *sperma* (seed) (Forster & Forster 1993). *Scoparium* means broom-like (Molloy 1975).

Leptospermum scoparium is known as red tea tree or red manuka due to it having dark-reddish brown wood (Molloy 1975). The hard and durable wood was used by the Maori to make implements (i.e. paddles, spade blades and bird spears) and weapons (Cooper & Cambie 1991). Manuka wood has been used for fencing and for tool handles, and it is much prized as firewood (Salmon 1996). Today, the wood is popular for burning in barbecues and used for smoking food, such as fish. Captain Cook and the early settlers, who made 'tea' from the leaves (Crowe 1997a, Brooker et al. 1998), gave the name 'tea-tree'. Captain Cook also successfully made beer using the leaves. No part of manuka is poisonous to man (Molloy 1975). Maori people used the leaves, bark and capsules to cure many common ailments such as dysentery, urinary complaints and diarrhoea (Macdonald 1973).

Leptospermum scoparium is found throughout the North and South Island of New Zealand and beyond the Three Kings Islands in the North and to Stewart Island in the South (Yin et al. 1984), from sea level to 1000 m (Salmon 1996). It also occurs in Australia (Dawson 1997) especially being common in Tasmania (Forster & Forster 1993). *Leptospermum scoparium* occurs in many habitats from coastal sand dunes to the cold, wet Fiordland Mountains and in rock outcrops, raw pumice and leached gravel's (Molloy 1975), thus exhibiting tolerance to a wide range of soil conditions. Characteristics that ensure the early and rapid establishment of *L. scoparium* on a wide range of sites include its wide ecological tolerance, vigorous growth and ability to colonise inhospitable sites especially those of low fertility and with low temperatures.

In general, manuka is very tolerant of drought, waterlogging and frost (Pollock 1986). For example, it can tolerate both extremes of moisture from very dry, such as dry pumice on the Central north Island Volcanic Plateau (Elder 1962), to very wet, such as the permanently wet terrace pakihi bogs in Westland (Rigg 1962; Mark & Smith 1975). Cook et al. reported that *L. scoparium* could tolerate waterlogging. In contrast, Bannister (1986) reported that it is drought resistant. NZERN (2002) state that they are highly frost tolerant. In horticultural publications, cultivars are classified generally as being slightly frost tender or hardy (Duncan & Davies 1985) but observations made by Warrington & Stanley (1987) confirm that present ornamental cultivars are tolerant of mild frosts throughout the year. They should not be regarded as hardy. They report that the frost hardiness temperature (i.e., the temperature that causes damage) and the lethal temperature (i.e. the temperature that causes death) in winter was -5 to -8°C, respectively. In summer, the frost hardiness temperature was between -2 and -5°C, and the lethal temperature was between -4 and -9°C. There is an overlap in the

distribution of the vegetatively similar manuka and kanuka (*Leptospermum ericoides* and *Kunzea ericoides*), but manuka has the wider tolerance for boggy ground and infertile soils (Burrell 1965;Molloy 1975).

Leptospermum scoparium has a long history of cultivation. It has been grown in England since 1772 (Dawson 1997). *Catalogue of the Plants Cultivated in the Royal Botanic Garden Kew* was published in 1789 and it listed that *L. scoparium,* although it was named as *Philadelphus scoparius*, was grown in Kew gardens. Seeds of *L. scoparium* was offered for sale in England as far back as 1776 (Cooper & Cambie 1991). Today, *Leptospermums* are cultivated in many countries, including the UK, France, Denmark, Israel, South Africa, the USA, Australia and New Zealand (Dawson 1997). The latest new Tea Trees are 'Joy', which have light pink to red-purple single flowers (Watson 2001a); 'Naoko", which have very light pink to red-purple semi-double flowers (Watson 2001b) and 'Emily NAO' which have very light pink to red-purple semi-double flowers (Watson 2001c). They are grown as ornamentals in gardens and for cut flowers.

Manuka is susceptible to manuka blight (*Eriococcus orariensi*), a sooty mould that grows on the honeydew excreted by a scale insect (Cave & Paddison 1999). A new genus and species of sooty mould (*Capnofrasera dendryphioides*) has recently been isolated from *Leptospermum scoparium* (Hughes 2003). Manuka is also susceptible to the lemon tree borer (*Oemona hirta*), which causes young branchlets to die off in early summer, and webworms (*Heliostribes atychioides*), which form irregular masses within the bush (Metcalf 2000). *Leptospermum scoparium* is the alternative host for the large cerambycid beetle whose primary host are the native beeches (Dugdale 1975).

Leptospermum scopariums essential oils have anti-bacterial and anti-fungal properties, and commercial production of the oil has been performed in the East Cape (Perry et al. 1997). The essential oil also contains leptospermone, a triketone, which has anthelmintic (remedy for expelling worms) (Brooker 1975), and insecticidal properties (Cooper & Cambie 1991). Honey made from manuka contains methyl syringate, which has shown to have antibacterial properties (Copper & Cambie 1991, Willix 1991). Manuka honey was tested against *Staphylococcus aureus* and showed a high antibacterial activity (Allen et al. 1991). Early results from a pilot trial at Otago's School of Dentistry indicate that confectionery made using a special manuka honey could help keep teeth healthy by inhibiting acid produced by oral bacteria (Technology Reports 2002).

Manuka is useful for restoration of native vegetation along roadsides, earthworks and eroded areas (Pollock 1986). It is also used for sand dune restoration (NZERN 2002). *Leptospermum scoparium* is a primary colonising species. Mark & Dickinson (1989) found it to be a dominant pioneer on the landslides in the Fiord Ecological Region of southwestern New Zealand but over time it became decreasingly important. *Leptospemum scoparium* shrubland tends to be developed from bare ground or from short open vegetation and its presence often indicates the destruction of previous vegetation by fire (Esler & Astridge 1974). Burning of tea tree usually results in re-invasion by the same species. When Maori, and later the Europeans, destroyed native forest by fire, *L. scoparium* was the dominant colonising species (Burrows 1973). In the Waitakere range tea tree became established through the clearing of land as a result of logging, burning by gum diggers, and burning and grassing for farming (Esler & Astridge 1974). *Leptospermum scoparium* makes an excellent nurse plant, thus providing the ideal environment for later successional native species to regenerate (Porteous 1993). It has been found that manuka stands, left alone, will revert to forest if the sites are suitable (Molloy 1975). The landslides that Mark & Dickinson (1989) observed are now covered with mature forest. *Leptospermum scoparium* failed to regenerate from the seventh decade. This would be due to insufficient light penetrating to permit growth of seedlings and so more shade tolerant species would overtop the *Leptospermum* (Burrows 1973). Successions through *L. scoparium* usually take less than 100 years, depending on the site (Porteous 1993). *Leptospermum scoparium* is useful for erosion control on erosion-prone hill country (Bergin et al 1993), as found on the East Coast of the North Island. It can also be useful in the control of erosion on roadsides, gullies and riverbanks (Crowe 1997b; NZERN 2002). Watson & O'Loughlin (1985) observed that mature *L. scoparium* stands provide reinforcement to the soil mantle and inhibit the development of shallow landslides, but this protection diminishes quickly after clearing of *L. scoparium.*

2.2.5.2 Seed Storage

Metcalf (1995) reports that *L. scoparium* seed stores well. Seed dry stored for 5 months germinated within 10 days, which is the same for fresh seed germinating. Metcalf (1995) also states that *L. scoparium* can be stored for a few months in open conditions without any attempt to control temperature or moisture levels. The seeds can be placed in a paper envelope. Grant (1966) reported that seed stored for over 18 months at room temperature showed no decrease in germination percentage and Mohan et al. (1984a) observed that the final germination percentage did not vary during the experimental period of 12 months. To store *L. scoparium* seed, Bergin & Van Dorsser (1988) state that cool dry storage (2°- 4°C) in

sealed containers is preferable. According to Turnbull & Doran (1987) *Leptospermum* demonstrate orthodox seed storage behaviour, i.e. if seed is stored at low moisture content (5%) and at a low temperature in a sealed container Leptospermoideae species can remain viable and vigorous for 5-20 years (Turnbull & Doran 1987). Ralph (1994) reports that Australian *Leptospermum* has a long seed life. Storage behaviour is consistent with argument for the seed as orthodox (Hong et al. 1998b).

2.2.5.3 Seed Germination

Generally, germination percentage for *L. scoparium* seed appears to be low, for example Burrell (1963, cited in Grant 1966) obtained 20% germination, Grant (1966) averaged 19% and Mohan et al. (1984a) had germination of 11%. According to Mohan et al. (1984b), because there is a large seed output, (200 to 500 seeds per capsule-Silvester, 1962, cited in Grant 1966), the actual number of viable seeds shed by *L. scoparium* is high. Prakash (1969) examined Australian *L. scoparium* seed and reported that only 15% contained embryos and the remaining seed showed a collapsed embryo sac. Prakash (1969) detailed the development of the dicotyledonous embryo and suggested that absence of fertilisation may have been the cause of embryo sac collapse, with pollination stimulating the growth of the nucellus and seed coat. A commercial packet of *L. scoparium* seed (New Zealand Tree Seeds) states that viable seed per gram is approximately >1,000.

Leptospermum scoparium usually germinate well if left uncovered according to Metcalf (1995). In contrast, Bryant (1994) suggests lightly covering the seed. The optimum germination temperatures for quick and even germination of *Leptospermum* are 15-24°C and at these temperatures it should take 10-35 days to germinate (Bryant 1994). Ralph (1994) reports that *Leptospermum* has a very high viability without any pre-treatments and that it is suitable for direct seeding. Bergin & Van Dorsser (1988) state that the seed readily germinates after sowing. A commercial packet of *L. scoparium* seed (New Zealand Tree Seeds) recommends sowing seed direct from the packet and keeping them moist until germinated

Germination for all of the sub-family Leptospermoideae is epigeal, with the thin cotyledons being raised above the ground. The seeds are non-endospermic (Turnbull & Doran 1987).

2.2.5.4 Seed Germination Experiments

Several experiments have been performed to demonstrate the effects that light has on germination of *L. scoparium* seed. Mohan et al (1984a) reported experiments on photoperiod

treatment applied to germination experiments (10,12,14,16 or 24 h light in 24 h cycle) at 25 $\mu Em^{-2}s^{-1}$ and demonstrated that there are no significance differences on germination between the photoperiods. They also looked at germination of *L. scoparium* grown in the dark and discovered that germination did occur in the dark but was half that of germination in the light.

Herron et al. (2000) performed several experiments on the light requiring needs of *L. scoparium. Leptospermum scoparium* germinated under a wide range of irradiances from 0.9 $\mu molm^{-2}s^{-1}$ (very dense shade) to 700 $\mu molm^{-2}s^{-1}$ (full daylight). In the dark only 5% of seed germinated and the promoting effect of red light (100 minutes of exposure to red light stimulated 90% germination) on *L. scoparium* seed germination was nullified by immediate exposure to far-red light, implying phytochrome was the pigment involved.

Silvester (1962, cited in Grant 1966) showed that *L. scoparium* would not germinate in darkness, but found that 0.5% and 0.25% of daylight was sufficient for germination.

Burrell (1963, cited in Grant 1966) stated that light had no effect on dormancy for *L. scoparium*, while Levy (no date, cited in Grant 1966) in contrast, suggested that *L. scoparium* had a light requirement for germination. Bieleski (1959, cited in Grant 1966) observed that light levels under stands of manuka were normally too low for regeneration of the species. Mark et al. (1989) observed that once a dense canopy of *Leptospermum* forms it precludes establishment of its own light-demanding seedlings. Burrows (1973) suggested that in dense stands of *Leptospermum* there is insufficient light penetrating to permit growth of seedlings.

Miller & Henzell (2000) subjected eight invasive weeds, that commonly invade New Zealand native forests, and four native plants (*Leptospermum scoparium* included) to 2 different light regimes, either total darkness (zero light for 24 h/day) or a 16 h light and 8 h dark cycle and concluded that light significantly increased germination of *Leptospermum scoparium.*

Grant (1966) stated that *L. scoparium* is a pasture weed in many parts of New Zealand. His experimental work was determining what factors favour the establishment of *L. scoparium* and the quantitative effect of farm management techniques on this process. By adding fertiliser to plots, thus encouraging grass to grow, and keeping the sward uncut, it was discovered that little or no light reached the soil and so few or no seedlings germinated. The number of seedlings was the highest on grass plots without applied fertiliser, and on

fortnightly cut plots. He concluded that light at ground level is a vital factor in the ingress of *L. scoparium* into pastures.

Grant (1967), Mohan et al. (1984a) and Herron et al. (2000) studied the effect temperature has on *L. scoparium* germination. Grant (1967) and Herron et al. (2000) performed similar experiments. They both studied the influence of temperatures, from 4°C to 35°C, on the germination of *L. scoparium.* Grant (1967) showed that at 20°C and under continuous light, germination was completed after 10 days, and averaged 19%. Above 20°C complete germination (19%) took 9 days, while at 15°C and 4.5°C, 16 and 43 days were needed, respectively. Below 20°C germination was reduced to 14%. Herron (1999) had no germination at 5°C, but the seeds successfully germinated at all other temperatures (from 10°C to 34°C). The higher the temperature the faster the rate of germination. Germination at 34°C with a T_{50} was 24 days. At this temperature germination was first observed on day 2 and the last seeds germinate on day 7. At 25°C and 20°C the time to 50% germination was 3 and nearly 6 days, respectively. At 18°C the rate of germination was 7 days. At 15°C germination slowed to 9.2 days and at 10°C, the time to 50% germination doubled to 18.6 days.

Mohan et al. (1984a) observed the effect of constant temperature on germination, with a photoperiod of 14 h at 30 $\mu Em^{-2}s^{-1}$. It was found that there was no difference in the final germination percentages between temperatures of 12°C to 25°C, but the lower the temperature the longer the lag phase and the slower the germination rate. It appears that *L. scoparium* seed can withstand cycles of wetting and drying prior to germination (Mohan et al. 1984a) as alternating periods of dehydration with periods of adequate moisture only slightly reduce the final percentage of seed germinating, but had a major effect on the overall rate.

Germination percentages can vary from tree to tree and also there can be seasonal variation in the germination of *L. scoparium* (Mohan et al. 1984b). Seasonal variation in the germination of *L. scoparium* was shown for seed collected from the Waitakere ranges. There was a significant marked seasonal effect on percentage germination in the field (in seed traps), under laboratory conditions and as a total of both. A highly significant model involving temperature and moisture regimes accounted for most of the variation. Overall germination was highest in early spring and lowest during late summer. Mohan et al. (1984a) showed that there was no significant effect on the germination percentage of seeds from capsules of various ages (using capsules from three consecutive seasons).

Freshly sown *L. scoparium* seed was found to germinate and survive best on bare ground in the Manawatu from July to September, while December to March germination was very low or zero (Grant 1966).

2.2.6 *Muehlenbeckia australis*

Plate 2.6: *Muehlenbeckia australis* in flower. (Sourced from Salmon 1999)

2.2.6.1 Species Description

Muehlenbeckia australis (Forst. f.) Meissn. (bush pohuehue, puka; large-leafed Muehlenbeckia) belongs to the Polygonaceae family, which consists of 51 genera and 1,150 species according to Mabberley (1987 – cited in Hong et al. 1998b). *Muehlenbeckia* is a genus consisting of 15 species of climbing or sprawling sub-shrubs and shrubs (Cave & Paddison 1999). At least two of the New Zealand species are endemic (Metcalf 2000). *Muehlenbeckia australis* is a stout liane up to 10 m tall with many interlacing branches forming tangled masses in the absence of support (Allan 1961; Cave & Paddison 1999). The main stem is up to 10 cm in diameter. The bark is grey (Allan 1961). The pale green, broad-ovate to broad-oblong leaves are 2 to 8 cm long and 1 to 3 cm wide.

The flowers are dioecious or polygamous consisting of axillary and terminal clusters of bracted racemes up to 5 cm long (Allan 1961, Metcalf 2000). The male flowers have 8 stamens and the pistil is obsolete while the female flowers have anthers reduced to thick staminodia or are obsolete. The flowers are cream and about 4 mm across (Salmon 1999). The perianth becomes enlarged and succulent in fruit (Metcalf 2000).

The fruit are trigonous, hard, brown to black nutlike achenes (Metcalf 2000; Webb & Simpson 2001). The ripe fruit are enclosed in fleshy white (sometimes-dry) perianth lobes, which aid dispersal (Allan 1961; Webb & Simpson 2001). Small birds such as blackbirds,

thrushes and white-eyes are major seedeaters of *Muehlenbeckia* (Dobson 1975) thus dispersing the seed (Burrows 1996b). Seed could also be dispersed by brushtail possum (*Trichosurus vulpecula*) as Cochrane et al. (2003) reported that *Muehlenbeckia australis* is one of the possums preferred foods in mixed Nothofagus fusca-N. menziesii forest of the north westland of New Zealand. Some *Muehlenbeckia* seeds, surrounded by dry perianths, probably disperse by falling to the ground (Burrows 1996b). The first signs of germination in the seeds is the splitting of the seed coat followed by radicle emergence (Burrows 1996b).

The juicy swollen flower, which holds the black seed, is edible. The small swollen flowers were very popular with Maori children (Colenso 1868). Australian *M. adpressa* is very similar to *M. australis* and Allan (1961) states that they are related. The fruits of *M. adpressa* have been made into confectionery, pies and puddings (Crowe 1997a).

Muehlenbeckia australis is distributed throughout New Zealand (Three Kings, North and South, Stewart and Chatham Islands) (Allan 1961; Cave & Paddison 1999). It is found in lowland to montane forest especially marginal, throughout (Allan 1961). Burrows (1996b) states that it is in the forests where it reaches the canopy. *Muehlenbeckia australis* is also found on Norfolk Island.

Muehlenbeckia australis is not in cultivation. Cave & Paddison (1999) state that it may be useful as a ground cover. It is best to grow in very poor soil, as it becomes a rampant weed in better conditions. Baars & Kelly (1996) performed an experiment to show the effects that light has on the establishment and survival of seedlings of *M. australis* under a mature undisturbed forest canopy. They concluded that *M. australis* is dependent on relatively high levels of irradiance for survival and growth. Its rapid responses to increased irradiance levels, along with its high growth rates, demonstrates that it is of a weedy nature. Despite the fact that *Muehlenbeckia* is a native climber it is capable of smothering vegetation (Baars & Kelly 1996; Burrows 1996c), especially at the forest margin (Porteous 1993), so if used in a revegetation project it would be best to plant on its own.

Zapyrasata calliphana Meyr. larvae mine into leaves of *M. australis* and *M. complexa* in New Zealand, Norfolk Island and Three Kings Island (Dugdale 1971). *Muehlenbeckia australis* is eaten by brush-tailed possum (Trichosurus vulpecula). *Mueklenbeckia australis* was a main component of the diet of possums living in the Otari reserve, Wellington (Purchas 1975).

2.2.6.2 Seed storage

Metcalf (1995) reports that *Muehlenbeckia* stores well. Hong et al. (1998b) report that *Muehlenbeckia australis* demonstrated an orthodox storage behaviour.

2.2.6.3 Seed germination

According to Metcalf (1995) raising *Muehlenbeckia* from seed is not difficult. In contrast Baars & Kelly (1996) state that *M. australis* is difficult to germinate. Metcalf (1995) recommends that the seed be cool-moist stratified for 2 months before sowing. Bryant (1994) reports that the optimum germination temperature range is 13-20°C. He recommended that the seed be lightly covered and that it takes 10-28 days for the seed to germinate at the optimum germination temperatures. Metcalf (1995) states that germination should occur within 1 month.

2.2.6.4 Seed germination experiments

Burrows (1996b) reported that seed took 35 days for the seed to begin germinating and 166 days to complete germination, with 97 % of seed germinating. Seed germinated in dark and soil treatments, although they were slower to germinate compared with the standard treatment. The final percentage of seed was relatively high, 72% and 68% respectively. Seed did not germinate in the coldest part of the winter so Burrows (1996b) believes that *M. australis* is affected by a degree of environmental constraint.

2.2.6.5 Other germination experiments

Burrows (1996b) demonstrated that the surrounding fruit of *M. australis* does not have an inhibitory affect on the germination of the seeds as the final germination percentage was only slightly lower than the standard treatment, 88% and 97% respectively.

2.2.7 *Myrsine australis*

Plate 2.7: *Myrsine australis* newly planted at Massey University (Turitea Campus).

2.2.7.1 Species Description

Myrsine australis (A. Rich.) Allan (mapou, mapau, red matipo) is classified within the family Myrsinaceae, a family consisting of about 32 genera and 1000 species of trees and shrubs which has about 10 species endemic to New Zealand (Salmon 1996; Cave & Paddison 1999; Metcalf 2000). *Myrsine australis* is a much-branched, densely foliaged shrub or small tree, 3 to 6 m tall. A distinctive feature of this tree is while the bark on the mature trunk is grey, the young branches and branchlets are red to reddish brown (Salmon 1996; Metcalf 2000). The narrow leaves (3 to 6 cm long and 1.5 to 2.5 cm wide Allan 1961), on red petioles up to 5 mm long, are pale green or yellowish green with strong undulate margins and hairy midveins (Salmon 1996; Cave & Paddison 1999; Metcalf 2000).

There are separate male and female flowers that are on different plants. The clusters of unisexual flowers occur along the bare branches and arise in the axis of the leaves (Sampson 1975). They are 1.5 to 2.5 mm in diameter and consist of four small sepals, four whitish petals, four stamens and a central ovary. The sepals are free or partly joined towards the base, and the petals are free or partly joined (Metcalf 2000). The smaller female flowers have a large fringed stigma (Salmon 1996). There is evidence that the unisexual flowers have evolved from bisexual ones, for the male flowers have sterile reduced ovaries and the female ones have small, non-functional stamens (Sampson 1975). Flowering is from December to

April (Sampson 1975; Salmon 1999). Flowering can be spasmodic, for example, Leathwick (1984) observed at Whirinaki State Forest, in 1980 that flowering was heavy resulting in an abundance of fruit in autumn 1981, but in the next summer no flowers were produced.

The fruits are drupes, that turn black when ripe (Salmon 1996). The small fruits (2 to 3 mm in diameter) have a fleshy interior and a central hard region (endocarp), which encloses a single-seed (Sampson 1975). The endocarp is buff brown, pale orange-yellow or pale brown and is 3.5-4.5 x 2.4-2.7 mm (Webb & Simpson 2001). Clusters of berries can be found from October through to February (Sampson 1975; Salmon 1999). It takes approximately a year for a mature fruit to develop from the ovary of a female flower so both flowers and mature fruits can be found on the tree at the same time (Sampson 1975; Leathwick 1984). Fruiting is irregular, with gaps of several years with no or few fruit between heavy fruiting years (Sullivan & Burrows 1995). It is difficult to distinguish between ripe and unripe fruit because of the gradual changes in fruit colour during ripening (Leathwick 1984). The fruit are eaten by birds, which disperse the seed (Burrows 1996d). Silvereye (*Zosterops lateralis*), tui (*Prosthemadera novaeseelandia*) and whitehead are attracted to the fruit (Crowe 1999). Kereru (*Hemiphaga novaeseelandiae*) were seen eating unripe fruit (Burrows 1996d). O'Donnell & Dilks (1994) reported that kereru and bellbird (*Anthornis melanura*) also eat ripe *M. australis* fruit.

Seedlings of *M australis* can be epiphytic as they have been found growing on the trunks of tree fern *Dicksonia squarrosa* on both Stewart Island and Bench Island (Stewart & Veblen 1979).

There are several Maori names for *M. australis*: the most commonly is mapau. Bushmen, in the past, misused the name by calling mapau 'maple' or 'red maple' because of the colour of the stems. Fortunately these misused names have died out (Metcalf 2000).

Maori used *M. australis* wood for digging sticks and composite adze handle sockets (Cooper & Cambie 1991). Maori also used the branches of the tree to perform karakia or incantations (Crowe 1999). The leaves yielded an infusion, which Maori used as an analgesic to treat toothache (Brooker 1975; Hedge et al. 1995). The timber has been used by cabinetmakers both in solid form and as a veneer. It was recommended for handles of chisels and other carpenter's tools and for wooden chairs because the wood is very strong (Crowe 1999).

Myrsine australis has antiviral properties which is due to the presence of triterpene saponins. All 8 oleanane-type saponins compounds isolated from *M. australis* exhibited antiviral activity against Polio type I and herpes simplex type I viruses (Bloor & Qi 1994). Two of the saponins exhibited cytotoxic activity against P388 leukaemia cells (Bloor & Qi 1994, Hegde et al. 1995). The leaves contain rutin, used in the relief of arthritic problems. It also contains embelin, which has been used elsewhere as a remedy for skin disease, intestinal worms and as a general tonic (Crowe 1999).

Mapou is found throughout the North, South and Stewart Islands and is found from sea level to 900 m (Metcalf 2000). Occurs in lowland to mountain forests especially around forest margins and scrubland (Allen 1961, Metcalf 2000). It is often rather local in its distribution (Metcalf 2000).

Myrsine seed was collected on Cook's third voyage to New Zealand by the gardener called David Nelson. These seeds were grown in many places through out the United Kingdom and Europe and planted in private and botanical gardens. It is noted that *Myrsine* was growing in Kew Gardens in 1934 and it was considered as being a hardy genus when grown out doors as it did not require any form of protection (Cooper & Cambie 1991). Although this plant is considered hardy it does not tolerate waterlogging or long periods of severe drought (Pollock 1986; Crowe 1999b). *Myrsine australis* is considered to be an excellent foliage plant and to keep a dense bushy shape when planted in the garden it does require pruning (Crowe 1997b; Cave & Paddison 1999). It grows rapidly in infertile soil with ample moisture (Pollock 1986). There is another form of Mapou called *M. australis* 'McKenzie's Gold'. This is a variegated form, with leaves that have a central yellow zone and green margins (Cave & Paddison 1991). It was discovered in a small remnant of bush on the hillside in the Pencarrow area of Wainuiomata township in 1979 (Metcalf 1982; Metcalf 2000).

The fungus *Vizella tunicata* is parasitic on leaves of *Pittosporum tenuifolium* and *M. australis* (Gadgil 1995).

Myrsine has a moderately small crown and is very wind-tolerant so it is well suited for use in low shelter. It is useful in intermediate stages of restoration and revegetation of native vegetation (Pollock 1986). It is a mid-successional to later-successional plant (NZERN 2002). Kennedy (1978) and Johnson (1978) demonstrated this. Kennedy (1978) described the native

vegetation succession on the small island east of Stewart Island. A loess deposit some meter's deep with soils enriched by guano caps it. Coastal shrubland of *Hebe elliptica, Olearia angustifolia* and *Senecio reinoldii* gives way to low forests of either *Myrsine australis* or *Myrsine chatamica* inland. On dunes in Fiordland National Park older dunes typically carried a low forest of *Myrsine australis, Hedycarya arborea* and *Griselinia littoralis* while the oldest dunes carried a high forest with *Metrosideros umbellata* and *Weinmannia racemosa.* This is typical native vegetation succession for this particular area (Johnson 1978). It can be used for sand dune restoration, slope stabilisation and as a windbreak (NZERN 2002). Its regenerative versatility is enhanced due to it being capable of producing root sprouts (Burrows 1994).

2.2.7.2 Seed Storage

There are no reports on the storage behaviour of *Myrsine australis* but *Myrsine africa* demonstrated orthodox storage behaviour and that seeds are maintained in the long-term storage facility (Plant Genetic Resources Centre), Ethiopia (Zewdie 1994 – cited in Hong et al. 1998b).

2.2.7.3 Seed Germination

Crowe (1999) reports that it grows easily from seed. Metcalf (1995) states that germination is slow and can take from 2 to perhaps 18 months.

2.2.7.4 Seed Germination Experiments

Burrows (1996d) reported that *M. australis* germination is intermittent or episodic spread over a long period. Seeds took 112 days to first germination and were complete by 290 days resulting in 100% germinating. A few seed germinated over winter and early spring but the majority germinated the following autumn. When seeds were placed in soil they germinated faster but with only 54% germinating. Seeds placed in the dark were slow to germinate and success was low (42%). Seeds that were kept dry (kept at room temperature and humidity in petri dishes) for 7 months then placed in the same conditions as the standard treatment, after 171 days, 100 % germinated within 16 days.

Metcalf (1995) recommends cool-moist stratification for 2-3 months before sowing and to germinate in cool to intermediate conditions. A commercial packet of *M. australis* seed (New Zealand Tree Seeds) recommends that the seed be kept moist in refrigerator at 2-5°C for 56 days before sowing.

2.2.7.5 Other Germination Experiments

Burrows (1996d) reported that the fruit has an inhibitory affect on the germination of *Myrsine* seed as only 16% of the seed germinated.

Buried seed germinates moderately well with 68% of seedlings reaching the soil surface between 8-12 months. 12% of the seed that germinated died underground (Burrows 1996d). He reports that *M. australis* could form seed banks of more than a year's duration.

2.2.8 *Phormium tenax*

Plate 2.8: *Phormium tenax* planted as part of a green corridor project at Makino Road, Feilding.

2.2.8.1 Species Description

Phormium tenax J. R. et G. Forst. (flax, swamp flax, harakeke, wharahi) belongs to the small Hemerocallidaceae family, which consists of 3 genera (Germplasm Resouces Information Network 2003). It is also placed in Agavaceae and Phormiaceae. Mabberley (1987 –cited in Hong et al. 1998b) includes Phormiaceae in the Agavaceae. *Phormium tenax* is not true flax but is in fact a lily. The flax name belongs to linseed flax, *Linum usitatissimum*, which is an annual from Europe, and the only point of resemblance is that both plants yield commercially useful fibre. True flax is grown more often for oil than for fibre in New Zealand (Wardle 1975). *Phormium* is a genus of two species of clump-forming perennials. *Phormium tenax* has long, erect sword-shaped leaves, 1 to 3 m long and 5 to 12 cm wide (Moore & Edgar 1970; Cave & Paddison 1999; Salmon 1999). The leaves are formed in fans. They have short stout rhizomes with thick fleshy roots (Metcalf 2000). *Phormium* 'Yellow Wave' is one of several cultivars that have been developed. It is a hybrid derived from *P. cookianum* (a cream variegated form) and *P. tenax* (a bronze, red-striped form) (Metcalf 2000). *Phormium* 'Yellow Wave' was selected from a batch of *Phormium* Rainbow Hybrid seedlings (Heenan 1991). It grows from 1 to 1.2 m with drooping foliage (Cave & Paddison 1999; Metcalf 2000). The leaves are heavily variegated with yellow. This cultivar was produced about 1967 (Metcalf 2000).

Phormium tenax reproduces asexually by the production of ramets (Harper et al. 1970) and sexually by large inflorescences (Craig & Stewart 1988). Flowers are hermaphroditic and

protrandrous (Craig 1989). In late spring, flowering stems develop at the bases of leaves and very rapidly shoot up to their full height (as much as 4.3 m). Each consists of a greenish-black central stem over 3 cm thick bearing 15 or more side branches some 20 cm long, which in turn bear up to 50 bisexual flowers (Wardle 1975). The flowers open up on October, November or December. They are dull red or yellowish and the perianth is curved (Metcalf 2000). The flowers stand erect and are 3 to 4 cm long and the tepals fit closely together to form a nectar-filled vessel (Wardle 1975). There are 6 stamens and the ovary is sessile. Honey eating birds, especially tuis, eagerly seek out the copious supply of nectar (Wardle 1975). Flax has a heavy nectar flow every three years (Van Eaton 1983). The visiting of tuis to *P. tenax* is probably one of the most common examples of ornithophily seen in New Zealand (Godley 1979). Godley (1979) believes that the orange pollen is carried accidentally by the birds from one plant to the other. Another bird, the introduced starling (*Sturnus vulgaris)* likely pollinates flax plants as they have been observed taking nectar from *P. tenax* (McCann 1956). *Phormium tenax* is thought to be predominately bird pollinated, but the nectar and pollen is utilised by a variety of native and introduced insect species including honey bees (*Apis mellifera)* (Murphy & Robertson 2000). Their study discovered that (in the Tongariro National Park New Zealand) fruit and seed set was significantly higher at sites with bird visitation. It is suggested that flax have a flexible pollination system that enables it to maintain a range of fruit and seed levels under different pollinator (e.g. birds and insects) regimes. Van Eaton (1983) recommends that *P. tenax* is planted to provide pollen for bees to overcome the spring deficit faced by many hives in Southland/South Otago. Bees use flax as a pollen source rather than a nectar source. Lloyd & Webb (1986) state that dichogamy, the separation of the presentation of pollen and stigmas in time within a plant, appears in the self-incompatible, protrandrous (stamen matures before carpel) *Phormium tenax* is capable of self-fertilisation but the plant preferentially outcrosses. Few selfed flowers produce seed (Craig 1989; Becerra & Lloyd 1992).

The fruit are dark brown capsules 5 to 8 cm long. They are erect, trigonous and three-valved (Moore & Edgar 1970; Metcalf 2000). The capsules are mature by early January. By early autumn, they have split and released their black, shiny, papery seeds to the wind (Wardle 1975). The seeds are elliptic, plate-like but slightly twisted and measure 9 to 10 mm long and 4 to 5 mm wide (Moore & Edgar 1970). Naturally outcrossed flowers produce mainly large seeds with large amounts of endosperm. Self-pollination from the same inflorescence produces many small seeds and very little endosperm. Compared with the latter, self-pollination between inflorescences of the same flowers produces larger seeds with

intermediate amounts of endosperm (Craig 1989). The New Zealand flaxseed contains oil with nutritional qualities (Morice 1971).

Flax plants in different localities and habitats can vary in appearance (Metcalf 2000). For example, the leaves of some plants droop for most of their length. Variations are generally thought to be inherited but some, including the stunted plants growing in exposed coastal places or peat swamps, where the water is sluggish and acid, may reflect the environment (Wardle 1975). Distribution, taxonomy, morphology and cultivation of *P. tenax* were investigated by Fuentealba (1975) and it was concluded that *P. tenax* is polymorphic. This is due to the degree of rigidity of the leaves and the fibre contents of the leaves. Leaves range from being erect to weeping. The content of fibre ranges from 10-16% according to the variety; of this, 70% is first quality fibre, although the proportion depends upon the length of the leaf, larger leaves producing a greater quantity of the first quality fibre.

This has been one of the most valuable and versatile of the New Zealand plants. Rope and millions of wool bales are only two of the products produced from flax (Macdonald 1973). In former times, before the coming of the white settlers, flax was the Maori people's principal fibre. The leaves were split and turned into baskets or kits that were used for food gathering, cooking and storage. Clothing such as cloaks, capes and sandals, and household items, such as mats and sieves were produced from the leaves. The name *Phormium* comes from the Greek word *phormion, which* means mat. Also, ropes and nets were produced (MacDonald 1973; Cooper & Cambie 1991). Maori were aware that there were different qualities of fibre produced by the different cultivars of *Phormium*. Cultivars with weak fibre were given the collective name Haro and cultivars with good quality fibre were given the name Tihore. Good quality fibre could only be scraped out with a nail while poor quality fibre was scraped out with a shell (Heenan 1991). When the white settlers arrived in New Zealand they too saw the value of *Phormium*. From about 1820, the settlers employed the Maori to prepare increasing quantities of flax fibre for export. In 1830 there was a boom with more than 2700 tons of flax being exported to over seas markets (Hargreaves 1975; Cooper & Cambie 1991). In the 1860s the invention of a scrapping and percussion machine to produce fibre by mechanical means, "ignited the market" (Hargreaves 1975). By 1940 the trading of flax had dwindled. In the early 1900s, thousand of acres of flax plants were growing in New Zealand. The Manawatu area was the main centre of activity. Local mills produced 14000 tonnes of flax each year (Cooper & Cambie 1991). The beginning of the end happened from about 1910 when yellow-leaf virus disease spread throughout the plantations, and also the draining of swamps to

establish high value dairy pastures (Wardle 1975). Today the flax industry is almost non-existent and flax is used mainly to make traditional Maori crafts and ornamental garden specimens. It is part of the cut foliage market (Waitomo News 1989). Maori women mainly produce traditional Maori crafts. They make plaited flax kit (Kete) to be sold in tourist and gift shops. The Maori also make decorative patterned panels called tukutuku panels by weaving dressed and dyed strips of flax (Pendergrast 1984). The chief use of the plant, other than in horticulture, is as windbreaks in exposed coastal places and along fence lines on farms.

The flowering stalks once they have released the seeds begin to dry. The dry stalks, that the Maori call korari, are extremely light. The Maori people bound together the stalks to make temporary rafts, or moki. It is noted that in 1853 Nathaniel Chalmers (he was the first European to reach the Wanaka district) and his Maori companions sailed down the Clutha river on a moki (Wardle 1975).

The Maori people used flax for medicinal purposes. Large leaves could be used as splints (MacDonald 1973). The gum that is found at the base of the leaves was used to cure burns, scalds, old sores, and ease rheumatic and sciatic pains. It was also applied to wounds to staunch the flow of blood and protect them from infection (Brooker 1975). Early settlers found relief from arthritic pain. Roots boiled in water and then drunk relieved constipation. The roots have purgative and anthelmintic properties. External use of the root liquid cured ringworm and was used on babies' skins for chafing. The gum, or jelly, of *P. tenax* has healing qualities and contains antiseptic properties (Macdonald 1973). There are many other medicinal uses. Because *Phormium* was used so much in Maori traditional medicine, especially the roots, Harvey & Waring (1987) investigated root extracts to discover what the properties were. Antifungal compound musizin, stypandrone, beta-sitosterol and hexaconasol were isolated and identified. The Maori people believed that the placing of flax leaves on the skin would allow demons to escape through them (Brooker 1975).

Parts of the flax are edible. The nectar was collected by the Maori and used either alone or to sweeten other dishes (Crowe 1997a). The Maori call flax-honey waikorari (Cooper & Cambie 1991). Nectar consists mainly of sugars (fructose, sucrose, and glucose) along with a small quantity of vitamins and minerals. Water forms about 55% (Brouk 1975). Flax nectar mixed

with water was one of the few drinks the Maori used apart from plain water (Tregear 1926). Seed oil is rich in linoleic acid and also contains palimitic, oleic and stearic acids (Cambie 1989).

Cucurbitacins (Cucurbitacin D, cucurbitacin I, isocucurbitacin D and 3- epiisoucurbatin D) have been isolated from the leaves, the later two being cytotoxic. They showed significant inhibitory activity against lymphocytic leukaemia in mice and against cells derived from human carcinoma of the nasopharynx (Kupchan et al. 1978).

Phormium tenax can be found in suitable habitats from Stewart Island to the North Cape of the North Island, and the offshore Chatham and Auckland Islands (Allen 1961).). It is also native on Norfolk Island. On the Auckland Islands it is apparently an introduction (Wardle 1975). *Phormium tenax* occurs from sea level to 1370-1400 m (Pollock 1986; Metcalf 2000). Flax is typically a plant of wet soils, abundant in coastal and lowland swamps. Swamp flax is less robust, except along water channels. It is plentiful on low-lying riverbanks and lake shores where water is abundant but not stagnant (Wardle 1975; Metcalf 1998). Flax does not establish in areas that are permanently submerged but established plants that do become flooded remain quite healthy (Wardle 1975). Flax is not confined to only growing in swamps or along river banks as it can also be found on hillsides, especially where there are seepages, and lower mountain regions (Metcalf 1998). *Phormium* swamps, in the early days, covered a large part of lowland New Zealand, especially on the Hauraki, Manawatu, Canterbury, and Southland plains. In Westland the largest remaining flax swamps occur. Present vegetation within the wetlands is a mixture of kahikatea (*Dacrycarpus dacrydioides*) swamp forest, *P. tenax* – dominated shrubland and rushland, and oligtrophic bogs. It is important to protect this area. Conservation issues in the wetland primarily reflect activities associated with drainage and other alterations of the water regime (Sorrell & Partridge 1999).

Phormium has along history of cultivation. The Maori who selected and named the plants made the first selections of flax plants by the quality and strength of their fibres (Wardle 1975). In contrast, the modern cultivars selected for ornamental horticulture since about 1860's, are chosen for the different growth forms and leaf colouring (Heenan 1991). There were three variegated varieties that the Maori did grow as ornamental plants as two of them had very poor quality fibre (Heenan 1991). Flax make very good garden plants that can be grown as shelter or hedging, in containers or as foliage contrast in a mixed planting (Cave & Paddison 1999).

It makes an excellent feature plant alongside water or terraces (Metcalf 2000). When growing flax in the garden it responds best to generous watering and it is best placed in permanently moist conditions (Bryant 1997). This species has a very wide habitat range and climatic tolerance. It grows well overseas as reported by Vitek & Dobes (2000) who discuss *P. tenax* naturally invading the Cantabrian coast of Spain. Flax will grow in damp or dry conditions such as very wet and water logged soils or dry hillsides. It can withstand strong and coastal winds and very tolerant of salt spray. It is extremely frost hardy (Pollock 1986; Cave & Paddison 1999). Warrington & Stanley (1987) report that the frost hardiness temperature and the lethal temperature in winter were -6/ -11°C, respectively. In summer, the frost hardiness temperature was between -2 and -5°C, and the lethal temperature was between - 4 and – 9°C. They concluded that *P. tenax's* lowland origin would not lead to natural selection of very frost tolerant forms. Metcalf (1972) regarded it as being 'half-hardy'. *Phormium tenax's* root system is not deep or wide spreading but it will grow and maintain roots below the water table (Pollock 1986). Propagation is by seed and by division into fans (Bryant 1997). The roots, when planting, must not be planted deeper than their original depth. This is especially important in very wet soil (Pollock 1986).

Phormium tenax is susceptible to many pests. The major pest is the Phormium yellow-leaf virus, which stunts the plant, causes leaves to yellow, eventually wilting and the whole plant collapses. A small native plant hopper (*Oliarus atkinsoni*) is the main means by which the virus is spread (Leifting et al. 1997) and it has been suggested that seed also carry it (Metcalf 2000). Infected plants should be destroyed and for propagation purposes it is recommended that only healthy plants be used. Observations by Ushiyama et al (1969) suggest that a mycoplasma-like organism rather than a virus cause the yellow leaf disease. The Phormium yellow leaf phytoplasma is associated with strawberry lethal yellow disease. The phytoplasma is closely related or identical in some cases (Anderson et al. 1998). *Phormium tenax* is more susceptible to the virus than *P. cookianum.* Larvae of two moths attack *P. tenax* - edge cutting flax moth (*Melanchra steropastis*), which feed along the leaf margins into which they cut deep, V-shaped incisions, and flax moth (*Orthoclydon praefectata*), which chew longitudinal furrows into the surface tissues of the leaves, thus exposing the fibres which eventually die leaving holes in the leaves (Metcalf 2000). Other insects to attack flax are the cabbage tree scales (*Leucaspis cordylinidis* and *L. stricta*), that form soft, white, encrusted patches on the undersides of the leaves. A fungal disease that attacks both form of *Phormium* is Phormium leaf spot (*Glomerella cingulata* and *Gloesporium phormii*), that produce areas of brownish or greyish dead tissue at the tips and along the margins of leaves (Metcalf 2000).

The New Zealand jumping spider (*Trite planiceps*) lives in the rolled-up leaves of *P. tenax* and similar plants (Taylor & Jackson 1999). They require the rolled-up leaves as part of their mating rituals.

An excellent use of flax in revegetation projects is next to waterways. It stabilises the banks of drains and small streams and the leaves shade the water thus helping to prevent weed growth. Fallen dead leaves remain attached to the rest of the plant and they can partially break the force of water impinging on the banks. *Phormium tenax* is excellent in areas that are subjected to seasonal waterlogging such as gully systems. It is also good used in projects that require some shelter such as wet lowland areas, coastal swamps or estuaries, and seasonally dry, wind swept hillsides (Pollock 1986). Pollock (1986) recommends the taller erect forms, including the cultivars *P. tenax* 'Goliath' (of unknown origin but grown in the Christchurch Botanic Gardens for many years – Metcalf 2000), 'Purple Giant' (unknown origins but plants have been seen in a few old gardens around Christchurch – Metcalf 2000), 'Variegatum' (one of the oldest cultivars grown in New Zealand – Metcalf 2000) and 'Williamsii Variegatum'. *Phormium tenax* is a pioneer/colonising plant and also in naturally a mid-successional plant in some areas (NZERN 2002). *Phormium tenax* has a potential for use in restoration plantings as it can act as a nurse plant facilitating the succession from pasture to forest (Porteous 1993; Reay & Norton 1999). They observed that in some areas of the Port Hills, Canterbury, *P. tenax* appears to be invading lightly grazed and ungrazed grasslands thus providing opportunities for indigenous woody species to establish that are not otherwise present because of competition. *Phormium tenax* is relatively fire-resistant (Porteous 1993; Crowe 1997b) and can be used to establish a buffer between a forest remnant and a potential source of fire. It has the ability to recover from fire by responding from the base of the plant (Porteous 1993). It can be useful for restoring sand dunes (NZERN 2002).

2.2.8.2 Seed Storage

Metcalf (1995) reports that the seed stores well. Seed can be cold moist stored for several months before sowing at a later time. Bergin & Van Dorsser (1988) recommend that cool, dry storage (2° - 4°C) is required for *P. tenax*. Hong et al. (1998b) reports that both *P. tenax* and *P. cookianum* have orthodox storage behaviour.

2.2.8.3 Seed Germination

Bergin & Van Dorsser (1988) state that flax seeds germinate readily. Sow seed when fresh Metcalf (1995). Germination usually occurs within about 4 weeks. Metcalf (1995) reported that seed that was cool-moist stored for 5 months germinated within 12 days. A commercial packet of *P. tenax* seed (New Zealand Tree Seeds) recommends that the seed be kept moist in refrigerator at 2-5°C for 42 days before sowing. Bryant (1994) suggests covering the seed and placing the tray in a dark place until germination. In some areas damping off and collar rot can be a problem. If damping off appears to be a problem then a suitable fungicide can be used. The choice of parent plant for the seed has an influence on the incidence of damping off (Metcalf 1995). The optimum germination temperatures for quick and even germination of *Phormium* are 13-25°C and at these temperatures it should take 10-35 days to germinate (Bryant 1994). A commercial packet of *P. tenax* seed (New Zealand Tree Seeds) states that viable seed per gram is approximately 100.

2.2.9 *Sophora prostrata*

Plate 2.9: *Sophora prostrata* bush growing on a hillside at Banks Peninsula. Inset: *S. prostrata* flower.

2.2.9.1 Species Description

Sophora is a genus of 52 species (Hong et al. 1998b) of trees and shrubs found in Asia, Americas, Australia and New Zealand (Cave & Paddison 1999). *Sophora prostrata* Buchan. (dwarf kowhai, prostrate kowhai) belongs to the Fabaceae family and is only found in New Zealand (Markham & Godley 1972: Taylor 1975b). *Sophora prostrata* is usually a low, rounded bush with densely intertangled rigid branches (Godley 1975) but in more sheltered places, such as, gullies, it can grow more erect, less tangled and attain 2 m or more (Allan 1961: Metcalf 2000). This is a divaricating kowhai with zigzag stems. Side buds grow out as soon as they are formed and push the main shoot apex aside. The axis of the branch is tipped over first to one side and then to the other as it develops, resulting in alternate branching (Taylor 1975b). Re-curved extremities of branches and stems form a dense canopy with a relatively smooth outer surface (Godley & Smith 1978 cited in Carswell & Gould 1998). The bark is orange to yellow-brown in colour. Leaves are up to 2.5 cm long consisting of 2 to 4 pairs of leaflets, with or without a terminal leaflet (Allan 1961: Metcalf 2000). Foliage is sparse. This species is not deciduous (Godley 1975)

The flowers are solitary or 2 to 3 together on short peduncles and are 2.5 cm long (Allan 1961: Metcalf 2000). It is not a profusely flowering shrub, with fewer flowers than other species of *Sophora*, and when grown in cultivation the flowers tend to be hidden inside the bush (Metcalf 2000). The flowers are yellow to orange consisting of 5 petals consisting of a

standard, wings and keel (Allan 1961: Godley 1975: Metcalf 2000). The petals are loosely held together to form a simple pendulous tube. Within the flower there are 10 stamens which are free and not joined as in most other legumes (Godley 1975). The pedicels are bent, so when compared with other species, the flowers hang up side down. Kowhai flowers tend to contain abundant nectar and pollen, thus attracting a wide range of visitors but the *S. prostrata* flowers are more tightly folded thus having fewer visitors (Godley 1975). *Sophora prostrata* flowers from late October until mid-November (Metcalf 2000). Kowhais do not usually flower for more than about 5 weeks (Godley 1979).

Shophora prostrata has narrowly winged pods, which are 2.5 to 4 cm long. There are 2–5 seeds per pod which are dark to reddish brown (Allan 1961: Metcalf 2000) and 5.4-8.2 mm long (Webb & Simpson 2001). Seeds of this species often have a small elliptic hilum, which is a distinguishing feature. Seeds or dry pods are wind-blown for short distances across the ground. Other species of *Sophora* are water dispersed (Fountain & Outred 1991) but it is unlikely that *S. prostrata* is water dispersed as the seeds are not buoyant in either fresh or seawater (Godley 1975: Hurr et al. 1999).

Sophora comes from the Arabic name Sophera that is a name given for some leguminous trees (Goldie 1976). The common name kowhai was derived from the colour of the flowers, as kowhai is the Maori word for yellow.

Maori people used the bark for curing gonorrhoea and ringworm (MacDonald 1973). Kowhai bark ash was incorporated with manuka ash and rubbed on bodies to cure skin rashes (Brooker et al. 1998). Bathing in water, which had been steeped with crushed bark, aided the recovery of bruises and fractures (MacDonald 1973). Bark of kowhai and manuka were boiled together and the liquid was drank in order to cure internal pains (Brooker et al. 1998). In contrast, Kowhais today are classed as being poisonous shrubs and trees and cause diarrhoea and abdominal pains (Crowe 1997a). The petals can be collected to obtain a natural plant dye of yellow, when added with alum mordant, and gold, when added with bichromate of potash (Crowe 1999).

Maori have long known the medicinal uses of *Sophora.* The occurrence of alkaloids with medicinal potential in kowhai is being explored (Cooper & Cambie 1991). Georgadze (1947) investigated the pharmacology of *Sophora* alkaloids.

The bark contains the phytoalexin 3-hydroxy-8, 9-methylenedioxy-pterocarpin while the leaves contain a mixture of flavonoid C- and O-glycosides. The roots of *S. prostrata* contain 14 flavonoid compounds (Iinuma et al. 1995). The alkaloid diosmin (hesperidin) was isolated from the flowers. Hesperidin is useful in haemorrhages both internal and external (Brooker et al. 1998). Perfume essences from the petals of kowhai have potential to be incorporated in the production of toilet soaps (Brooker et al. 1998). Due to the phenolic constituents found in *S. prostrata* seed coat and leaves it is readily distinguishable from the other species of *Sophora* (Markham & Godley 1972).

Shophora prostrata is found between Marlborough and South Canterbury on the lowlands and hills of the eastern South Island (Allan 1961; Goldie 1976; Metcalf 2000) from 76 m to 760 m (Metcalf 2000). It will grow in full sun or semi-shade and requires good drainage as it will grow poorly or die if grown in soil that becomes waterlogged in winter (Crowe 1997b; Cave & Paddison 1999). *Sophora prostrata* is frost hardy (Crowe 1997b). Warrington & Stanley (1987) confirmed that during winter the frost hardiness and lethal temperature was approximately - 6 and -11°C respectively. In summer, the frost hardiness temperature was between -2 and -5°C, and the lethal temperature was between - 4 and – 9°C. In general, *S. prostrata* is hardier than other species of *Sophora*.

Sophora, especially *S. microphylla* and *S. tetraptera*, have a long history of cultivation. *Sophora* was listed in the *Catalogue of the plants Cultivated in the Royal Botanic Garden Kew* (published in 1789). No reference was made regarding *S. prostrata* being grown at Kew. Seeds of *S. tetraptera* were taken back to England by Joseph banks in 1771 and raised in Chelsea in 1774 (Cooper & Cambie 1991). London nurserymen Loddigies and Grimwood offered both *S. microphylla* and *S. tetraptera* for sale in England in 1783. *Sophora prostrata* can be suitable for planting in a rock garden (Crowe 1997b). The colourful flowers make kowhai a popular garden plant especially if the gardener wants to attract birds to the garden.

Each year the kowhai loses most of its leaves to the caterpillar of the kowhai moth (*Mecyna maorilis*) (Dugdale 1975; Bryant 1997). *Sophora* spp. are prone to attack by larvae of *Stathmopoda aposema* (a type of moth) (Sullivan & Burrows 1995). Another insect that attacks kowhai is the kowhai scale insect (*Eriococcus kowhai*), which is particularly devastating as an attack can result in the death of the tree. A fungal disease that is common in Otago is witches' broom of kowhai (*Uromyces edwardsiae*), which form noticeable witches'

brooms on the branchlets, and cause the seed pods to become inflated and deformed (Metcalf 2000).

Sophora prostrata is a pioneering/colonising to mid-successional plant. Is useful for slope stabilisation, sand dune restoration and grown as a hedge to act as a windbreak (NZERN 2002).

Sophora prostrata, in general, appears not to maintain any substantial seed bank on the plant or in the soil (Webb 1993). Due to the seed and pod being light the wind allows pods to disseminate easily.

2.2.9.2 Seed Storage

Metcalf (1995) reports that *Sophora* store well. Hong et al. (1998b) reports that all species examined demonstrate orthodox storage behaviour. *Sophora microphylla*, a species native to New Zealand, had 100% germination after 10 years storage at the Seed Bank, Royal Botanic Gardens Kew (Hong et al. 1998b). Several *Sophora* species retained considerable viability after being dry-stored for 24-40 years (Norton et al. 2002).

2.2.9.3 Seed Germination

Metcalf (1995) recommends before sowing *Sophora* seed they should be either scarified, chipped or given hot water treatment (77-90°C), especially older seeds. Bryant (1994) also recommends that the seed be scarified. As an alternative he suggests that the seed be soaked for at least 48 hours before they are sown. If seeds are picked when they are slightly green and sown immediately germination is usually quick resulting in a high germination percentage. Webb (1993) reported that some seed does germinate without being subjected to scarification and on closer inspection it was found that the only seeds that germinated were taken from younger seedpods. Metcalf (1995) reported that germination of older seed, given hot-water treatment, occurred within 1-3 months, but if the seed was very old, 12 months later the seed looked as good as when it was sown. A commercial packet of *S. prostrata* seed (New Zealand Tree Seeds) recommends pouring boiling water over the seed, being left to cool and soaked for 24 hours. Metcalf (1995) recommends that if hot-water treatment is to be performed on *S. prostrata* then it should only be 50°C as it has a softer seed coat than the other species of *Sophora*. Bryant (1994) suggests covering the seed and placing the tray in a dark place until germination. The optimum germination temperatures for quick and even germination of

Sophora are 15-25°C and at these temperatures it should take 14-42 days to germinate (Bryant 1994).

2.2.9.4 Seed Germination Experiments

Webb (1993) performed several germination experiments using *S. prostrata* and *S. microphylla*. The first sets of experiments were about how these two species may overcome seed coat impermeability in nature. Three areas were considered: fire, microbial activity, and abrasion by riverbeds. *Sophora prostrata* seed is sensitive to fire. Some seeds will germinate after exposure to fire but the vast majorities were destroyed. It was thought that seed coat breakdown in the soil was attributable to microbial decomposition of the seed coat. Webb (1973) discovered that whole seeds of *S. prostrata* are resistant to microbial activity. Webb (1993) concluded that older *S. prostrata* seed probably undergoes an abrasion process in the soil in order for the seed coat to become permeable. As *S. prostrata* is not buoyant it is unlikely that the seed coat be broken by abrasion of the riverbeds, therefore, Webb (1993) did not perform this part of the experiment.

3

MATERIALS AND METHODS

3.1 Seed Collection

Mature fresh fruits were collected from several plants of each species either locally in the Manawatu (*Carex trifida* Cav., *Leptospermum scoparium* J.R. et G.Forst., *Myrsine australis* (A.Rich.) Alan, *Muehlenbeckia australis* (Forst.f.) Meissn., *Phormium tenax* J.R. et G.Forst., and *Sophora prostrata* Buchan.), northern Wairarapa (*Coprosma robusta* Raoul, *Hebe stricta* (Benth.) L.B.Moore), or southern Wairarapa *(Cyperus ustulatus* A.Rich.), southern North Island, New Zealand (Table 3.1).

Fruits were individually harvested by hand and placed in paper bags, except for *Coprosma robusta* and *Myrsine australis*, which were placed in sealed plastic containers. Once harvested, the seeds were subjected to various and appropriate cleaning techniques developed by the author (Appendix B).

3.2 Seed Storage

After cleaning, all seeds were placed into sealed, dried storage (water impermeable 12/20/50 µm laminated polyester/aluminium foil/ polythene packets) at 5°C until required for germination and other experiments. As required, seeds were sub-sampled from each packet and the packet re-sealed. Storage time was variable.

3.3 General Experimental Procedures

In all experimental work the following protocols were used to determine seed moisture content (SMC), seed viability, and germination.

Table 3.1 The nine species of plants, their collection site locations, the map grid reference (NZMS 260), and seed collection dates. (Seed collected in 2001 was used for the initial germination experiment, stratification experiment and effects of storage. To demonstrate the effect temperature has on seed germination 2001 seed of *Hebe*, *Leptospermum, Muehlenbeckia, Phormium* and *Sophora* and 2002 seed of *Carex, Coprosma* and *Cyperus* was used.)

Species	Location	Map grid reference	Seed collection dates
Carex trifida	Massey University Turitea Campus Palmerston North	T24/323878 40°23′20″ 175°37′25″	17 May 2001 15 May 2002
Coprosma robusta	Balance Road, Northern Wairarapa	T24/494922 40°20′50″ 175°49′25″	15 Apr 2001 01 Apr 2002
Cyperus ustulatus	Okau, Southern Wairarapa Coast	U26/680750 40°50′ 176°14′	29 Apr 2001 15 Apr 2002
Hebe stricta	Centre Road, Northern Wairarapa	T24/492924 40°20′45″ 175°49′20″	12 Aug 2001
Leptospermum scoparium	Massey University Turitea Campus Palmerston North	T24/323878 40°23′20″ 175°37′25″	28 Jun 2001
Muehlenbeckia australis	Pohangina Road, Manawatu	T23/453031 40°15′00″ 175°45′55″	19 Apr 2001
Myrsine australis	Massey University Turitea Campus Palmerston North	T24/323878 40°23′20″ 175°37′25″	16 Apr 2001 01 Apr 2002
Phormium tenax	Makino Road, Feilding	S23/283076 40°13′20″ 175°34′10″	1 Apr 2001
Phormium 'Yellow Wave'	Massey University Turitea Campus Palmerston North	T24/323878 40°23′20″ 175°37′25″	20 Apr 2001
Sophora prostrata	Massey University Turitea Campus Palmerston North	T24/323878 40°23′20″ 175°37′25″	9 Apr 2001

3.3.1 Seed moisture content (SMC)

SMC was determined using the constant air oven method for samples normally of 25 or 30 seeds (ISTA 1999) (Table 3.2). Seeds were dried at 103 ± 2°C for 17 ± 1 h, then placed into a desiccator for approximately ½ hour to cool. Seed moisture loss was calculated on a fresh weight basis. SMC was determined immediately after harvest (to give some indication of the moisture content of the seeds when shed from the parent plant), and after the final cleaning process before seeds were placed into storage. As the seed were stored in sealed foil packets, the latter SMC was assumed to be the moisture content for all experiments.

Table 3.2 The number of seeds used for each seed moisture content test (x 4 replicates) and relevant details concerning extraction processes prior to the seed moisture content (SMC) test.

Species	Number of seeds	Notes
Carex spp	25	Seed was removed from utricle.
Coprosma robusta	25	The exocarp and mesocarp were removed after harvesting by placing the drupes in a sieve, gently crushing by hand, and rinsing off the flesh. The endocarp surface was towel dried to remove free water.
Cyperus ustulatus	25	Seed was removed from utricle.
Hebe stricta	Not determined	
Leptospermum scoparium	0.015 g	Capsules were hand-split, thus releasing seeds immediately after being harvested.
Myrsine australis	25	As for *C. robusta*.
Muehlenbeckia australis	25	Seed was extracted from the surrounding fleshy panicle and towel dried to remove free moisture.
Phormium tenax	25	Seed was extracted from capsule by hand.
'Yellow Wave'	30	Seed was extracted from capsule by hand.
Sophora prostrata	20	Seed was extracted from capsule by hand.

3.3.2 Seed Viability

Seed viability was determined using the topographical tetrazolium test (ISTA 1999). Seed was taken from storage after 2-3 months, preceding the stratification experiment. Four replicates of 25 seeds (pooled) were preconditioned by rolling them in moistened seed germination paper (Anchor Paper Company, St. Paul, Minnesota, USA). The roll was placed in a jar with approximately 2 cm of water in the bottom. The jar and roll were placed in a plastic bag at 20 ± 2°C for 24 h. After 24 h a scalpel was used to make an incision in each seed, to expose the embryo (Table 3.3). Seeds were placed in 1% phosphate-buffered 2,3,5-triphenyl tetrazolium chloride solution at 20 ± 2°C for a further 24 h, in the dark (light-excluding photographic film bag). Seed was classified as viable if both the radicle and cotyledon(s) showed uniform red staining.

3.3.3 Seed Germination Experiments

Seed germination was determined for four replicates of 50 seeds (collected in 2001) for each species. There were four treatments. Seeds were either dusted with thiram fungicide (using the ratio of 1 seed volume for every 50 seeds) or left undusted, and these treatments were placed either in the light or the dark (for *Sophora prostrata*, seeds were scarified by chipping with a sharp scalpel). Each replicate treatment was placed on two moist seed germination blotters (Anchor Paper Company) which were placed into a sealable plastic box. Boxes were kept

either in the light (the total irradiance in the photosynthetically active radiation (PAR) range (400 – 700 nm) was measured using a Lambda LI-COR 185 meter with an LI-190 SA quantum sensor to obtain measurements of the photosynthetic photon flux density (PPFD) in $\mu molm^{-2}s^{-1}$) with a continual 24 h, cool white 58 W fluorescence light source (PAR received was between 0.07 $\mu mol\ m^{-2}\ s^{-1}$ and 3.15 $\mu mol\ m^{-2}s^{-1}$ depending on where the box was placed in the 20°C room). Each replicate was treated as a block and treatments were randomised within that block.

Table 3.3 The table prescribes procedures for preparation of pre-moistened seeds before staining.

Species	Incision
Carex spp	Longitudinal incision made above the area containing the embryo.
Coprosma robusta	Seed transversely cut below the radicle. Cut longitudinally along the top of the endocarp.
Cyperus ustulatus	Same as for *Carex* spp.
Muehlenbeckia australis	Longitudinal incision along side of the embryo.
Myrsine australis	Endocarp removed. Longitudinal incision along side of the embryo.
Phormium tenax	Longitudinal incision along side of the embryo.

Note: Seeds of *Hebe stricta, Leptospermum scoparium* and *Sophora prostrata* were not subjected to a tetrazolium test as they had 100% germination in the germination test.

Seed germination was monitored weekly, except for *Leptospermum scoparium* and *Hebe stricta* (monitored every two days) and *Sophora prostrata* (every four days). Dark treatments were counted under a weak green safe light (PAR up to 0.17 $\mu mol\ m^{-2}s^{-1}$). The light had previously been found safe for use in the classic 'Grand Rapids' lettuce Red/Far Red response experiments (D. Fountain, pers. Comm.). A seed was considered to have germinated when the radicle measured approximately 1 mm for all species. Germination was scored when cotyledons were visible (*Coprosma robusta*, *Hebe stricta*, *Muehlenbeckia australis* and *Myrsine australis*), the first true leaves were apparent (*Sophora prostrata*), the coleoptile was visible (*Carex trifida* and *Cyperus ustulatus*), or the coleoptile and first true leaf visible (*Phormium tenax*).

3.3.4 Seed Stratification Experiments

Seeds were dusted with thiram and placed on moist germination blotters as described in the initial germination method. Seeds were stratified by placing four replicate boxes of each species in a 5°C controlled germination room with a 24 h continual 58 W cool light source for periods of 4, 8, and 12 weeks before transfer to the standard conditions of the germination

room (20 ± 2°C, 24 h continual light source). Four replicate boxes of each species, which were set up at the end of the stratification period, served as unstratified controls.

Data were analysed by analysis of variance (ANOVA) using SAS for Windows (Release 8.2 (TS 2mo), SAS Institute, Cary, NC, USA). Prior to analysis data were checked for normality and homogeneity of variance using the proc univariate procedure in SAS.

Additional *Myrsine* Seed Stratification Experiments

2001 and 2002 *Myrsine australis* seed were assessed in 2002 using the previous stratification experiment protocols. Due to the lack of seed, seed germination was determined for four replicates of 15 seeds.

In addition to the above experiment, *Myrsine* seed of 2002 was stratified for 4, 5 and 6 months (07/08/02, 11/07/02, 06/06/02, respectively). Protocols previously mentioned were used except four replicates of 50 seeds were used for each stratification treatment. Seed were transferred to the standard conditions of the germination room (20 ± 2°C, 24 h continual light source) on the 04/12/02. Seed was left to germinate for a period of 2 months, after which, 50% of the seed in each replication of each treatment had the endocarp removed and the other 50% the endocarp was left intact. The seed was put back into the standard conditions in order to germinate.

In addition to the above experiments, four replicates of 50 *Myrsine* seeds had the endocarp removed and placed into the standard conditions of the germination room (20 ± 2°C, 24 h continual light source) for a period of 6 months (9/02/03 to 12/07/03). They were then stratified for a period of one and half months (12/07/03 to 30/08/03) by being placed into a 5°C controlled germination room with a 24 h continual 58 W cool light source, after which, they were returned to the standard conditions of the germination room.

3.4 The Effect of Seed Storage

After 12 months storage, in sealed water impermeable 12/20/50 µm laminated polyester/aluminium foil/ polythene packets, at 5°C, seed viability for *Carex trifida*, *Coprosma robusta*, *Cyperus ustulatus*, *Muehlenbeckia australis*, *Phormium tenax* and *Sophora prostrata* was determined using the topographical tetrazolium test (ISTA 1999).

Seed viability of *Hebe stricta* and *Leptospermum scoparium* was assessed using the germination test.

3.5 The Effect of Temperature on Germination

Note: *Myrsine* was not included, as the dormancy mechanisms were not known at this stage.

This experiment was conducted using a Grant Temperature Gradient Plate that was situated in a 20 ± 2°C controlled germination room with a 24 h continual 58 W cool light source. The plate had a possible range of 0°C to 100°C, and a stability of ± 1°C. For all experiments, the germination plate was set to run with a temperature gradient in one direction from 4°C to 35°C. For every 25 mm along the plate there was a change of 1°C in one direction. A data logger with two probes positioned at each end of the plate recorded temperatures over time, to check deviations from the set values.

Packing, Kimpac®, was soaked in water and placed on the base of the plate, followed by three layers of wet Anchor Steel Blue seed germination blotters. In order for the temperatures to stabilise the plate was left for several days. The light source was the same as that used in the germination experiments. The actual light received by the seeds (measured underneath the two layers of clear glass on top of the plate) was approximately around 4.9 μmol $m^{-2}s^{-1}$.

Before being subjected to the effects of temperature seed viability was determined using the topographical tetrazolium test (ISTA 1999). Between June 2002 and January 2003 each species was subjected to the Grant Temperature Gradient Plate (Table 3.4). Before being subjected to the temperature plate some of the seed species required dormancy to be broken. *Sophora prostrata* was scarified using a laboratory scale scarifying drum (Forsberg) with a diameter of 15 cm and a length of 13 cm. The drum was lined with medium grit (P150) sandpaper. The seeds were placed in the drum and scarified for a period of 8 seconds at 1425 RPM. After being placed on the gradient plate seeds that did not imbibe were chipped with a scalpel (data not shown). *Muehlenbeckia australis, Phormium tenax, Carex trifida, Cyperus ustulatus* and *Coprosma robusta* were stratified for a period of time at 5°C (33 days, 98 days, 106 days, 106 days and 100 days respectively) as recommended from the stratification experiment (Table 4.4).

Table 3.4 Dates that seeds were placed on the Grant Temperature Gradient plate and the number of seeds used

Species	Date started	Date finished	No. of seeds
Sophora prostrata	27 May 2002	1 Aug 2002	25
Hebe stricta	7 Aug 2002	4 Sep 2002	50
Leptospermum scoparium	7 Aug 2002	4 Sep 2002	0.015 g
Muehlenbeckia australis	6 Sep 2002	14 Oct 2002	25
Phormium tenax	14 Oct 2002	11 Nov 2002	20
Carex trifida	26 Nov 2002	6 Jan 2003	25
Cyperus ustulatus	26 Nov 2002	6 Jan 2003	25
Coprosma robusta	7 Jan 2003	Not completed	25

Seeds were placed onto the blotters at positions corresponding to 10 different constant temperatures: 6°C, 8°C, 10°C, 14°C, 18°C, 20°C, 22°C, 24°C, 26°C, 30°C, and 32°C. Each seed lot was placed onto an area 150 mm along the temperature gradient, and 10 mm across the gradient (so all were within 0.4°C). For each treatment there were four replicates. Due to the nature of the plate it was impossible to randomise the treatments, therefore, the experiment did not have a statistical design.

Counts began when seeds began to germinate. There were a lot of experiments to complete in the time available for this project and therefore a time restriction had to be placed on the length of time each experiment was run. Consequently, all experiments, except the *Sophora prostrata* experiment, were run with a total allowable time limit of approximately one month. *Sophora prostrata* went for approximately 2 months. Fortunately for most temperature experiments this was satisfactory time to display anticipated trends. Due to these time limits some of the treatments were concluded before germination was complete. At regular intervals the blotters were re-moistened. The germination percentage results were graphed, and the standard errors of the means (SE) were calculated and plotted:

$$\text{where } SE = \sqrt{\frac{S^2}{n}}$$

n = number of replicates; s = standard deviation

For rate of germination a "time to 50% radicle emergence" (T_{50}) was calculated. The following formula was given by Coolbear et al. (1984):

$$T_{50} = t_i + \left[\frac{\frac{(N+1)}{2} - n_i}{n_j - n_i} \right]$$

N = final number of seeds that germinate
t_i = count prior to 50% radicle emergence
t_j = count subsequent to 50% radicle emergence
n_i = number of seeds germinated at time t_i
n_j = number of seeds germinated at time t_j
Where $n_i < (N+1)/2 < n_j$ and n_i & n_j are adjacent counts

The times to 50% germination were also graphed.

3.6 Fixation and Evaluation of Seed Material for Light Microscopy

Whole seeds of *Carex*, *Coprosma*, *Cyperus*, *Hebe*, *Leptospermum*, *Muehlenbeckia* and part seeds of *Myrsine*, *Phormium* and *Sophora* were fixed in a primary fixative (3% glutaraldehyde and 2% formaldehyde buffered in 0.1 M phosphate (PO_4) buffer at pH 7.2) for two hours at room temperature. The sections were washed, at room temperature, by immersing them in fresh 0.1 M phosphate buffer and then repeated two more times. The samples were then fixed in the secondary fixative (1% osmium tetroxide (OsO_4) in 0.1 M phosphate buffer) for one hour at room temperature.

The samples were then washed two times as previously stated in 0.1 M phosphate buffer and the dehydrated in a graded acetone series:

1. 25% acetone: fifteen minutes
2. 50% acetone: fifteen minutes
3. 75% acetone: fifteen minutes
4. 95% acetone: fifteen minutes
5. 100% acetone: fifteen minutes
6. 100% acetone: one hour.

All the samples were infiltrated in a 50/50 percent acetone/resin mixture (Procure™ 812 epoxy resin). The samples and mixture were in a sealed container and were stirred overnight at room temperature. The container was opened and the acetone allowed to evaporate, leaving the samples infiltrated with resin. Samples were transferred into 100% resin and stirred for a further seven hours at room temperature. After this time period samples were embedded in fresh resin in a silicone rubber mould and cured at 60°C for 48 hours.

The samples were removed from the moulds and trimmed to give a parallel cutting surface. One μm sections were cut from the blocks using a Leica Ultracut R ultramicrotome. Sections were heat mounted on glass slides and stained with 0.1 M phosphate buffered 0.05% Toluidine Blue. The slides were then assessed using a Zeiss Axioplan compound light microscope. Photographs were taken with a MC100 35 mm format camera.

4

RESULTS

4.1 Fruit and Seed Description

4.1.1 *Carex trifida*

Germination is hypogeal. Seed reserves are stored in the plentiful endosperm.

Plate 4.1: *Carex trifida* seed heads.

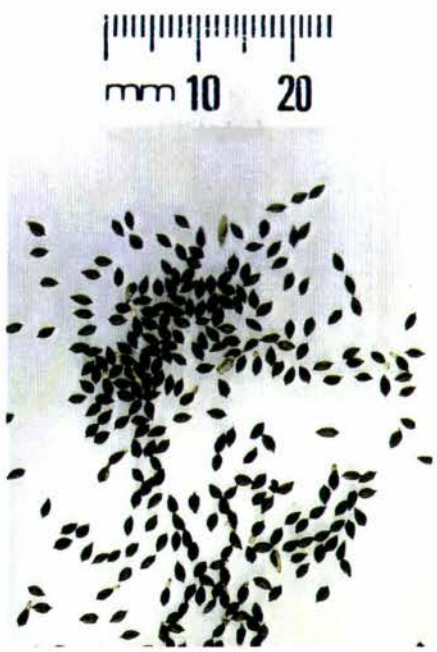

Plate 4.2: *Carex trifida* seeds that are trigonous in shape.

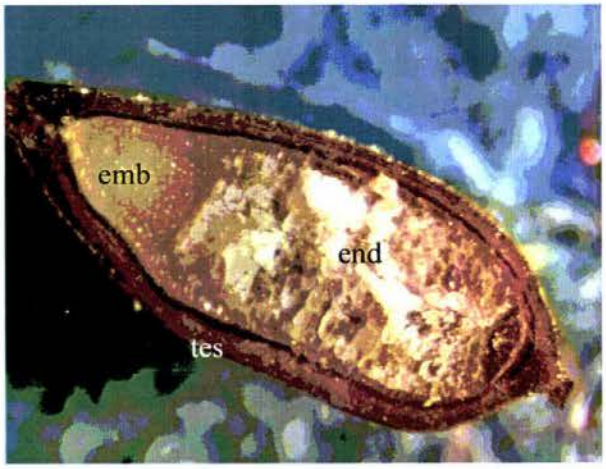

Plate 4.3: *Carex trifida* seed cut in half longitudinally, showing embryo (*emb*), testa (*tes*) and endosperm (*end*), which fills most of the seed. (x 40)

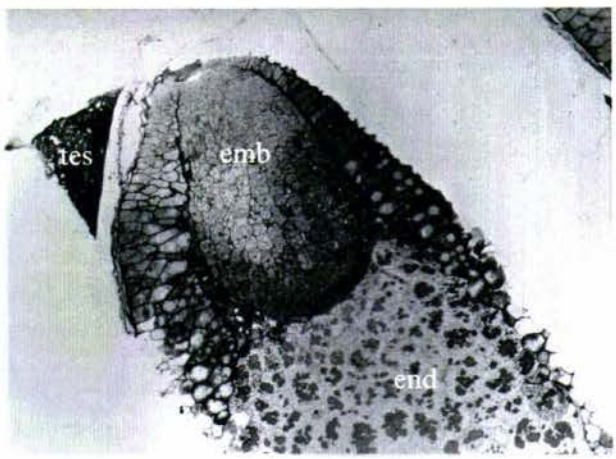

Plate 4.4: Light microscopic oblique section of *Carex trifida* seed, showing embryo (*emb*), testa (*tes*) and endosperm (*end*). (x 125)

4.1.2 *Coprosma robusta*

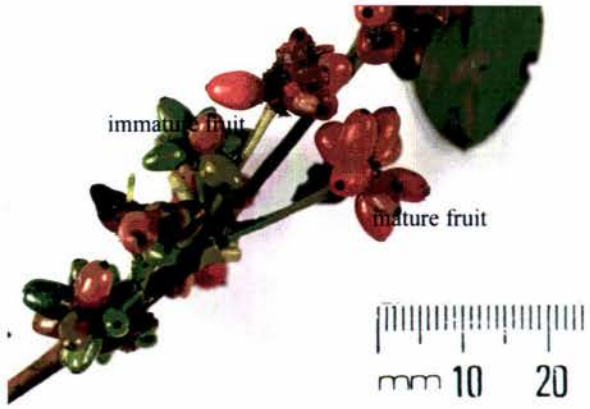

Plate 4.5: *Coprosma robusta* mature and immature fruits.

Plate 4.6: Seeds of *Coprosma robusta*.

Plate 4.7: A *Coprosma robusta* seedling and germinating seed. Germination is epigeal and the cotyledons start photosynthesising when they rise above the soil.

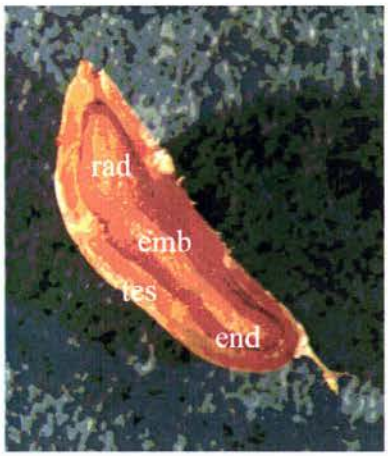

Plate 4.8: A *Coprosma robusta* seed cut in half longitudinally, showing embryo (*emb*), radicle (*rad*), testa (*tes*) and endocarp (*end*). Endosperm surrounds the entire embryo. Seed reserves appear to be stored in both the cotyledons and endosperm. (x 16)

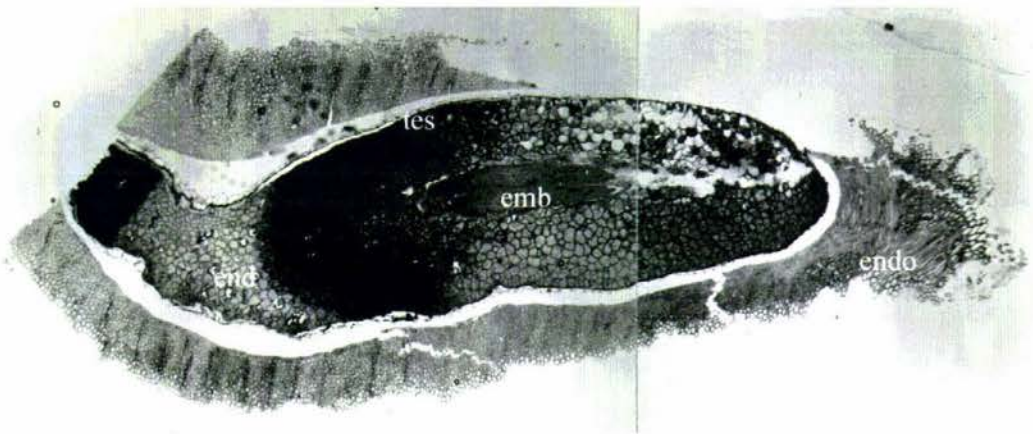

Plate 4.9: Light microscopic section of *Coprosma robusta* seed, showing embryo (*emb*), testa (*tes*), endocarp (*endo*) and endosperm (*end*). Note, the embryo is damaged. (x 63)

4.1.3 *Cyperus ustulatus*

Germination is hypogeal. Seed reserves are stored in the plentiful endosperm.

Plate 4.10: Seeds of *Cyperus ustulatus*.

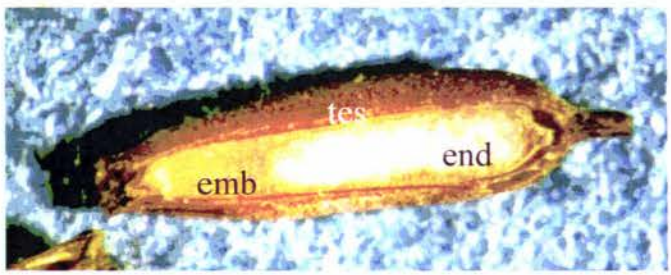

Plate 4.11: A *Cyperus ustulatus* seed cut in half longitudinally, showing embryo (*emb)* testa (*tes*) and endosperm (*end*). (x 25)

4.1.4 *Hebe stricta*

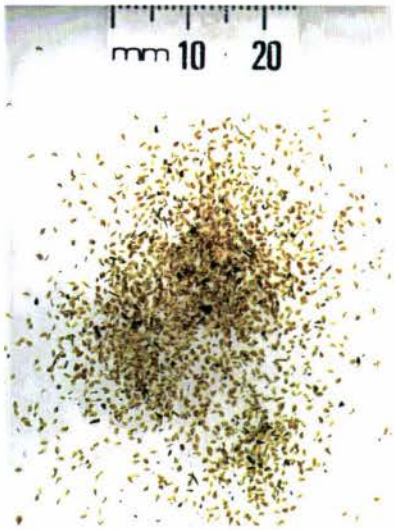

Plate 4.12: Seeds of *Hebe stricta*.

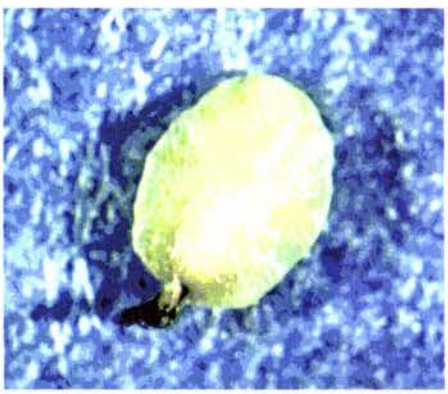

Plate 4.13: An entire, non-endospermic, *Hebe stricta* seed. Reserves are found in the two cotyledons. Note the visible embryo.

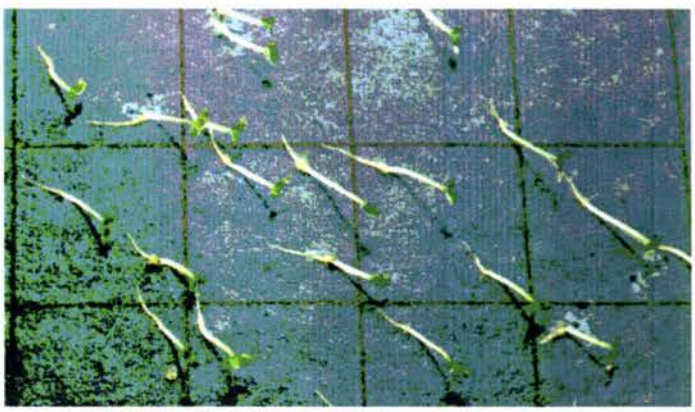

Plate 4.14: *Hebe stricta* seedlings growing towards the light. Germination is epigeal.

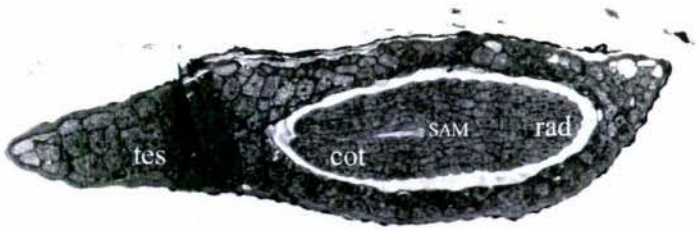

Plate 4.15: Light microscopic section of *Hebe stricta* seed, showing two cotyledons (*cot*), a shoot apical stem (*sam*), testa (*tes*) and radicle (*rad*). (x 125)

4.1.5 *Leptospermum scoparium*

Germination is epigeal. Seed reserves are stored entirely in the two cotyledons.

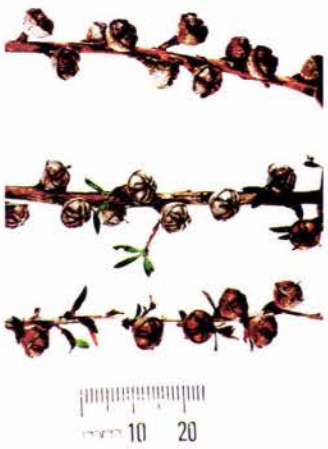

Plate 4.16: *Leptospermum scoparium* capsules.

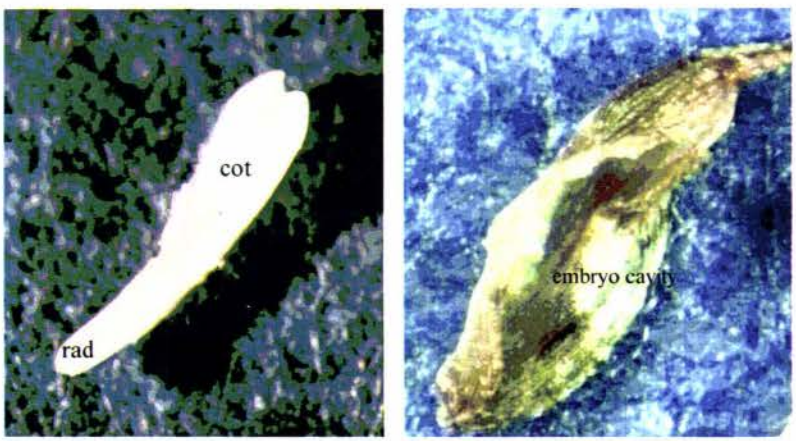

Plate 4.17: Linear *Leptospermum scoparium* embryo with cotyledons (*cot*) and radicle (rad) - left. Golden seed coat from which embryo on left has been removed – right. The embryo completely fills the non-endospermic seed. (x 25)

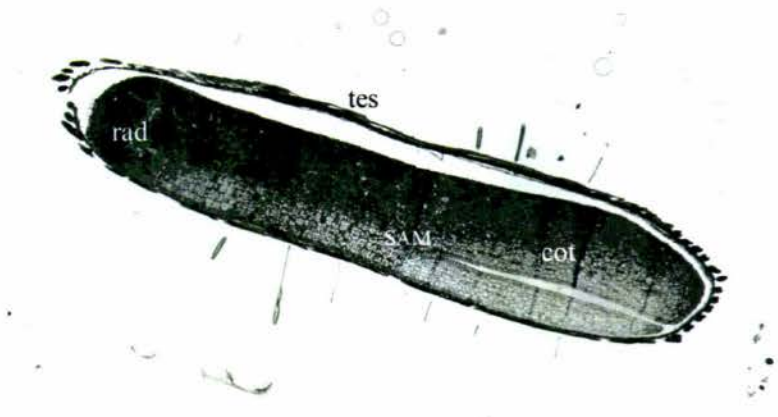

Plate 4.18: Light microscopic section of *Leptospermum scoparium* seed, showing two cotyledons (*cot*), a shoot apical stem (*sam*), testa (*tes*) and radicle (*rad*). (x 63)

4.1.6 *Muehlenbeckia australis*

Plate 4.19: Seeds of *Muehlenbeckia australis*.

Plate 4.20: Trigonous nut-like achene of *Muehlenbeckia australis*.

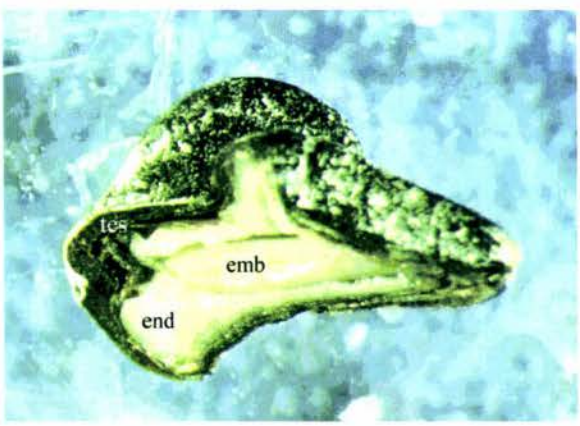

Plate 4.21: *Muehlenbeckia australis* seed cut in half longitudinally, showing embryo (*emb*) and endosperm (*end*). (x 25)

Plate 4.22: A *Muehlenbeckia australis* seedling. Germination is epigeal and the cotyledons start photosynthesising when they rise above the soil. Seed reserves are in the endosperm and the cotyledons.

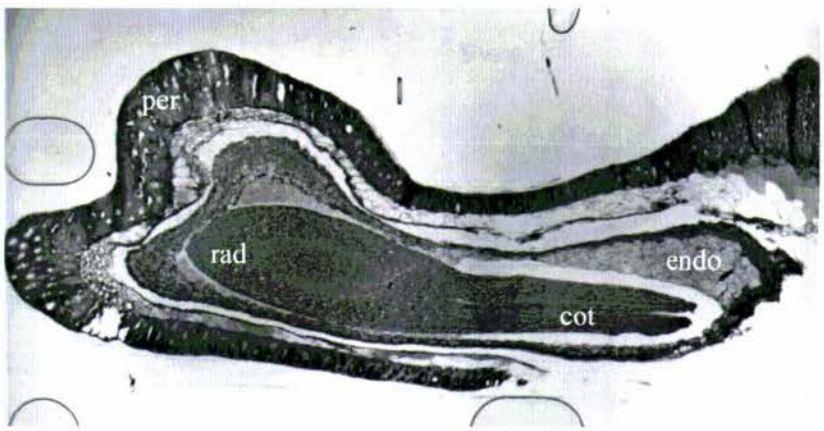

Plate 4.23: Light microscopic section of *Muehlenbeckia australis* seed, showing two cotyledons (*cot*), radicle (*rad*),endosperm (endo) and pericarp (*per*). (x 63)

4.1.7 *Myrsine australis*

Germination is epigeal

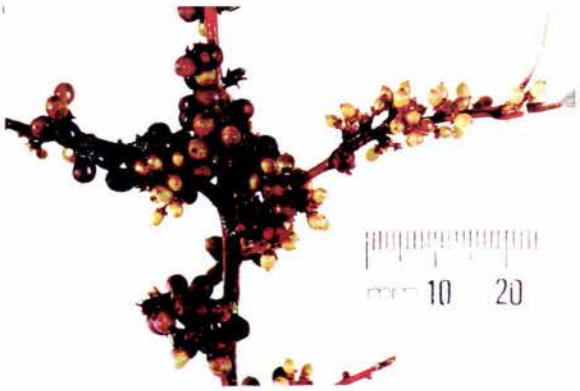

Plate 4.24: *Myrsine australis* mature (black), semi-mature (red) and immature (yellow/green) fruits.

Plate 4.25: Seeds of *Myrsine australis*.

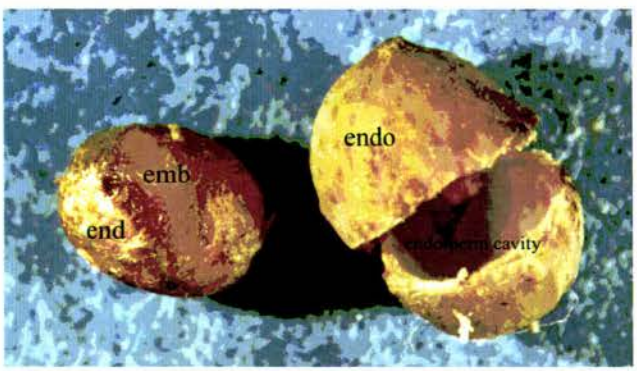

Plate 4.26: *Myrsine australis* seed is endospermous. Endosperm (*end*) and embryo (*emb*) – left, is removed from endocarp (*endo*) - right. (x 16)

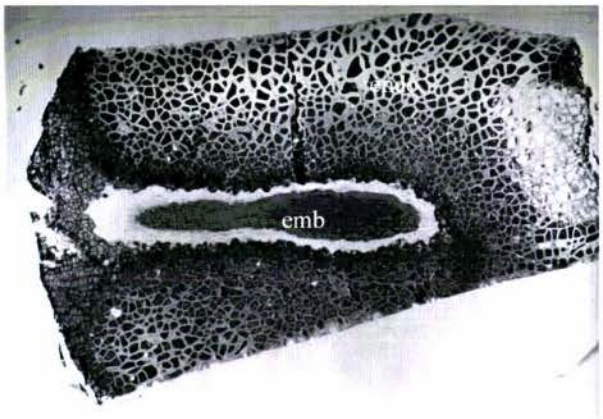

Plate 4.27: Light microscopic section of partial view of *Myrsine australis* seed, showing endosperm (*end*) and embryo (*emb*) (x 63)

4.1.8 *Phormium tenax*

Plate 4.28: *Phormium tenax* seed and capsules

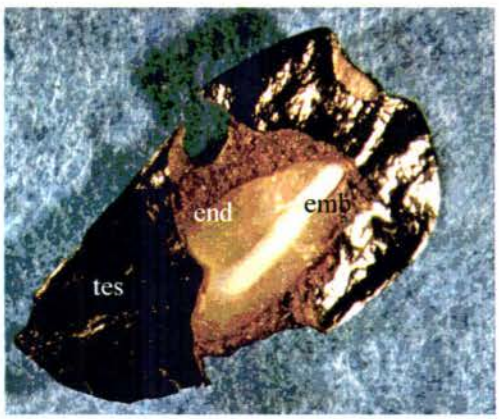

Plate 4.29: Transverse *Phormium tenax* seed section exposing endosperm (*end*) and linear embryo (*emb*). Some testa (*tes*) still intact.

Plate 4.30: A *Phormium tenax* seedling and germinating endospermic seed. Germination is epigeal. Seed reserves are in the endosperm. Note: coleoptile stays attached to the seed coat and first true leaf is evident.

4.1.9 *Sophora prostrata*

Germination is hypogeal. Seed food reserves are found solely in the coteledons.

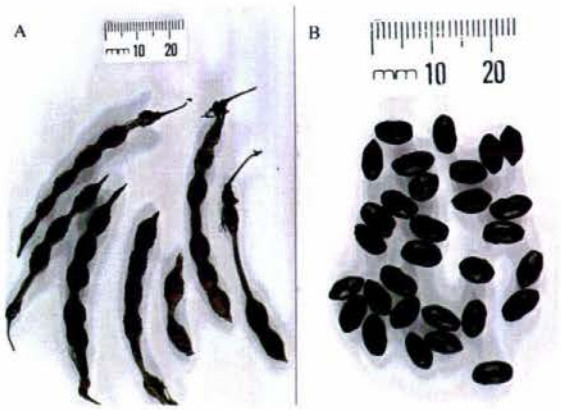

Plate 4.31: *Sophora prostrata* pods and seeds.

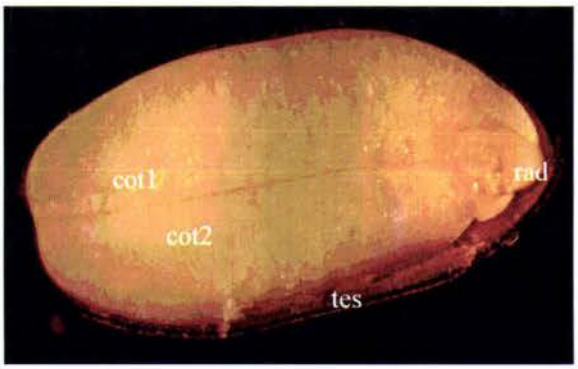

Plate 4.32: A *Sophora prostrata* seed cut in half longitudinally, showing the embryo, consisting of two cotyledons (*cot1* & *cot2*), that fill the entire seed, radicle (*rad*) and testa (*tes*). (x 10)

4.2 Seed Moisture and Viability

At harvest *Carex trifida*, *Cyperus ustulatus*, *Leptospermum scoparium*, *Phormium tenax*, *Phormium* 'Yellow Wave', and *Sophora prostrata* had seed moisture contents of 11-13% and when placed into storage (following limited temperature drying) their moisture contents (8-12%) did not alter greatly. After 18 weeks of storage, viability was high for all of these species (Table 4.1). *Coprosma robusta* had a moisture content of (31%) at harvest but was successfully dried to moisture content of 12% without loss of viability (Table 4.1). *Muehlenbeckia australis*, which had a moisture content of 33% at harvest, dried down to a moisture level of 12% but viability was low (43%). *Myrsine australis* also had high moisture content at harvest (30%), which was reduced to 17% before storage, with 92% viability (Table 4.1).

Table 4.1 Seed moisture content at harvest and storage, and seed viability preceding the stratification experiment. Tz, tetrazolium test; n.d., not determined. Seed viability plus dead seed does not always equal 100%. Empty seed is the difference.

Species	Seed Moisture Content		Viability (Tz) %	Dead %
	Harvest %	Storage %		
Carex trifida	13	8	90	6
Coprosma robusta	31	12	80	17
Cyperus ustulatus	13	9.5	73	16
Hebe stricta	n.d.	n.d.	n.d.	n.d.
Leptospermum scoparium	11	8	n.d.	n.d.
Muehlenbeckia australis	33	12	43	39
Myrsine australis	30	17	92	4
Phormium tenax	13	10	96	4
'Yellow wave'	11	9	96	4
Sophora prostrata	12	10	n.d.	n.d.

4.2.1 The Effect Of Seed Storage after 12 Months

After 12 months, seed viability declined for all species except *Hebe stricta*, *Leptospermum scoparium* and *Sophora prostrata* (Table 4.2). Seed viability of *Carex trifida* and *Phormium tenax* had substantial declines from 90% to 60% viable seed and 96% to 77% viable seed, respectively. In contrast *Coprosma robusta* had a small decline in seed viability from 80% to 72% viable seed. Even though *Muehlenbeckia australis* has low seed viability initially, what seed that was viable remains so, as it only had a small decline in seed viability. *Hebe stricta* and *Leptospermum scoparium* were not subjected to the tetrazolium test instead they were subjected to the germination test and after 12 months storage had 100% germination. *Sophora prostrata* was subjected to the tetrazolium test after 12 months and it was found to have 96%

seed viability. This, when compared with the initial germination experiment (96% germination), demonstrates that after 12 months storage there is no decline in seed viability. Due to lack of *Myrsine australis* seed it was not possible to determine seed viability after 12 months storage.

Table 4.2 Seed viability after 12 months storage. Tz, tetrazolium test; n.d., not determined. Seed viability plus dead seed does not always equal 100% due to presence of empty seed.

Species	Viability (Tz) % After 12 Months	Dead % After 12 months
Carex trifida	60	37
Coprosma robusta	72	28
Cyperus ustulatus	57	39
Hebe stricta	100	0
Leptospermum scoparium	100	0
Muehlenbeckia australis	38	61
Myrsine australis	n.d.	n.d.
Phormium tenax	77	21
Sophora prostrata	96	4

4.3 Seed Germination

Very low germination percentages were recorded in the light and dark treatments for *Muehlenbeckia australis* and *Phormium tenax*, but 100% germination was achieved in the light treatment for *Hebe stricta*, *Leptospermum scoparium* and *Sophora prostrata* (Table 4.3). *Sophora prostrata* also reached 100% germination in the dark treatments. Eighty percent of *Coprosma robusta* germinated but the germination were spread throughout the two years. There was no germination in any of the germination treatments for *Carex trifida, Cyperus ustulatus* and *Myrsine australis.*

Table 4.3 Seed germination data for light and dark (thiram and non-thiram) treatments. †, dormancy within the population precluded complete germination by one year (end of experiment was 29 August 2002 except for *Coprosma* and *Myrsine*, which were terminated 1st March 2003)); n.d., not determined. Tz, tetrazolium test (to determine viable ungerminated seed). *Hebe* and *Leptospermum* dark treated seed were placed in the light at the conclusion of the experiment in order to determine viable seed. % Germinated, fresh ungerminated plus dead seed does not always equal 100%. Empty seed is the difference.

				Percentage Of Seeds Germinated											
				Light						Dark					
		Light		Thiram			Non - Thiram			Thiram			Non - Thiram		
Species	Starting Date	Days to 1st Germination	Days to Complete Germination	% germ	% fresh ungerm Tz	% dead	% germ	% fresh ungerm Tz	% dead	% germ	% fresh ungerm Tz	% dead	% germ	% fresh ungerm Tz	% dead
C. trifida	15 Aug 2001	0	†	0	77 ± 3	23 ± 3	0	74 ± 7	26 ± 7	0	80 ± 5	20 ± 5	0	77 ± 5	22 ± 7
C. robusta	25 May 2001	19	†	80 ± 7	17 ± 3	1 ± 1	61 ± 5	23 ± 16	6 ± 2	0	80 ± 6	20 ± 6	0	82 ± 5	18 ± 5
C. ustulatus	25 May 2001	0	†	0	55 ± 10	45 ± 10	0	55 ± 8	45 ± 8	0	62 ± 5	25 ± 5	0	54 ± 3	29 ± 1
H. stricta	24 Aug 2001	4	10	n.d.	n.d.	n.d.	100	0	0	n.d.	n.d.	n.d.	7 ± 4	85 ± 7	8 ± 4
L. scoparium	30 Oct 2001	4	23	n.d.	n.d.	n.d.	100	0	0	n.d.	n.d.	n.d.	3 ± 1	97	0
Mueh. australis	25 May 2001	26	†	5 ± 1	10 ± 1	85 ± 1	8 ± 3	8 ± 1	82 ± 6	0	13 ± 1	87 ± 1	0	10 ± 2	90 ± 2
M. australis	25 May 2001	0	†	0	88 ± 3	12 ± 3	0	89 ± 2	11 ± 2	0	90 ± 4	10 ± 3	0	90 ± 4	10 ± 3
P. tenax	15 Aug 2001	12	†	11 ± 1	67 ± 3	22 ± 1	16 ± 4	70 ± 2	14 ± 2	1 ± 1	84 ± 7	15 ± 6	1 ± 1	82 ± 8	17 ± 8
'Yellow wave'	25 May 2001	12	†	13 ± 4	64 ± 5	20 ± 2	14 ± 2	52 ± 2	27 ± 2	6 ± 2	77 ± 3	17 ± 5	7 ± 3	69 ± 3	24 ± 3
S. prostrata	20 Aug 2001	7	15	100	0	0	100	0	0	100	0	0	100	0	0

Note: Figures shown are the mean ± standard error.

Table 4.4 Final percentage germination of seeds of *Carex trifida*, *Coprosma robusta*, *Cyperus ustulatus*, *Muehlenbeckia australis*, *Myrsine australis*, *Phormium tenax*, *Phormium* 'Yellow Wave' after stratification at 5°C for 0 (set up after the stratification period), 4, 8 or 12 weeks and subsequent incubation at 20°C. Means in the same row sharing the same letter are not significantly different ($P > 0.05$) The percentage of seeds germinating during stratification (i.e., at 5°C) is given in parentheses.

	Stratification period (weeks)			
Species	0	4	8	12
Carex trifida.	0a	26b	69c	90d
Coprosma robusta	4a	23b	65c	85d (22)
Cyperus ustulatus	0a	34b	26b	64c
Muehlenbeckia australis	11a	28a	25a	31a (10)
Myrsine australis	0a	0a	0a	4a
Phormium tenax	7a	34b	82c	94d (48)
Phormium 'Yellow wave'	8a	57b	81c (3)	95d (76)

4.3.1 *Carex trifida*

Seed viability was 90% (Table 4.1) but no untreated seed germinated. In contrast, stratification for 12 weeks gave 90% germination (Table 4.4). Seed viability was high at the end of the experiment for all treatments (Table 4.3).

4.3.2 *Coprosma robusta*

Seed germinated in the light in both non-thiram and thiram treatments (61 ± 5% and 80 ± 7%, respectively). At the end of the experiment most seed that had not germinated in all treatments remained viable (Table 4.3) No germination occurred in the dark treatment. No abnormal germination was observed in any treatments. Stratification increased germination to 85% (12 weeks). After 12 weeks stratification 22% of the seeds germinated at 5°C before being subjected to 20°C (Table 4.4).

4.3.3 *Cyperus ustulatus*

No germination was seen in any of the germination treatments (Table 4.3), but a high percentage (73%) of the seed was viable at the commencement of the experiment (Table 4.1). More than 50 % of the seed was viable at the end of the experiment for all treatments (Table 4.3). Stratification for 4 or 8 weeks enabled around 30% of the population to germinate, but 12 weeks stratification was needed before the majority (64%) of the population germinated (Table 4.4).

4.3.4 *Hebe stricta*

In the light, germination of *Hebe stricta* began 4 to 5 days after the seed was set to germinate, and 100% germination was reached after 10 days (Table 4.3). In the dark treatment only 7% of the population germinated (Figure 4.1). After 26 days seeds from the dark treatment were transferred to the light and a further 85 ± 7% of them germinated. No abnormal germination was seen.

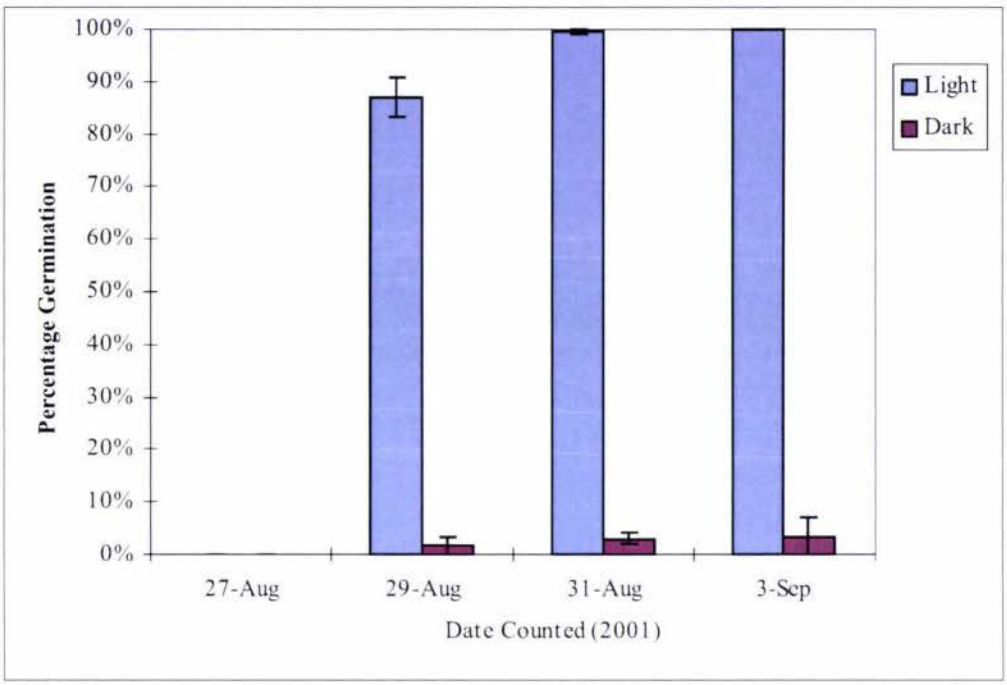

Figure 4.1 The effect of light on the germination percentage of *Hebe stricta* seed.

4.3.5 *Leptospermum scoparium*

In the light treatment, germination began 4 days after the seed was set to germinate and 100% germination of full seeds was reached after 23 days (Table 4.3). Embryo-less seeds were ignored in calculation of percentage germination. In the dark treatment, germination did not begin until after 7 days and only 3% of the population germinated. After 30 days, no further germination was observed (Figure 4.2). When transferred to the light, a further 97% of the seed germinated within 7 days. No abnormal germination was observed

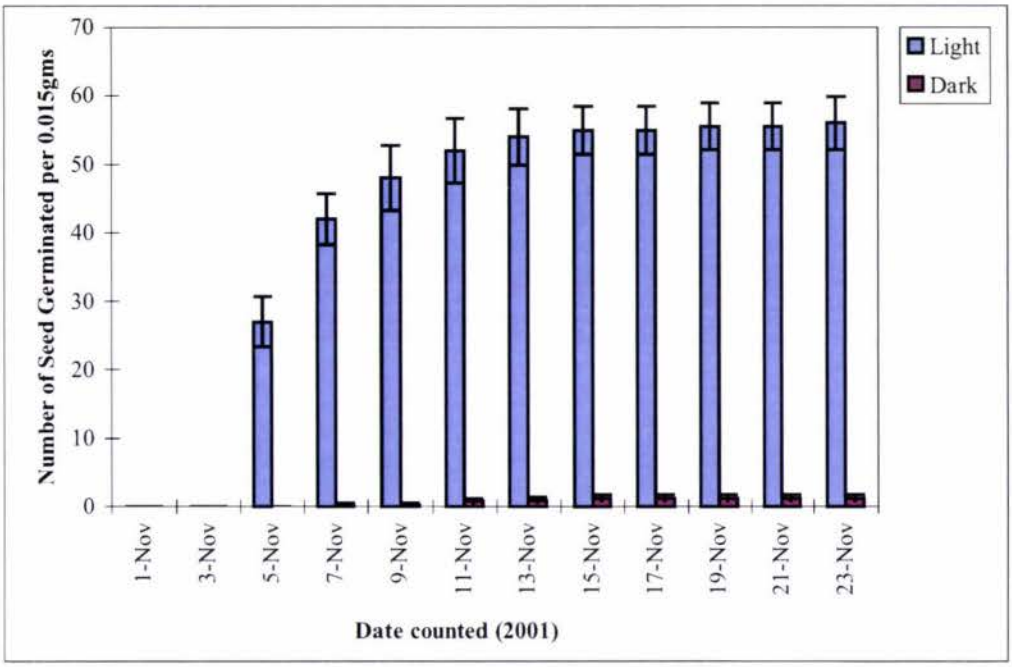

Figure 4.2 The effect of light on the germination percentage of *Leptospermum scoparium* seed.

4.3.6 *Muehlenbeckia australis*

In the light treatment 5 ± 1% and 8 ± 3 of the seeds germinated in the thiram and non-thiram treatments, respectively. There was no germination in the dark (Table 4.3). The viability test indicated that at least 43% of *Muehlenbeckia australis* seed was viable (Table 4.1). The remaining seed did not contain an embryo, was attacked by insects before harvest or were dead. There was substantial fungal growth on the seeds, irrespective of fungicide treatment, and seeds that germinated tended to rot. At the conclusion of the experiment there was a very high percentage of dead seed in all treatments (Table 4.3). Stratification did not improve germination (Table 4.4).

4.3.7 *Myrsine australis*

There was no germination in the light or dark after two years incubation. At the conclusion of the experiment viability of fresh ungerminated seed was high in all treatments (88% to 90% - Table 4.3). After 12 weeks stratification 4% of seeds germinated but there was no further increase to one year of incubation. Viability was high (92%) at the commencement of the stratification experiment (Table 4.1).

Additional *Myrsine* seed stratification experiments

There was no germination in either the 2001 or 2002 seed in any of the stratification treatments (4, 8,12 week and 4, 5, 6 months – data not shown). After a period of 6 months

(kept in constant light and at constant 20°C), half the seeds of the 4, 5, and 6 month stratified seed had the endocarp removed and the other half the endocarp was left intact. Within two days, the seed that had the endocarp removed had 51 ± 3%, 55 ± 3% and 48 ± 6% germination, respectively. Within one month the germination percentage had increased to 92 ± 3%, 88 ± 4%, and 96 ± 3%, respectively. There was no germination in the intact seed.

Seed that had not been subjected to stratification but had only the endocarp removed had some germination (32 ± 10%) after 6 months but this was increased (91 ± 2%) after a period of stratification.

4.3.8 *Phormium tenax*

An initial flush of seeds germinated in the light with 16 ± 4% (non-thiram treatment) and 11 ± 1% (thiram treatment). In the dark, 1 ± 1% (non-thiram and thiram treatments) germinated. At the conclusion of the experiment viability of fresh ungerminated seed remained high (Table 4.3). Stratification for 4 and 8 weeks significantly improved germination, but 12 weeks stratification was required before the majority (94-95%) of the population germinated (Table 4.4). After 12 weeks stratification 48% of the seeds germinated at 5°C. Results for the cultivar 'Yellow Wave' were similar (Table 4.3 and 4.4).

4.3.9 *Sophora prostrata*

After 15 days there was 100% germination in all treatments (Figure 4.3).

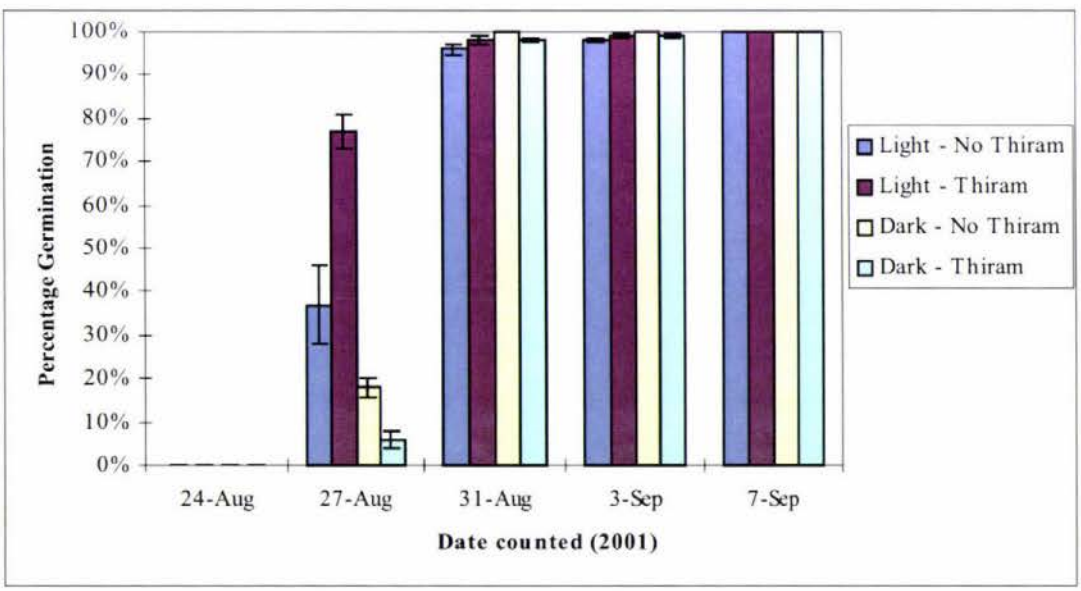

Figure 4.3 The effect of light on the germination percentage of *Sophora prostrata* seed.

4.4 The Effect of Temperature on Germination

Note: *Myrsine* was not included, as the dormancy mechanisms were not known at this stage.

4.4.1 *Carex trifida*

There was no germination of stratified seed at 6°C, 10°C, 14°C, 18°C, 30°C, and 32°C, but there was some germination at 22°C to 26°C. The experiment was terminated before all treatments had finished germinating (Figure 4.4). It is not possible to define optimum temperature ranges for germination due to the low percentage of germinating seed (Figure 4.5). All treatments had low germination percentages. The higher the temperature the slower the rate of germination although there were no significant differences between the treatments (Figure 4.6).

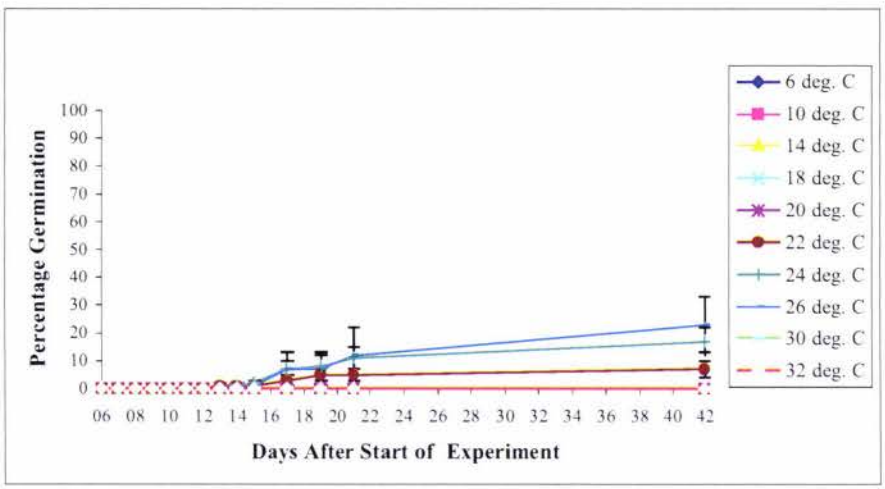

Figure 4.4 The effect of temperature on the germination percentage of *Carex trifida* seed.

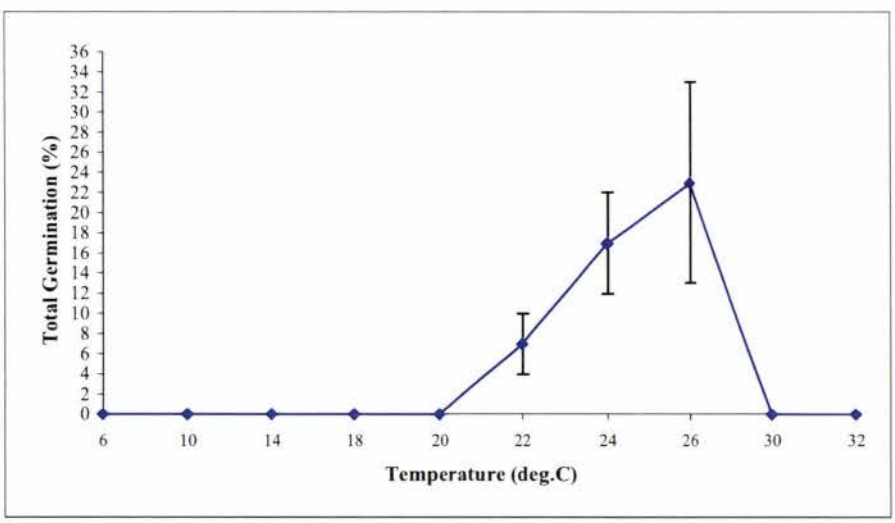

Figure 4.5 Temperature dependence of germination of *Carex trifida*.

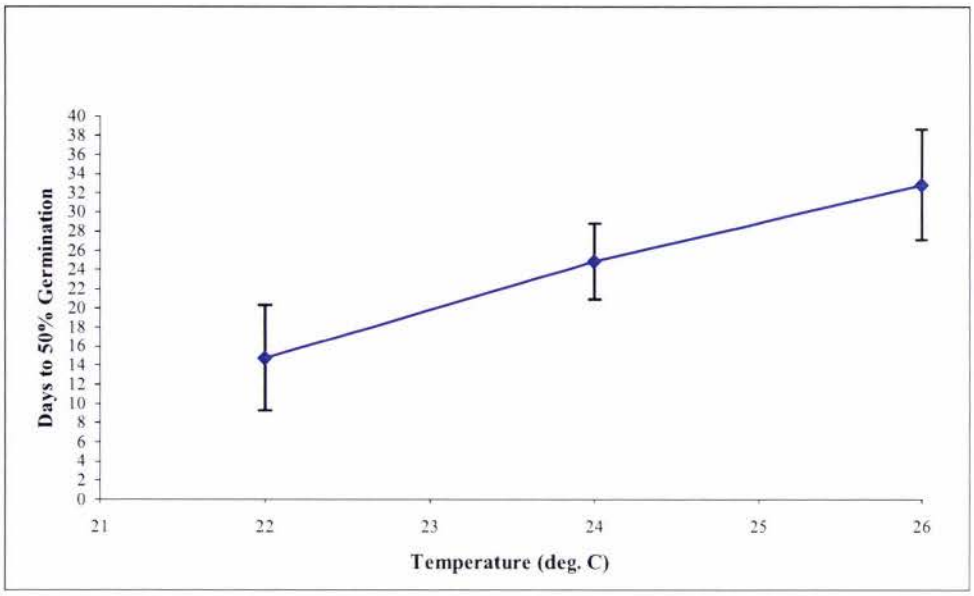

Figure 4.6 The effect of temperature on germination rate of *Carex trifida* seed.

4.4.2 *Coprosma robusta*

There is no data for *Coprosma robusta* due to the Temperature Gradient Plate malfunctioning.

4.4.3 *Cyperus ustulatus*

There was no germination at 6°C, 10°C, and 14°C but some seed germinated at all other temperatures from 18°C to 32°C. The experiment was terminated when there was no increase in germination over a period of 21 days (Figure 4.7). Figure 4.7 show variations of tri-phasic sigmoidial germination curves for all temperature treatments. Germination percentages increased as the temperature increased but at 32°C the germination percentage decreased. The optimum temperature range for germination is between 24°C to 30°C (Figure 4.8). The higher the temperature the faster the rate of germination and shorter the lag phase (Figure 4.7 and 4.9). Germination at 32°C was the most rapid with a T_{50} of 4.7 days. At this temperature, germination was first observed on day 6 and the last seed germinated on day 10. The time to 50% germination at 30°C was 5.1 days. It took 6.7 and just over 7 days at 26°C and 24°C, respectively to achieve 50% germination. At 22°C the rate of germination was 8.4 days and at 20°C the rate of germination was slightly slower although not significantly so, being 9.2 days. At 18°C it slowed to 12 days.

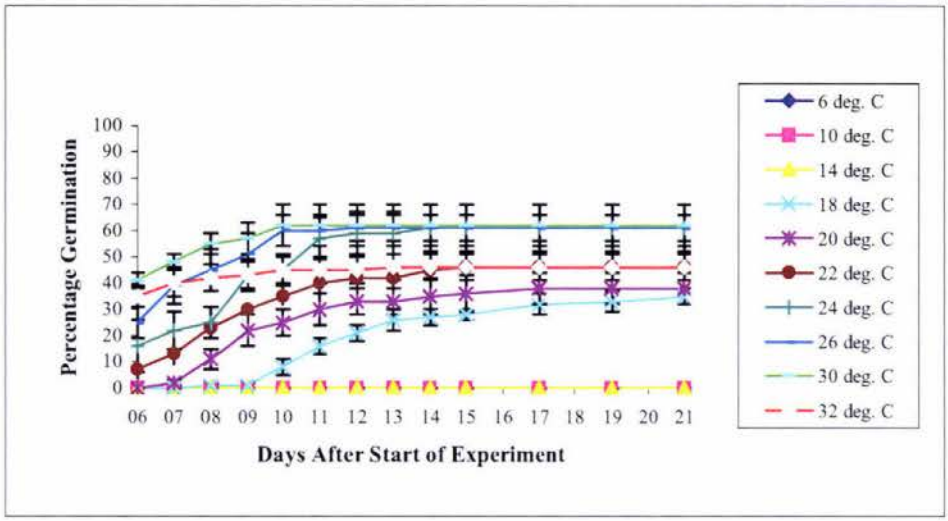

Figure 4.7 The effect of temperature on the germination percentage of *Cyperus ustulatus* seed.

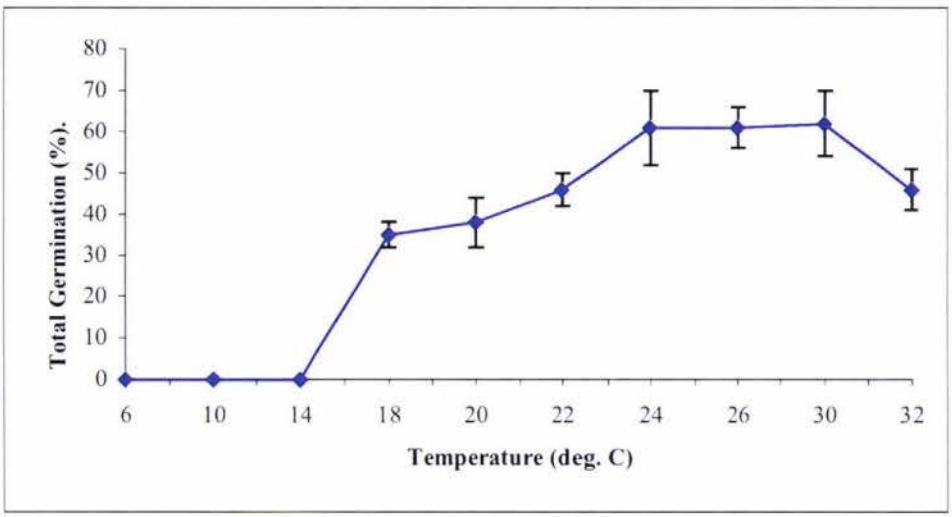

Figure 4.8 Temperature dependence of germination of *Cyperus ustulatus*.

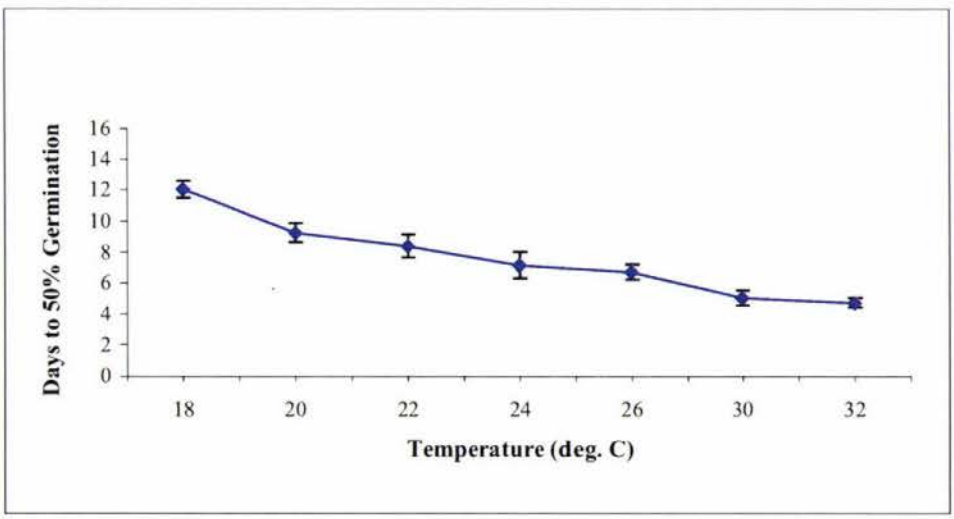

Figure 4.9 The effect of temperature on germination rate of *Cyperus ustulatus* seed.

4.4.4 *Hebe stricta*

There was no germination at 30°C and 32°C but seed successfully germinated at all other temperatures from 6°C to 26°C. The experiment was terminated after 28 days due to time restrictions (Figure 4.10). Figure 4.10 show variations of tri-phasic sigmoidial germination curves for all temperature treatments. At 6°C to 24°C total germination percentages were between 83% and 97% (Figure 4.10 and Figure 4.11) but at 26°C there was a significant decrease in total germination percentages. The optimum temperature range for germination is between 6°C and 24°C (Figure 4.11). The rate of germination increased as the temperature increased and the lag phase was shorter (Figure 4.10 and 4.12) but at 26°C the rate began to slow. Germination at 20°C and 22°C was the most rapid with a T_{50} of 5.7 days. At this temperature, germination was first observed on day 5 and the last seed germinated on day 23. The time to 50% germination at 24°C and 26°C were 6.4 days and 8.15, respectively. It took 6.3 days and just over 7 days at 18°C and 14°C, respectively to achieve 50% germination. At 10°C the rate of germination slowed to 12.4 days, and the time to 50% germination was nearly doubled to 23.2 days for the 6°C treatment.

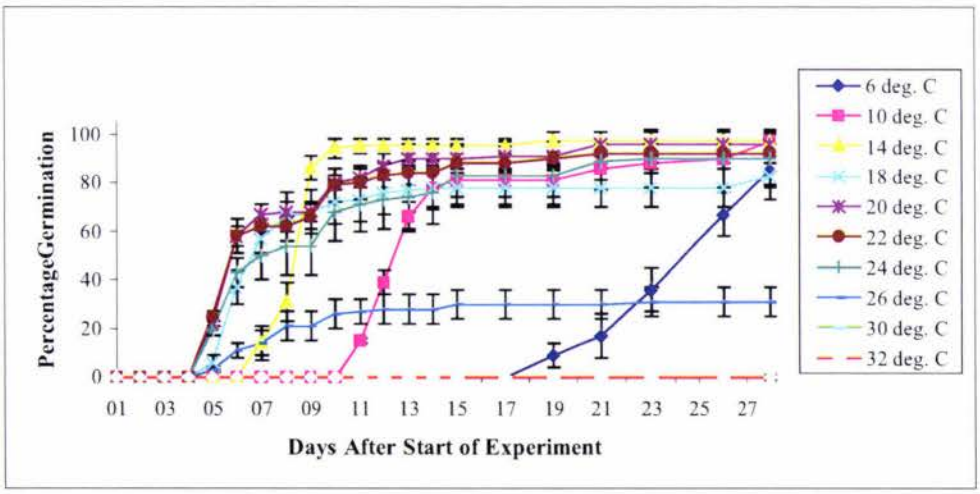

Figure 4.10 The effect of temperature on the germination percentage of *Hebe stricta* seed.

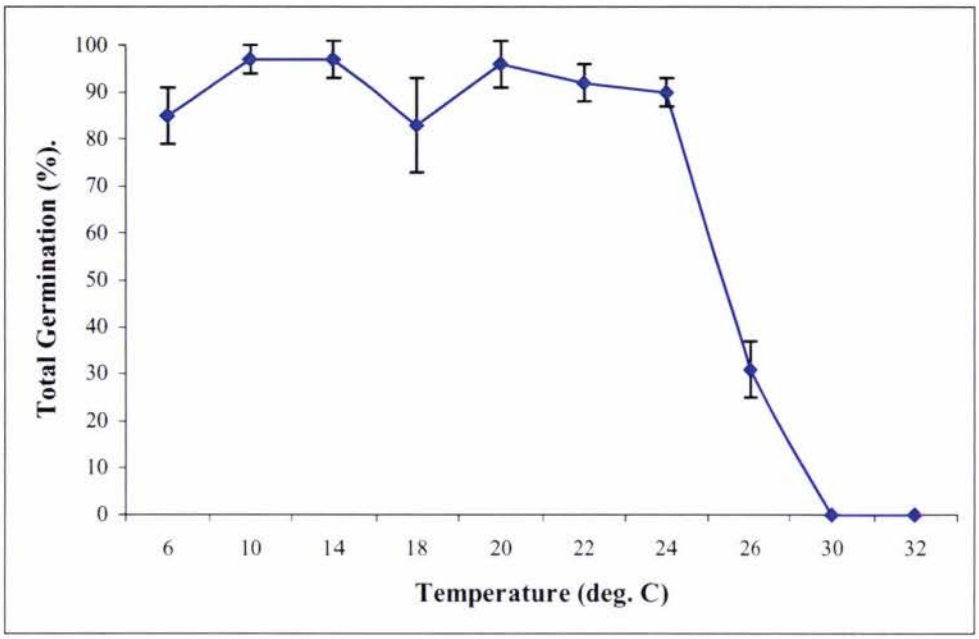

Figure 4.11 Temperature dependence of germination of *Hebe stricta*.

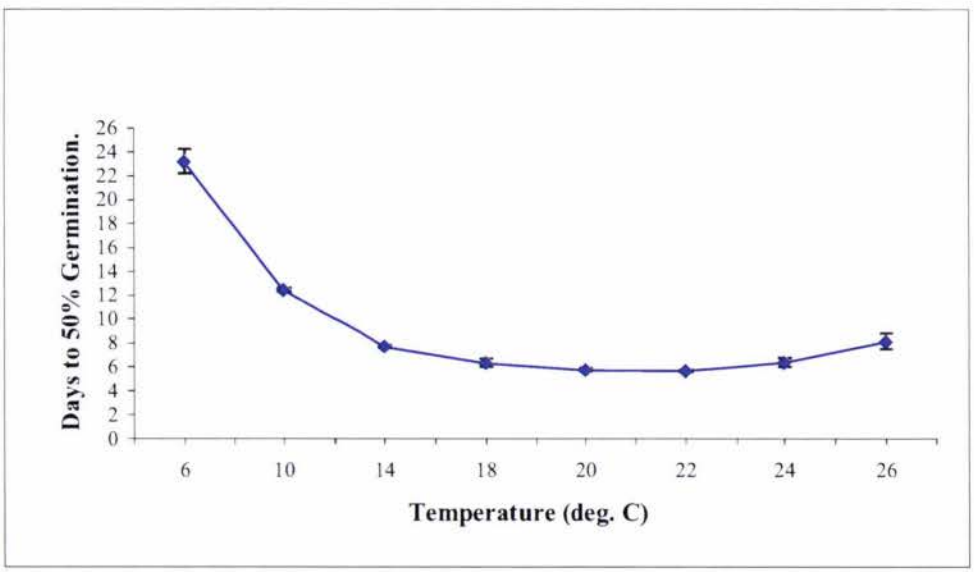

Figure 4.12 The effect of temperature on germination rate of *Hebe stricta* seed.

4.4.5 *Leptospermum scoparium*

At 6°C there was no germination but at all other temperatures from 10°C to 32°C seed successfully germinated. The experiment was terminated after 28 days due to time restrictions but within this time frame most of the treatments had 100% germination (Figure 4.13). Figure 4.13 show variations of tri-phasic sigmoidial germination curves for all temperature treatments.

Total germination was high for all treatments except for the 6°C treatment (Figure 4.13 and 4.14). The optimum temperature range for germination is between 10°C to 32°C (Figure 4.14). The rate of germination increased as the temperature increased and the lag phase was shorter (Figure 4.13 and 4.15). Germination at 30°C and 32°C was the most rapid with a T_{50} of 2.6 days. The time to 50% germination at 24°C and 26°C were 3.8 days and 3.4, respectively. It took 5.3 days and 4.9 days at 20°C and 22°C, respectively to achieve 50% germination. At 18°C germination rate was slightly slower taking 6.8 days. At 14°C the time to 50% germination was nearly doubled to 12.6 days, and at the 10°C treatment germination was very slow, taking 20.5 days to achieve 50% germination.

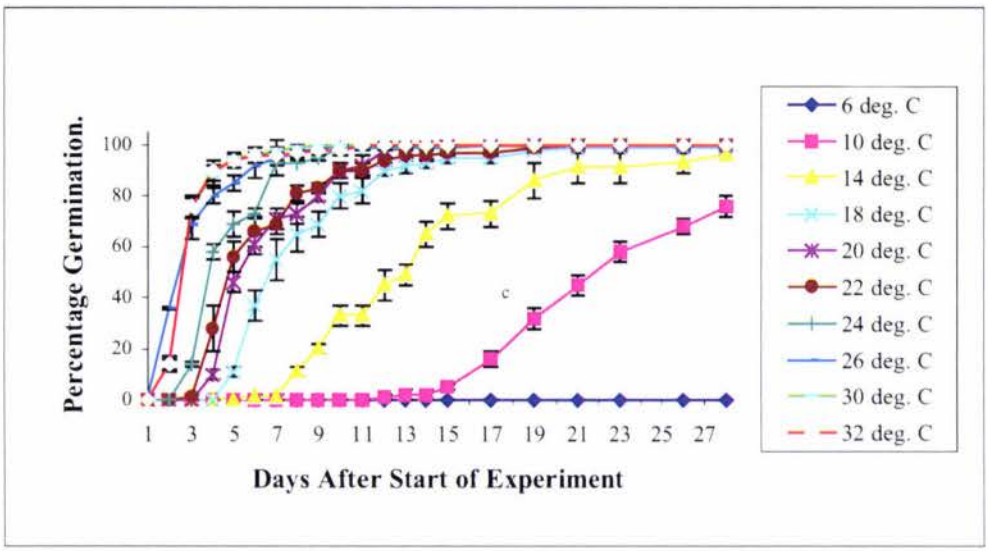

Figure 4.13 The effect of temperature on the germination percentage of *Leptospermum scoparium* seed.

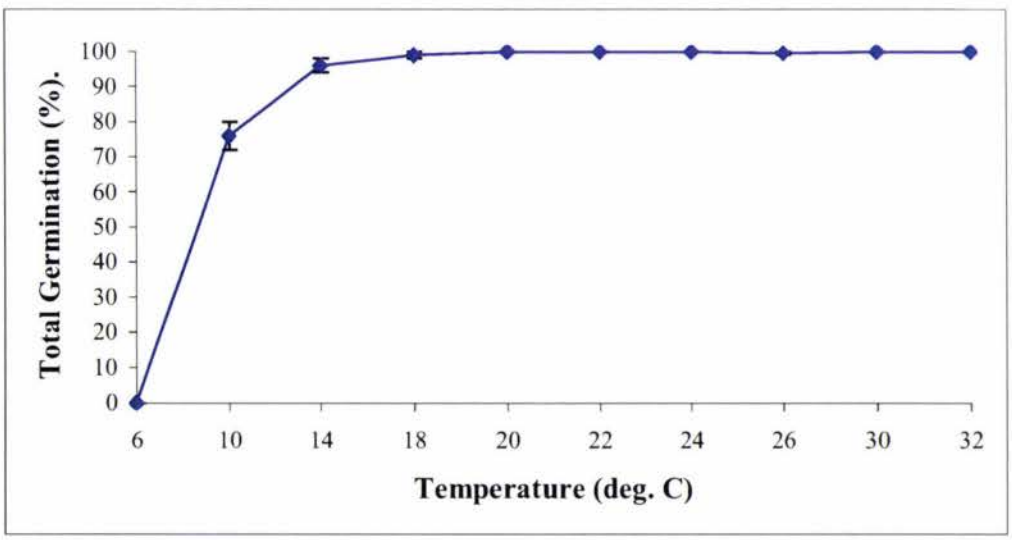

Figure 4.14 Temperature dependence of germination of *Leptospermum scoparium*.

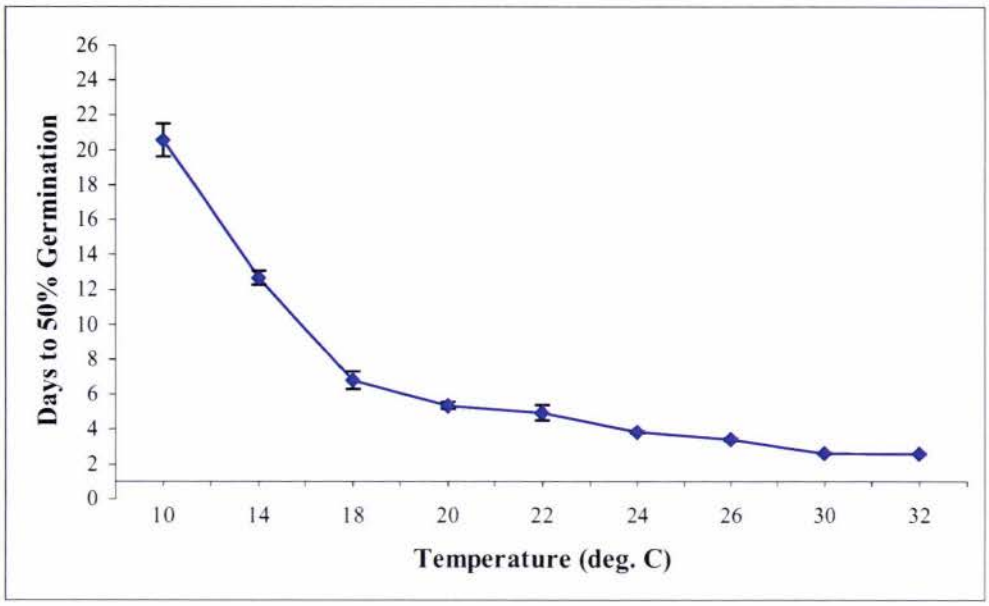

Figure 4.15 The effect of temperature on germination rate of *Leptospermum scoparium* seed.

4.4.6 *Muehlenbeckia australis*

There was some germination in all treatments, although germination between the replications was highly variable. The experiment was terminated after 39 days due to time restrictions (Figure 4.16). Figure 4.16 show variations of tri-phasic sigmoidial germination curves for all temperature treatments. Total germination increased as the temperature increased, up to 20°C, and then decreased (Figure 4.16 and 4.17). The optimum temperature range is not clear due to the variability of the data (Figure 4.17). The rate of germination increased as the temperature increased although there were no significant differences between 20°C to 32°C (Figure 4.16 and 4.18). The time to 50% germination was slow for all treatments. Between 20°C to 32°C time to 50% germination was between 17 to 19 days. At the lower temperatures, 6°C to 18°C, time to 50% germination was between 25 to 31 days.

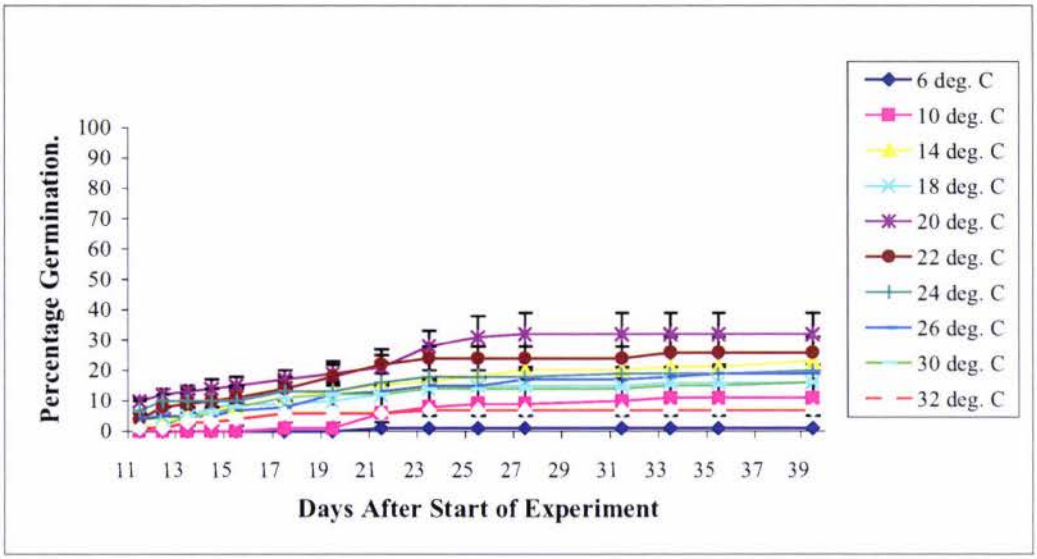

Figure 4.16 The effect of temperature on the germination percentage of *Muehlenbeckia australis* seed.

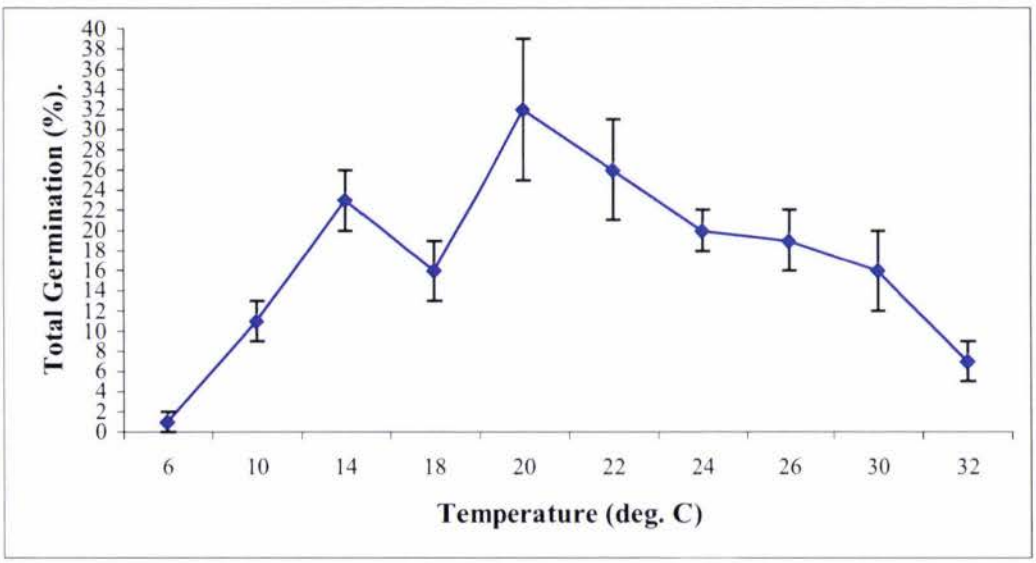

Figure 4.17 Temperature dependence of germination of *Muehlenbeckia australis*.

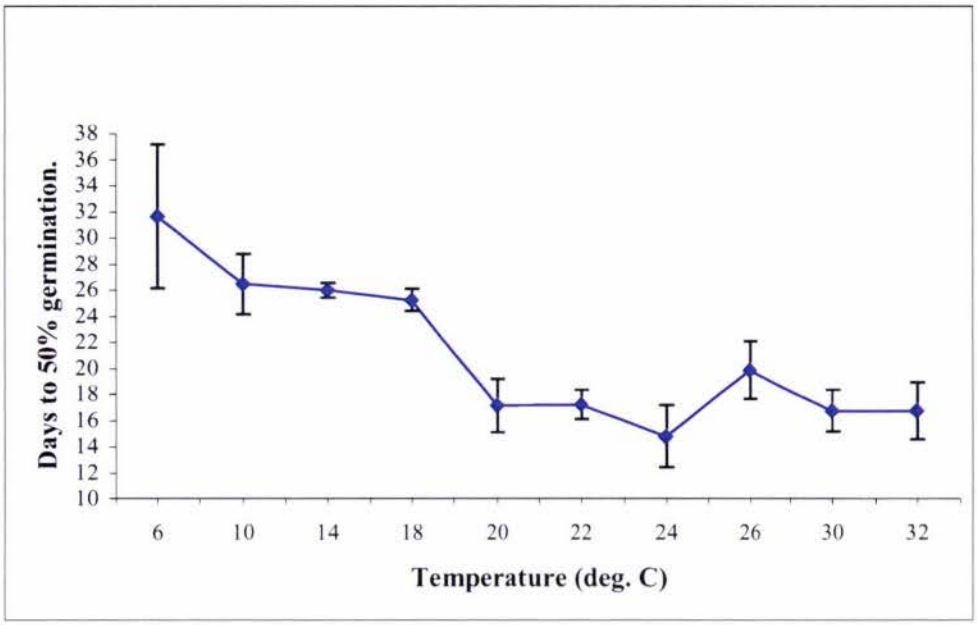

Figure 4.18 The effect of temperature on germination rate of *Muehlenbeckia australis* seed.

4.4.7 *Phormium tenax*

All treatments had some germination. The experiment was terminated after 31 days due to time restrictions but within this time frame some of the treatments had high germination percentages (Figure 4.19). Figure 4.19 show variations of tri-phasic sigmoidial germination curves for all temperature treatments. Total germination increased as the temperature increased, evened out between 14°C to 22°C, and then decreased (Figure 4.19 and 4.20). The suggested optimum temperature range for germination is between 14°C to 22°C (Figure 4.20). The rate of germination increased as the temperature increased (Figure 4.21). Germination at 32°C was the most rapid with a T_{50} of 2.3 days. The time to 50% germination at 30°C and 26°C were 3.5 days and 5.4, respectively. It took 4.8 days and 5.8 days at 24°C and 22°C, respectively to achieve 50% germination. At 20°C and 18°C germination rate was slightly slower taking 6.6 days. At 14°C the time to 50% germination was 10.2 days, and at the 10°C treatment germination was the slowest, taking 11.2 days to achieve 50% germination.

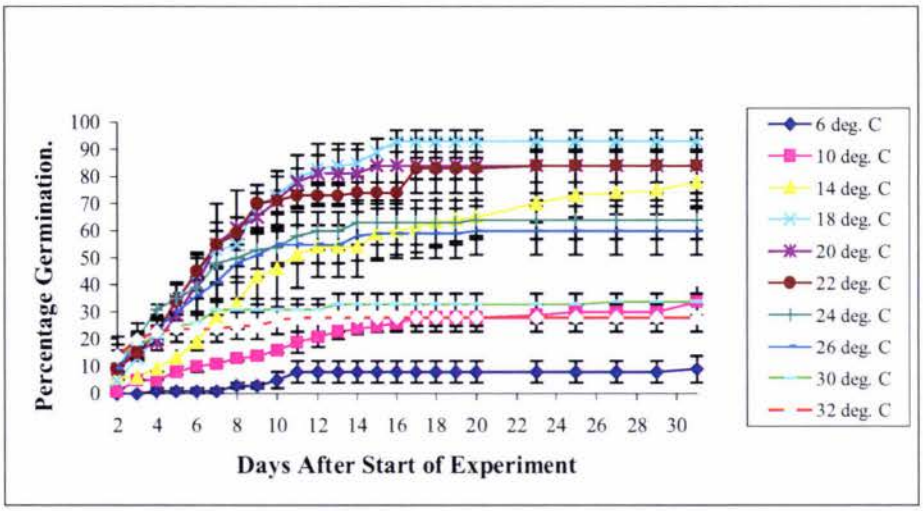

Figure 4.19 The effect of temperature on the germination percentage of *Phormium tenax* seed.

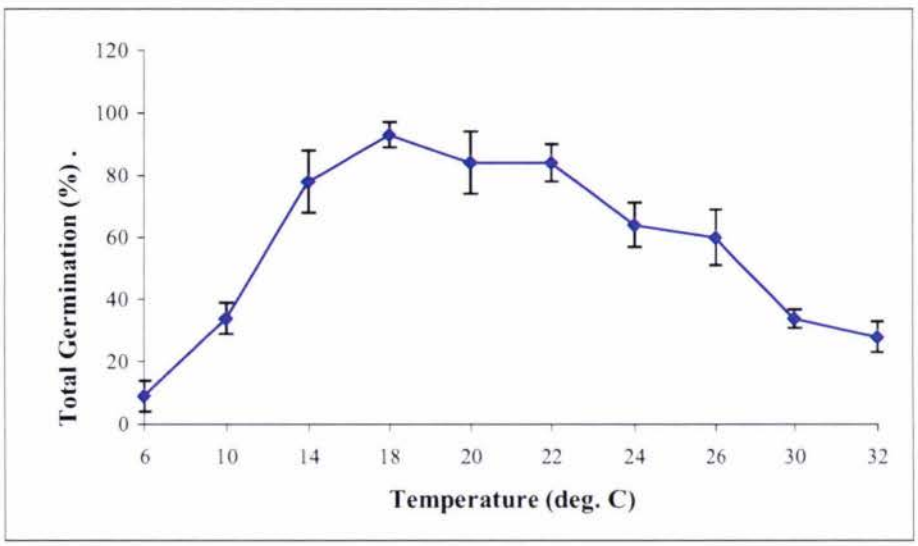

Figure 4.20 Temperature dependence of germination of *Phormium tenax*.

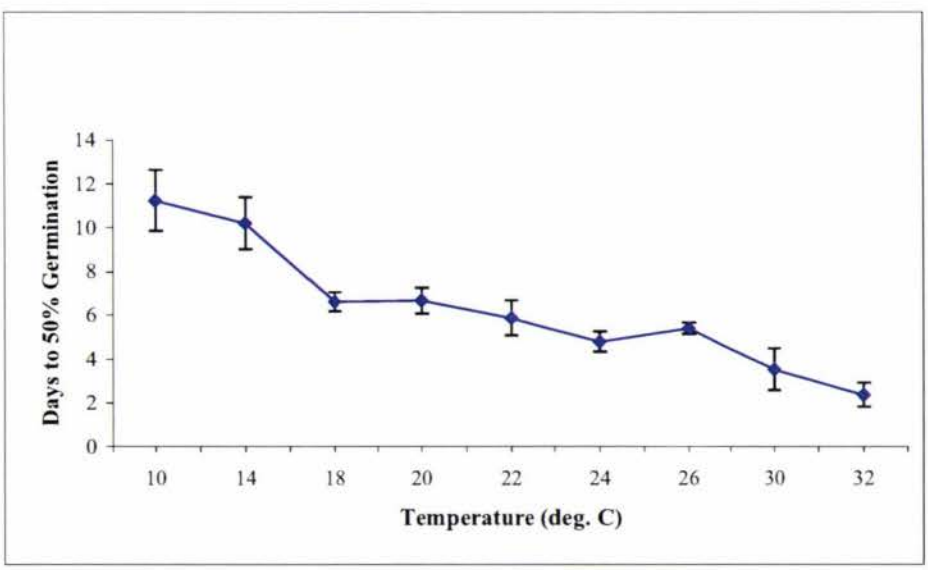

Figure 4.21 The effect of temperature on germination rate of *Phormium tenax* seed.

4.4.8 *Sophora prostrata*

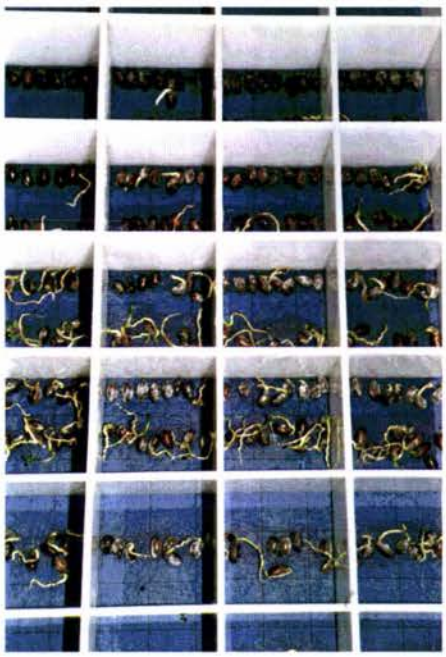

Plate 4.33: *Sophora prostrata* seed germinating on the thermogradient plate. (Photo: C.R. McGill)

At 6°C and 10°C there was no germination but at all other temperatures from 14°C to 32°C seed successfully germinated. The experiment was terminated after 69 days due to time restrictions but within this time frame most of the treatments had high germination percentages (Figure 4.22). Total germination increased as the temperature increased, up to 22°C, and then decreased (Figure 4.22 and 4.23). The optimum temperature range for germination is 18°C to 24°C (Figure 4.23). Generally, the rate of germination decreased as the temperature increased (Figure 4.24). Germination at 18°C was the most rapid with a T_{50} of 11.1 days. The time to 50% germination at 14°C was slower, taking 18.0 days. It took 12.3 days at 20°C to achieve 50% germination. At 22°C germination rate more than doubled taking 28.4 days. At 24°C the time to 50% germination nearly doubled to 47.6 days, and at the 26°C and 30°C germination slowed even more, taking 53.4 days and 55.0 days to achieve 50% germination. At 32°C germination rate slightly increased to 47.4 days.

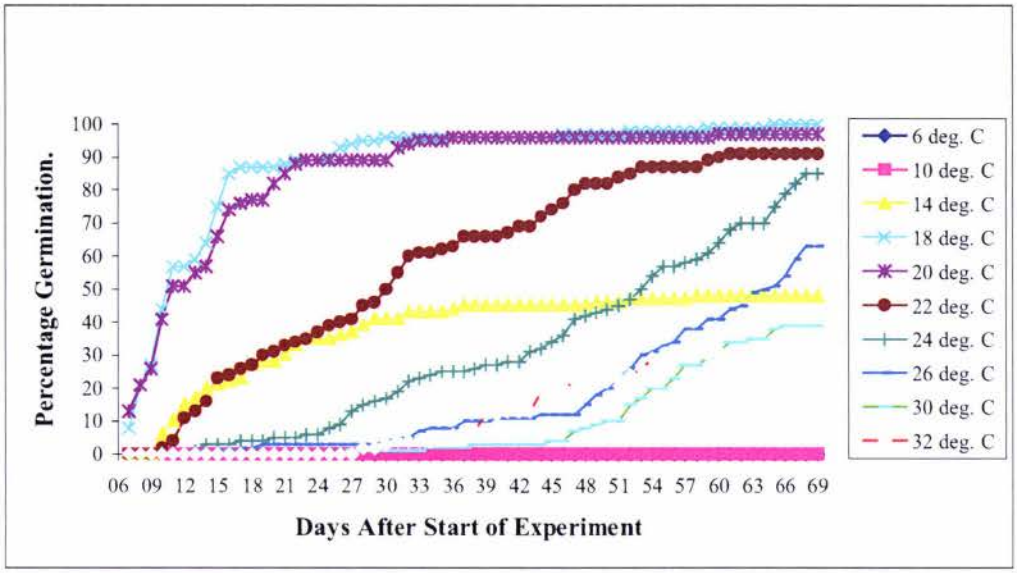

Figure 4.22 The effect of temperature on the germination percentage of *Sophora prostrata* seed.

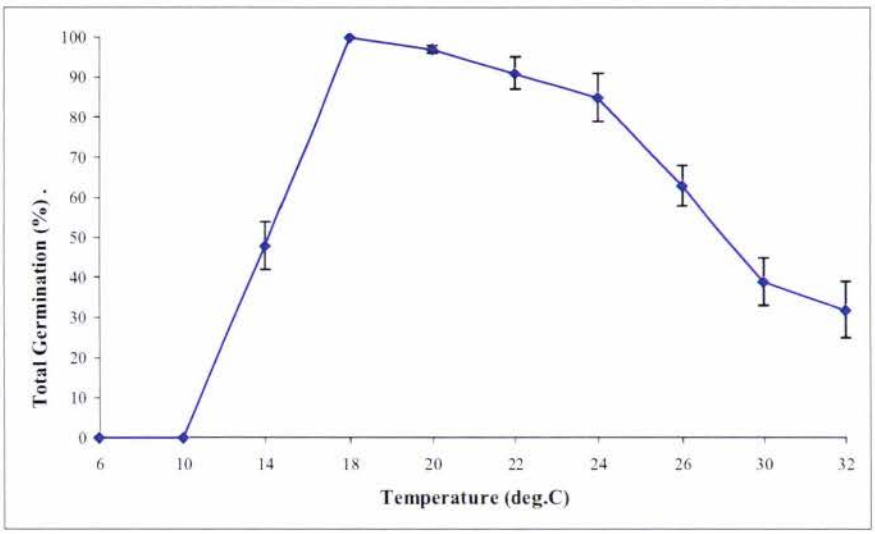

Figure 4.23 Temperature dependence of germination of *Sophora prostrata* seed.

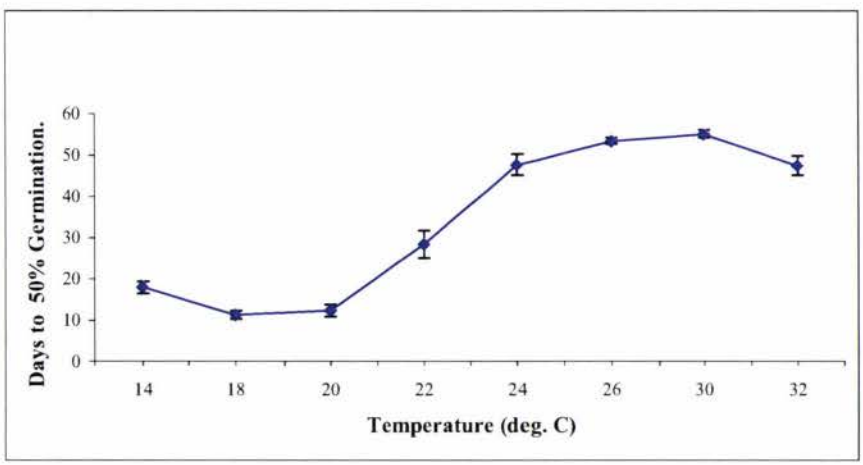

Figure 4.24 The effect of temperature on germination rate of *Sophora prostrata* seed.

5

DISCUSSION

5.1 Seed Moisture and Seed Storage

After developing seeds reach physiological maturity, they either desiccate (for orthodox seed to a moisture content of 5-15%) or bypass complete desiccation (recalcitrant seeds) (Hartmann et al. 1997). A recalcitrant seed loses viability after drying, while orthodox seeds tolerate drying (Bewley & Black 1994). *Carex trifida*, *Cyperus ustulatus*, *Coprosma robusta*, *Leptospermum scoparium*, *Myrsine australis*, *Phormium tenax* and *Sophora prostrata* appear to be orthodox, as they remained viable when desiccated to moisture contents of <20%. After 12 months in dry cool storage they all remained highly viable (Table 4.2). Hong et al. (1998a, 1998b), who do not report on the species *Carex trifida*, *Cyperus ustulatus*, *Myrsine australis* and *Sophora prostrata*, report that similar species are probably orthodox.

There are conflicting reports regarding *Coprosma robusta*. Metcalf (1995) reports that *Coprosma* seed does not keep particularly well in dry storage and Bergin & Van Dorsser (1988) recommend moist, cool storage at 2°- 4°C, which suggests that *C. robusta* might demonstrate recalcitrant seed storage behaviour. Hong et al. (1998b) questions if this species is recalcitrant. After 12 months dry storage, with moisture content of 12%, *C. robusta* maintained high seed viability (72%) (Table 4.2), which would indicate that it is orthodox in nature.

New Zealand Tree Seeds recommend that *Cyperus ustulatus* (Cyperaceae) seed be kept moist in the refrigerator (2-5°C) for 28 days before sowing. This would act as a period of stratification, thus increasing the chance of germination, rather than a storage requirement. After 12 months dry-cool storage 57% of the *C. ustulatus* seed remained viable (Table 4.2). It would be of interest to store seed in moist-cool conditions for 12 months and compare with the dry-cool storage conditions. Budelsky & Galatowitsch (1999) studied seed storage and germination conditions in order to maintain seed viability and stimulate germination rates of five species of *Carex* (Cyperaceae). They compared moist-cold long-term storage with dry-cold long-term storage and reported that moist-cold long-term storage was associated with a high level of viability in all five species after 2.5 years. Therefore, they recommend that

Carex seeds should be stored under moist-cold conditions to maintain seed viability over time.

Hebe stricta stores well according to Metcalf (1995). Results from this study show that *H. stricta*, after 12 months dry-cool storage at moisture content of 5 ± 1% had 100% germination (Table 4.2). Simpson (1976) reported similar results for *H. stricta* var. *stricta* demonstrating that there was 100% viability after 10 months dry-storage at room temperature. As *H. stricta* seed remained viable a low moisture content it is most likely orthodox.

For *Muehlenbeckia australis* the situation was more complex with seeds at 12% SMC exhibiting 43% viability. The possibility of recalcitrance in remaining seed was ruled out by observations of immature embryos and seed predation. It is also unlikely that *M. australis* is recalcitrant, as Metcalf (1995) observed that it stores well. Hong et al. (1998b) reported that *M. australis* was orthodox and could be stored for a long period of time at –18°C or less in air-tight containers with a seed moisture content of 5 ± 1%.

The data presented are in agreement with Metcalf (1995) who reported that *Phormium tenax* could be cool-moist stored for several months and that seed stored for 5 months germinated within 12 days. A high percentage (48%) of *Phormium tenax* seed germinated at 5°C in the 12-week stratification period, so if seed was to be stored I recommend that it be stored with low seed moisture content at 5°C.

After 12 months dry storage *Sophora prostrata* retained considerable viability (96%). This is not surprising as considerable longevity of legume seeds is well established (Priestly 1986). A recent report by Norton et al. (2002) clearly showed that several *Sophora* species stored for 24-40 years, under dry conditions, retained considerable viability.

Establishing whether seeds are orthodox or recalcitrant can give guidance for seed storage and indicate whether they will remain persistent in a seed bank. This has implications for sustainable revegetation applications.

5.2 Germination

5.2.1 *Carex trifida*

Carex trifida required stratification before seed germinated (Table 4.4). This concurs with Schutz (2000) who found that in almost all species of *Carex* evaluated, dormancy was broken by stratification at low temperatures. Not all *Carex* spp. require stratification to break dormancy, as Ralph (1994) reported that some Australian *Carex* spp. germinate readily. For some New Zealand *Carex* seed, Metcalf (1995) recommended a period of stratification to increase germination percentages. Schutz & Rave (1999) reported that there was almost no germination in darkness prior to stratification, and the germination in the light (after stratification) was considerably higher in all but two species compared with that in darkness for 28 species of *Carex*; thus, light and stratification are required for germination of these species. Schutz & Rave (1999) concluded that the *Carex* species studied had broadly similar germination response patterns and the fact that they were released from high levels of primary dormancy by low-temperature stratification suggests that they are spring germinators.

These results indicate that in *Carex trifida* the levels of dormancy are variable within the seed population with stratification period determining the germination percentage (Table 4.4). Evans & Cabin (1995) state that this is a mechanism that probably evolved to reduce the risk of reproductive failure of seed crops, thus spreading germination over time. Metcalf (1995) reported that, in general, *Carex* spp. have erratic germination. This is likely due to the variable levels of dormancy within the seed populations.

The range of temperatures at which seed will germinate appears to be relatively small as the 2002 *Carex* seed only germinated at 22°C, 24°C and 26°C. In contrast, a high percentage of the 2001 seed germinated at 20°C in the stratification experiment. Both the 2001 and 2002 seed were subjected to 12 weeks stratification so it is suggested that the 2002 had deeper dormancy (in the 2002 seed the highest percentage of seed that germinated (23%) was at 26°C even though it had a seed viability of 73 ± 4% (Figure 4.4 & Table 4.2). Between-year variations in dormancy have been reported at population level (e.g. Evans & Cabin 1995; Andersson & Milberg 1998; Qaderi & Cavers 2002). Germination patterns tend to have a genetic basis, but can be markedly modified by environmental conditions during the seed-ripening period (Schutz & Rave 2003).

Establishing the optimum germination range is not possible due to the low percentage of seed that germinated. There is a trend illustrating temperature sensitivity, as *Carex trifida* does not germinate at the low temperatures (Figure 4.4 & 4.5). This is in agreement with Schutz (2002) who reported that *C. frigida* required cold stratification to release dormancy, but germination was restricted to high temperatures (>15 deg. C). This is in contrast to what Metcalf (1995) states who recommends cool conditions when germinating *Carex* seed.

As *Carex trifida* seed appears to require light for germination, a period of cold to break dormancy and higher temperatures to germinate it could build-up a large seed reservoir in the soil. An example of a *Carex* spp. that forms a persistent soil seed bank is the weed *Carex albata* Boott. (Watanabe et al. 2001). It invades and dominates sown pastures in Japan. For *Carex trifida* the formation of soil seed bank would be of a benefit for persistence and maintenance of revegetation projects, especially on roadside cuttings.

5.2.2 *Coprosma robusta*

There was germination in the light but it was slow and spread over two years (Table 4.3). Burrows (1995) stated that seed germination is spread out over a period of time but his seed germination was spread out over a shorter time period. This may be due to the seed being germinated fresh (not been subjected to storage conditions), therefore, dormancy mechanisms had not been established. Metcalf (1995) had a delay in germination of untreated *C. robusta* seed, which took 4 to 5 months to start germinating. Spread of germination indicates, most likely, that should early-germinated seedlings be subjected to a periodic environmental risk, those germinating later could survive. *C. robusta* did not germinate in the dark (Table 4.3). Miller & Henzell (2000) found that *Coprosma repens* germinated better in the light, but their results were inconclusive due to low germination. In contrast, Burrows (1995) recorded germination of *C. robusta* in both light and dark treatments (90% and 60%, respectively).

Burrows (1995) indicated that seed extracted from the fruit had rapid germination onset and was completed (90% germinated, 10% dead) within 10 weeks, but for seed still in the fruit there was less germination (72%), suggesting that the fruit may inhibit germination. He concluded that *Coprosma* fruit exudate does have an inhibitory effect on germination after subjecting lettuce seed to the exudate and demonstrating that the rate of germination and root and shoot growth were effected. Stratifying seed still in fruit may increase germination percentage and could be performed in future research. This might lead to seed/fruit being sown without the need for cleaning.Burrows (1995) harvested seed in February while seed for

this experiment was harvested in mid-April. Although a different location and growing season make comparisons difficult, it may be that the delayed harvest of seed allowed the development of embryo-based dormancy, but without detailed information on the developmental sequence in *Coprosma robusta* seed this is speculative. Schutz & Rave (2003) stated it is possible to have differences in the germination responses between populations. It may be due to a genetic basis or reflect phenotypic variability acquired as the fruit matured (Gutterman 1992).

Stratification was able to alleviate dormancy in this species, and again there was a population response with length of stratification determining germination percentage (Table 4.4). Bergin & Van Dorsser (1988) and Metcalf (1995) recommend a period of chilling before germinating the seed. Due to equipment failure it was not possible to report how temperature affected germination for this species. Bryant (1994) reported that the optimum germination temperatures for quick and even germination are between 15-22°C. Burrows (1995) reported that *Coprosma* is capable of germinating in relatively low temperatures of autumn-winter but the rates slow as the temperatures lower. He did not report if seeds had been subjected to a period of chilling. Seed germination of other species belonging to Rubiaceae are affected by temperature. For example, *Genipa americana* (semi-deciduous tree from South America) germinates between 20°C to 35°C, with the highest germination at 30°C and no germination at 10, 15 or 40°C (Nascimento et al. 2000); *Anthocephalus chinensis* (fast growing Himalayan Indian tree) germination percentage was significantly higher at 32°C than at 24°C and 28°C and germination commenced earlier at 32°C (Anuj et al. 2000)

Clearly *Coprosma robusta* germination rate increases when subjected to a period of chilling but seed will still germinate, eventually, over a period of time, if it has not been subjected to a period of chilling (Table 4.3). This work demonstrated that *C. robusta* is also responsive to light. It would be of interest to repeat the dark germination treatments using seed that had been subjected to a period of stratification.

Seed is probably persistent in a soil seed bank. A high percentage of imbibed seed that had been in the dark for nearly two years was still viable (Table 4.3), which might indicate that seed could imbibe in the soil but if not subjected to light will not germinate but remain viable. Burrows (1995) reported that *Coprosma* which sprang up after forest disturbance does so from seed crop of the previous fruiting season.

5.2.3 *Cyperus ustulatus*

The germination behaviour of *Cyperus ustulatus* is similar to that of *Carex trifida* and *Coprosma robusta* in that after 12 weeks stratification a reasonably high level of germination was obtained (Table 4.4). Again, germination response was a function of stratification period. Chozin & Yasuda (1991) examined the progeny of *Cyperus iria* and *Cyperus microiria* and found that dormancy was broken by stratification for a period of 1 month. The species studied here is thus similar in its cold requirement to *C. iria* and *C. microiria*. Metcalf (1995) also suggested that *C. ustulatus* required a period of cool-moist stratification in order to break dormancy.

Cyperus ustulatus did not germinate in the dark (Table 4.3). Similar results were found for similar species *C. erythrorhizos*, *C. flavicomus* and *Fimbristylis autumnalis* (annuals found on the mudflats at Lake Barkley, USA), which germinated to a maximum of 7%, 4% and 0%, respectively, in darkness (Baskin et al. 1993). This seed had been buried for 25 months and when exhumed was subjected to light treatments to test the germinability of the seed resulting in 91-100% or the seed germinating.

The range of temperatures at which seed will germinate was relatively large with the optimum germination range being (24°C to 30°C) (Figure 4.7 & 4.8). At this stage it is important to note that the time limit on experimentation may have been a significant limitation. There is a trend illustrating temperature sensitivity, as *Cyperus ustulatus* did not germinate at the low temperatures (6°C to 14°C) (Figure 4.7). As temperature increased germination rate increased (Figure 4.9). High temperature requirement for germination probably prevents them from emerging at the very beginning of the growing season.

Cyperus ustulatus commonly grows in moist sand-flats or coastal swamps. Both these areas would be prone to flooding, which if the seed germinated when flooding occurs would have a lesser chance of survival. It is likely dormant when flooding occurs. *C. erythrorhizos and C. flavicomus* remain dormant until mudflats are exposed and temperatures in the growing season increase. Further research should be performed to establish if there is a correlation between flooding, dormancy and germination.

5.2.4 *Hebe stricta*

The germination of *Hebe stricta* is clearly light dependent and therefore exhibits dormancy according to the definition used in this study. In complete darkness, at a constant 20°C germination was 7% after 10 days, while 100% of seeds kept in the light germinated (Figure 4.1). Simpson (1976) reported similar results for *H. stricta* var. atkinsonii (but at a constant 25°C), where germination in the light was 86% while dark germination was 4%. Other *Hebes* that had significant differences between light and dark germination (at 25° C) *are H. elliptica* (Light (L)= 98%, Dark (D)= 6%); *H. pimeleoides* (L= 22%, D= 2%); *H. pinguifolia* (L= 60%, D= 2%); and *H. salicifolia* (L= 100%, D= 21%) (Simpson 1976). Secondary dormancy was not induced by keeping imbibed *H. stricta* seeds in the dark. Seeds of the dark treatment were transferred to the light conditions where the seeds rapidly germinated, obtaining 100% germination (data not shown). Seeds subjected to adverse environmental stimuli after dispersal can develop secondary dormancy. According to Bewley & Black (1994) a loss of sensitivity to light can accompany secondary dormancy. Other authors have similarly found that *Hebes* require light to germinate. Widyatmoko & Norton (1997) reported that *Hebe cupressoides* germination percentage and germination rate increased with increasing irradiance; germination percentage in the dark was 12% and in "full light" was 84%. Fountain & Outred (1991) noted that seed of some species of *Hebe*, e.g., *H. speciosa* and *H. salicifolia*, require light for germination and Simpson (1976) reported that many species of *Hebe*, other that have not been mentioned previously, germinate readily at 25°C in light. Simpson (1976) states "that while light is necessary for germination for those species that respond to a temperature of 25°C this requirement is overcome for some species by exposure to low temperature, e.g. *H. raoulli*". This statement might be misleading, as although the seed was kept in the dark, the seed was exposed to light for short periods once a week to be examined for germination, which might be enough to break dormancy. To break dormancy some seeds only require exposure to white light for a few seconds, for example, *Verbascum thapsus* only requires 5 s of white light to stimulate germination to 50% of the full control (Gross 1985).

Hebe stricta demonstrated high seed germination percentage over a range of constant temperatures (Figure 4.10), except at 30°C and 32°C where there was no germination. Simpson (1976) supports this for some other species of *Hebe*, for example, *H. hulkeana* had 85%, 100%, 85%, 100%, 60% and 72% germination (in the dark) at 0°C, 5°C, 10°C, 12°C, 15°C and 25°C, respectively and *H. raoulii* had 80%, 80% and 100% germination at 10°C, 12°C and 15°C, respectively. What is interesting is that *H. raoulii* had no germination at 0°C,

5°C and 25°C, which indicates that *Hebe* spp have differing sensitivities to temperature. Several species (e.g. *H. albicans*, *H. buchananii* and *H. decumbens*) tend to germinate at the low temperatures while others germinate at the high temperatures, as previously mentioned. In general most mountain species require colder temperatures to germinate while seed of species from lowland and lower mountain habitats germinate at higher temperatures of 20°C to 25°C. In this study there was a significant drop in germination percentage between 24°C and 26°C (Figure 4.11). The rate of germination for *Hebe stricta* was affected by temperature (Figure 4.12). Generally, as the temperature increased so did the rate. Bryant (1994) reports that the optimum germination temperatures for quick and even germination of *Hebe* are 13-24°C and at these temperatures it should take 14-28 days to germinate. Results of this study found that the optimum germination range was much wider (6°C to 24°C).

As *Hebe stricta* requires light to germinate and the seed is small, allowing it to be readily buried, it could form a persistent soil seed bank.

5.2.5 Leptospermum scoparium

The germination of *Leptospermum scoparium* seed is, like *Hebe stricta*, light dependent. Other authors (Grant 1966; Mohan et al. 1984a; Herron et al. 2000; Miller & Henzell 2000) have similarly found little or no germination in *Leptospermum scoparium* seed incubated in darkness. I therefore confirm their earlier reports with experiments performed under the standard conditions reported here.

Leptospermum scoparium had maximum seed germination over a range of constant temperature (Figure 4.13 and 4.14). Grant (1967) who reported that germination was reduced below 20°C obtained similar results. In contrast, Mohan et al. (1984) and Herron (1999) had maximum germination at lower temperatures. The optimum germination temperatures for quick and even germination of *Leptospermum* are 15-24°C and at these temperatures it should take 10-35 days to germinate (Bryant 1994). As the temperature increased there was abnormal seedling development. At the temperatures recommended by Bryant (1994) seedlings were normal and most likely to grow into a normal plant. A number of other species in the family Myrtaceae exhibit similar germination temperature requirements. For example, *Eucalyptus nitens* germinated over a wide range of temperatures (13°C to 33°C) (Humara et al. 1996) and *Metrosideros polymorpha* seed germinated in all constant temperatures tested (10°C to 34°C) with germination percentage and rate being the greatest at 22°C (Drake 1993).

In conclusion, *L. scoparium* appears not to exhibit between year variation in light dormancy, therefore, germination patterns would have a genetic basis rather than influenced by environmental conditions. *Leptospermum scoparium* grows in a wide range of habitats, from cold to hot regions of New Zealand, hence it might be expected to have a wide temperature germination range.

5.2.6 *Muehlenbeckia australis*

There was very little germination in the initial germination experiment (Table 4.3), in the light, even though 43% of the seed was viable. This maybe a result of extremely low light irradiance (between 0.07 μmol m^{-2} s^{-1} and 3.15 μmol m^{-2} s^{-1} depending on where the box was placed in the 20°C room), which represents very dense shade. Baars & Kelly (1996) report that *Muehlenbeckia australis* is dependent on relatively high levels of irradiance for survival and growth but their research only reported on the growth of the whole plant. They did not test germination of the seed. Ku et al. (1996) reported that *Polygonum hydropiper*, which belongs to the same family as Muehlenbeckia, requires strong light for germination. Very little seed that germinated in the present study developed into a normal seedling and this maybe, again, due to low light levels. *Muehlenbeckia australis* is capable of high rates of growth but only if there is lots of light available (Baars & Kelly 1996). There was no germination in the dark (Table 4.3). These data are in conflict with germination results reported by Burrows (1996b) who found that *Muehlenbeckia australis* reached 75% germination in the dark and 97% germination in light, which is substantially more than the germination achieved in this study in either the light or dark. Clearly the dormancy status of the seedlots studied must have differed. Possible reasons for this are ecotype variation, location, and collection time.

Muehlenbeckia australis did not show a stratification requirement. Although the actual germination percentage (31%) was low, under light conditions the percentage of viable seed germinating was high (58-72%). *Muehlenbeckia australis* demonstrated some germination over a range of constant temperatures (Figure 4.16). At 6°C, 10°C and 32°C there was very little germination (Figure 4.16). Burrows (1996b) had no germination in the coldest part of the Canterbury winter, which indicates that this species is affected by a degree of environmental degree. Germination rate is slow (Figure 4.16 and 4.18). Bryant (1994) reports that the optimum germination temperatures range are 13-20°C. The data of this study was too variable

to establish the optimum germination range but the highest germination percentage was at 20°C.

Dormancy release of other species in the family Polygonaceae has been reported. For example, *Polygonum aviculare* L. require temperature fluctuations (Batlla et al. 2003) and *P. hydropiper* and *P. lapathifolium* require temperature fluctuation and a short period of chilling (Araki & Washitani 2000).

Light quality and fluctuating temperatures could be areas for future research.

5.2.7 *Myrsine australis*

In the initial germination experiments there was no germination in the light or the dark but the seed was viable as revealed by the tetrazolium test (Table 4.3). While the viability of *Myrsine australis* was high (92%), only minimal germination occurred after 12 weeks stratification. It is not clear if this is a stratification response or simply an indication that dormancy in at least a portion of the population is being alleviated over time, or a combination of the two. It was possible that much longer stratification was required but seed that was subjected to 4, 5, and 6 months stratification did not germinate. The stratification experiment was repeated in 2002 with 2001 seed (to give an indication if dormancy been alleviated over time) and 2002 seed to see if there is any differences between different seed lots. This resulted in no germination for either the seed lots even though the seed was viable. Burrows (1996d) reported that *Myrsine australis* was slow to germinate and suggested that the embryo may still be developing after seed fall. Metcalf (1995) also reported that *Myrsine* seed, even after being subjected to stratification, could take 2 to 18 months to germinate.

The hard endocarp appears to act as a mechanical barrier to radicle emergence as in all treatments there was swelling in the area where the radical emerges but the endocarp did not break. This phenomenon is found in olive seeds resulting in low germination (Sotomayer-Leon & Caballero 1990). They devised a method where they broke the endocarp to remove mechanical dormancy. Fifty percent of the seed, of the present research, had the endocarp removed and 50% of the seed left intact. Within one month seed that had the endocarp removed had high germination percentage while the intact seed had no germination. There were no significant differences between the 4, 5, and 6-month periods of stratification. In another experiment 2002 seed that had not been subjected to a period of chilling but had the endocarp removed had some germination (32%) but this was increased to (91%) after a period

of chilling (1 month). Similar results were obtained for other species that have an endocarp, for example, *Prunus mahaleb* intact and endocarp-removed seeds that were stratified (120 days 4 deg C) were compared. Percentage germination was 92.5, 100 and 78.8 for seeds with the endocarp removed, from unripe, ripe and overripe fruits, respectively, and 31.3, 22.5 and 11.3 for seeds with the endocarp present (Carrera et al. 1986). It is possible that *Myrsine australis* requires an after-ripening period in order for the embryo in the seed to mature (as the swelling of the seed did not appear till several months after imbibition), chilling requirements and removal of the endocarp (thus relieving mechanical dormancy). Burrows (1996c) indicated that *Myrsine australis* required winter low temperature to overcome a biochemical block to germination. Fountain & Outred (1991) suspected that *Myrsine australis* is quiescent, meaning that no specific priming treatments are needed to initiate embryo growth and that seeds germinate after seed fall. Data from this work do not support that observation. It is likely that seedlings growing under parent plants observed by Fountain & Outred (1991) were, in fact, from seeds from previous growing seasons and been subjected to a period of cold as well as the endocarp being broken. As many different species of birds eat the fruit it is likely that the seed is scarified. Burrows (1996c) concluded that the dormancy of *Myrsine australis* seed could permit seed to persist either at the soil surface or beneath the soil for up to 2 years.

In future research, soaking in GA or acid could break dormancy, as it is not easy to mechanically remove the endocarp. Carrera et al. (1986) successfully improved the germination of non-stratified (with the endocarp present) *P. mahaleb* seed from 0 (untreated controls) to 80% by soaking in GA at 1000 p.p.m. Acid could break a weak region of the endocarp as reported by Li XiaoJie et al (1999). Sulfuric acid broke seed dormancy of *Rhus aromatica* that has a similar fruit structure to *Myrsine.*

5.2.8 *Phormium tenax*

A small percentage of the *Phormium tenax* population was not dormant, as there was some germination in all treatments (light and dark) (Table 4.3). *Phormium tenax* seed requires a period of chilling to overcome dormancy in the majority of the population. Eight to 10 weeks stratification was required as a pre-treatment to break dormancy (Table 4.4). These results are similar to *Carex trifida* in that the stratification period determines the germination percentage (Table 4.4) and that the levels of dormancy are variable. Germination is often spread over time to reduce risk of reproductive failure of the seed crop (Evans & Cabin 1995).

Germination of other species found in the Hemerocallidaceae family is also promoted by chilling. For example, *Hemerocallis dumortierii* (daylily native to Korea Peninsula) required at least 14 days chilling to obtain high germination percentage (97%) (Sim YongGu et al. 1996) and commercial growers recommend storing *Hermocallis* seeds (moist) in the refrigerator for at least 6-8 weeks before planting.

Phormium tenax demonstrated some germination over a range of constant temperatures (Figure 4.19). The mid-range of temperatures (14°C to 22°C) resulted in high germination of seed. Bryant (1994) who obtained an optimum germination range from 13°C to 25° reported similar results. At these temperatures *Phormium* seed took 10-35 days to germinate.

5.2.9 *Sophora prostrata*

Species of *Sophora* have a coat-imposed dormancy and require scarification in order to allow water uptake (Stilinovic & Grbic 1988; Fountain & Outred 1991; Wang 1991; Metcalf 1995). The data presented on *Sophora prostrata* support these observations, and also indicate that there is no light requirement for germination in addition to the seed coat imposed dormancy. This is supported by Webb (1993) who reported that *S. prostrata* germinates well in the dark even at different temperatures. The seeds of *S. prostrata* would probably be described as non-photoblastic, i.e. light is neither essential (positively photoblastic) during germination nor does it inhibit (negatively photoblastic) germination (a concept described by Bradbeer 1988). Non-sensitivity to light is a characteristic of larger seeded species (Thompson & Grime 1983). Everitt (1983) obtained similar results for *Sophora secundiflora* and reported that light was not required for germination and no other dormancy mechanisms were observed other than the hard seed coat.

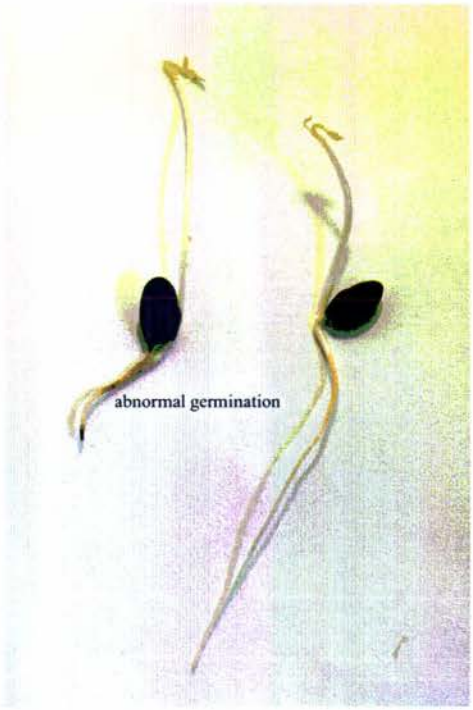

Plate 4.34: *Sophora prostrata* seed germinated in the dark, undergone etiolation.

Germinated seedlings of *Sophora prostrata*, in the dark, undergo etiolation. That is, they have tall, thin weak stems with very small-unexpanded foliage, which is yellowish white in colour (Plate 4.34). This could indicate that seedlings compete well in poor light environments as Bradbeer (1988) states that etiolation is an important adaptation. It allows germinating seedlings to elevate their photosynthetic organs above the soil surface and shading vegetation to reach irradiation sufficient to maintain the seedling in photosynthetic autonomy. According to Webb (1993) *S. prostrata* seems to germinate under the dense canopy of its parents (Plate 2.9), therefore, it appears to be shade tolerant. Various species of *Mimosa* (leguminous shrubs) germinate just as well beneath the canopy as in open areas (Orozco-Almanza et al. 2003).

The germination responses indicate sensitivity to temperature. Although the range which seeds germinate is relatively large (14°C - 32°C) the optimum germination range is quite narrow (18°- 24°C) (Figure 4.22 and 4.24). Webb (1993) reported similar results for the range of temperatures that *S. prostrata* will germinate at but the optimum temperature range was narrower (10°C - 20°C). Webb (1993) ran the experiment for 35 days while this study went for 69 days and he stated that if the experiment went longer then there may have been a wider optimum temperature range. Bryant (1994) concludes that the optimum germination temperatures for quick and even germination of *Sophora* are 15-25°C and at these

temperatures it should take 14-42 days to germinate. Generally, as temperature increased the germination rate slowed (Figure 4.23). This might be as a result of microbial attack and germination was stopped as a result rather than as a result of the high temperatures. Species that rapidly and completely germinate across a wide range of temperatures should germinate more reliably under variable soil conditions than species that exhibit a narrow range (MacGraw et al. 2003).

Other species from the Fabaceae family show similar germination behaviour. For example, *Aeschynomene americana* (legume grown in North Australia) did not germinate at low temperatures (8°C) and total percent germination was high at temperatures from 16°C to 36°C. The ability to germinate at a broad range of temperatures indicates that this species is well adapted to northern Australia (MacDonald 2002). The optimum temperature for seed germination of several *Mimosa* spp. is between 20°C and 35°C (Orozco-Almanza et al. 2003). McGraw et al. (2003) reported that 10 species of legumes native to Missouri, USA, germinated poorly when temperatures were lower than 15°C and that many of the species attained maximum germination percentage and fastest germination rate across a range of temperatures rather than at a single optimal temperature. Seed germination of *Daldergia nigra* has a broad temperature range and is also insensitive to light, therefore, it can germinate both in understoreys and gaps where the mean temperature is high (Ferraz-Grande & Takaki 2001).

5.3 CONCLUSIONS

The results of the present study show that, of the species tested, *Carex trifida*, and *Cyperus ustulatus* require a period of low temperature stratification in order to break dormancy. The majority *Phormium tenax* require a period of chilling to break dormancy, and *Coprosma robusta* and *Muehlenbeckia australis* require a period of chilling to increase germination rate. *Myrsine australis* requires the removal of the endocarp and a period of chilling. This information is critical to the use of these species in re-vegetation studies or applications. *Hebe stricta* and *Leptospermum scoparium* seeds require light in order to germinate. Scarification of *Sophora prostrata* seed is required in order to break dormancy. A summary of the key data obtained for each species is shown in Table 5.1.

Table 5.1 Seed moisture content (SMC) at storage, seed viability preceding the stratification and seed viability after 12 months storage, requirements to break dormancy and time to 50% germination at 20°C and 26°C. Tz, tetrazolium test; n.d., not determined

Species	SMC Storage %	Initial Viability (Tz)%	12 month Viability (Tz)%	Germination Requirement	*T_{50} 20°C	*T_{50} 26°C
Carex trifida	8	90	60	Stratification	0	32.85
Coprosma robusta	12	80	72	Stratification (increases germination rate) & Light	n.d.	n.d.
Cyperus ustulatus	9.5	73	57	Stratification	9.26	6.73
Hebe stricta	n.d.	n.d.	100	Light	5.78	8.15
Leptospermum scoparium	8	n.d.	100	Light	5.36	3.43
Muehlenbeckia australis	12	43	38	Stratification (increases germination rate) & Light	17.18	19.88
Myrsine australis	17	92	n.d.	Stratification and Endocarp Removed	n.d	n.d.
Phormium tenax	10	96	77	Stratification	6.68	5.42
Sophora prostrata	10	n.d.	96	Scarification	12.34	53.44

* T_{50} at 20°C and 26°C following dormancy breaking treatment

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Appendix A:

Hydroseeding experiment conducted winter 2002

Introduction

Hydroseeding is the technique of applying a mixture of seeds, fertilisers (usually slow release fertilisers), mulches to protect seeds (bulk material to act as a carrier for other constituents e.g. binders), reduce soil moisture loss and provide initial erosion protection, adhesives and other additives in one or several operations, on to steep road batters and other sites (Kraayenord 1986; Coppin et al. 1990).

Hydroseeding roadsides for erosion control is being performed in many countries (Carr et al., 1980; Salkever 1994; Andres et al. 1996; Florineth & Gertgraser 1998; Andres et al., 2000). Andres et al. (2000) states that topsoiling and subsequent hydroseeding are the main practices designed to reclaim motorway embankments. Megahan (2001) looked at three erosion controls treatments on road cuttings on forest roads in the mountains of Idaho. The methods used were dry seeding, hydroseeding and terracing with hydroseeding. The areas where hydroseeding was used showed statistically significant reductions in erosion. Andres et al. (1996) also found good results in controlling erosion on motorway slopes in Spain, using the hydroseeding technique.

Hydroseeding can be performed in conjunction with other planting practises in order to obtain better slope stabilisation. Florineth & Gertgraser (1998) shows that after hydroseeding has established a ground cover, deciduous tree seedlings can be planted into the hydroseeded layer.

Hydroseeding equipment mounted on its transport (Photo: C.R. McGill)

Aim

To assess the suitability of a panel of New Zealand indigenous plant species (*Carex trifida*, *Coprosma robusta*, *Cyperus ustulatus*, *Hebe stricta*, *Muehlenbeckia australis*, *Myrsine australis*, *Phormium tenax*, *and Sophora prostrata*) for roadside land restoration using hydro-seeding technology. The effects of different cover crops in combination with different rates of fertiliser application on the establishment of native plants were assessed.

Introduction

Experiments conducted in 2001 established the most appropriate ways to treat seeds in order to break dormancy mechanisms, for example, stratification (Table 4.4). These results were used as a guide to pre-treat seeds before hydroseeding in winter 2002.

Materials and Methods

The experiment was carried out at one roadside cutting field site situated on State Highway 57 (4 km from Massey University). Ideally, several field sites should have been used but there was a lack of new roadside cuttings to be found. The field site was split into smaller plots with treatments established by hand to investigate a range of treatment combinations. The field experiment was fully replicated five times (to accommodate within-site variation).

For each indigenous plant species, the experiment was a randomised complete block design, with three levels of fertiliser (0, 0.1 or 1.0 times the commercially used rate of 200 kg/ha 12:10:10 Nitrophoska) in factorial combination with four "levels" of cover crop (no cover crop, Crested Dog's Tail, Annual Moata Ryegrass, or Browntop each at sowing rates typical of commercial practices). There were 12 treatment combinations (Table a.1) and these treatments were randomised within each of the five blocks.

Table a.1 The 12 treatment combinations

Treatment number	Level of fertiliser used	Cover crop
1	0	0
2	0	Browntop
3	0	Crested Dog's Tail
4	0	Annual Moata Ryegrass
5	0.1	0
6	0.1	Browntop
7	0.1	Crested Dog's Tail
8	0.1	Annual Moata Ryegrass
9	1	0
10	1	Browntop
11	1	Crested Dog's Tail
12	1	Annual Moata Ryegrass

In this experiment, it was assumed that competition between seedlings of the indigenous plant species would be minimal, allowing the species to be sown together, yet analysed separately.

Site conditions: The ground used for this experiment was reasonably uniform and level, and free of vegetation (sprayed with Roundup prior to establishment of the experiment) and topsoil (to avoid competition from pasture and other species already established on site).

Establishing treatments: Each block measured 6 m x 8 m, with 12 connected 2 m x 2 m plots. Plot corners were pegged so that plots could be easily located over the following months. Cover crop and fertiliser treatments were applied to each 2 m x 2 m plot by sowing and incorporating by hand before the indigenous plant seeds were sown. Within each plot, a central 1 m x 1 m area was pegged, into which the indigenous plant seeds were sown.

Mulching: After cover crop and fertiliser treatments had been applied, and the indigenous plant seed sown in the central 1 m^2 plot, the entire area was mulched with woodfibre and trackifer slurry at a commercial rate. This was applied by a clean hydro-seeder at a rate that did not disturb the treatments.

Sowing rates: The rate of sowing of viable (seed viability was determined using the topographical tetrazolium test (ISTA 1999)) indigenous plant seed depended on the size (seed weight) of each species. These rates were as following:

Seed size	Weight range (g/1000 seeds)	Rate (viable seeds/m^2)	Total needed (per 60 1 m^2 plots)
Light	<0.1	100	6,000 (e.g. *Leptospermum* and *Hebe*)
Intermediate	0.1 – 1.0	50	3,000 (e.g. *Carex* and *Cyperus*)
Heavy	>1.0	25	1,500 (e.g. *Coprosma*, *Muehlenbeckia*, *Myrsine*, *Phormium*, and *Sophora*.

Monitoring of germination: Cover crops, fertiliser, indigenous plant seeds and mulch were applied on the 23rd August 2001. The trial site was monitored once a week for 6 months for germination of cover crops and indigenous plants.

Results

Over the 6 month period there was no obvious germination of cover crops or indigenous plants. It is thought that due to the extreme weather conditions the hydro-seeding trials were unsuccessful. The topographical tetrazolium tests indicated high viability of all seed used. The cover crops were subjected to germination tests at Massey University Seed Testing Station preceding the experiment. Moata Ryegrass and Browntop had high germination percentages (94% and 91%, respectively), which would indicate if conditions were more favourable in the field they should of germinated. Crested Dog's tail had a low germination percentage of 21% so it would be expected not to have a high germination percentage in the field. In the future, it would be advisable to perform some glasshouse trials where the seed that has been pre-treated (to break dormancy characteristics) be incorporated with the slurry, grown under ideal conditions, in order eliminate or prove that the slurry effects the seed.

Whilst the trial area may not have validated the germination and dormancy research, Rural Supply Technologies Ltd (hydro-seeding company) have applied it on a commercial scale, which is showing good success rates. This in turn is proving the value of the seed germination and dormancy-breaking research.

Appendix B: Cleaning descriptions and the equipment that was used for each species studied.

Species	Cleaning Description
Carex trifida	Seed heads were air-dried at ambient room temperature for a period of 1 month, then threshed through a Westrup (LA-H) Dehuller with a screen size of 2 mm × 2 mm. The seeds were then processed through an air-screen cleaner (Burrows Office Clipper Tester and Cleaner) using a round shaped screen with an aperture of 1.59 mm (top) and an oblong-shaped screen with an aperture of 5.99 mm × 0.73 mm (bottom). Seeds were then processed through a Westrup (LA-H) Indented Cylinder Separator with a 2.75 mm indent to remove chaff and remaining husks. Lastly, a South Dakota seed blower with constant airflow of 1.07 m s^{-1} removed any remaining seed husks and light seed.
Coprosma robusta	Immediately after harvesting, mature fruits were placed in a plastic container containing distilled water and placed in the 20 ± 2°C constant temperature room to ferment, for a period of 4 days. Water was decanted off and the flesh and skins surrounding the seeds were rubbed off by hand and the cleaned seeds were left overnight at room temperature spread out on dry, 38 lb regular weight seed germination paper (Anchor Company), in order to dry off excess water. Seeds were rubbed between rubber mats to dislodge any dry debris attached to the seeds, and then the debris was removed in a South Dakota seed blower (airflow 4 m s^{-1}).
Cyperus ustulatus	Seed heads were air-dried at ambient room temperature for 3 weeks then threshed through Westrup (LA-H) Dehuller-screen size 2 mm × 2mm. Seeds were then processed through the air-screen cleaner with oblong shaped screen sizes 4.22 mm × 0.82mm (top) and 0.54 mm × 0.54 mm (bottom) and then processed through the Westrup (LA-H) Indented Cylinder Separator with a 2.75 mm indent followed by a 1.25 mm indent, which takes out grains of sand and round, inert particles. Lastly, seeds were processed through the South Dakota seed blower with a constant airflow of 4 m s^{-1} to remove husks and light seed.
Hebe stricta	Seed heads were placed in a paper bag and kept at ambient room temperature for 1 week. This enabled capsules to open, thus releasing seeds. Seed and debris were sieved (aperture of 1mm^2) which separated seeds from the debris. The sieving procedure was repeated several times.
Leptospermum scoparium	Branches with capsules attached were collected. Seed from several seasons were collected (probably from the 1998-99, 1999-00 and 2000-01 seasons) as all remained on the bushes. The branches with attached capsules were placed in a paper bag and dried at ambient room temperature for 1 month. The capsules opened, thus releasing seeds, which collected at the bottom of the bag. The seed was separated by, firstly, sieving (sieve aperture 1 mm^2) and then placed in a seed cleaning device (Contab LC m Microblower type 35, 220 V). Seeds were blown for 3 minutes with a constant airflow (9 setting) to remove further debris and empty seed. Seeds were then re-blown at the same setting (debris was subjected to a germination test to discover if any of the seed blown off was "full" but there were none).
Muehlenbeckia australis	Seed heads were air-dried at ambient room temperature for 1 month, threshed through Westrup (LA-H) Dehuller-screen size 2 mm × 2 mm and processed through the air-screen cleaner using round shaped screen sizes of 2.98 mm (top) and 1.59 mm (bottom). Then they were processed through the Westrup (LA-H) Indented Cylinder Separator with a 2.75 mm indent followed by a 1.25 mm indent, which removes grains of sand and round, inert particles. Lastly, seeds were processed through the South Dakota seed blower with a constant airflow of 4 m s^{-1}, which takes out husks and light seed.
Myrsine australis	Mature, fresh fruit were placed on dry 38 lb regular weight seed germination paper and left for three days to air-dry at ambient room temperature. To remove fruit skins, fruits were rubbed between two rubber mats. Loose skins were removed with a South Dakota seed blower with airflow of 4 m s^{-1}.
Phormium tenax	Capsules were spread out on a bench, where they were air-dried for a period of 2 weeks. Capsules split open and seed fell out or was shaken out by hand. Seeds were separated from the debris by hand.
Sophora prostrata	Pods, containing seed, were individually harvested by hand. Once harvested, pods were placed in a cardboard box and left at ambient room temperature for a period of 1 month. Pods were threshed through a Westrup (LA-H) Dehuller with a screen size of 10 mm × 10 mm. The seed were then cleaned through an air-screen cleaner using round shaped screens with holes 5.16 mm (top) and 3.77 mm (bottom).

A preliminary experiment was performed prior to *Leptospermum scoparium* seed being processed through the microblower in order to compare the germination percentage before and after processing. It has been stated in past papers (Grant 1966; Mohan et al. 1984) that the overall germination percentage for *L scoparium* seed is low. Four replicates of 100 seed were placed on Anchor Steel Blue seed germination blotter. The blotters were placed in clear plastic boxes, placed in 20 ± 2°C germination room. Germination obtained was 2.95 ± 0.62 % per 100 seeds. The remaining seeds were without embryos. All 'full' seed germinated.