Regeneration mechanisms in Swamp Paperbark (*Melaleuca ericifolia* Sm.) and their implications for wetland rehabilitation

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Declaration

I, Randall William Robinson, declare that the PhD thesis entitled Regeneration mechanisms in Swamp Paperbark (*Melaleuca ericifolia* Sm.) and their implications for wetland rehabilitation is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work

Randall William Robinson

28 August 2007

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Summary

This study investigated three aspects of the life history of Swamp Paperbark (*Melaleuca ericifolia* Sm.) that have implications for the ecology, management and restoration of wetlands occupied by the species: i) seed germination responses and tolerances; ii) clonal growth characteristics; and iii) safe sites for recruitment.

Laboratory studies included the responses and tolerances of seed to three key environmental factors: light; temperature; salinity; and the potential interactions they may have on germination. Germination percentages were used as indicators of success. Darkness, moderate temperatures (~ 20^0C) and low salinity levels (< 2 gL^{-1}) were found to be the most ideal germination conditions. Additional studies were carried out on secondary structures, hypocotyl hairs, which were shown to influence establishment success of seedlings. The conditions found ideal for germination proved to be suitable for hypocotyl hair formation.

Field and laboratory studies were carried out to determine the allocation of resources to reproductive effort and seed production in *M. ericifolia*. Comparative studies were carried out between two sympatric *Melaleuca* species with contrasting life histories and reproductive strategies (clonal vs. non-clonal) to determine if there were differences in reproductive capacity and commitment of resources to either sexual or asexual reproductive effort. There was low germinability of the clonal species *M. ericifolia* (< 40%) when compared to the non-clonal *M. parvistaminea* (> 70%). Germinability of *M. ericifolia* was reduced as population size decreased and distance to nearest population and degree of disturbance increased.

Laboratory and field studies were undertaken to investigate the growth characteristics and ecological significance of the clonal growth form. Genetic methods were used to determine the genetic diversity and clonal intermingling in existing populations. Individual genets were found to contain thousands of stems and cover areas greater than 3,000 m^2. Intermingling of the genets was not found. Air-photograph interpretation and structural analysis of individual clones were used to determine colonisation rates, longevity and time since recruitment. Lateral growth rates were generally found to be rapid, up to 0.5m per year. The largest plants found (3,274 m^2), were determined to be approximately 52 years old.

Safe sites for germination and recruitment were determined using historical aerial photographs and climate data combined with on-ground confirmation and characterisation of conditions. Microtopographical relief provided by hummocks within the wetland provided suitable safe sites for recruitment by modifying light, salinity and moisture levels to a range suitable for germination and hypocotyl hair production. Recruitment was however, restricted to a limited range of climatic conditions that diluted salinity levels but did not inundate newly germinated seedlings: flood conditions in spring followed by average rainfall in summer.

Recommendations for landscape-scale rehabilitation of wetlands using *M. ericifolia* were formulated. The implications of the findings of this study on current ecological restoration theory and practice are discussed. Germination from seed in the highly modified conditions found in many wetlands in South-eastern Australia is problematic due to the specific climatic and on-ground conditions needed for successful

recruitment. The findings of the growth and genetic studies of *M. ericifolia* indicate that planting of nursery grown stock is possible and even preferable if the growth characteristics of the plant are taken into consideration. Present planting methods used for non-clonal terrestrial species, hand planting large numbers of seedlings at close spacings, is inappropriate for *M. ericifolia.* A planting method that carefully selects planting sites, uses smaller numbers of plants and factors in clonality and lateral growth rates (time) would reduce restoration costs and improve long-term survival of planted stock.

Chapter 1

Introduction

Swamp Paperbark *(Melaleuca ericifolia* Sm.*)* is a colony-forming clonal tree species that grows in near-coastal wetlands in south-eastern Australia. The distribution and abundance of this species has decreased markedly with the clearing or draining of many of the wetlands in which it formerly occurred (Bowkett and Kirkpatrick 2003). Community groups, non-government organisations and government authorities are committing considerable effort and finances to the restoration of *M. ericifolia* and the wetlands in which it occurs. Closely related species, occurring in similar situations throughout Australia and overseas, are also the subjects of large-scale restoration efforts (Turner and Lewis 1997; de Jong 1997; de Jong 2000).

Assisted regeneration of *M. ericifolia* and related species is, at present, totally reliant on the use of manually planted nursery-raised seedlings. Conventional plantings of *M. ericifolia* follow terrestrial planting schemes for non-clonal species, using high numbers of individuals planted on 2-3 m spacings or closer (de Jong 2000; Greening Australia 2003). The process of natural regeneration, the preferred ideal in the long-term, is not well understood and alternative techniques have not been developed or tested to improve planting success. In the short – medium-term reliance on manual planting remains a reality although it is by no means the preferred option (Cole 1998; Van der Valk 1998; de Jong 2000). A full characterisation of the life history attributes of the species, including regeneration mechanisms such as seed germination

establishment and clonal lateral spread is necessary if more naturalistic regeneration methods are to be attempted.

The evolution of the clonal growth form (vegetative outgrowths sprouting at a distance from the original plant) is usually attributed to the high natural variability of the wetland habitat and severely limited resources (Fischer and van Kluenen 2001). Clonality confers two advantages to wetland plants. Firstly, the clonal growth form allows plants to circumvent sexual reproduction in an environment where seedling recruitment events may be extremely rare or risky. Second, as many stems in an individual clone of *M. ericifolia* remain connected, they retain their ability to transfer air, nutrients and water between stems. Stems growing in ideal conditions have the theoretical ability to support stems growing in otherwise unsuitable conditions, conferring an ecological fitness not available to non-clonal plants (Hutchings 1999).

The allocation of resources to asexual versus sexual reproduction has been linked to the heterogeneity of the environment or limited resources (Cain *et al.* 1996) leading to a delay in reproductive maturity. In extremely resource-limited environments, such as wetlands and deserts, the clonal life form is particularly well developed (Song and Dong 2002; van Groenendael *et al*. 1997) and plant longevity may be extreme (Vasek 1980). Several clonal and non-clonal *Melaleuca* species are co-extensive in southern Australia and provide an opportunity to undertake comparative studies.

Development of the clonal growth form allows efficient capturing of resources and great longevity in some plants, but may lead to a disadvantage in regard to sexual reproduction and severely reduced genetic diversity within a population (Wherry

1972). Novel or infrequent ecological challenges may arise that result in senescence of existing individuals leading to further erosion of genetic diversity or even local extinction. Decisions in relation to the conservation of *M. ericifolia* are limited by a lack of knowledge regarding the genetic diversity and number of individuals within existing populations. The clonal life form in *M. ericifolia*, and the potentially large number of individual stems contained in a genet, may lead to unwise seed collection or propagation techniques. Seed production between genets can vary considerably but may be relatively uniform within the genet (*pers obs*.). Not understanding the underlying clonal nature of the species and the potentially large area covered by a genet may lead seed collectors to deduce that they are dealing with a genetically diverse colony instead of one genetically uniform plant.

This study questions the appropriateness of presently used methods for regeneration of *M. ericifolia* and, by extension, similar swamp-growing clonal species. It would appear that present assisted regeneration is not based on knowledge of the species used or the basic ecology of the system being restored (Van der Valk 1998). Existing restoration of brackish wetlands is based on horticultural principles, is expensive and is applicable to the small scale only and likely to be unsuccessful (Mitsch 1998; Mitsch and Wilson 1996). Two major questions identified in preliminary work on *M. ericifolia* (de Jong 2000; Van der Walk 1998) relate to:

- the ecological fitness of the plants established through present manual planting techniques and;
- the relevance of present planting techniques to a clonal species such as *M. ericifolia*.

1.1 General Ecological Background to the Project

1.1.1 *Melaleuca*

Melaleuca is one of several large and diverse genera of shrubs and trees including *Eucalyptus, Leptospermum* and *Callistemon* that make up the family Myrtaceae. Australia contains seventy-five native genera and over 1,400 species of Myrtaceae, which is nearly half the total number of species in the world (Jeanes 1996). Forests and woodlands dominated by *Melaleuca* cover nearly 90,513 km^2 in Australia (National Land and Water Resource Audit (2001) (Figure 1.1).

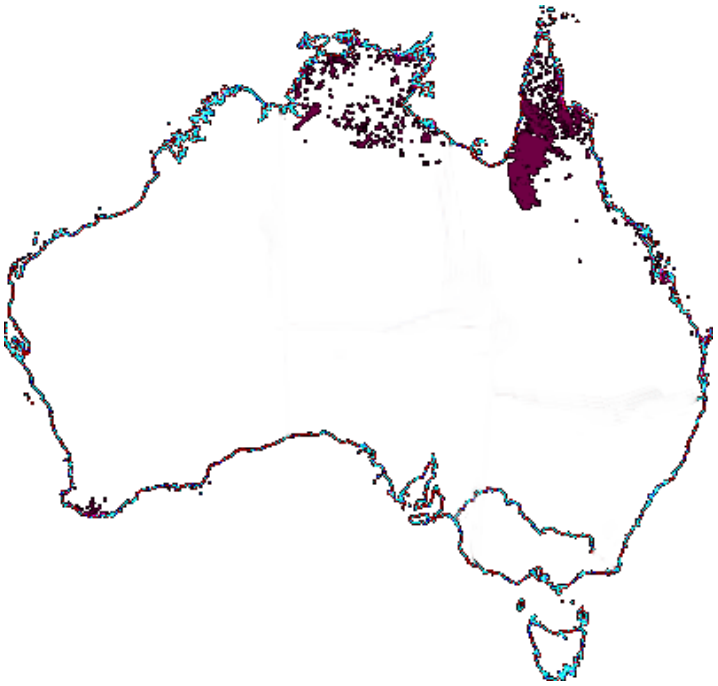

Figure 1.1 Distribution of *Melaleuca* woodlands and forests in Australia. Adapted from National Land and Water Resource Audit (2001). Woodlands, Forests

Swamp Paperbark (*Melaleuca ericifolia*), the species under investigation, is one of a genus of approximately 240 species of trees and shrubs with a primary distribution in Australia but represented in New Guinea through to South-east Asia (Spencer 1996). Many *Melaleuca* species are associated with wetlands or areas with impeded drainage. All *Melaleuca* species have seed stored in woody capsules on the plants. Only a few of the species found in eastern Australia produce lateral vegetative growth by suckering from the roots (Byrnes 1984) and these species occur in seasonally inundated situations (swamps).

The most primitive species of *Melaleuca* occur in inundated tropical lowlands with *Melaleuca* generally replacing *Eucalyptus* as the dominant tree species (Barlow 1988). This suggests a warm climate derivation of *Melaleuca*. There is a record of *M. ericifolia* in southern Australia from a fossilised forest in western Tasmania dating from the late Pleistocene (1.2 million years before present) (Rowell *et al.* 2001) and an even earlier possible record from the Mid Cenozoic (25 million years before present) (Lange 1978) when temperatures in this area were approximately 1-1.5^{0}C higher than present.

The genera *Melaleuca, Callistemon* and *Eucalyptus* are part of a larger flora that has evolved within Australia (Australian element) and have adapted to changing climate, particularly decreasing temperatures, increasing aridity and seasonality (Hill *et al.* 1999) and decreasing soil fertility. These factors have lead to increasing habitat differentiation and speciation with most of the present broad vegetation formations and many genera well established by the Late Cenozoic (Hill *et al.* 1999).

1.1.2 Adaptations to soils and climate

Scleromorphy, characterised by small, hard leaves, short internodes and small plant size, is a characteristic of *M. ericifolia*. Evolution of scleromorphy in *Melaleuca* and other genera in the Australian element of the Australian flora, is a specific adaptation to low nutrient levels in the soil (Specht 1972; Johnson and Briggs 1981) and in particular, low soil phosphorus levels (Beadle 1968) and not an adaptation to aridity as originally assumed. Xeromorphy (morphological adaptation to aridity) is a secondary trait conferred by plant adaptations to low nutrient status soils.

Most species of *Melaleuca* have woody capsules that release seed after the parent branch is killed, either by fire or other means. The development of serotinous fruits (woody, late-opening capsules) in *Melaleuca* and similar genera is generally viewed as an adaptation to and protection from fire (Specht 1981). Gill (1993) clearly reports, however, that the greatest richness of woody fruits is more closely related to mineral-poor soils. Many of the species that produce serotinous fruits do not have other seed dormancy factors and are relatively short-lived in the soil seed bank (Ashton 1985; Gill 1993). There is some evidence that fire may temporarily increase soil nutrients to allow germination and survival of woody-fruited species (Specht *et al.* 1958; Ashton 1976) but this is not conclusive.

A second competitive advantage conferred by serotiny is predator satiation (Silverton and Charlesworth 2001). The predator satiation hypothesis is usually applied to trees that produce mast (large seed crops, followed by small or non-existent seed crops), flooding the system with copious amounts of seed and therefore overwhelming the

ability of predators to harvest all seed. This strategy ensures that at least some seed and seedlings survive. A similar effect is achieved post-fire in Australian species that have woody persistent fruits that are held in the canopy and released *en masse* after fire (Ashton 1979). While some annual seed rain takes place in *Melaleuca*, through the death of stems, this may not be of sufficient magnitude to outpace the harvesting rate by granivores (ants).

1.1.3 Vegetative growth

A particularly noticeable feature of *M. ericifolia* is its clonal growth form, with individual plants able to cover large areas and produce numerous stems. In evolutionary terms, the clonal growth form is very ancient and occurs in a wide range of plants (Mogie and Hutchings 1990). van Groenendael *et al.* (1997) and Hatton (2005) estimated that well over two-thirds of wetland plants exhibit the clonal growth habit.

The clonal growth form, based on lateral vegetative reproduction, is highly mobile, allowing for wide-ranging utilisation of nutrients, space and other resources without going through sexual reproduction in potentially inhospitable sites (Silvertown and Charlesworth 2001). There appears to be a trade-off between clonal growth and sexual reproduction, with decreased sexual reproduction associated with increased clonal growth as has clearly demonstrated in the wetland genus *Mimulus* (Scrophulariaceae) (Sutherland and Vickery 1988). Clonal growth is delayed until after flowering in *Heiracium* (Asteraceae) (Bishop *et al.* 1978) but the reverse is true

in some bamboo species (Poaceae) (Silvertown and Charlesworth 2001). The trade-off between sexual reproduction and clonal growth in *M. ericifolia* is not at all clear.

The particular way that a plant produces new lateral growths (ramets) can determine the distribution and intermingling of separate plants (genets) (Harper 1978). Plants that produce short and frequently branched connections between ramets generally spread along a front or phalanx (Silverton and Charlesworth 2001). Conversely, plants with long-spacers and little branching, progress in guerrilla mode, infiltrating individuals of the same or other species (Lovett Doust 1981). The phalanx mode of growth tends to occur in low-nutrient, high-light habitats (van Groenendael *et al.* 1997). The guerrilla mode of growth is more closely allied with soils where nutrients or moisture are not evenly distributed. It is not understood if the growth form of *M. ericifolia* is guerrilla or phalanx.

Species utilising either the phalanx or guerrilla mode of growth may retain the connections between the ramets. These physical attachments allow all the stems in a plant to function as one, allowing transport of nutrients, oxygen and water between spatially separate and potentially disparate but physiologically integrated ramets (Marshall 1990). The physical attachments between stems may be of particular importance in *M. ericifolia*. If, as is thought, individual plants with multiple connections occupy large parts of an environmental gradient, *e.g.* soil moisture, these established plants would not be adversely affected should other parts of the plant be inundated. Conversely, clonality may allow plants to expand into areas normally too dry to support *M. ericifolia*, with the dry area stems being supported by those in moister habitats. Ramets of *Fragaria chiloensis* (Rosaceae) growing in well lit, dry,

nutrient-poor habitats are known to share resources with connected ramets in shaded, well-watered, nutrient-rich habitats to the benefit of both (Alpert and Mooney 1986).

The factors affecting senescence and death in *M. ericifolia* are not known. Theoretically, unless the habitat conditions change dramatically, clonal plants are potentially immortal. There are some studies of the longevity of individual clonal species. While the longest-lived clone of a woody plant so far identified is *Lomatia tasmanica* (Proteaceae), in western Tasmania, with an age of approximately 43,600 years old (Lynch *et al.* 1998) there are many others that are very old. A clonal hybrid Eucalypt, also in Tasmania, has conservatively been estimated to be 900 years old (Tyson *et al.* 1998). Individual Creosote Bush (*Larrea tridentata*) plants in the Mojave Desert have been estimated to be 6,000-11,000 years old (Vasek 1980). Slightly older is the Box Huckleberry (*Gaylussacia brachycera*) in Pennsylvania at approximately 12,000 years old (Wherry 1972). Plants of *M. ericifolia* while exhibiting a similar growth habit to these last examples are unlikely to be as old as these previous examples as the Gippsland Lakes environment, as presently configured, is less than 6,000 years old (Bird 1965).

1.1.4 Genetic diversity

Long-term management and conservation of *M. ericifolia* populations, including determining planting densities for restoration plantings, is dependant on the identification of the number of individuals and genetic diversity within naturally occurring populations. Degree of genetic diversity can vary widely between and within species (Hamerick and Godt 1990). While genetic diversity is widely held to

be desirable, species that primarily reproduce vegetatively are commonly found to have low genetic diversity (Simonich and Morgan 1994; Holsinger, 2000; Rivera-Ocasio *et al.* 2002; Nuortila *et al.* 2002). Low genetic diversity in plants is not, however, directly related to reproductive success. This is especially the case in clonal plants (Eckert 2001) with many of our most common weeds being clonal. *Salvinia molesta* (Salvineaceae), a sterile hybrid and one of the world's most abundant wetland weeds, relies exclusively on vegetative reproduction (Loyal and Grewal 1966).

It may not be always be readily apparent when the population of a long-lived clonal species has fallen below a critical threshold for sexual reproduction (Fehrig 2001). This is especially the case with species with long generation times, in which the critical extinction event may not become evident for hundreds of years (Armbruster *et al.* 1999). For some out-crossing clonal species, populations, which in fact are individuals, may give a false sense of security to conservation managers. This senario was the case for Box Huckleberry (*Gaylussacia brachycera*) prior to specific population studies (Wherry 1972) that determined the populations to be clones. If pollination requirements are not met, the plant becomes functionally asexual and is at risk of extinction.

When trying to identify clones in the field, the inherent variability of the foliage and other morphological characteristics within and between clones often provides little guide to determining the extent of any given clone, but this is not always the case. Genetic testing and analysis allow positive identification of individual clones at a level of discrimination that is not possible with traditional morphological approaches (Tyson *et al.* 1998). Genetic analysis also gives an indication of the size of the

individual plant from which the level and type of recruitment over time can be determined as well as the rate of mortality. Knowing the number of individuals in a population confers an ability to determine genetic diversity and conservation of genetic diversity (Aitken *et al.* 1998).

No genetic studies have been carried out to date on *M. ericifolia* or indeed other *Melaleuca* species to determine natural population densities. Genetic testing has not been carried out on what have been assumed to be individual plants that cover large tracts of land. Conservation of the species in the long term is dependent on the identification of the number of potentially sexually reproducing plants in a population and across the species' distribution. Although it is not envisioned that the extremes found in some studies (one plant per 16 ha for *Gaylussacia brachycera* (Wherry 1972) or one plant per 1.2 sq. km for *Lomatia tasmanica* (Lynch *et al.* 1998) will be found in *M. ericifolia*, at present there is no indication of the number of individual clones in a population, their size or longevity.

Genetic testing can indicate the degree of clonal intermingling (the degree of overlap between adjacent plants). Studies in woody clonal species worldwide indicate that degree of clonal intermingling varies widely among species from complete separation of individuals to total mixing of ramets (Zhang *et al.* 2002; Van Kluenen *et al.* 2000) with phalanx species tending not to intermingle. Lack of clonal intermingling, should this prove true in *M. ericifolia*, would suggest alteration to present planting densities and configuration of plantings. Specifically, if individual *M. ericifolia* plants planted at close spacings do not overlap, the ecological fitness conferred by the clonal growth form will be negated.

Molecular markers have been used as the most reliable tool to determine the number of individuals in populations of clonal species. Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR)-based marker method that increases the number of markers without limit (Torimaru *et al.* 2003) and has been used to determine clonal diversity in a large number clonal species in Australia and overseas (Kreher *et al.* 2000; Widen *et al.* 1994; Williams *et al.* 1990). While RAPD markers have proved useful, recent work at the Royal Botanic Gardens Melbourne by Elizabeth James (RBG Melbourne pers comm.) indicate that Inter Simple Sequence Repeat (ISSR) allows for greater numbers of markers within an individual sample, at much reduced cost (Godwin *et al.* 1997).

1.1.5 Sexual reproduction

Seed production and gemination has been little studied in *M. ericifolia*. The main study of *M. ericifolia* was by Ladiges *et al.* (1981). They found that germination of *Melaleuca* seed was inhibited by submergence. *Melaleuca ericifolia* also failed to germinate at salinity levels above 14 g/L^{-1} although the range of salinities tested in this study was limited. Interestingly, germinated seed was able to survive in water for several weeks by floating on the surface. Ladiges *et al.* (1981) used a standard germination temperature known to effect germination in a wide variety of plants. Salter (2001) investigated the synergistic effects of salinity and water regime on seedling survival, but not germination, as part of an honours project.

In a study of regeneration of a swamp in which *M. ericifolia* occurred, germination of seed and survival of seedlings was rare and limited to sites with specific conditions, namely weed free and constantly moist but not inundated (de Jong 2000). de Jong's (2000) study does not record the period of emergence for *M. ericifolia* but does indicate that seed was planted in mid-summer. The likelihood of *Melaleuca* being reliant on higher temperatures for germination of seed is likely, based on the tropical derivation of the genus. No studies have been carried out to determine the tolerances and ideal temperature and light conditions for germination. None of the above germination studies investigated the potential synergistic effects of combining temperature, light and salinity as would be found under field conditions.

Reproduction from seed in a long-lived clonal species may be of little importance in the short – medium-term, or possibly even to the long-term survival of the species. This is because only sufficient numbers of recruits are needed to replace plants that senesce and die, or to colonise new habitat (Rea and Ganf 1994). Numerous examples exist of widespread and abundant, long-lived clonal, species with little or no record of seedling recruitment (Peirce 1998; Nuortila *et al.* 2002). For example, some of the world's most problematic weeds are not known to reproduce sexually, including Salvinia (*Salvinia molesta*) and Water Hyacinth (*Eichhornia crassipes*) (Room and Julien 1995; Wright and Purcell, 1995), the latter only reproducing sexually in it's native habitat (Barrett 1980).

The age at which plants reach their reproductive maturity is closely correlated to plant/adult longevity and availability of resources (Takada and Caswell 1997; Geber 1990). Delay of reproductive maturity can have a competitive advantage in nutrient

restricted sites by increasing the amount of nutrients and energy available for seed production (de Jong *et al.* 1987). Under horticultural conditions there appears to be significant differences in the reproductive maturity in the sympatric species *M. ericifolia* and Rough-barked Honey-myrtle (*M. parvistaminea)* although this has not been proven in the field. Longevity of both species is not known but there would appear to be potential differences in longevity based on growth form. *M. ericifolia* has the ability to spread laterally by means of vegetative growths from the roots, whereas Rough-barked Honey-myrtle lacks this ability.

1.1.6 Rehabilitation approaches

Approaches to the rehabilitation of wetlands has become increasingly polarised with various authors arguing for intervention or non-intervention (van der Valk 1998; Mitsch 1998). de Jong (2000) attempted to strike a mid-point between these two competing and incompatible theories by suggesting that some intervention (planting) may be required in wetlands where clearing and grazing have been the main form of degradation.

Despite altered hydrology being cited as the most common form of disturbance in wetlands (Streever 1997), there has been little investigation into altering or restoring hydrological processes in coastal wetlands (de Jong 2000). Synergistic effects among altered hydrology, clearing, grazing and salinity are likely to have a significant impact on plant growth (Kozlowski 1997). Recent work initiated in Victoria, of which this project is a part, is investigating the management of high-value wetlands subjected to multiple environmental threats (Boon *et al.* 2005).

1.2 Aims of this project

This project forms part of a larger overall grant-funded project investigating the management and rehabilitation of brackish wetland facing multiple threats based at Dowd Morass on the Gippsland Lakes at Sale, Victoria. Other related PhD projects based at both Victoria University and Monash University are specifically investigating the effects of water regime on ecological health, the effects of water regime and salinity on keystone species and invertebrate/plant interaction in relation to management. A fuller documentation of Dowd Morass and the overall project can be found in two handbooks prepared by the research team (Boon *et al*. 2005; Boon *et al*. 2007)

The aims of this project were to:

- **Determine the number, distribution and intermingling of clones** in a naturally occurring population of *M. ericifolia*. Genetic testing will be carried out using Inter Simple Sequence Repeat (ISSR) will be used. Individual clones will be determined using a hierarchical analysis function in Microsoft Excel 2000 (Microsoft Corporation, Troy, New York).

- **Determine the rate of colonisation** of *M. ericifolia* over a 46-year time frame from historical aerial photography. Rate of colonisation will be calculated by averaging growth rates of a number of clones of *M. ericifolia* determined through genetic testing and assisted by aerial photograph interpretation and

visual assessment on the ground. Existing aerial photography dating back 46 years will be used to map extension of lateral growth of selected clones.

- **Carry out a comparative study of the viability of the clonal and non-clonal species** *M. ericifolia* and *M. parvistaminea* and between populations of *M. ericifolia* across a majority of its range. Comparative analysis will be used to determine the trade-off between allocation of resources to sexual reproduction versus lateral vegetative growth.

- **Determine the ideal conditions and tolerance levels under which germination** of *M. ericifolia* can be achieved and the individual and potential synergistic effects of light, temperature and salinity (factors) on key germination indicators (percentage germination, percentage recovery and germination after inundation with salt water). Standard graduated germination tests will be carried out in the laboratory using growth cabinets. Data will be analysed utilising a general linear model three-way ANOVA with fully orthogonal design using version 11 of SPSS. Key germination indicator recorded will be; total percentage germination.

- **Determine the sensitivity and tolerance levels** of single-celled structures (hypocotyl hairs) to salinity, temperature and light. These structures have been shown to be critical to establishment of a range of wetland species (Baranov 1957; Polya 1961; Matsuo and Shibyama 2002).

- **Determination of safe site for recruitment** of *M. ericifolia*. A range of methods will be used including historical aerial photography, historical climate data and on ground survey and assessment. Data collected will be compared to the germination tolerances and parameters identified in previous chapters.

- **Formulation of recommendations for landscape-scale rehabilitation** of brackish wetlands utilising *M. ericifolia* will be formulated using the findings of this and other studies.

Chapter 2

The study site

2.1 Introduction

Fieldwork was carried out at Dowd Morass on the south-western shores of Lake Wellington near Sale, Victoria (38°07'S 147°10'E) (Figure 2.1). The study site is a > 1,500 ha wetland on public land and is presently managed by Parks Victoria. Dowd Morass makes up part of the overall Gippsland Lakes Ramsar site and is listed on the register of the National Estate (DSE 1999). The water levels at Dowd Morass are managed and have been kept artificially high for at least the last 20 years, with one purposeful drawdown event (Schulz pers. comm.). Levee banks within Dowd Morass have recently been restored to allow two distinct hydrological regimes to be maintained.

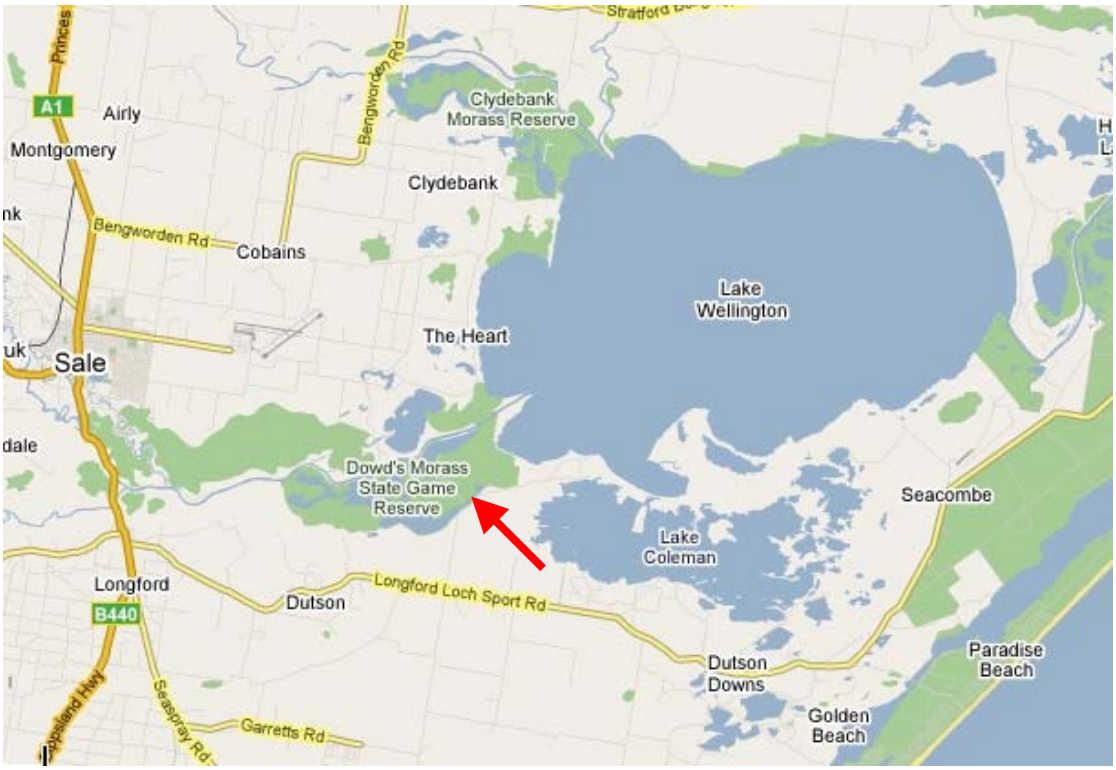

Figure 2.1 Map of the Gippsland Lakes, Victoria. Lake Wellington is the large lake at the western edge of the lakes complex with Dowd Morass (red arrow) located on the southwest edge of Lake Wellington (copyright Google 2008, MapData Sciences Pty. Ltd. PSMA).

2.2 History of Dowd Morass

Alienation of the land at Dowd Morass, primarily for the purposes of grazing, started in approximately 1888 and continued until 1942 (State Rivers and Water Supply Commission 1972). In 1968 large sections of the easternmost section of Dowd Morass were converted to the Dowd Morass Wildfowl Reserve leaving most of the western part of the wetland in private ownership. Between 1959 and 1975 attempts to purchase the land in the western sections of the wetland were unsuccessful.

In 1973, while the western sections were still in private ownership, further alienation of the wetland occurred when a series of levee banks approximately 0.9-1.9 m Australian Height Datum (AHD) were constructed (Figure 2.2). These levees almost completely separated the eastern and western sections of the wetland (Figure 2.2). The levees were constructed "with a view to drainage and development for agricultural purposes" (SRWSC 1972), to prevent overbank flows from the Latrobe River and to prohibit brackish water from Lake Wellington entering the western side of the morass (Keith Heywood, *pers. comm.*). Two artificial drains were constructed in the early 1970s to establish a hydraulic connection between Dowd Morass and the Latrobe River.

In 1975 the State Government of Victoria purchased the western part of the Dowd Morass wetland and created a State Game Reserve incorporating all of Dowd Morass. Breaches were created in the levees to improve water circulation within the morass and to restore a more natural hydrology and water regime. In 1987, the managing agency (Parks Victoria) installed gated culverts on the larger of the two drains (Drain

1, Figure 2.2) so that water levels could be artificially managed (Sinclair Knight Merz 2003).

The recent history of Dowd Morass and the wetlands of the Gippsland Lakes are most strongly influenced by a range of human-induced changes. At the time of settlement, Dowd Morass was a primarily freshwater wetland, filling via floodwaters from the Latrobe River and periodically with water from the variably saline Lake Wellington. Water levels within Dowd Morass would have naturally fluctuated with seasonal variations to rainfall and evaporation. The natural wetting and drying cycles prior to European settlement are estimated to have happened on a five-year cycle with complete drying out (drawdown) about every five years (Parks Victoria 1997).

Five major human-induced changes have significantly altered the spatial and temporal water regime and water quality of the Gippsland Lakes and the surrounding wetlands.

1. The formerly forested catchment of the Latrobe River has undergone major land-use change from the mid 19th century. Primary amongst these changes is the conversion of large tracts of forest to agricultural land with subsequent alterations to water tables, nutrients and sediment inputs to the river (Gutteridge Haskins and Davey 1991).

2. There have been major impoundments of waters of the Latrobe River, particularly Lake Glenmaggie (1926) and the Thomson Dam (1983). Both of these dams have reduced variability of flow within the river and reduced frequency of smaller flood events (Grayson 2003).

3. The creation of a permanent connection between the ocean and the Gippsland Lakes at Lakes Entrance altered the salinity regime of the lakes system and lowered water levels in the lakes by approximately 60 cm. The permanent opening of the Gippsland lakes to the ocean has particularly impacted on Lake Wellington and the adjacent wetlands Dowd Morass and Heart Morass, which were primarily freshwater wetlands prior to European settlement (Gippsland Lakes Task Force 2004).

4. The construction of the internal levee banks and drains significantly altered the hydrological regime of Dowd Morass. The drains allowed flooding or draining of the wetlands from the Latrobe River (Figure 2.2). Several of the compartments created by the levees have water levels maintained at artificially high levels and have been prevented from experiencing natural drawdown. The lack of internal connectivity created by the levees has maintained distinct water and salinity regimes for each compartment (Boon *et al*. 2007).

5. The water regime at Dowd Morass has been actively managed since 1975 when the wetland was declared a State Game Reserve. Flooding of Dowd Morass was commenced in 1975 to prevent saline intrusion from Lake Wellington and to provide suitable habitat for waterfowl (Schulz *pers. comm.*). Since that time water levels have been maintained at an artificially high level except when there was a trial drawdown during the summer of 1997-98. This drawdown was prompted by evident deterioration in the condition of *M. ericifolia* adults and lack of recruitment of young seedlings (Schulz *pers comm.*).

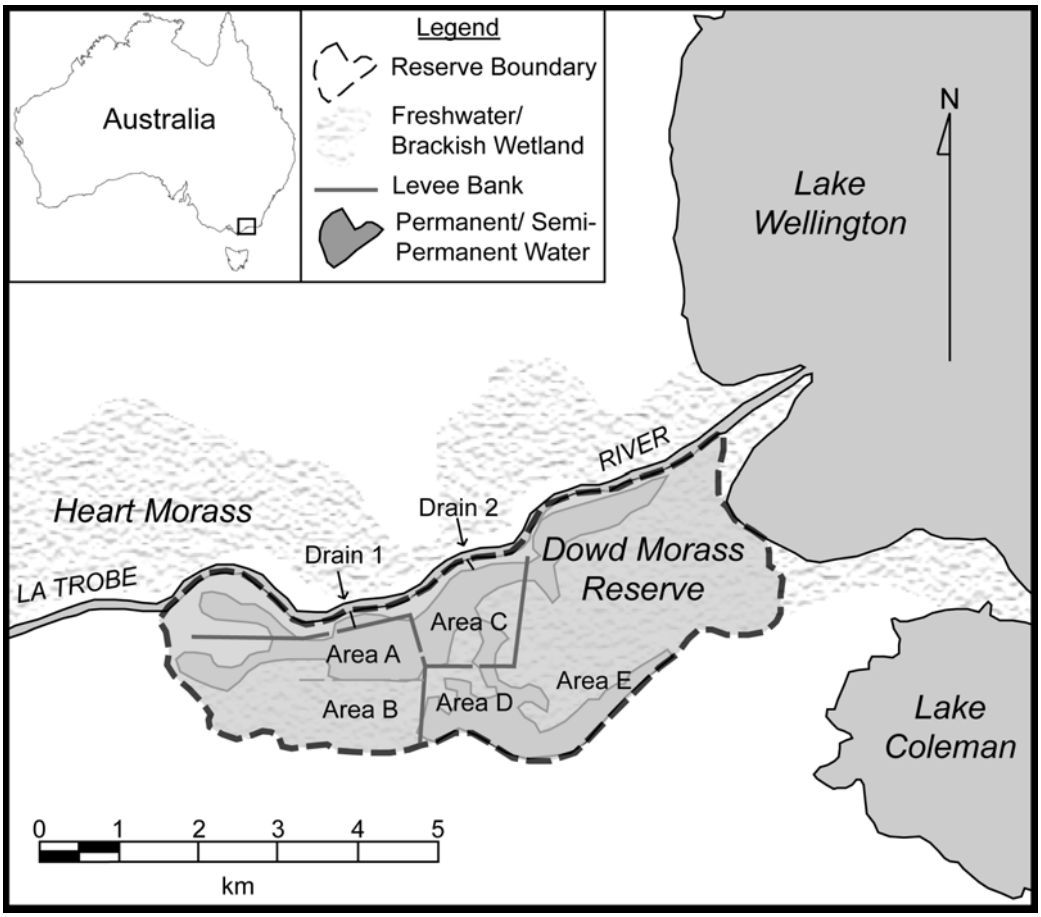

Figure 2.2 Dowd Morass, showing the location on internal levees and the resultant division of the wetland into five discrete zones. Taken from Boon *et al*. (2007).

2.2.1 Water levels over past ~ three decades

As noted earlier, a series of levee banks approximately 0.9-1.9 m AHD were constructed in Dowd Morass in 1973. These levees almost completely separated the eastern and western sections of the wetland and radically altered the natural water regime of the wetland. Analysis of aerial photographs by Boon *et al.* (2007) showed that surface water covered only 12 % (182 ha) of the wetland in 1964. Water covered 7 % (121 ha) of the wetland when the levees were constructed in 1973.

The breaches in the levee walls created in 1975, while having some effect on restoring water regime, were not successful in restoring pre-levee water regimes. By 1982 the extent of open water at Dowd Morass increased to 31 % of the total area (515 ha) this being a conservative estimate due to the difficulty of detecting the presence of surface water beneath the canopy of Swamp Paperbark.

Various episodic spot measurements of water levels in Dowd Morass are available from 1992 to 2003 but as they have not been calibrated, should be interpreted with caution (Figure 2.3). These data do, however, support the notion that Dowd Morass has been flooded permanently since at least 1992, with the exception of the drawdown in 1998. These data also demonstrate that water levels in Areas A – E of the Morass have fluctuated between 0.2 and 0.6 m over this period. Moreover, water levels in Areas A – E rose and fell in concert, suggesting these areas are now relatively well connected hydrologically.

Staff from Parks Victoria were concerned, in the mid 1990s, that near-permanent inundation was having adverse impacts on Swamp Paperbarks in Dowd Morass. A drawdown of water levels was initiated in March 1997 by draining water through the gated culverts to the Latrobe River. Water levels were completely drawn down during the summer of 1998 and the wetland was dry for 173 days. This drying time was not considered by Parks Victoria staff as sufficient to achieve the management goals of restoring the Swamp Paperbark community.

In March 1998, the managing agency opened the gated culverts joining Dowd Morass with the Latrobe River in order to allow water to flow into the wetland. The Latrobe River was low at that time and there was only a small flow into the Morass. During a major flood of Lake Wellington in mid-1998, brackish water from Lake Wellington backed up the Latrobe River and entered Dowd Morass via the Dardenelles and via overbank flow along the Latrobe River. The effect on wetland salinity of this saline intrusion can be seen in Figure 2.5. Since reflooding in 1998, water levels in Dowd Morass have been maintained between 0.3 and 0.8 m, deeper even than pre - drawdown levels (typically 0.2-0.6 m).

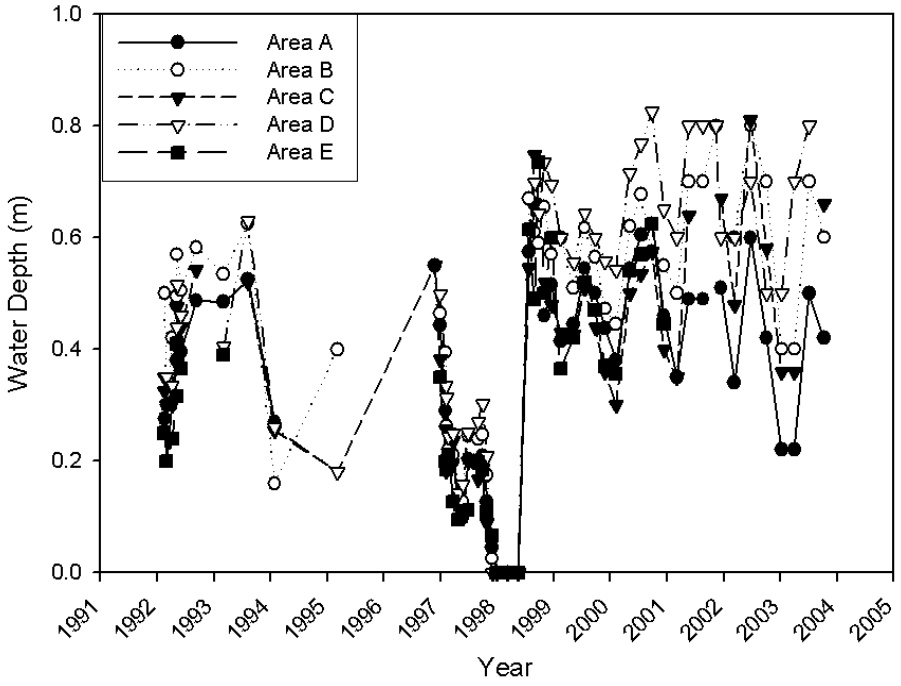

Figure 2.3 Recent (since 1991) patterns in water levels in various sections of Dowd Morass. (Taken from Boon *et .al* .2007)

2.2.2 Salinity regimes over past ~ three decades

Spot measurements of electrical conductivity suggest that the salinity of water in Dowd Morass fluctuated between <1,000 and over 20,000 μS cm^{-1} between 1992 and 2003 (Figure 2.4). The effects of the saline intrusion from Lake Wellington are evident in Figure 2.5, with salinities reaching 20,000 μS cm^{-1}in late 1998 and early 1999.

After the short-lived drying event and saline intrusion in 1998, the average salinity of surface water in Dowd Morass has increased and become more variable. Prior to 1998 surface water salinities were generally below ~ 8,000 μS cm^{-1} excluding one sampling period in 1995. Differences across sites within the wetland also have become evident, with Area E > Area D > Area C > Area B > Area A (Figure 2.4). This pattern may reflect the influence of saline intrusions from Lake Wellington extending into Areas E, D and C but exerting little effect in Areas A and B.

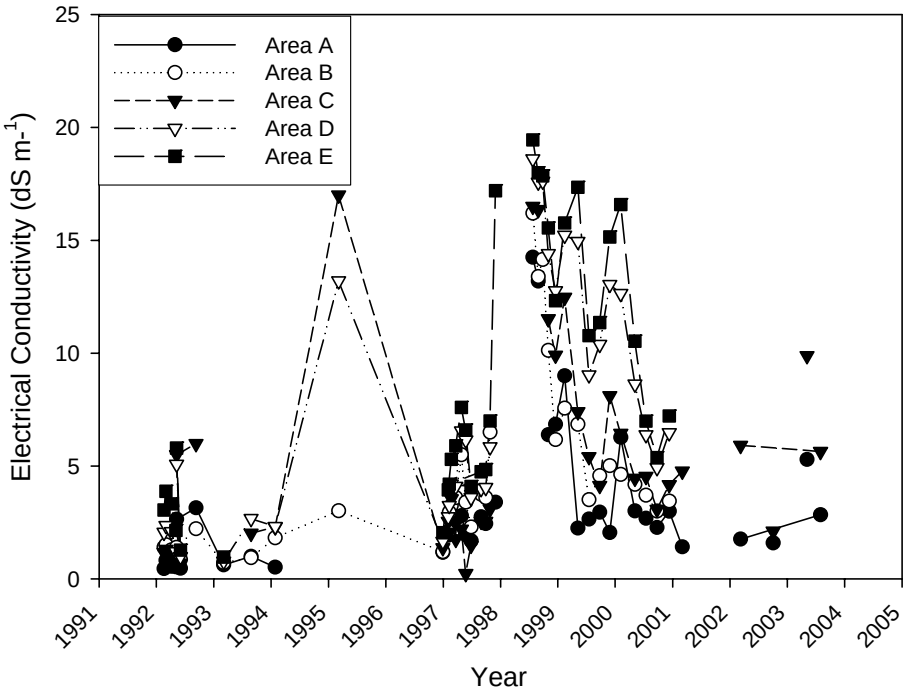

Figure 2.4 Recent (since 1991) salinity patterns in various sections of Dowd Morass (Taken from Boon *et al.* 2007)

2.3 Water quality in Dowd Morass

The report by Sinclair Knight Merz (2001) contained a full analysis of all water-quality monitoring data for Dowd Morass from 1991 to 2001. Table 2.1 shows a summary of these data.

Table 2.1 Summary of water-quality data for Dowd Morass, pooled over all sites, from 1991 to 2001. These data were obtained from the Environment Protection Agency (EPA), Parks Victoria and WaterWatch, and analysed by Sinclair Knight Merz (2001).

Variable	Mean ± standard error (n)	Maximum	Minimum
Water temperature	19 ± 1 (219)	35	9
pH	6.6 ± 0.1 (214)	8.9	2.8
Dissolved oxygen (mg L^{-1})	8.5 ± 0.3 (25)	10.5	5.3
Salinity (mS cm^{-1})*	4.02 ± 0.33 (223)	19.45	Not given
Turbidity (NTU)	91 ± 10 (127)	580	< 1
Total phosphorus (mg L^{-1})	0.23 + 0.02 (138)	1.55	0.02
Total Kjeldahl nitrogen (mg L^{-1})	1.58 ± 0.2 (9)	2.40	0.73
Ammonium (mg L^{-1})	0.04 ± 0.02 (7)	0.10	< 0.01
Nitrate plus nitrite (mg L^{-1})	0.09 ± 0.04 (16)	0.67	< 0.01

* seawater = ~ 50 mS cm^{-1}

The skew and substantial range in most of the water-quality variables is most notable. For example, the mean salinity was just over 4.0 mS cm^{-1} but the median was only 2.12 mS cm^{-1} and the maximum-recorded value was nearly 20 mS cm^{-1}. Similarly, water-column pH varied between 2.8 and 8.9, turbidity from < 1 to nearly 600 NTU, and total phosphorus from near the limit of detection to 0.23 mg P L^{-1}. The pH of the water column periodically dropped to less than 3 pH units, possibly due to the underlying acid sulfate soils.

2.3.1 Project data (Boon *et al.* 2007)

Measurements of a number of important water-quality variables were taken as part of the overall project carried out by the wetland ecology groups of Monash and Victoria Universities (Boon *et al.* 2007). Concentrations of total nitrogen and total phosphorus

in the water column of Dowd Morass varied widely from area to area (Table 2.2). The highest nutrient concentrations were detected in Area B, the site of the ibis rookery. The two species of ibises that nest at Dowd Morass are the Straw-necked Ibis (*Threskiornis spinicollis*) and the Australian White Ibis (*Threskiornis molucca*); Figure 2.5 shows the rookery in mid 2006 and Figure 2.6 shows the poor water quality, indicated by the algal bloom, in this region of the wetland.

Table 2.2 Water-column nutrient data for four areas at Dowd Morass. Means ± standard errors are shown, n=5 (Taken from Boon *et al.* 2007)

Date of sampling and wetland area	**Total nitrogen (mg N L^{-1})**	**Total phosphorus** (mg P L^{-1})
June 2003		
A	0.59 ± 0.06	0.03 ± 0.005
B	2.82 ± 0.20	0.39 ± 0.05
C	0.52 ± 0.06	0.02 ± 0.0004
D	1.62 ± 0.26	0.04 ± 0.011
November 2003		
A	3.32 ± 0.40	0.23 ± 0.06
B	4.30 ± 0.25	0.41 ± 0.08
C	3.62 ± 0.19	0.22 ± 0.022
D	2.67 ± 0.10	0.07 ± 0.007

Figure 2.5 Rookery in Area B of Dowd Morass in mid 2006. Photo courtesy of Professor Paul Boon, Victoria University.

Figure 2.6 Algal bloom in Area B (the rookery) at Dowd Morass. The plant growing on the Swamp Paperbark hummock is *Chenopodium glaucum.* Photo courtesy of Matthew Hatton, Victoria University.

2.4 *Sediment quality in Dowd Morass*

2.4.1 Carbon, nitrogen and phosphorus contents

Sediments in Dowd Morass have about 10-15 % w/w carbon and 0.7-1.2 % w/w nitrogen (Table 2.3). Phosphorus concentrations are also high, typically over 0.5 mg g DW^{-1} (= 0.05 % w/w) (Boon *et al.* 2007).

Table 2.3 Carbon, nitrogen and phosphorus content of sediments in four areas at Dowd Morass (Boon *et al*. 2007).

Wetland area	Nutrient content (mg g DW^{-1})			C:N:P ratio (by mass)
	Carbon	**Nitrogen**	**Phosphorus**	
A	128	7.0	0.20	640:35:1
B	151	6.9	0.87	173:8:1
C	94	7.0	0.58	162:12:1
D	153	12.3	0.61	250:20:1

2.4.2 Soil salinity

Sediments in Dowd Morass are often extremely salty. Table 2.4 shows the soil moisture, soil electrical conductivity and *in situ* soil salinity for sediments in Areas B and D, as well as along the shoreline of the wetland, from 2003 to 2006. By way of comparison, seawater has an electrical conductivity of about 50 mS cm^{-1}; thus the value 30.8 $\pm$ 1.7 mS cm^{-1} recorded for Area D in 2006 represents an *in situ* soil salinity of well over one-half seawater.

Table 2.4 Soil moisture, electrical conductivity and in situ soil salinity for sediments in three zones of Dowd Morass from 2003 to 2006. Means ± standard errors are shown, n=5.

Sediment variable	Depth (cm)	Wetland area	Date			
			2003	**2004**	**2005**	**2006**
Soil moisture (mL g DW^{-1})	0-10	B	2.3 ± 0.2	2.5 ± 0.1	2.4 ± 0.2	3.6 ± 1.0
		D	2.2 ± 0.5	2.1 ± 0.1	3.0 ± 0.3	2.2 ± 0.4
		Shoreline	2.5	2.2 ± 0.1	2.4 ± 0.5	2.6
	10-20	B	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1
		D	2.2 ± 0.2	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1
		Shoreline	2.2	2.0 ± 0.1	2.0 ± 0.2	2.3
Soil EC (mS cm^{-1})	0-10	B	3.8 ± 0.4	6.6 ± 1.2	12.4 ± 0.8	11.1 ± 2.1
		D	3.6 ± 0.9	6.2 ± 0.5	13.2 ± 1.4	13.4 ± 2.5
		Shoreline	4.2	5.7 ± 0.5	10.2 ± 2.0	9.8
	10-20	B	3.0 ± 0.3	5.9 ± 0.5	7.5 ± 0.7	8.6 ± 1.5
		D	3.8 ± 0.6	8.4 ± 1.0	9.7 ± 1.0	12.6 ± 0.7
		Shoreline	2.9	5.1 ± 0.6	6.3 ± 0.5	9.6
In situ soil salinity (mS cm^{-1})*	0-10	B	8.2 ± 0.7	13.1 ± 0.9	24.2 ± 2.1	17.1 ± 3.6
		D	8.3 ± 1.1	16.7 ± 1.6	22.2 ± 1.0	30.8 ± 1.7
		Shoreline	8.3	13.0 ± 1.1	21.4 ± 0.8	18.9
	10-20	B	7.1 ± 0.6	12.4 ± 0.9	17.1 ± 1.4	19.7 ± 2.5
		D	8.3 ± 0.8	20.6 ± 1.7	23.3 ± 1.7	29.0 ± 1.6
		Shoreline	6.5	12.6 ± 1.2	15.4 ± 0.6	20.7

* seawater = ~ 50 mS cm^{-1}

2.4.3 Soil pH and the presence of acid-sulfate soils

Acid-sulfate soils are soils that produce sulfuric acid (H_2SO_4) when exposed to the air (National Working Party on Acid Sulfate Soils 2000). In Australia, potential and/or actual acid sulfate soils are found along almost the entire coastline with the main exception being the steep limestone cliffs of the Great Australian Bight (National Working Party on Acid Sulfate Soils 2000). Acid-sulfate soils are especially common along the eastern seaboard and there are many examples where their disturbance has created severe environmental problems: Trinity Bay East near Cairns (Qld) and

Tuckean Swamp near Ballina (NSW) are the most well known examples (Hagley 1996; Powell and Martens 2005).

The sulfuric acid produced when acid-sulfate soils are activated moves through the soil, stripping iron, aluminium and manganese, as well as dissolving, in the worst cases, heavy metals such as cadmium. This noxious mixture makes the soil highly toxic and, combined with the very low pH (< 3), renders the growth of most plants impossible (Fitzpatrick *et al.* 2000). Acid-sulfate soils generally do not present a serious management problem as long as they are kept waterlogged. They become problematic when wetlands surface soils dry out and oxidise.

If potential or actual acid-sulfate soils are present, it may be unacceptable to instigate a strong wetting and drying cycle in hydrologically-altered wetlands because of the risk of severe damage to downstream estuarine ecosystems should the wetland drain even partially and the sediments start to oxidise. Johnston et al. (2003), for example, reported extensive fish kills in the Clarence River estuary of northern NSW were caused by an oxygen-depletion event which was, in turn, caused by anoxic and iron-rich surface waters draining from two acid-sulfate soil backswamps.

Re-establishing more natural wetting and drying regimes is planned for a number of wetlands in the Gippsland Lakes, especially those in the Lake Wellington wetlands complex (e.g., Dowd Morass, The Heart Morass). The existence of actual or potential acid-sulfate soils in these areas may present the single most important factor limiting the degree to which these wetlands can be episodically dried out and reflooded. Revegetation attempts are also likely to be compromised by the presence of acid-

sulfate soils. It is possible also that acid release, perhaps combined with acute oxygen depletion, could account for some of the fish kills experienced in the Gippsland Lakes (John Ginivan, DSE, *pers. comm.*).

Investigations by Boon *et al*. (2007) did show that actual and potential acid-sulfate soils occur in the Lake Wellington wetlands and probably also in wetlands and other coastal areas across the entire Gippsland Lakes region (Table 2.5, Figure 2.7). In recognition of the likelihood of acid-sulfate soils being distributed widely around the Gippsland Lakes area and having the potential for major environmental impacts, Dowd Morass has been selected as a routine monitoring site as part of the CSIRO's national acid-sulfate soils monitoring framework.

Table 2.5 Titratable peroxide activity (TPA) results for 12 sediment samples from Dowd Morass (Taken from Boon *et al*. 2007).

Wetland area	Location	TPA (mol H^+ tonne sediment^{-1})
A1	515684 / 5777786	195
A3	515254 / 5777448	73
A5	515822 / 5777514	25
B2	515408 / 5776653	55
B3	515363 / 5776889	53
B5	515533 /5776796	67
C1	516067 / 5777645	22
C2	516262 / 5777905	21
C4	516713 / 5778441	16
D2	516610 / 5777309	29
D3	516391 / 5777334	31
D5	516122 / 5776983	26

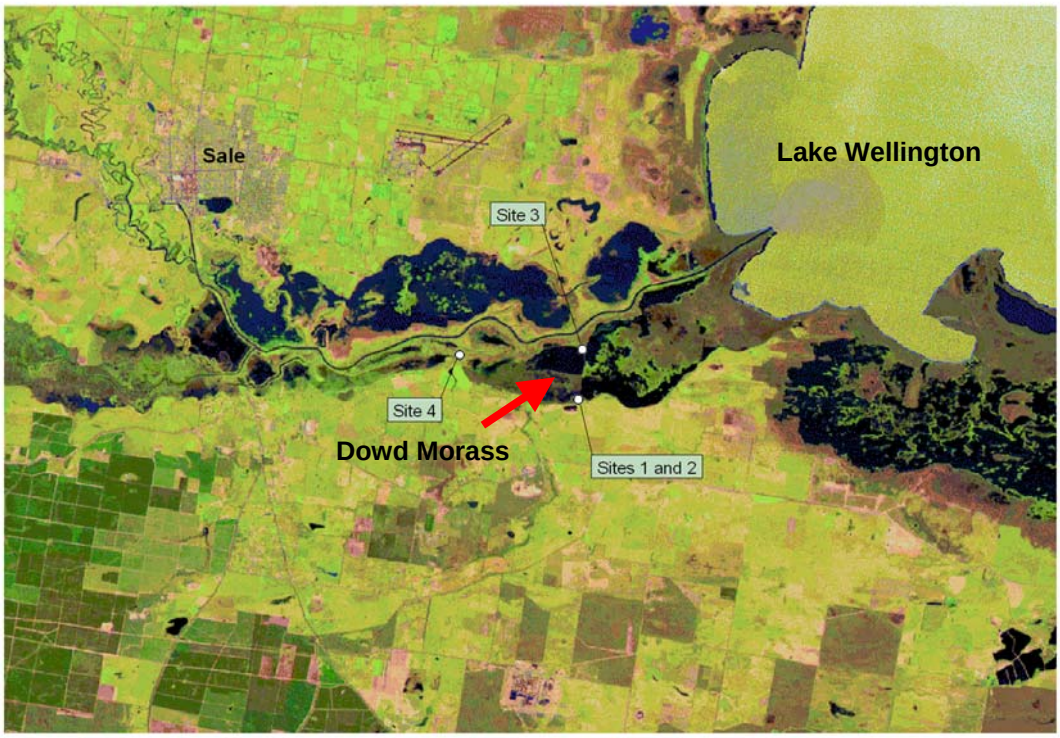

Figure 2.7 Location of the four sites used for a complete sulfidic analysis of Dowd Morass sediments (Crawford 2006).

2.4.4 Heavy metals

A limited range of analyses for heavy metals in the sediments of Dowd Morass are available from samples taken by Boon *et al.* (2007) (Table 2.6).

This limited amount of sampling suggests that sediments in Dowd Morass are not heavily contaminated with heavy metals. Only nickel concentrations exceed the International Standards on Quality Control (ISQC) or low trigger values proposed in the most recent Australian and New Zealand Environment Conservation Council (ANZECC) guidelines (ANZECC-ARMCANZ 2000). Moreover the guidelines suggest that trigger values should be relaxed when sediment organic carbon content is

markedly higher than 1 %: as the sediments have 10-15 % w/w carbon contents, even the values for nickel are not likely to be problematic.

Table 2.6 Concentrations of heavy metals in two areas of Dowd Morass. Samples were taken in late 2004 (Boon *et al*. 2007).

Heavy metal	**Concentration in sediments (mg kg DW^{-1})**		**Relevant ANZECC trigger value[17]**
	Area B	**Area D**	
Cadmium	< 0.2	< 0.2	1.5
Zinc	44	50	200
Copper	16	21	65
Lead	30	39	50
Chromium	34	48	80
Barium	320	610	NA
Nickel	28	34	21
Antimony	< 1	< 1	2
Arsenic	10	16	20
Boron	75	150	NA
Mercury	< 0.1	< 0.1	0.15
Selenium	2	< 1	NA

Ten of the 17 samples taken at Dowd Morass had a mercury concentration above the limit of detection (0.05 mg kg DW^{-1}) (Boon et al. 2007). These data would suggest very slight contamination of the wetland's sediments with mercury, possibly as a consequence of gold mining in the catchment or from agricultural land uses.

2.5 Vegetation of Dowd Morass

There are four main vegetation communities found at Dowd Morass

- Low closed-scrub to woodlands of *Melaleuca ericifolia* (Swamp Paperbark);
- Swards of *Phragmites australis* (Common Reed);

- Submerged beds of *Vallisneria americana* (Eelgrass); and
- Small areas of mud flats containing salt-tolerant plants such as; *Disphyma clavellatum* (Rounded Noon-flower), *Distichlis distichophylla* (Salt-marsh Grass), *Hemichroa pentandra* (Trailing Hemichroa) and *Sarcocornia quinqueflora* (Beaded Glasswort).

Historically, the area now occupied by Dowd Morass would have been part of a large estuary. Over time, through closure of the estuary, sedimentation and change in hydrological process, successional change in vegetation from open-water communities to woody vegetation has taken place (Bird 1961; Bird 1965). This is evidenced in recent times at Dowd Morass by the change from Reed communities to Closed-scrub composed of *M. ericifolia* in the period from 1957 until present.

In a comprehensive study of successional change to vegetation in the Gippsland Lakes system, Bird (1962) predicts directional change from open-water through to woody vegetation and on to saltmarsh vegetation as salinity levels of the Gippsland Lakes increases. The management implications of this are that each of the prior seral stages of vegetation will be replaced as conditions change over time creating a highly mobile set of vegetation communities of temporary duration. Bird (1962) predicts that the long-term prospects for *M. ericifolia* in the Gippsland Lakes system are limited with eventual extinction of the species and replacement with a Saltmarsh community.

Three of the four major vegetation communities are shown in Figures 2.8 - 2.10.

Figure 2.8 Stands of Swamp Paperbark, *Melaleuca ericifolia*. Area B, Dowd Morass, Sale, Victoria. Photo courtesy of Professor Paul Boon, Victoria University.

Figure 2.9 Dense swards of Common Reed, *Phragmites australis*, in the background with submerged beds of Eelgrass, *Vallisneria americana*, in the forground. Area D, Dowd Morass, Sale, Victoria. Photo courtesy Kay Morris, Monash University.

Figure 2.10 Aerial photograph of a section of Dowd Morass, showing areas of Common Reed (CR) and Swamp Paperbark (SP). Individual clumps of Swamp Paperbark are clearly visible (an example is marked with the red arrow). Photo courtesy of Parks Victoria.

Chapter 3

Clonality in *Melaleuca*: a study of population structure and dynamics using molecular analyses and historical aerial photographs

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Abstract

Determining the extent of clonality in *M. ericifolia* is critical to the understanding of structure and dynamics of populations of this species. Inter Simple Sequence Repeats (ISSR) were used to determine genet size and degree of intermingling, while historical aerial photographs are used to determine lateral expansion rates and longevity. Individual 'dome-shaped patches' of *M. ericifolia* were determined to be individual genets with the ISSR approach, and there was no evidence of intermingling of the 10 genets sampled. Genet size after 46 years ranged from 1,174-3,274 m^2, additional plants not included in the genetic testing were speculated to be between 80-100 years old and were considerably larger in size. The implications of these findings, most notably lack of intermingling and growth rates, are discussed in relation to survival of genets in a wetland with variable water regime and configuration of plantings to maximise success and minimise costs.

3.1 Introduction

The identification of individual genets of clonal plants is critical to the understanding of population structure and dynamics, particularly for determining genet size, competitive relationships and genetic diversity within populations of clonal plants (Kennington and James 1997; Kreher *et al.* 2000). The extent and the type of clonality exhibited by the plant (*e.g*. phalanx vs. guerrilla) have major implications for survival, reproduction and competitive ability. Extensively clonal species are generally assumed to have lowered sexual reproduction due to increased energetic and nutrient costs of seed production (Sutherland and Vickery 1988; Lovett Doust 1989; Reekie 1999; Eckert 2001). Alternatively, clonal plants may have increased competitive ability in environments where sexual reproduction is limited due in part to resource sharing between ramets (Rea and Ganf 1994; Barsoum 2002; Peltzer 2002). Clonality is a major characteristic of wetland plants in Australia, with well over two-thirds being classified as clonal (van Groenendael *et al*. 1997; Hatton 2005).

The development of the clonal growth form has arisen several times in the genus *Melaleuca* (Craven and Lepschi 1999) and more widely in the family Myrtaceae (Lacey 1983; Kennington and James 1997; Tyson *et al.* 1998). Within the genus *Melaleuca,* regeneration strategies range from seed-only regenerators (*e.g. M. parvistaminea*), those that resprout only from the main trunk (*e.g*. *M. uncinata, M. quinquenervia*) through to extensively clonal species that resprout from stems and roots (e.g. *M. ericifolia, M. halmaturorum* (Jeanes 1996; Craven and Lepschi 1999; Holliday 2004). Although there is occasional mention of the regenerative ability of various *Melaleuca* species in the

literature, there is no comprehensive investigation of clonal regeneration capacity for any of the species.

Clonality is often regarded as an adaptation to spatial and temporal heterogeneity of environmental conditions (Kleign and van Groenendael, 1999). Some of the conditions that lead to environmental heterogeneity (patchiness) in the situations in which *M. ericifolia* grows are fire, salinity, water level (drought and flooding), soil pH, and impacts from breeding colonies of of birds. In evolutionary terms, the clonal growth-form is very ancient and occurs in a wide range of plants (Mogie and Hutchings 1990) and environments (Klimes *et al.* 1997), but is particularly well developed in habitats that are particularly hostile to sexual recruitment (Rea and Ganf, 1994; Barsoum, 2002). Spatial and temporal availability of germination conditions or seedling recruitment sites may be the evolutionary driver favouring clonality. Additionally, artificially prolonged flooding has been shown to shift vegetation composition to one based primarily on clonal species (Ernst and Brooks 2003).

The ability of the clonal growth form to effectively capture resources without having to proceed through sexual reproduction in potentially inhospitable sites confers a degree of competitive advantage in spatially and temporally heterogeneous environments (Silvertown and Charlesworth 2001). The shift to clonal growth and corresponding reduction in sexual reproduction has been linked with increasing heterogeneity of the environment and decreases in available safe sites for germination; this being clearly demonstrated in the wetland genus *Mimulus* (Sutherland and Vickery 1988). Sexual recruitment in M. *ericifolia* is rarely recorded and presumed to be episodic in nature and limited to specific conditions (de Jong 2000). The impact of clonality coupled with

reduced sexual reproduction may have long-term impacts on genetic structure of populations, genetic diversity within populations and evolutionary capability (Ellstrand and Roose 1987; Widen *et al*.1994).

The production of lateral growths (ramets) generally follows two main configurations that can dictate the distribution and intermingling of separate plants (genets). Phalanx species produce short and frequently branched connections between ramets spreading along a front that excludes other genets (Harper 1977; Silverton and Charlesworth 2001). Guerrilla species produce long-spacers with little branching allowing plants to infiltrate neighbouring individuals of the same or other species (Harper 1977; Lovett Doust 1981). Ecologically, the two growth forms tend to occur separately although it is not uncommon for both forms to occur in the same wetland or for individual species to exhibit both forms at various stages of their life. The phalanx mode of growth tends to occur in low-nutrient, high-light habitats (van Groenendael *et al.* 1997) with a low degree of spatial and temporal heterogeneity of environmental conditions (Kleijn and Van Groenendael, 1999). The guerrilla mode of growth is more closely allied with soils in which nutrients and moisture are not evenly distributed. Anecdotal evidence suggests that *M. ericifolia* is a phalanx species but it is not known with certainty which mode of growth *M. ericifolia* exhibits.

While the clonal growth form is widely recognised and studied in herbaceous plants, there are far fewer ecological studies of the clonal growth form in woody plants. Notable exceptions include *Populus tremuloides* and *Larrea tridentata* in western USA, Quercus species in Florida, *Gaylussacia brachycera* in eastern USA, *Lomatia tasmanica* in Australia (Lynch *et al*. 1998) and *Hedysarum laeve* in China (Wherry 1972; Vasek 1980;

Abrahamson and Layne 2002; Peltzer, 2002; Zhang *et al.* 2002;). Expansion rates and site capture by these species is highly variable ranging from several centimetres per year for *Larrea tridentata* (Vasek 1980) to several metres per year for *Populus tremuloides* (Krasny and Johnson1992).

The aims of this component of the study were to determine the extent of clonality in *M. ericifolia* and the degree on intermingling of genets. The use of a time series of aerial photographs of high resolution was seen as a way of tracking the growth of individual genets over a 46-year time frame. The use of molecular analysis to identify these individual genets would allow accurate tracking and to assess degree of intermingling. Inter simple sequence repeats (ISSRs) are components of a marker system that accesses variation in the numerous microsatellite regions dispersed throughout the plant genome and is suitable for species where little information is available on the genome (Zietkiewicz *et al.* 1994). Primers target di- and tri-nucleotide repeat motifs, which are characteristic of microsatellites in the nuclear genome. Generally one to three ISSR primers are sufficient for identifying individuals (Wolfe and Liston 1998; Esselman 1999). The coupling of molecular analysis with aerial photography was seen to provide a degree of accuracy not available if only one of these methods was used.

There is anecdotal evidence that *M. ericifolia* is extensively clonal and that it is a phalanx species (*pers. obs.*). The characteristically dome-shaped configuration of populations is typical of the phalanx manner of growth. Extensive ramet production from the roots and the formation of a front of ramets lends further evidence to the above observations (Figure 3.1, Figure 3.2). If this is the case, what are now thought to be populations will in fact prove to be individual genets. Competitive exclusion, a characteristic of the phalanx

growth form, would imply the lack of intermingling of genets. Some of these 'populations' of *M. ericifolia* are extensive, covering hundreds of m^2. The implications of extensive clonality and large size of genets would mean that few plants would occupy large area, reducing potential genetic heterogeneity of true populations. For conservation managers, genetically homogeneous populations that are in fact individual genets would imply that present conservation measures are preserving very little genetic diversity. Similarly, genetically homogeneous populations would have implications in regard to the collection of seed for restoration projects; genets would have to be identified to ensure all seed was not collected from one plant. Further, present planting methods involving large numbers of genetically distinct plants at close spacings may reduce the competitive advantages conferred by the clonal growth form. Genetic homogeneity will be evident not only in molecular analyses but in the evaluation of individual patches seen in the historical series of aerial photographs.

3.2 Methods

The ability of *M. ericifolia* to form adventitious shoots was confirmed by physically examining exposing roots connecting ramets in individual patches of *M. ericifolia* and tracing these roots back to mature stems. (Figure 3.1). The clonal characteristic is common between all populations, a Tasmanian example has been used in Figure 3 as it was clearer than examples from Dowd Morass where herbaceous plants obscured ramets and connections. The characteristic dome shape of the patches provided anecdotal evidence of the vegetative derivation of individual patches (Figure 3.2).

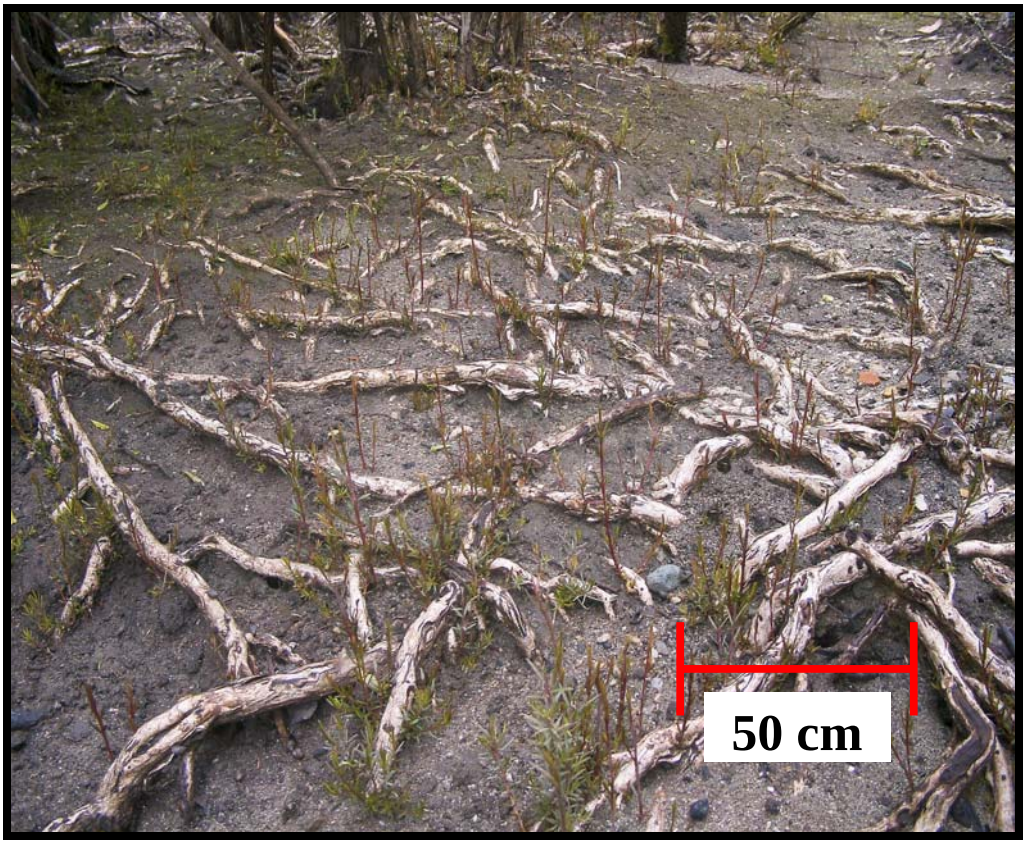


Figure 3.1 Exposed root system of a mature patch of *M. ericifolia* exhibiting strong production of vegetative growth (ramets) from exposed roots, Narawntapu National Park, Tasmania.

Figure 3.2 Individual patch of *M. ericifolia* at Wilson's Promontory National Park, Victoria, exhibiting the characteristic dome shape, with tallest stems in the centre of the patch (approximately 8m high) grading down in height to the leading edge (paler green outer ring - approximately 1 m high). Surrounding plants are Common Reed (*Phragmites australis*). Total patch width approximately 50 m. Photo courtesy of Ms. Deborah Reynolds, Victoria University.

3.2.1 Sample collection and molecular analysis

To determine if these individual patches were derived from one or several genets, sampling was carried out on a grid pattern on two large patches at Dowd Morass (45 m x 120m and 30m x 68m) containing what appeared to be, from the circular patterning within the patches, three genets and two genets (Figure 3.3). The grid was arranged to ensure that samples were taken in the centre and edges of the patches and on either side of what appeared to be joins between the putative genets (Figure 3.3 a-b). A grid pattern was chosen in preference to random sampling as a grid more clearly determined the distribution of individual genets within a patch and clearly elucidates whether the plant is of guerrilla or phalanx growth habit (Chen *et al.* 2002). An additional five individual ring-shaped patches of plants were sampled to determine if these were individual genets as there was anecdotal evidence of sexual reproduction (true seedling having alternate leaves, ramets opposite leaves) within the senescent interiors of these large patches of *M. ericifolia* (Figure 3.3 c).

Approximately 200 g of actively growing stem tips were collected for each sample from individual aerial branches, half of which was placed in sealed plastic bags kept on ice and transported to an -80^0 C freezer. The other half was sealed in plastic containers and desiccated with silica gel, also kept on ice and transported to a 4^0C refrigerator until processed. Frozen material was collected and saved to ensure availability of material should the preferred desiccated material prove unsuitable.

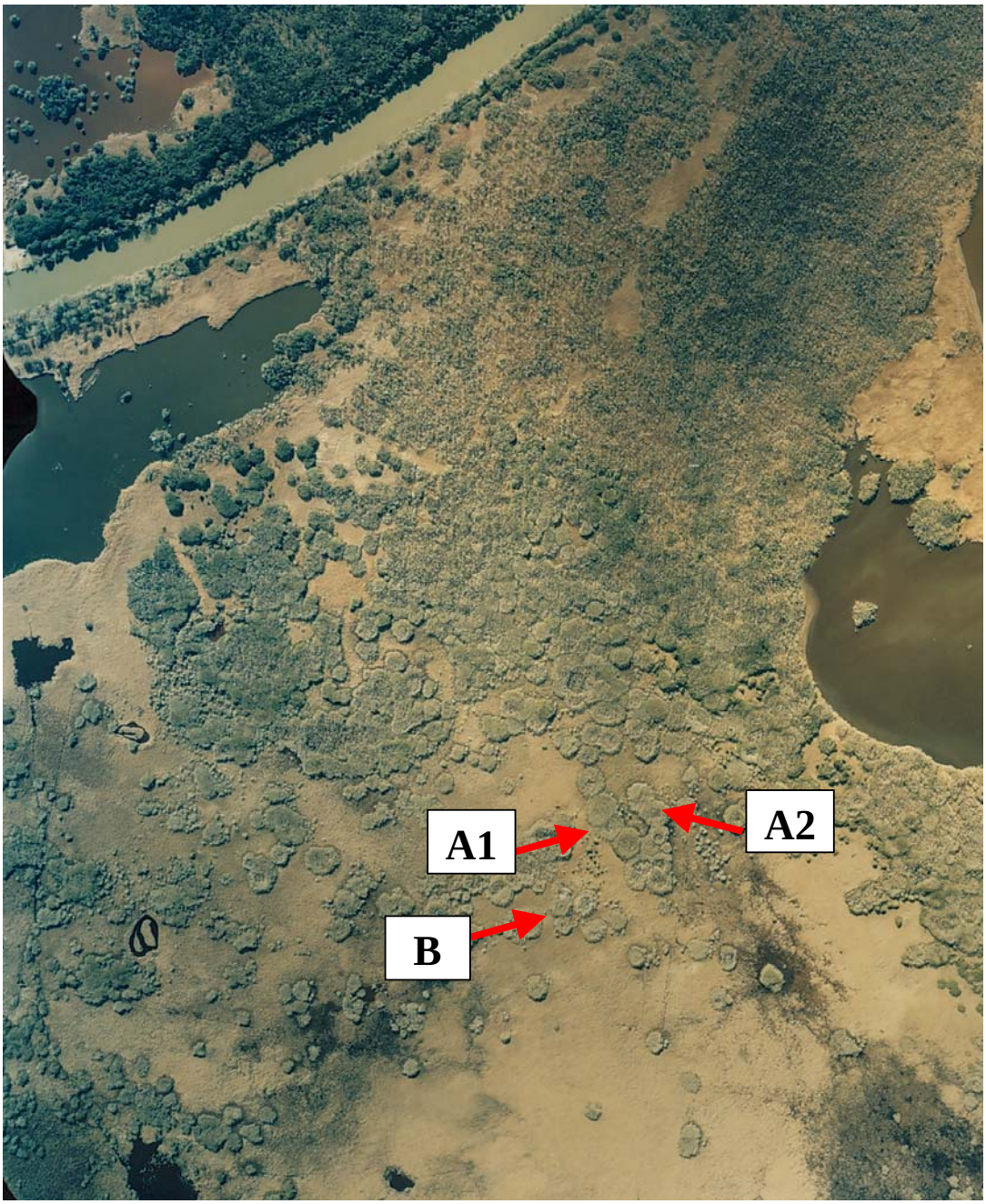


Figure 3.3 Location of sampled patches of M. ericifolia at Dowd Morass, Sale, Victoria. A = patches sampled for determination of genetic diversity of patches and intermingling of genets (close-up Figures 3.3a and 3.3b). B = Doughnut shaped patches sampled to determine if regeneration within the patch represents vegetative or sexual regeneration (close-up Figures 3.3c).

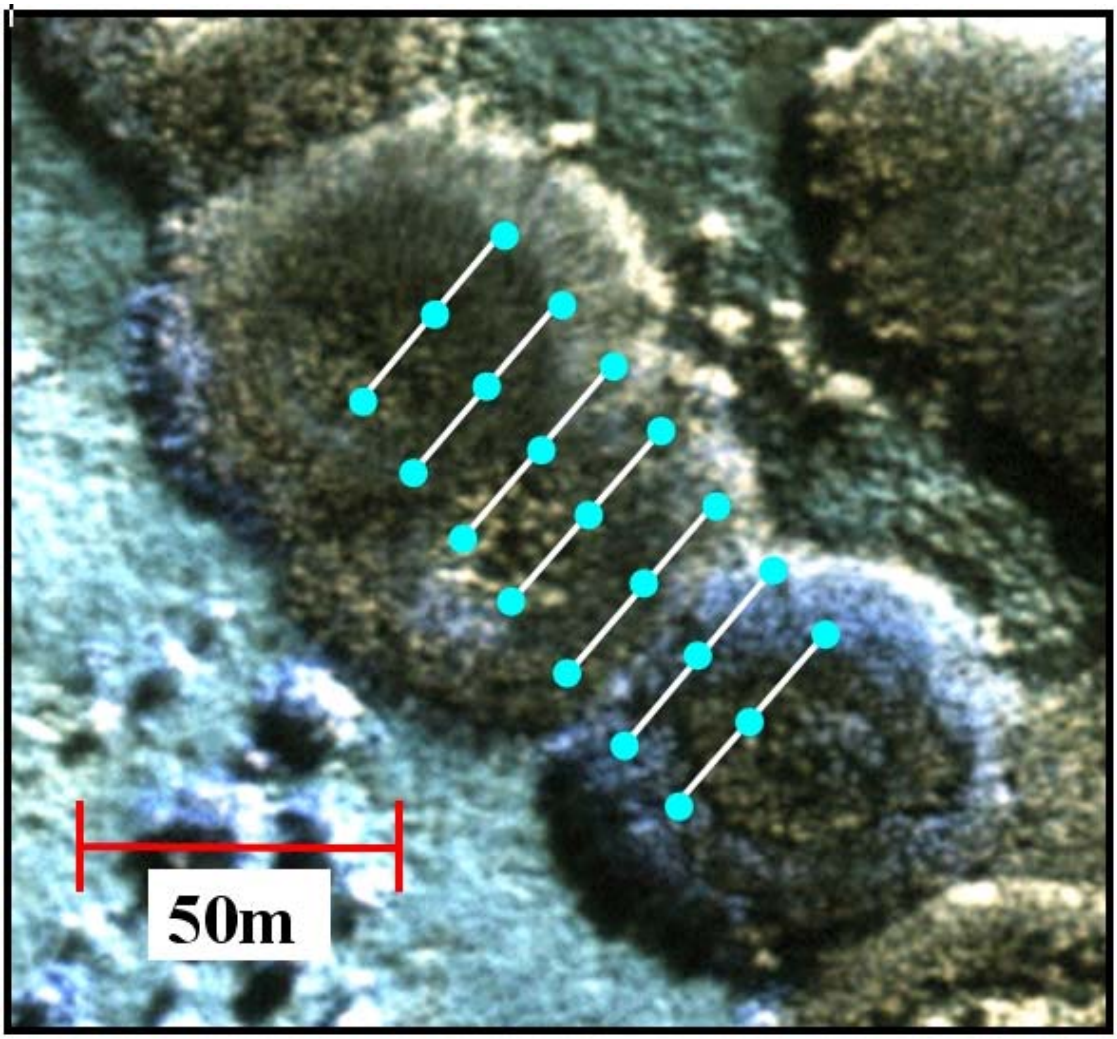


Figure 3.3a *Melaleuca ericifolia* patch A1 showing sample points

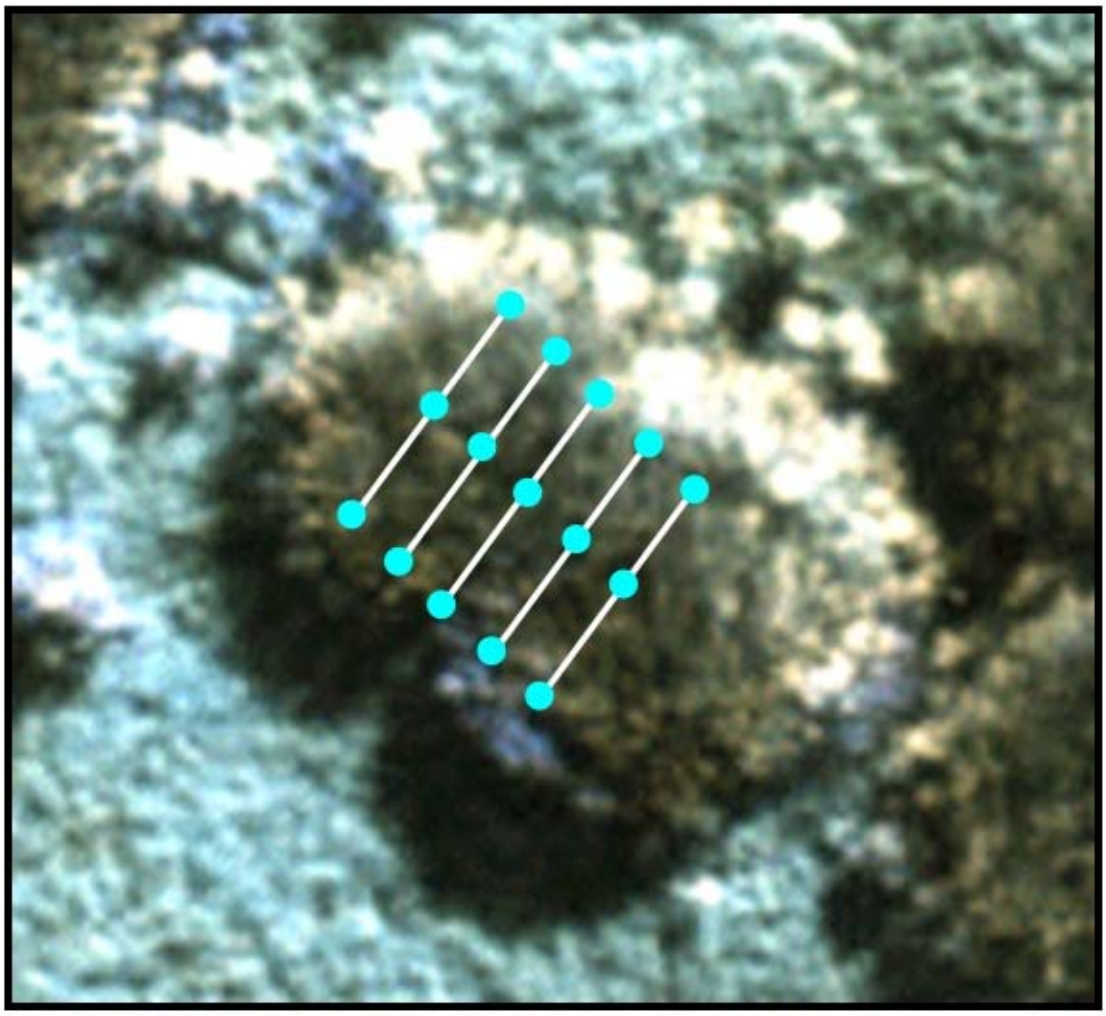


Figure 3.3b *Melaleuca ericifolia* patch A2 showing sample points

Figure 3.3c Doughnut-shaped *Melaleuca ericifolia* patches (B) showing sample points. Lighter coloured edges represent actively expanding ‘front’, darker centres represent senescent older stems and area of regenerating young growths.

DNA isolation

DNA was isolated from leaf material dried in silica gel using QIAGEN Dneasy plant DNA extraction minikits (QIAGEN Pty. Ltd. Doncaster, Victoria. Aust.). Samples (20 mg) of dry tissue were ground with a small amount of acid-washed sand and DNA isolated according to the manufacturer's instructions.

Primer screening

Primers considered promising for *Melaleuca* were screened and those that gave clear bands that were reproducible and could be scored readily were used to amplify DNA from all samples. In all, five primers were used: DatA, 888, BDBLz, HB15, and 814.

DNA amplification

DNA was amplified in 20 μl reactions containing 10 μl QIAGEN HotStart Master Mix (8 μl H_2O, 1 μl 10 μM primer, 1 μl DNA (20 mg). Polymerase chain reactions (PCR) were performed in an Eppendorf MasterCycler® gradient thermal cycler using the following profile: 95^0C for 15 min (1 cycle); 94^0C for 45 s, 72^0C for 1 min (35 cycles); final extension step of 72^0C for 10 min with an indefinite soak at 4^0C.

PCR products were visualised on 2.0% agarose gels stained with ethidium bromide and photographed under UV light. Gels were photographed with a Kodak EDAS 290 digital camera and Kodak 1D software. Each sample was scored manually using digital images and assigned a multilocus phenotype allowing comparison of all samples. When using

dominant markers such as ISSRs, the term phenotype is generally used rather than genotype (the underlying genetic basis). This is because a plant that, for example, is homozygous for the dominant allele (AA) cannot be distinguished from a plant that is heterozygous (Aa) but a homozygote recessive is distinguishable (aa).

PCR products were scored as present (1) or absent (0) for each individual which was then assigned a multilocus phenotype based on the combined PCR product patterns of all ISSR primers. The probablility of identical patterns having arisen independently via sexual reproduction was then calculated following the method described by Parks and Werth (1993). Due to slight amplification problems (not all fluoresced evenly) all primers were run twice. Poorly amplified samples were excluded from the scoring.

Data analysis

In clonal species such as *M. ericifolia*, the analysis of genetic diversity data can be misleading due to the individual genet being sampled multiple times although each sample is treated as if it were an separate individual. Determining a statistical basis for distinguishing if individual samples are derived from asexual reproduction from a single zygote or if the same phenotype was produced independently via sexual reproduction is difficult, particularly if individuals of the same phenotype are clustered (Parks and Werth 1993; Widen et al. 1994).

To overcome this difficulty, the approach used was to calculate the probability, *P*, (Eq. 1), once a particular phenotype was found, of obtaining that same phenotype, assuming

sexual reproduction, in (n-1) subsequently sampled individuals (Parks and Werth 1993; Sydes and Peakall 1998).

$$P = (P_{gen})^{n-1}$$

Where n is the total number of individuals with the same multilocus phenotype and P_{gen} = probability of obtaining the observed multilocus phenotype via sexual reproduction. If *P* is small, it can be concluded that the most likely explanation for the observed cluster of individuals of the same phenotype is common derivation through asexual reproduction.

For dominant markers such as ISSRs where only two phenotypes are possible (presence or absence of a particular fragment), P_{gen}, represented as P_{dgen} (Sydes and Peakall 1998), is calculated using Eq. (2).

$$P_{dgen} = \text{II}x_i$$

Where x_i is the frequency of whichever phenotype (band presence or absence) was observed at locus *i* in the individual being considered. This approach has been used by a number of authors to analyse data from suspected clonal species (Parks and werth 1993; Widen *et al.* 1994; Sydes and Peakall 1998).

As *M. ericifolia* has been recorded as having multiple ramets arising from large inter-connected roots (Figure 3.1), analysis of ISSR data was carried out according to the above equation to give the probability of phenotypes having arisen independently more than once. A complete set of the data and calculations is provided in Appendix 1.

3.2.2 Analysis of aerial photographs

The molecular analysis allowed for the determination and mapping of individual genets using aerial photographs. Aerial photographs of Dowd Morass, taken in 2003 at a scale of 1:6,000, were obtained from Parks Victoria. These images were scanned at a resolution of 1,200 dpi using a Powerlook 2100XL flatbed A3 scanner (Umax Technologies Inc., Dallas, USA). Each image was rectified using the Leica Photogrammetry Suite (LPS) component of Erdas Imagine^{TM} v. 8.7 software (Leica Geosystems, Heerbrugg, Switzerland). Erdas Imagine^{TM} uses a digital elevation model (DEM), ground control points (GCPs), and camera calibration data to remove geometric distortions existing in the original images. Since the vertical topographic variation within the sampled section of the wetland is less than one metre, the DEM used in the rectification process was assumed to be flat. The processing of the images was carried out by Michael Roache as part of the overall project into the management of Dowd Morass.

The geographical coordinates of the ground control points were determined in the field using a Garmin GPS instrument (72 version 2.03 hand-held unit, Garmin, Olathe, USA). At least five GCPs were identified on each photograph from the 2003 series. The rectified images were resampled using a nearest-neighbour algorithm and projected in the Universal Transverse Mercator (UTM) co-ordinate system. Images were saved in digital Bitmap (BMP) file format and then inserted into an AutoCAD R2002 drawing file (.dwg format) (Autodesk Pty Ltd.). An area of approximately 10.5 ha on the aerial photograph was randomly selected and geo-referenced, using three fixed points on-the-ground. Individual genets of *M. ericifolia* were measured in the field in February 2004 to obtain

scale. Using these known dimensions the scale of the base photo was adjusted to match on-ground measurements and set to 1:100.

Individual genets were identified and traced using the AutoCAD command *polyline* in each bitmap image and an individual number was assigned to each genet. The *polyline* command is a connected sequence of line segments that creates a single object. Drawing a polyline creates an enclosed boundary around an object, assigning the object a physical size. The AutoCAD command *getarea* was used to calculate the area enclosed within the boundary of each plant patch. The *getarea* command generates an area result in square metres, to one decimal place. The area data obtained from each calculation was then tabulated alongside each plant patch. This process was repeated for each plant patch identified on the aerial photograph series.

Determination of growth rates and longevity based on aerial photography interpretation

A series of aerial photographs of Dowd Morass taken in 1957, 1964, 1973, 1978, 1982 and 1991, at a scale of 1:6,000 to 1:20,000 (Table 3.1) was obtained from the Land Information Centre, Laverton, Victoria and Parks Victoria, Government of Victoria. These photos were scanned and rectified using the same procedure listed above.

Table 3.1 Source and characteristics of aerial photographs used in this study. VLIC is the Victorian Land Information Centre (Laverton, Victoria) and PV is Parks Victoria.

Date	Source	Series	Approximate scale	Emulsion
May 1964	VLIC	Lake Wellington Project	1:16,000	Black and White
April 1973	VLIC	Dutson	1:8,000	Black and White
Feb 1982	VLIC	Sale M/S 8321	1:42,500	Black and White
Nov 1991	VLIC	Sale M/S 8321	1:25,000	Colour
Aug 2003	PV	GL Ramsar Wetlands	1:6,000	Colour

An area containing all genetically tested patches was selected from the 2003 image and from each historical aerial photograph. These images were cropped and the image size adjusted so that all images were the same size (389 x 328 pixels). Plant specimens with known dimensions (obtained at the site in February 2004) were used as a point of reference to obtain scale. Using these known dimensions the scale of the base photo (2003) was adjusted to match on-ground measurements and the scale was set to 1:100. Each subsequent bitmap image was adjusted by applying the same scale ratio.

Plant patches identified as individual genets using the molecular method and eight additional patches with similar configurations were identified on each bitmap image and traced using the AutoCAD command *polyline.* An individual number was assigned to each selected genet. The area data obtained from each calculation was then tabulated alongside each plant patch. This process was repeated for each plant patch identified on the aerial photograph series. Age of genets was determined by backward tracing of the expansion of the identified patches from the most recent aerial photograph to the oldest

until individual patches were no longer able to be located or identified. The smallest size of genet able to be reliably identified was just less than 50 m^2. Genets were selected from an area of Dowd Morass that has been subjected to the least amount of human interference, particularly to water regime. It was deemed that these genets would represent the underlying growth rates of the species better than those subject to artificially raised or lowered water levels. Additional considerations in the selection of genets were the clarity of photographs and the ability to clearly identify individuals over the time period of the study.

3.3 Results

3.3.1 Vegetative reproduction

Physical examination of the exposed roots systems of mature plants revealed extensive connective networks of roots between both mature ramets and mature and immature ramets (Figure 3.1). While it was impossible to trace all the connections within individual patches it was possible to trace connections between individual ramets. Most of the root systems observed were very shallow, generally 20-30cm deep with the majority of larger roots within 5-10cm of the soil surface (Figure 3.4). Young, actively-growing ramets arose directly off larger (> 1 cm diameter) roots. Exposure and tracing of the root system of young growths on the edges of patches confirmed their connection to larger previously established ramets (Figure 3.1).

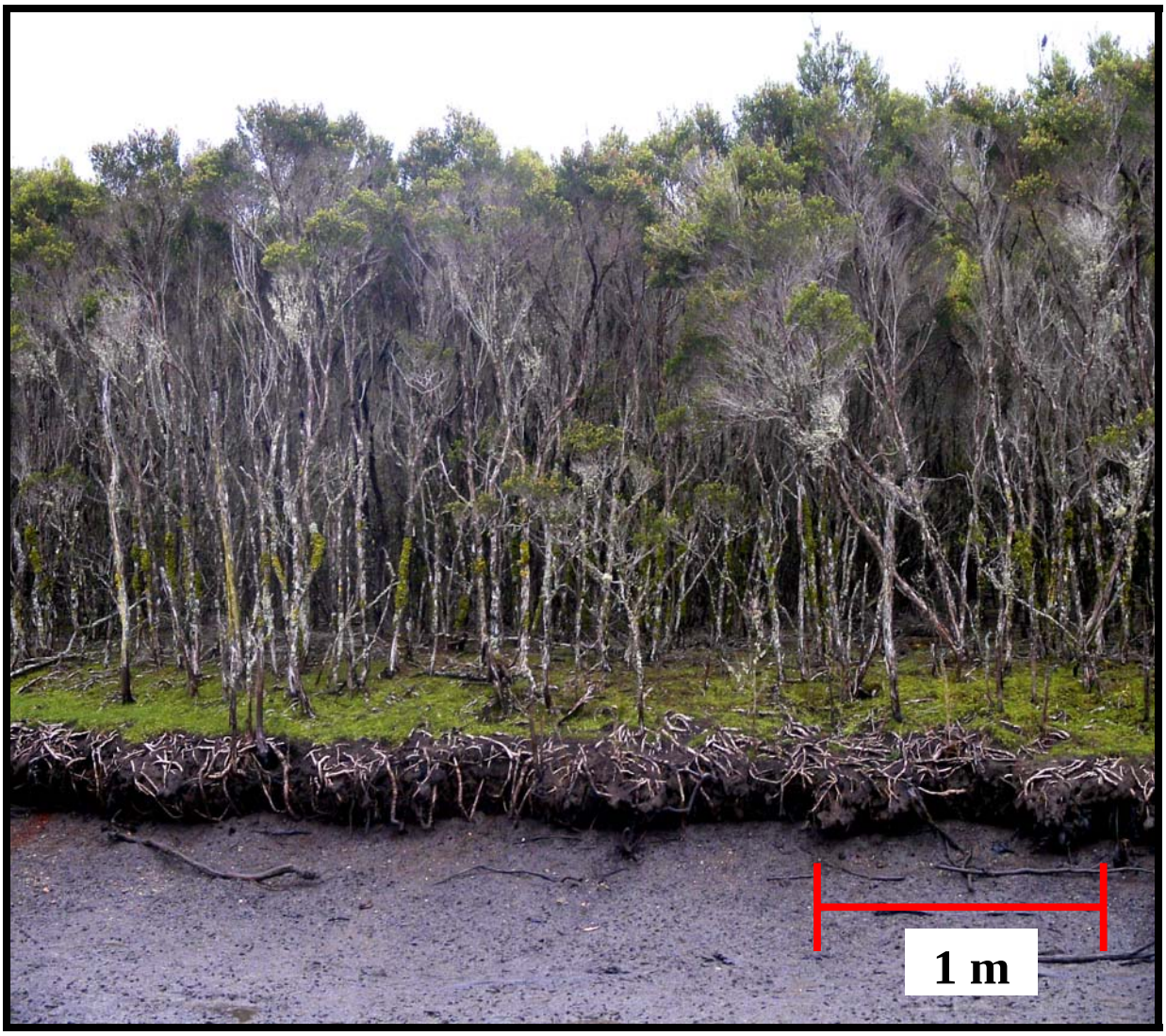


Figure 3.4 Exposed edge of a patch of *M. ericifolia* showing the depth of the extensive network of roots, Narawntapu National Park, Tasmania.

3.3.2 ISSR analysis

The use of ISSR confirmed visual and physical assessments of clonality (section 3.3.1), with individual genets being easily determined both on-the-ground and from aerial photographs. Sampling of the two large patches (60 m x 120 m and 55 m x 60 m) indicated that, as predicted, they were derived from three and two genets, respectively (Figure 3.5, 3.6). Unique multilocus phenotypes within both patches exhibited distinct and exclusive clustering with no indication of intermingling. Two samples within genet two (ladder 9 and 11) failed to amplify clearly in either run, giving misleading results (Figure 3.6). This was attributed to the sample being composed of slightly older material than the other samples or a processing error.

Sampling of the five individual ring-shaped patches strongly suggests derivation from individual genets. Two of the individual patches (genet 6 and 10) had one anomalous sample occurring within the regenerating centre where the original ramets had died. There was little dissimilarity (one allele difference) between the phenotype of these two anomalous sample and the phenotype of the surrounding plant (primer 814) and it was not possible to determine if this was a somatic mutation, a distinct genet, contamination or improper amplification during processing of the sampled material (Figure 3.6). The same results were obtained after running the samples twice. Whatever the explanation, there would appear to be a genuine difference between these two anomalous samples and the surrounding samples.

The probabilities of getting the same sequences occurring more than once was exceedingly low for all phenotypes identified in this study with the exception of phenotypes 6 and 10 (Table 3.2).

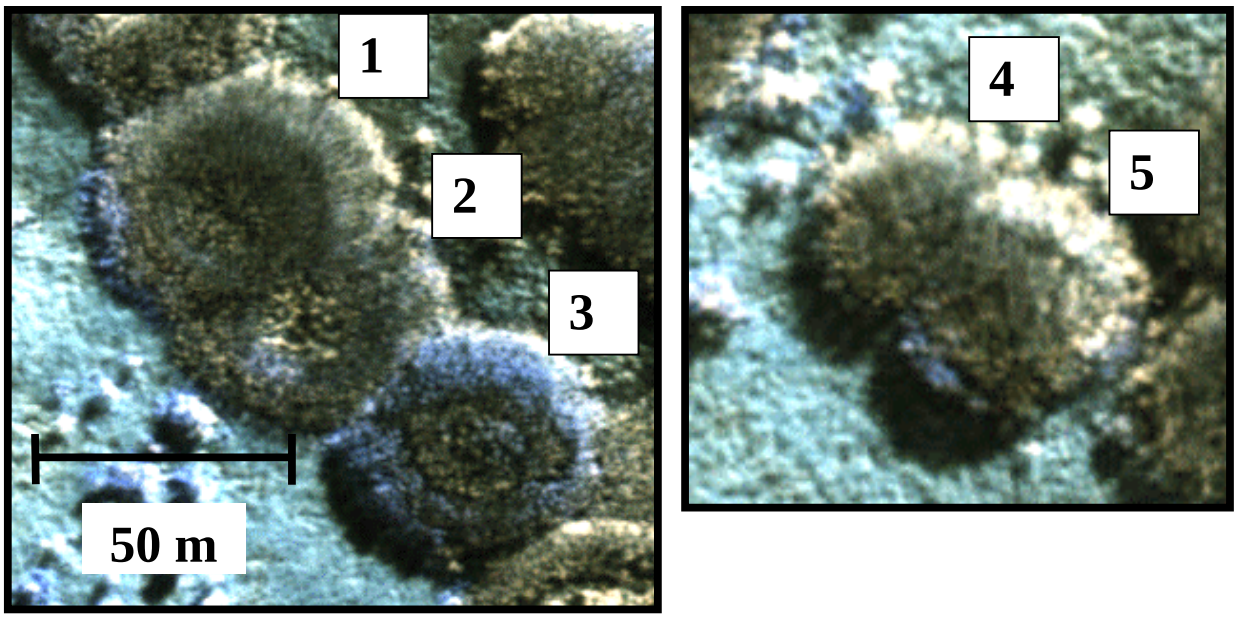
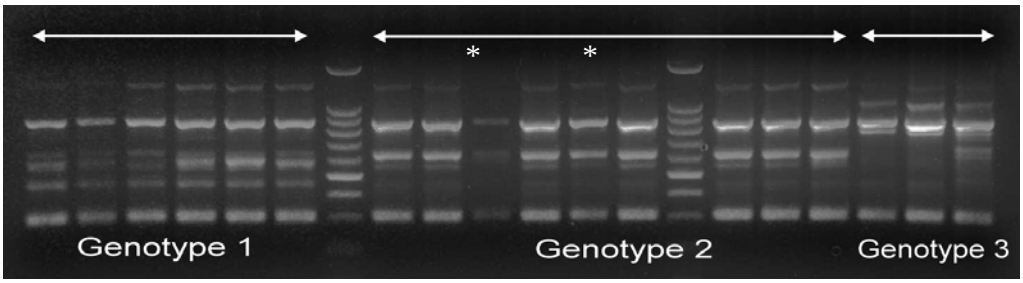
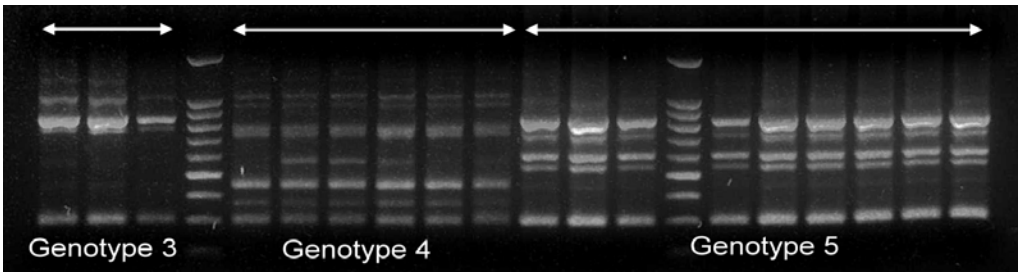
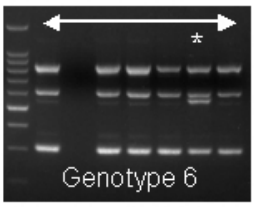
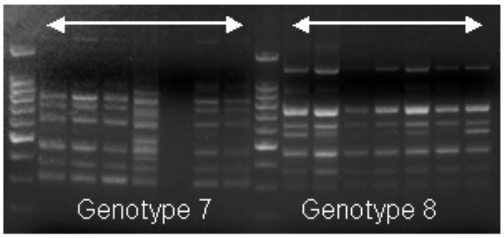


Figure 3.5 Two large patches of *M. ericifolia* showing the individual genets (1-5) determined by ISSR.









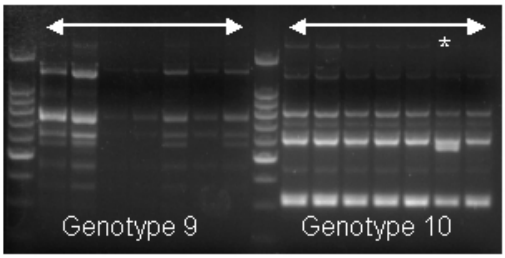


Figure 3.6 ISSR DNA profiles for all samples using primer 814. Groups appearing between "ladder" bands represent individual genets. * = Samples that contain anomalous bands.

Table 3.2 Probability of observed phenotypes occurring (Pgen) based on allele frequencies in the whole data set and the probability, given that they have been observed, of obtaining the observed number of samples with indistinguishable phenotypes from sexual reproduction.

Phenotype	n	Pgen	$P=(Pgen)^{(n-1)}$
1	6	4.62E-15	2.12E-72
2	7	3.96E-13	3.84E-75
2A	2	2.29E-14	2.29E-14
3	6	1.81E-13	1.95E-64
4	6	1.12E-17	1.73E-85
5	9	3.72E-13	3.70E-100
6	6	8.79E-13	5.24E-61
6A	1	5.24E-61	1
7	7	1.20E-15	3.00E-90
8	7	2.22E-15	1.19E-88
9	7	7.25E-13	1.45E-73
10	6	3.87E-13	8.63E-63
10A	1	4.92E-14	1

Under both sampling regimes (patches and ring-shaped patches) the genets covered large areas (1-3,275 m^2; 2-1,645 m^2; 3-2,535 m^2; 4-1,320 m^2; 5-1,922 m^2; see also Table 3.3) in 2003 and were composed of hundreds of individual ramets. Several genets growing in close proximity to each other exhibited apparently competitive relationships, with the more rapidly expanding genet crowding adjacent genets, and the apparently less competitive genets growing laterally away from the competitive front. Phenotypic variations could be observed on the ground that corresponded to genotypic variation confirmed by genetic testing allowing visual identification of individual genets (Figure 3.7)

Figure 3.7 Visual differentiation of phenotypes – phenotype 4 (left) with small bright green leaves and upright, white stems. phenotype 5 (right) with olive green leaves and spreading, grey stems. Lack of intermingling of genets is visible in centre of photo. Phenotype 4 and 5 correspond to genets 4 and 5 in Figure 4.5.

3.3.3 Growth rates and longevity, determined from aerial photographs

The use of historical aerial photographs allowed for the determination of growth rates of individual genets of *M. ericifolia* over a period of 46 years (Figure 3.8). There was a clear colour/greyscale difference between *M. ericifolia* and *Phragmites australis* on the images. The distinctive round shape of the *M. ericifolia* genets assisted greatly in isolating individual genets.

Mean expansion rates of individual genets of *M. ericifolia* over the 46-year study period varied widely, ranging from just over 3.5 m^2 to nearly 9 m^2 per year (Table 3.3). On average this represented an expansion rate of just over 5.4 m^2 per year and just less than 0.5 m of lateral extension annually. Expansion rate, however, was not, evenly distributed over the 46-year sample period (Figure 3.9, 3.10). After an initial rapid establishment phase between 1957 and 1964, growth slowed in the following period, 1964 – 1973. Between 1973 and 1991 expansion continued at an exponential rate, after which there was a considerable slowing of lateral spread (Figure 3.10).

Table 3.3 Size of 18 individual genets of *M. ericifolia* at the end of each sample period at Dowd Morass over 46 years (1957-2003). Measurements are in m^2.

Genet	1957	1964	1973	1978	1982	1991	2003
1	NA	53	63	108	407	732	2240
2	NA	102	193	297	536	1419	1614
3	NA	261	359	455	741	1526	1817
4	NA	153	240	350	606	1373	1922
5	NA	100	260	417	652	1206	1320
6	NA	64	128	211	255	953	1490
7	NA	234	391	610	829	1952	2549
8	NA	266	388	504	954	1961	3274
9	NA	137	329	530	823	1608	1645
10	NA	291	460	754	854	2145	2535
11	NA	45	423	622	885	1086	1632
12	NA	109	204	340	518	932	1528
13	NA	125	281	436	580	1265	1934
14	NA	51	58	116	438	786	1407
15	NA	191	235	359	641	821	1174
16	NA	262	378	54	822	2207	2219
17	NA	82	231	377	575	797	1837
18	NA	74	134	401	803	2018	2131

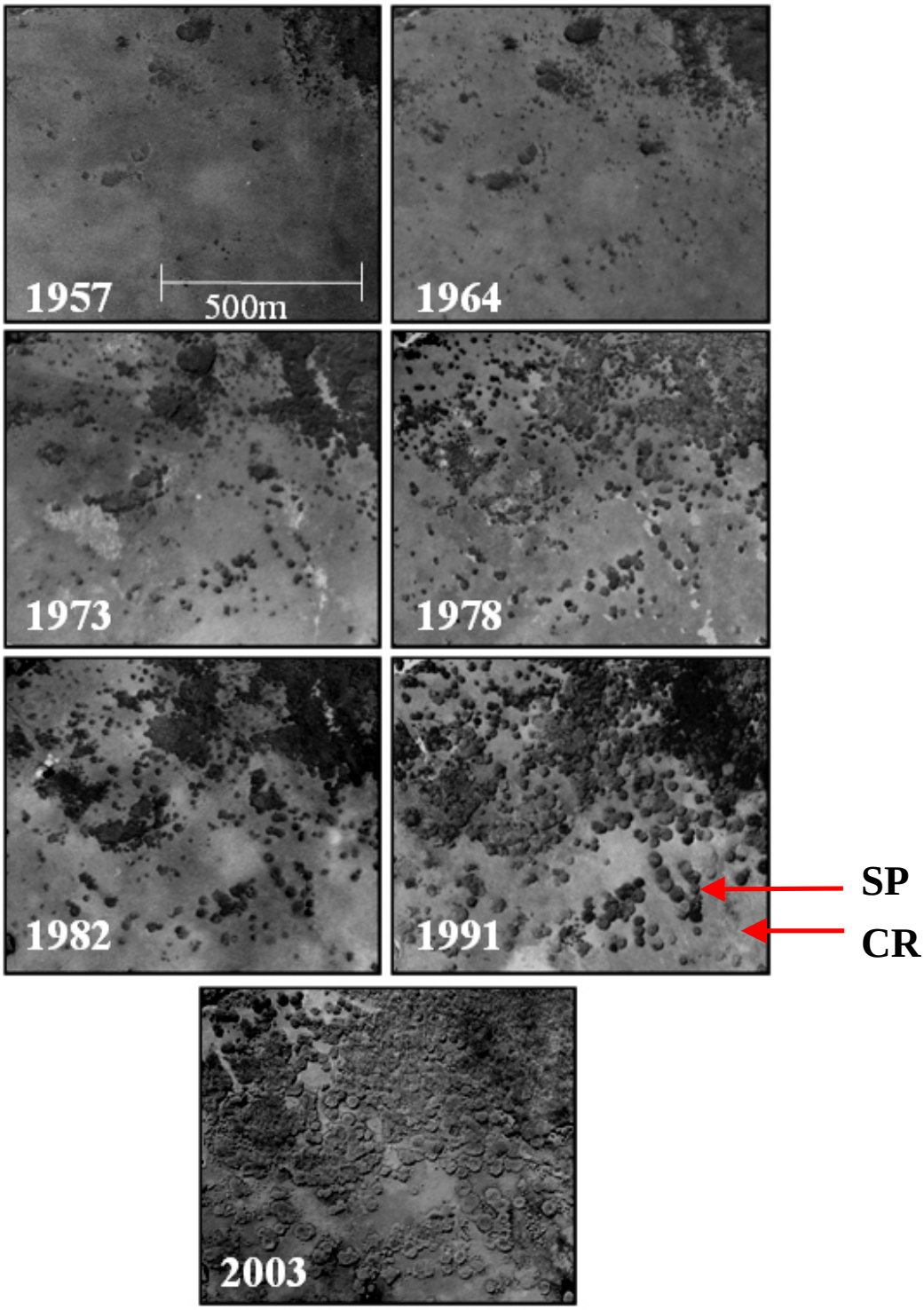


Figure 3.8 Historical aerial photographs of a section of Down Morass, from which growth rates and longevity of genets of *M. ericifolia* were derived. Pale sections (CR) of photos represent *Phragmites australis* (Common Reed); dark patches (SP) represent *M. ericifolia* (Swamp Paperbark).

The aerial photographs indicated that individual genets of *M. ericifolia* reached large sizes after 46 years, ranging from 1,174 to 3,274 m^2. While there was no direct evidence of longevity of individual genets of *M. ericifolia*, evidence of a marked slowing of expansion rate and the development of ring-shaped genets (where the centres had died out) suggest that individual ramets may senesce after approximately 40 years. Large genets, up to 40-60 m across, identified on the photographs from 1957, were still observed in the aerial photographs when compared to 2003. These individuals, based on expansion rates identified in this study, would range in age between 80 and 100 years old. The ongoing replacement of older ramets with daughter ramets within the genet makes aging of these older individuals problematic. Ages considerably older than 100 years may be obtained but remain undetected if lateral expansion is not possible and only internal replacement of ramets takes place.

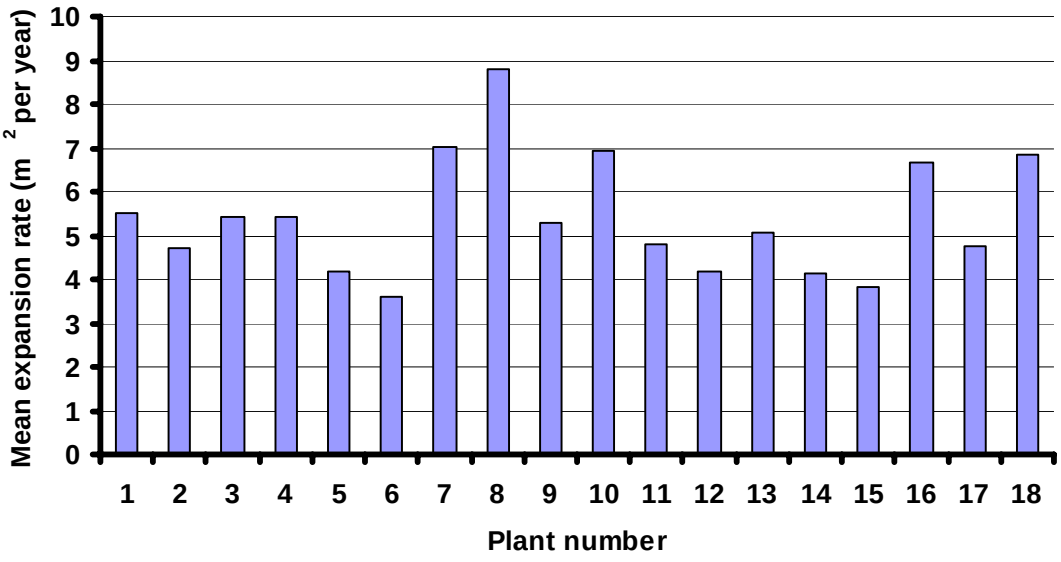


Figure 3.9 Mean expansion of individual *M. ericifolia* genets over 46 years (1957-2003) at Dowd Morass, Sale Victoria.

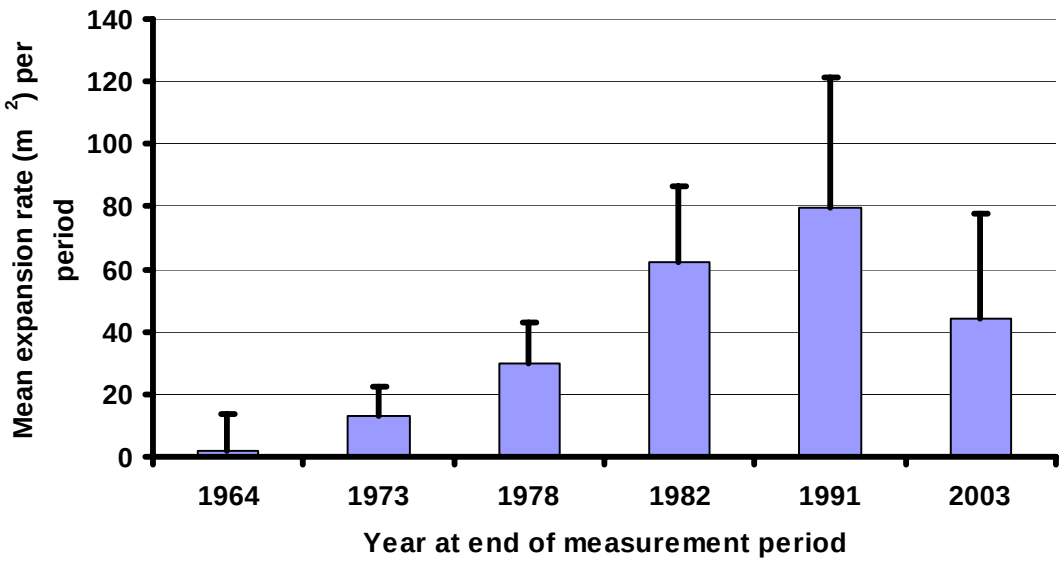


Figure 3.10 Mean expansion of all genets of *M. ericifolia* during various periods over 46 years (1957-2003) at Dowd Morass, Sale, Victoria. (n = 18, error bars represent standard deviation)

3.4 Discussion

3.4.1 Genet size and growth type

ISSR proved useful in determining individual genets in *M. ericifolia,* with Primer 814 differentiating each putative genet. Other primers could not be used to differentiate genets. The DNA-based markers (ISSRs) used in this study identified clear clustering of distinct multilocus phenotypes, indicating that *M. ericifolia* exhibits the characteristics typical of the 'phalanx' mode of clonal growth (Lovett Doust 1981), and most notably a dense advancing front of closely spaced ramets. Similar results have been reported for the strongly clonal *Eucalyptus argutifolia* (Myrtaceae) in Western Australia (Kennington and James 1997) and in a range of other clonal *Eucalyptus* species throughout Australia (Lacey 1983; Tyson *et al.* 1998; Smith *et al.* 2003) and *Melaleuca* and *Populus* species overseas (Kempermen and Barnes 1976; Miwa *et al.* 2001). The *Eucalyptus*, *Melaleuca* and the *Populus* species listed above all exhibited the phalanx mode of growth. These results confirm anecdotal evidence that large dome-shaped patches of *Melaleuca ericifolia* are composed of one genet and that adjoining genets exhibit no intermingling even though they may grow in close proximity and form larger patches.

Genet sizes measured in this study, 1,320 to 3,275 m^2, were markedly larger than reported for other phalanx clonal species of Australian Myrtaceae, *eg*. *Eucalyptus argutifolia* (529 m^2) (Kennington and James 1997) and *E. amygdalina* X *risdonii* (19 m^2) (Tyson *et al.* 1998). Genets of *Melaleuca cajuputi* in Thailand obtained large sizes (530 m^2) (Miwa *et al.* 2001), but were still less than half the size of the smallest *M. ericifolia* genets at Dowd Morass. Although large, genets of *M. ericifolia* are relatively small

compared with some other notable clonal species, such as *Lomatia tasmanica* (1.2 km wide (Lynch *et al.* 1998)), *Pteridium aquilinum* (1.2 km wide (Parks and Werth 1993), *Zostera marina* (6,400 m^2 (Reusch *et al.* 1999) and *Populus tremuloides* (43.3 ha (Kemperman and Barnes 1976).

Species demonstrating either the phalanx or guerrilla mode of clonal growth may retain connections between the ramets. This characteristic was exhibited in *M. ericifolia* (Figure 3.1). These physical attachments allow all the ramets within a genet to function as one, facilitating resource sharing, particularly nutrients, oxygen and water between individual and potentially widely separated ramets (Marshall 1990). The physical attachments between stems may be of particular importance in *M. ericifolia,* which grows in an environment with highly variable water regimes and salinities. The ecological implications for individual genets composed of large numbers of ramets with semi-permanent to permanent connections are many-fold, most notably in relation to rapid site capture and efficient nutrient foraging and utilization.

Organisation of physiologically integrated ramets in the phalanx growth form has particular advantages in environments that are temporally heterogeneous (Eriksson and Jerling 1990). Integrated ramets allow plants such as *M. ericifolia*, once established from seed, to colonize sites that would be unavailable if seedling recruitment alone were the only method of colonization (Alpert 1995) with older ramets subsidising daughter ramets. Should connected ramets occupy large areas over a gradient, the entire genet would not be permanently affected should part of the plant be inundated, desiccated or subject to unfavourable salinity levels, a common feature of the areas occupied by *M. ericifolia*. Additionally, extensive clonality may allow plants to expand into areas normally too dry or too wet to support mature *M. ericifolia*, with the dry area ramets being subsidised by

those in moister habitats and vice versa, increasing the ecological amplitude of the species. Ramets of *Fragaria chiloensis* growing in well lit, dry, and nutrient poor habitats are known to share resources with connected ramets in shaded, well-watered, nutrient rich habitats to the benefit of both (Alpert and Mooney 1986). Extensive carbohydrate reserves in the underground storage organs also allow rapid regeneration after perturbations such as fire and flood (James 1984).

The confirmation, through the use of molecular analysis, that large dome-shaped patches of *M. ericifolia* are predominantly if not exclusively one genet significantly alters how conservation of this species needs to be approached in future. The lack of integration of adjacent genets and the large number of closely-spaced ramets supports anecdotal evidence that *M. ericifolia* possesses the phalanx manner of clonal growth. The rapid expansion rate and large size obtained by individual genets in a relatively short period of time implies that few genets are able occupy a given space at any given time. These factors combined alter perceptions of the true diversity of what would have previously been considered populations. This study clearly elucidates that there are in fact only about 7 plants per hectare in a naturally occurring population, not hundreds or even thousands as previously thought.

3.4.2 Implications for revegetation

While the benefits of extensive physiologically integrated ramets and expansive clonality are many, there are several distinct disadvantages in the longer-term. Reduction or loss of genetic diversity or sexual reproduction in populations of clonal plants is well documented (Widen *et al.* 1994; Jelinski and Cheliak 1992; Kreher *et al*. 2000; Eckert

2001). The evolution of the clonal growth form is believed to be a consequence of limited opportunity for sexual recruitment (Mogori *et al.* 2003). Habitat change, including alteration to water regimes and salinity levels can move the environment beyond the ecological tolerances of the species forcing clonal plants to rely on asexual reproduction for continued survival (Honnay and Bossuyt 2005; Wesche *et al.* 2005) and on sexual reproduction for colonisation of new sites (Rea and Ganf 1994). The existing conditions of the wetlands of the Gippsland Lakes have, through permanent opening of the intermittent opening to the sea and human induced alteration to the water regime through the installation of levee banks, severely impacted the ecological conditions of the wetlands, increasing salinity levels and varying from historical wetting and drying patterns (Bird 1962; Greyson 2003).

In the short-term, altered water regimes and salinity level changes seems to have benefited *M. ericifolia* in the wetlands of the Gippsland Lakes, allowing expansion of existing genets and recruitment of new genets (Raulings *et al.* 2006). However, longer-term effects of these altered regimes are becoming apparent with decline of existing genets and reduced sexual and asexual reproductive capacity (Raulings *et al.* 2006). The requirements for sexual recruitment need to be understood to allow rehabilitation of these now highly managed and degraded wetlands. Some studies investigating the hydrology, microtopography and planting technique of *M. ericifolia* in the Gippsland Lakes wetlands have been carried out (Raulings *et al.* 2006) although the planting techniques trialled have been largely unsuccessful. Specific germination and recruitment condition information is needed to allow planned intervention and site manipulation to achieve maximum success. Knowledge of expansion rates and interactions of individual genets has major implications for spatial and temporal appropriate planting techniques. Present

planting techniques that focus on dense plantings of individual plants (genets), usually on 2 x 2 to 3 x 3m centres, primarily to achieve rapid canopy cover, may be particularly inappropriate for the long-term survival of *M. ericifolia* in brackish-water wetlands. If hand-planting of seedlings is to be used, it may be more appropriate to plant far fewer plants under ideal establishment conditions and allow the rapid expansion rates of the species to come into play. Counts of numbers of genets per hectare, based on the work carried out in this study, would indicate that there are as few as three genets per hectare and that on average there are 7 per hectare. Present planting densities utilising 3 m centres between planted seedlings utilises approximately 1,110 plants to revegetate one hectare. Fewer, well-placed plants coupled with time represents both a significant cost saving and probably a superior ecological outcome.

Chapter 4

Comparison of two contrasting life forms of *Melaleuca* in south-eastern Australia.

Abstract

The viability of seed and the trade-off between sexual and asexual reproduction can vary widely between plants of the same genus occupying different aspects of an environmental gradient in wetlands. Viability can vary among plants of the same species across the range of that species in response to different environmental parameters. A comparative study was carried out between two co-occurring members of the genus *Melaleuca* (*M. ericifolia* and *M. parvistaminea*) with contrasting growth type (rootstock regenerator vs. seed-only regenerator). Additional viability testing was carried out on 23 populations across the southern part of the range of *M. ericifolia* in Victoria and Tasmania in order to test the hypothesis that a range of factors including population size, distance to nearest population and alterations to habitat may play a role in viability over and above inherent viability. Seed weight and viability of the rootstock regenerating *M. ericifolia* was consistently lower (<30 µg, < 38 %) than the seed-only regenerating *M. parvistaminea* (>30 µg, > 70 %). Across the range of *M. ericifolia*, viability varied from 0 – 38%, with large, relatively undisturbed populations having higher percentage germination. Populations of *M. ericifolia* affected by isolation, limited gene flow, disturbance and secondary salination had markedly reduced viability regardless of area covered by the population.

4.1 Introduction

Melaleuca ericifolia (Swamp Paperbark) and *Melaleuca parvistaminea* are two members of the large serotinous genus *Melaleuca* (Myrtaceae) that occurs primarily in Australia (Spencer 1996). There is considerable overlap in the distribution of the two species and until recently both were considered varieties of *M. ericifolia* (Albrecht 1987). Differences in regeneration and reproductive ability are two of the main distinguishing features between these taxa: *Melaleuca parvistaminea* is a seed-only regenerator while *M. ericifolia* forms extensive colonies of vegetatively reproduced stems through root suckering (Albrecht 1987). Both species occur in wetlands, but are usually spatially and ecologically segregated: *M. parvistaminea* occurs in areas in south-east Australia with only intermittent, short-term inundation in primarily freshwater swamps (Figure 4.1), whereas *M. ericifolia* occurs in wetter habitats that may be flooded for many months, ranging from fresh to brackish swamps (Figure 4.2). Accordingly, it is not uncommon to find both species growing in the same wetland but spatially separated along an elevational gradient. The co-occurrence of these two species in many wetlands throughout south-eastern Australia provides an ideal opportunity to investigate the hypothesis that there is a trade-off between sexual and asexual reproduction and that this may be expressed as reduced seed viability in the clonal species.

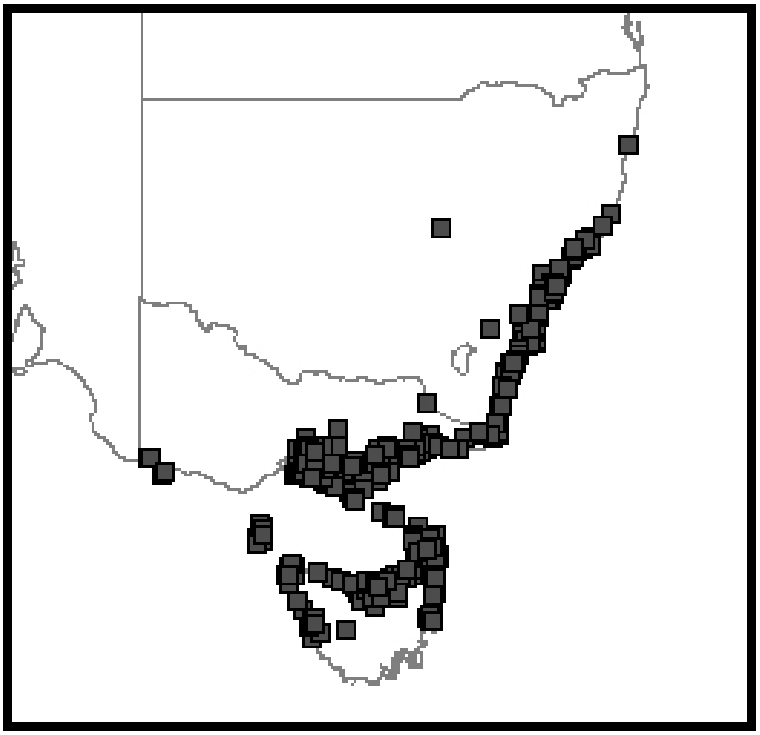


Figure 4.1 Distribution of *Melaleuca ericifolia* in Australia (Australian Virtual Herbarium)

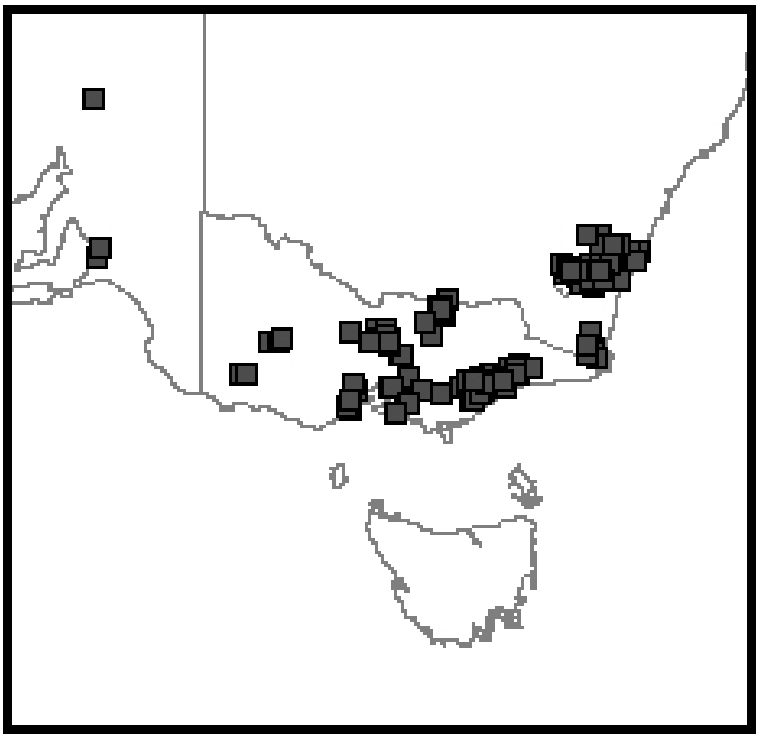


Figure 4.2 Distribution of *Melaleuca parvistaminea* in Australia (Australian Virtual Herbarium).

It is commonly assumed that there is a trade-off between sexual and asexual reproduction in plants due to the high costs (energetic and nutrient) of sexual reproduction (Lovett Doust 1989; Reekie 1999; Eckert 2001). There are, however, conflicting findings regarding resource allocation to sexual reproduction in clonal plants, with some authors finding reduced commitment to sexual reproduction (in *Mimulus*: Sutherland and Vickery 1988) and others no reduction in sexual reproductive outputs (Rea and Ganf 1994).

While there are some seed viability studies of several populations of *M. ericifolia* in South Gippsland (Ladiges *et al.* 1981; Robinson *et al.* 2006) there are no comprehensive studies of this species over the majority of its range from northern New South Wales through to western Victoria and Tasmania. Such studies are essential if generalisations about trade-offs between sexual and asexual reproduction are to be made. No references to the seed viability of any populations of *M. parvistaminea* were found.

The aims of this component of the thesis are to:

1. Compare the seed viability in the clonal *M. ericifolia* to the non-clonal *M. parvistaminea*,
2. Determine the importance of site isolation and population size on the seed viability of *M. ericifolia* and *M. parvistaminea*, and
3. Determine the importance of site degradation on the seed viability of *M. ericifolia* and *M. parvistaminea*.

4.2 Methods

4.2.1 Seed collection

Seed capsules were collected between April 2004 and November 2005 from 12 – 25 adult trees (different clones) scattered throughout various populations of *M. ericifolia* across its range in Victoria and Tasmania, including several Bass Strait Islands (Table 4.1). Seed was collected from seven populations of *M. parvistaminea* in South Gippsland that were known to be natural occurrences (Table 4.2) were the range of this species overlaps with *M. ericifolia*. The characteristic phalanx (dense advancing front) growth form and dome shape was used to determine individual plants of *M. ericifolia*; seed was collected from widely separated plants that were clearly not related clonally. Approximately 12 individual plants were sampled scattered throughout each population of *M. parvistaminea*. Approximately 500 seed capsules were collected from each putative individual of each species. Seed loses viability after approximately 1 year, the short period of storage, mostly over the cooler winter months was not viewed as materially altering overall viability.

Information was collected for each of the populations of *M. ericifolia*, including locality (latitude and longitude), adjacent disturbance and an estimate of area covered by the species. Area of population for *M. ericifolia* was visually assessed and classed into categories shown in Table 4.3. Site salinisation and distance to nearest population was calculated using site records from the Waterwatch and the National Herbarium Melbourne

Table 4.1 Populations and location of seed collection sites for *M. ericifolia* across southern Australia. Localities marked with an * are potentially introduced populations.

Population location	Latitude	Longitude	Population size (ha)
Little River (Victoria)	37° 56' 17"	144° 28' 33"	>1 ha
Cape Nelson (Victoria)	38° 24' 39 '	141° 23' 41	1-5 ha
*Mount Wellington (Tasmania)	42° 53' 09"	147° 15' 32"	>1 ha
Heritage Golf Course (Victoria)	37° 40' 55"	145° 21' 05"	>1 ha
Brushy Creek (Victoria)	37° 46' 00"	145° 17' 00"	1-5 ha
Yanakie Peninsula (Victoria)	38° 48' 52"	146° 14' 09"	1 ha
Dowd Morass (Victoria)	37° 28' 58"	149° 40' 38"	> 1000 ha
*Kemps Marsh (Lake Sorell) (Tasmania)	42° 47' 31"	147° 33' 26"	1-5 ha
Spadoni Reserve (Victoria)	37° 28' 58"	149° 40' 38"	1-5 ha
Boggy Creek (Victoria)	37° 42' 00"	145° 33' 12"	1-5 ha
Cades Road (Victoria)	37° 33' 38"	149° 07' 13"	10-50 ha
Apsley Marshes (Tasmania)	41° 59' 59"	148° 15' 53"	10-50 ha
Lightwood Creek, Point Nepean Nation Park (Victoria)	38° 25' 5"	144° 56' 19"	5-10 ha
Garfield (Victoria)	38° 05' 06"	145° 36' 38"	5-10 ha
Cann River (Victoria)	37° 32' 35"	149° 07' 07"	10-50 ha
Gypsy Point (Victoria)	37° 28' 58"	149° 40' 38"	10-50 ha
Yarram (Victoria)	38° 37' 55"	146° 40' 05"	10-50 ha
Cape Barren Island Population 1 (Tasmania)	40° 24' 43"	148° 01' 42"	10-50 ha
Cape Barren Island Population 2 (Tasmania)	40°26' 28"	148° 08' 14"	10-50 ha
Flinders Island Population 1 (Tasmania)	39° 57' 10"	148° 10' 49"	50-100 ha
Flinders Island Population 2 (Tasmania)	40° 13' 17"	148° 17' 40"	50-100 ha
Gladstone (Tasmania)	40° 55' 01"	147° 54' 30"	50-100 ha
Narawntapu National Park (Tasmania)	41° 10' 72"	146° 36' 70"	100-1000 ha

Table 4.2 Populations and locations of seed collection sites for *M. parvistaminea* in South Gippsland, Victoria

Population location	Latitude	Longitude	Population size (ha)
Rosedale	38°10'4"	146°57'24"	1-5 ha
Maffra	37°56'2"	146°48'41"	1-5 ha
Fernbank	37°52'31"	147°16'16"	1-5 ha
Providence Ponds	37°57'46"	147°19'18"	5-10 ha
Heyfield	37°56'33"	146°43'34"	5-10 ha
Briagalong	37°49'9"	146°58'39"	5-10 ha
Sale Common	38° 8'2"	147°5'0"	5-10 ha

Table 4.3 Classification of population size of *M. ericifolia* across southern Australia.

Classification	Population size
Very small	Under 1 ha
Small	1-5 ha
Medium	5-10 ha
Medium/Large	10-50 ha
Large	50-100 ha
Very Large	100-1000 ha
Expansive	Greater than 1000 ha

Capsules were stored in paper bags at 20^0C for one week. The bags were lightly shaken to release seed from the capsules and the contents sieved to remove the empty capsules and other detritus. Seed was placed in clean paper bags for a further three days to remove excess moisture, then transferred to sealed glass containers and stored at 20^0C in darkness until used, usually within 2 months of harvest. Preliminary germination trials began in late April 2004, with the main trials carried out in July 2004 and December 2005.

4.2.2 Seed viability

The number of seeds per gram was determined by taking five samples of known weight (0.003-0.007 g), which were counted by eye. The average number of seeds was calculated from the mean of the aggregated totals of the five samples. The weight of individual seeds was determined by dividing the number of seeds per sample by the weight of the sample (Association of Official Seed Analysts 1990).

Viability testing followed procedures outlined by the Association of Official Seed Analysts (1990), except that the number of seeds per replicate run was increased from 25 to 100 to improve statistical rigour (Robinson *et al*. 2006). A small pilot study was carried out before the main trials to determine statistical power and the number of replicates needed to detect significant responses to environmental variables (Zar 1999). These trials indicated that four replicates, each using 100 seeds, yielded a power of >0.99.

Seeds were surface sterilized by placing them in small sealed muslin bags and plunging the bags in 10 % w/v sodium hypochlorite solution for 20 seconds and then rinsing them three times in distilled water. For each viability-trial replicate, 100 seeds were evenly spaced in a grid pattern on a disc of Whatmans #3 filter paper in a 9-cm diameter petri dish. Each paper disc was wetted with 8 mL of distilled water and the dish sealed with Labfilm to reduce moisture loss. A total of four replicate petri dishes (i.e., 400 seeds) were used for each viability test. Seeds were germinated and then incubated in growth cabinets with day-time temperatures of 20^{o}C and night-time temperatures of 10^{o}C. A 12:12 hour light:dark cycle was used. A bank of fluorescent tubes designed for plant growth provided light, emitting a PAR of 40 μE m^{-2} s^{-1} at the level of the seeds. All replicates were randomly shuffled daily within the cabinet to randomise spatial variability, and germination was measured after 7, 14 and 21 days. The trial was terminated at 21 days because no additional germination was recorded after Day 14. Germination was judged by the emergence of the base of the hypocotyl from the testa.

4.3 Results

4.3.1 Melaleuca ericifolia

Viability of seed from various population of *M. ericifolia* varied widely, from 0 % for two isolated populations in western Victoria and one from southern Tasmania through 32 % for populations from East Gippsland and 33–38 % for the majority of Tasmanian and Bass Strait Island populations (Table 4.4). Germination percentage was closely correlated with seed weight (R^2 = 0.933, see Figure 4.3) with little deviation from a linear relationship.

Populations with lower viability had a large percentage of unfilled seed. The three populations with zero germination were excluded from data analysis (rows 1-3 Table 4.4). There was little relationship between viability and population size with three larger populations, Apsley Marshes, Cades Road and Dowd Morass (10-50 ha and > 1000 ha, respectively) exhibiting a clear deviation from the mean (Figure 4.4). Populations under 5 ha also exhibited wide divergence from the mean (Table 4.4 and Figure 4.4). Features in common with all sites with very low viability were the degree of adjacent human disturbance, secondary salinisation, and isolation from the nearest population.

Table 4.4 Population size, seed weight and viability of various populations of *Melaleuca ericifolia* in Victoria and Tasmania, Australia

Population location	Population size (ha)	Number of seeds per mg	Mean Seed weight (µg)	Percent germination (%)
Little River (Victoria)	> 1 ha	0	0	0
Cape Nelson (Victoria)	1-5 ha	0	0	0
Mount Wellington (Tasmania)	> 1 ha	0	0	0
Heritage Golf Course (Victoria)	> 1 ha	61.5	16	0.3
Brushy Creek (Victoria)	1-5 ha	66.4	15	0.5
Yanakie Peninsula (Victoria)	1 ha	55.3	18	5.5
Dowd Morass (Victoria)	> 1000 ha	55.6	18	6.0
Kemps Marsh (Lake Sorell) (Tasmania)	1-5 ha	53.6	19	6.0
Spadoni Reserve (Victoria)	1-5 ha	53.4	19	6.3
Boggy Creek (Victoria)	1-5 ha	49.9	20	9.3
Cades Road (Victoria)	10-50 ha	49.2	20	12.8
Apsley Marshes (Tasmania)	10-50 ha	47.3	21	14.5
Lightwood Creek Point Nepean National Park (Victoria)	5-10 ha	42.5	23	21.5
Garfield (Victoria)	5-10 ha	45.2	22	22.8
Cann River (Victoria)	10-50 ha	43	23	23.3
Gypsy Point (Victoria)	10-50 ha	41.4	24	27.0
Yarram (Victoria)	10-50 ha	39.2	26	32.0
Cape Barren Island Population 1 (Tasmania)	10-50 ha	39	26	32.3
Cape Barren Island Population 2 (Tasmania)	10-50 ha	38.7	26	33.0
Flinders Island Population 1 (Tasmania)	50-100 ha	34.2	29	34.0
Flinders Island Population 2 (Tasmania)	50-100 ha	34.4	29	34.5
Gladstone (Tasmania)	50-100ha	37.9	26	35.8
Narawntapu National Park (Tasmania)	100-1000 ha	37.7	26	38.0

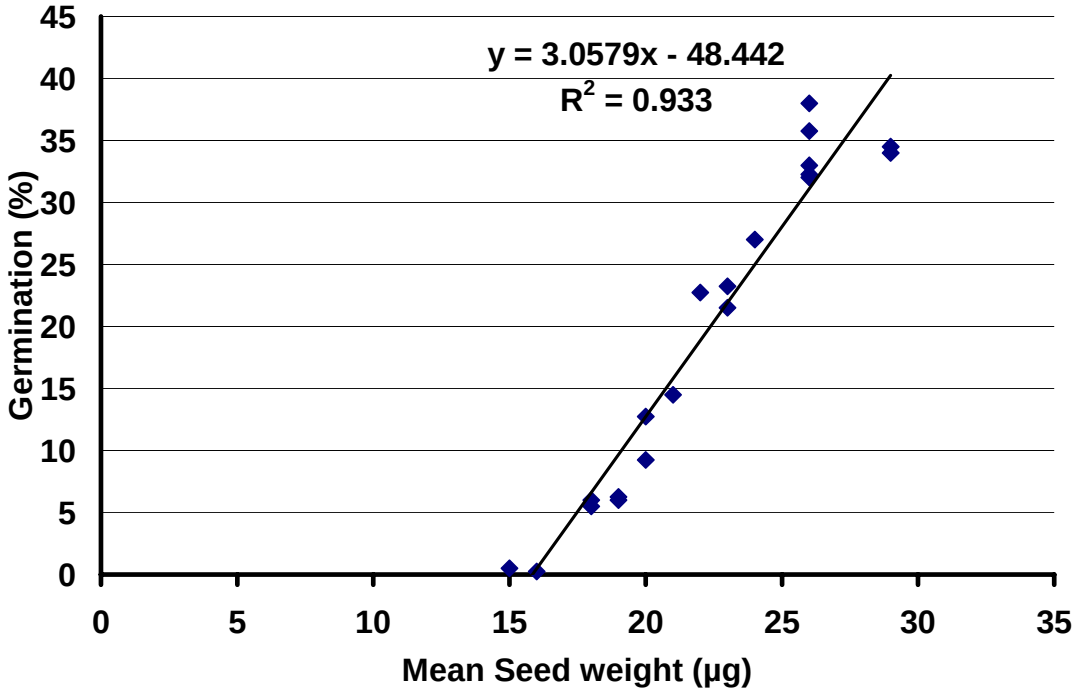


Figure 4.3 Linear regression of seed weight versus germination rate for *Melaleuca ericifolia*.

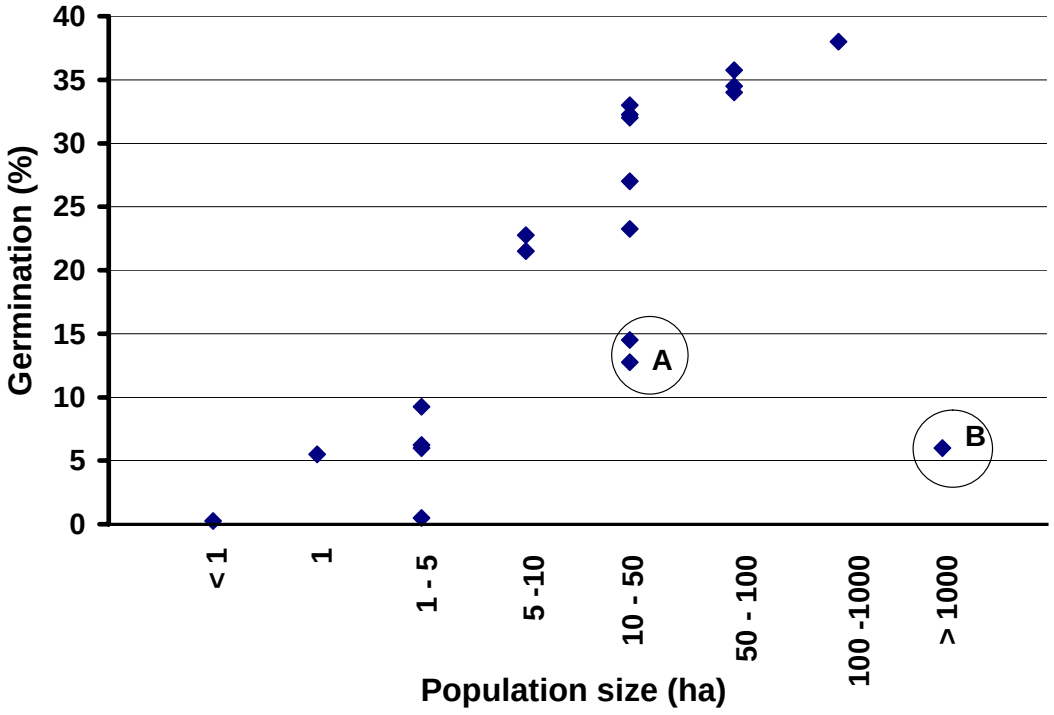


Figure 4.4 Germination rate for various population sizes of *Melaleuca ericifolia* in Victoria and Tasmania. A – Apsley Marshes and Cades Road; B – Dowd Morass.

4.3.2 *Melaleuca parvistaminea*

Viability of seeds from the various populations of *M. parvistaminea* was much higher than for *M. ericifolia,* and varied from 70 – 80 % regardless of population class (Table 4.5). Although most larger population classes had better overall germination, there was overlap in germination rates between the various population size classes, unlike the case with *M. ericifolia* (Table 4.4). Lower germination rates were not associated with disturbance to the sites. *Melaleuca parvistaminea* seeds were consistently heavier (31-33 µg**)** than *M. ericifolia* seeds (15-29 µg**)** (Figure 4.5).

Table 4.5 Population size, seed weight and viability of various populations of *Melaleuca parvistaminea* in South Gippsland, Victoria, Australia.

Population location	Population size (ha)	Number of seeds per mg	Mean seed weight (µg)	Percent germination (%)
Rosedale	1-5 ha	31.6	32	70
Maffra	1-5 ha	31.3	32	75.5
Providence Ponds	5-10 ha	31.1	32	76.5
Fernbank	1-5 ha	32.2	31	78
Heyfield	5-10 ha	30.2	33	79.5
Briagolong	5-10 ha	30.7	33	80
Sale Common	5-10 ha	30.5	33	80.5

4.3.3 Comparison of *M. ericifolia* and *M. parvistaminea*

There was a clear distinction in germination rates and seed weights between populations of *M. ericifolia* and *M. parvistaminea*. Average seed weights of *M. parvistaminea* were always above 30 µg seed^{-1} whereas those of *M. ericifolia* were

below 30 µg seed^{-1}. Even greater differentiation was seen in overall germination of the two species with *M. ericifolia* varying from 0–38 % and *M. parvistaminea* varying from 70–80% (Figure 4.6).

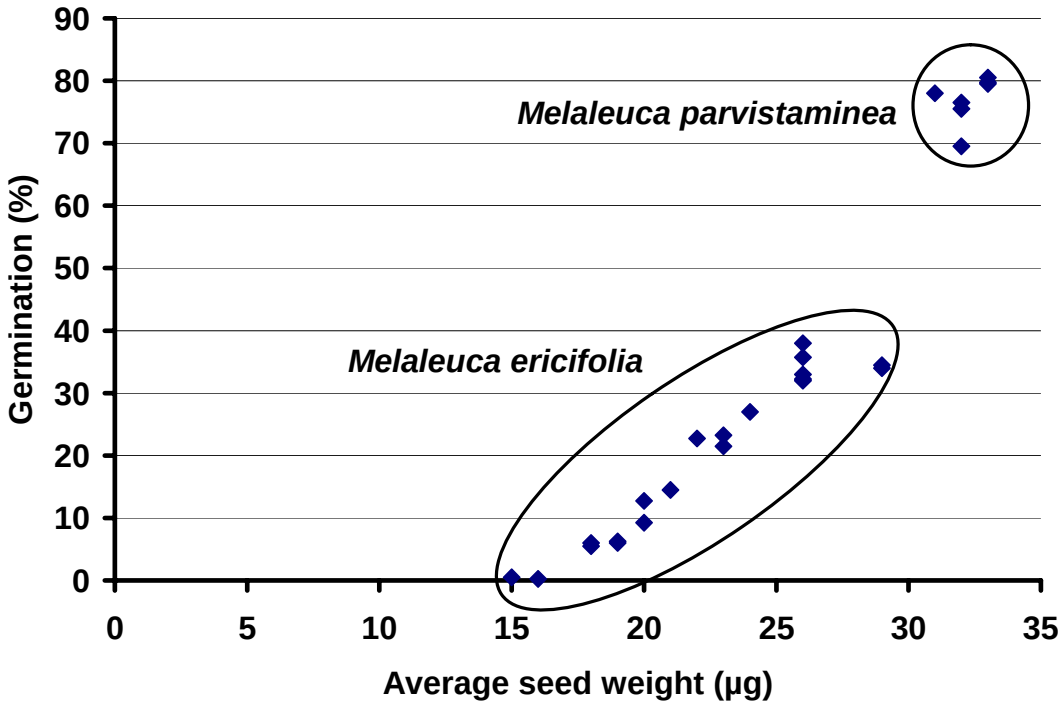


Figure 4.6 Comparison of germination rates and seed weights of various populations of *M. parvistaminea* and *M. ericifolia*.

4.4 Discussion

4.4.1 Trade-offs between sexual and nonsexual reproduction

The clear dichotomy in germination rates in relation to regeneration characteristics of *M. parvistaminea* and *M. ericifolia* (Figure 4.6) suggests that there is a trade-off in resource allocation between these two species. This result confirms findings of a number of other studies that indicate resources are primarily directed to seed production in obligate seed-regenerating species and to vegetative reproduction in rootstock-regenerating species (Armstrong 1982; Sutherland and Vickery 1988). Seed size and germination rates were consistently higher in the obligate seed-regenerating species *M. parvistaminea* than in *M. ericifolia*, and smaller average seed size and lower overall germination rates were consistent across the range of provenances of the rootstock-regenerating species *M. ericifolia.*

Reduced seed production and viability does not imply reduced genetic diversity in populations (Ellstrand and Roose 1987). Lamont and Weins (2003) undertook a pair-wise comparative study across a range of generic pairs of species, mainly in Australia, that exhibited a similar dichotomy in regeneration method and ecological segregation to *M. ericifolia* and *M. parvistaminea*. They found that 30 of the 33 pairs of woody species tested had lower viable seed production in the resprouting species. They postulated however, that lowering of potential sexual reproduction in resprouting plants did not indicate lowering of genetic diversity within a given population but did not indicate why this would be. Widen *et al*. (1994) reported no loss of genetic diversity in a review of 45 mainly herbaceous clonal species from a range of habitats, including wetlands, in the Northern Hemisphere.

Sexual reproduction after the initial establishment of vegetatively reproducing, clonal plants may not be important to survival in the short to medium term (Eriksson 1989; Silverton 1982; Harper 1978). For example, of the 68 clonal species investigated by Eriksson (1989), 41 were not observed to recruit into existing established populations. Even amongst clonal species that do regenerate from seed, for example *Solidago canadensis*, changes in population structure over time may favour those individuals with the strongest ability to reproduce vegetatively (Hartnett and Bazzaz 1985). Sexual reproduction would only be needed upon the death of the parent plants or to colonise new environments.

4.4.2 Other factors influencing sexual reproduction in *M. ericifolia*

Low fecundity in *M. ericifolia* and the lack of consistent observation of seedlings within existing populations in the wild would indicate that plants are diverting resources to vegetative, rather than sexual, reproduction. Variations in viability, from 0 % to 38 %, across the range of *M. ericifolia* indicate that there are other processes occurring that influence sexual reproductive success. The three populations of *M. ericifolia* that had zero seed viability (Little River; Cape Nelson: Mt Wellington, Table 4.4) all occurred at the very extremes of the species' distribution in western Victoria and southern Tasmania: the Little River population is approximately 70 km from the nearest *M. ericifolia* population, the Cape Nelson population 300 km, and the Mount Wellington population 200 km. At all three sites there is no obvious morphological variation within the population and, although seed capsules were found on the plants, none contained seeds with embryos. It may be that these three

individual populations actually represent individual plants that have seeded into the site and now persist vegetatively. The largest of the three populations, at Cape Nelson, covered 1-5 ha (see Table 4.1). Three populations that exhibited low viabilities, Dowd Morass, Cades Road and Apsley Marshes (6, 12 and 14 % respectively) were all relatively large (10-1000 ha) and were within 50 – 100m of other existing populations. All three sites are highly disturbed, occur in primarily agricultural landscapes and suffer from secondary salinisation (Grayson 2003; Ramsar 2006).

Most of the populations of *M. ericifolia* with germination rates < 10 % were highly disturbed remnants, usually adjacent to roadsides or in formerly grazed land. It may be that these populations represent colonisation by relatively few individuals. Fragmentation and limited re-colonisation of this type adversely affects genetic diversity, reduces pollination and variously affects hydrology and potentially increases nutrient levels depending on surrounding land use (Henriquez 2004; Renison *et al.*2004; Wooller and Wooller 2004; Cunningham 2000; Morgan 1999) with consequent effects on germination capacity and seed size.

Although *M. ericifolia* can grow in brackish swamps, there is a great deal of variation in the salinity tolerance of different genets (Ladiges *et al.*1981) and in the salt sensitivity of various life stages (Robinson *et al.* 2006; Salter *et al.* 2007). Prior to European settlement, all three of the sites listed above (Dowd Morass, Cades Road Swamp and Apsley Marshes) were freshwater swamps. Present conditions of the ground and surface water at least at the first two sites, Dowd Morass and Cades Road, have become highly saline with levels up to half sea water (> 20 g L^{-1}, Grayson 2003; Waterwatch data). Reduced flowering, seed set and viability with increasing salinity

levels is widely recognised as affecting reproductive success in a range of species including those that naturally occur in salt marshes and brackish situations (Boscaiu *et al.* 2005; Labidi *et al.* 2004; Redondo *et al.* 2004).

Secondary salinised sites pose difficult and perhaps intractable problems for sexual reproduction in woody taxa. The underlying conditions that produce secondary salinisation are complex and the impacts usually cover extensive areas. In the case of Dowd Morass, removal of nearly 90 % of the original flow of fresh water from the rivers that feed the Morass has allowed intrusion of salt water from the sea (Grayson 2003). Periodic flushing of Dowd Morass with fresh water no longer occurs and salinity levels have been progressively rising. Major alterations to salinity and water regime have been recognised as contributing to the collapse of existing vegetation communities within several major wetland systems in Australia (Kingsford 2000).

Collapse of the existing *M. ericifolia* dominated swamp, first identified by reduced seed viability in this study but more recently from rapid and extensive plant death (Boon *et al*. 2007), could see complete removal of *M. ericifolia* from most of this wetland system and in time, local extinction. Dowd Morass is one of several Ramsar-listed wetlands surrounding the Gippsland Lakes. The colonial nesting birds, Ibis (*Threskiornis* spp.*)* and Cormorants (*Phalacrocorax* spp.*),* are reliant on *M. ericifolia* for nesting and roosting at Dowd Morass. The loss of *M. ericifolia* would have significant impacts on these two bird species, making the habitat unsuitable for them. If present changes to vegetation induced by salinisation of Dowd Morass continue, predictions made by Bird (1962) that complete alteration of the vegetation to a salt marsh will become true. Loss of the existing vegetation and suitable sites for nesting

of colonies of birds would inevitably lead to the loss of the Ramsar listing of these wetlands.

It would appear that the least disturbed and potentially most viable populations of *M. ericifolia* are in East Gippsland, the Bass Strait Islands and some sites in Tasmania. The viability of seed from these populations varied from 20 % to 38 %. Similar seed viabilities, ranging from 3-28 %, have been found in the rootstock regenerator (resprouting) *Melaleuca quinquenervia* in Queensland and South Florida (Reyachhetry *et al.*1998; Browder and Schroeder 1981; Meskimen 1962). *Melaleuca quinquenervia* is an ecologically equivalent species to *M. ericifolia* and indeed replaces it in near-coastal freshwater to brackish wetlands in northern NSW and Queensland.

4.5 Conclusions

Seed viability of most populations of the rootstock-regenerating species *M. ericifolia* was markedly lower than the co-occurring seed-only regenerator, *M. parvistaminea*. While low seed viability would appear to be detrimental to the continued survival of *M. ericifolia*, this is at least partially compensated for by extensive vegetative reproduction. However, very low viability rates were detected for isolated and highly fragmented populations of *M. ericifolia*, as well as those affected by secondary salinisation, these factors inhibiting the ability of *M. ericifolia* to sexually reproduce (Eckert 2001). Loss of sexual reproduction potential is particularly critical in sites such as Dowd Morass and Cades Road that experience salinisation, as this may lead to eventual loss of the entire paperbark population. In support of this conclusion, Bowkett and Kirkpatrick (2003) predicted that long-term survival of *M. ericifolia* in Tasmania would only occur in the largest populations of the species in the Tamar Valley which would be most resistant to ecological change.

To conserve and maintain the sexual viability of populations of *M. ericifolia* the underlying causes of decreased viability of seed, below that which is naturally occurring, needs to be addressed. Interference with naturally isolated populations by introduction of additional genets and therefore genetic diversity (*eg.* by revegetation activities) could alter vegetation dynamics of the sites and competitive abilities of *M. ericifolia*. Increasing the competitive ability of *M. ericifolia* in this way could threaten the existing vegetation communities of these sites. Conversely, introduction of genetic material between sites in highly fragmented remnants could increase the competitive

ability of genetically weakened remnants, provided sexual reproduction takes place, contributing to the conservation of the species locally.

Chapter 5

Germination characteristics of *Melaleuca ericifolia* Sm. (Swamp Paperbark)

Abstract

Seed collected from Dowd Morass, a secondary-salinised Ramsar-listed wetland of the Gippsland Lakes region in eastern Victoria, showed very low viability (< 6 %), with less than 50 % of the seeds germinating even under ideal laboratory conditions. Greatest germination occurred with surface-sown seed, germinated in darkness at a mean temperature of 20^{o}C and salinity < 2 g L^{-1}. At 20^{o}C, maximum germination occurred at a salinity of 1 g L^{-1}; germination fell rapidly at a near constant rate with increasing salinity. Lower temperatures, while moderating the inhibitory effects of salinity, markedly reduced germination; higher temperatures increased the inhibitory effects of salinity and light and reduced overall germination rates. Seeds subjected to brief inundation with saline water germinated rapidly if flushed by, and subsequently grown under, freshwater conditions. Specific timing of management interventions, particularly manipulations of water regime to control salinity regimes, are required if germination of *M. ericifolia* on the landscape scale is to be successful. Even so, the low overall viability of the seed would present difficulties to large-scale, seed-based rehabilitation efforts.

5.1 Introduction

Swamp paperbark (*Melaleuca ericifolia* Sm.) is a small clonal tree in the Family Myrtaceae which grows in coastal (freshwater and brackish-water) swamps across southern and eastern Australia, from Tasmania through to northern New South Wales (Jeanes 1996). Since the distribution and abundance of this species has decreased markedly with the clearing or draining of wetlands in which it formerly occurred (Bowkett and Kirkpatrick 2003), a high priority of natural-resource management agencies and non-government organisations throughout Australia is the rehabilitation of high-value coastal wetlands that contain, or did contain, *M. ericifolia* and other *Melaleuca* species (de Jong, T.J. 1997; de Jong, N. 2000). While most *Melaleuca* species are reliant on seed to produce new individual plants (e.g., *Melaleuca parvistaminea*, *Melaleuca quinquenervia*), *M. ericifolia* is unusual in that it is extensively clonal, producing physically independent ramets across time and space (Ladiges *et al*. 1981).

The development of the clonal growth form is commonly seen as a response to limited opportunities for seed to germinate and seedlings to recruit into the population (Barsoum 2002; Pan and Price 2002; Sachs 2002). The investment in clonality, at the apparent expense of sexual reproduction, allows plants to survive and persist in conditions that would be hostile to non-clonal plants (Jurik 1985; van Kluenen *et al.* 2000; Eckert 2001; Barsoum 2002). The extensive clonal growth exhibited by *M. ericifolia* might act as a buffer to all but the most challenging environmental conditions. Major cataclysmic events, including flood, drought, fire and inundation by sea water, occur periodically in the habitats occupied by the species and are

potentially lethal to both adult and juvenile *M. ericifolia* (Ladiges *et al*.1981; Salter 2001; Grayson 2003). While short to medium-term survival is ensured by the clonal growth form, colonisation of new sites or re-colonisation of existing sites after mortality of existing *M. ericifolia* plants is presumed to be reliant on germination from seed (de Jong N. 2000).

Although *M. ericifolia* seed seems to have no specific inherent germination inhibitors apart from containment within woody capsules, it is possible that short-term, salt- and temperature-induced dormancy may take place, as has been reported for *Melaleuca quinquenervia* (Serbesoff-King 2003). Viability, however, is highly variable across the range of *M. ericifolia*, with a large percentage of unfilled or otherwise damaged seed within the capsules, a trait shared with *M. quinquenervia* (Ladiges *et al.* 1981; Rayachhetry *et al*. 1998; J. Salter pers comm.). Seed of *M. ericifolia* and *M. quinquenervia* loses viability rapidly once released from the plant, with germinability greatly reduced after one year (Woodall 1983; Bodle and Van 1999; Rayamajhi *et al*. 2002; J. Salter pers comm.). Germination of *M. ericifolia* seed at Coomonderry Swamp in New South Wales (Australia) has been reported to be episodic, and survival of the germinants is reliant on a narrow range of site conditions (de Jong N. 2000). This conclusion is consistent with the analysis of a series of historical air photographs of *M. ericifolia* swamps in western Gippsland, which suggest there are gaps of several decades between successful recruitment events.

Despite its widespread distribution across coastal areas in eastern and southern Australia and its priority listing for rehabilitation efforts, it is not clear what factors control the success of seed germination and plant establishment in *M. ericifolia*. The

coastal environment in which most *M. ericifolia* grows suggest that salinity will play a major role, both in terms of absolute effects of salt and the effects of short-term exposure to saline water (Ladiges *et al.* 1981). In common with many other taxa, temperature and light intensity are also likely to be important (Gul and Weber 1999; Khan and Gulzar 2003). Finally, the depositional nature of coastal wetlands suggests that burial may play a role; burial has been shown to be a critical factor controlling germination success in other wetland and terrestrial plant species (Van *et al*. 1998). The only published research on the individual effects of key environmental variables - temperature, light, burial and salinity - on germination in *M. ericifolia* is by Ladiges *et al*. (1981). Although interactive effects among these factors have not been investigated at all, there are documented effects of the interactive effects of environmental variables, particularly light, salinity and temperature, on the germination of a range of brackish wetland species (Gul and Weber 1999; Khan and Gulzar 2003).

The aim of the present study was to quantify the primary and interactive effects of key environmental parameters - salinity, temperature, light, burial and substrate type - on the germination of *M. ericifolia*. This information will be useful in providing explanations for historical patterns in changes to vegetation in coastal wetlands and in developing better strategies and protocols for rehabilitating these areas at the landscape scale. In particular, this study will help in explaining the characteristics needed to determine safe sites for germination and establishment.

5.2.1 Seed collection

Seed capsules were collected in April 2004 from 25 adult trees scattered throughout the population of *M. ericifolia* at Dowd Morass. Individual plants were determined by visual assessment of growth configuration; a characteristic dome shape. Seed was collected from widely separated (> 100 m) plants that were presumed to be un-related clonally. Capsules were stored in paper bags at 20^0C for one week. The bags were lightly shaken to release seed from capsules, and the contents sieved to remove empty capsules and other detritus. Seed was placed in clean paper bags for a further 3 days to remove excess moisture and transferred to sealed glass containers and stored at 20^0C in darkness until used. Preliminary germination trials began in late April 2004, with the main trial carried out in July 2004.

5.2.2 Seed viability

Viability testing followed procedures outlined by the Association of Official Seed Analysts (1990), except that the number of seeds per replicate run was increased from 25 to 100 to improve statistical rigour. A small pilot study was carried out before the main trials to determine statistical power and the number of replicates needed to detect significant responses to the environmental variables (Zar 1999). These trials indicated that four replicates, each using 100 seeds, yielded a power of >0.99.

Seeds were surface sterilized by placing them in small sealed muslin bags and plunging the bags in 10 % W/V sodium hypochlorite solutions for 20 seconds and then rinsing them three times in distilled water. For each viability-trial replicate, 100 seeds were evenly spaced in a grid pattern on a disc of Whatmans #3 filter paper (Whatman laboratory Division, Maidstone, Kent, England) in a 9-cm diameter petri dish. Each paper disc was wetted with 8 mL of distilled water and the dish sealed with laboratory film to reduce moisture loss. A total of four replicates (i.e., 400 seeds) was used for each viability test, which were undertaken in growth cabinets with daytime temperatures of 20^{o}C and night temperatures of 10^{o}C. A 12:12 hour light:dark cycle was used. Light was provided by a bank of fluorescent tubes designed for hydroponic use, that emitted a PAR of 40 μmol m^{-2} s^{-1} measured at the level of the seeds. All replicates were shuffled daily within the cabinet to randomise placement effects. Germination was measured after 7, 14 and 21 days. The trial was terminated at 21 days because no additional germination was recorded after Day 14. Germination was judged by the emergence of the base of the hypocotyl from the testa.

The weight of individual seeds was determined by counting the number of seeds in a 1 mg subsample (Association of Official Seed Analysts 1990). The average number of seeds was calculated from the mean of the aggregated totals of five subsamples.

5.2.3 Interactive effects of salinity, light and temperature on germination

Seed was sorted to ensure potential "germinability" by removing any obviously unfilled seeds (i.e., those not containing embryos). Unfilled seeds were identified and discarded on the basis of their noticeably paler colour and transparency when viewed

with back-lighting under a dissecting microscope. As with the seed viability trials, seed was surface sterilized with 10 % sodium hypochlorite solution for 20 seconds, then rinsed three times in distilled water.

The set of environmental conditions used for laboratory trials was chosen to mimic the range of conditions occurring in the field. For example, water-column salinity at Dowd Morass varies from near fresh (< 0.2 g L^{-1}) to over half seawater (19 g L^{-1}), so the suite of laboratory salinities not only covered this range but extended to a full seawater treatment. Long-term climate records show the mean daily air temperature at the nearby Sale East military base to range from a daily minimum of 3^{o}C in July to a daily maximum of 25^{o}C in January and February (Bureau of Meteorology 1988). The following conditions were used to assess prime effects and interactions among the three environmental variables of salinity, light and temperature: i) salinity (0, 1, 2, 4, 8, 16 or 32 g L^{-1}, made up with a commercially available sea-salt mixture (Red Sea Heidelberg Aquarium Supplies, Melbourne, Victoria); ii) darkness (constant darkness versus a 12:12 hr light:dark cycle at a PAR of 40 μmol m^{-2} s^{-1}); and iii) temperature (constant 10^{o}C, 20^{o}C or 30^{o}C).

Four replicate petri dishes were used per light/temperature/salinity treatment, with 100 seeds per petri dish. As in the seed viability trials, seeds were arranged in a grid pattern on a Whatmans #3 filter paper disc and covered with 8 mL of water at the appropriate salinity for each trial. In total, 168 petri dishes were used and 16,800 seeds tested.

5.2.4 Effects of preliminary exposure to salt on germination

Approximately 16 mg of sterilised seed, representing about 100 potentially germinable seeds, were placed in 50 mL containers filled with various concentrations (0, 1, 2, 4, 8 or 16 g L^{-1}) of reconstituted sea water as described above and soaked in the dark at 20^{o}C for a range of time periods (1, 2, 4, 8 or 16 days). At the end of each period of preliminary saline exposure, seed in each container were washed three times with distilled water and sown with distilled water as per the viability tests described earlier. Germination was recorded on the day seeds were transferred to distilled water and for each subsequent day until no further germination occurred.

5.2.5 Effects of seed burial and substrate type on germination

Approximately 16 mg of seed were sown in 15-cm diameter pots filled with milled peat moss (Nature Land Brand, TAS Seaweed Pty. Ltd, Devonport, Tasmania). Pots were filled with substrate to within 1 cm of the top of the pot and gently tapped down to even the surface. All pots were thoroughly wetted and then stood in 6 cm of distilled water in trays and placed in a lightly shaded glasshouse at the Trust for Nature (Cottlesbridge, Victoria). Seed was scattered evenly over the surface and then either left uncovered or covered with 1, 3 or 6 mm of just-damp peat moss passed through a 1-mm mesh-size sieve. All pots were lightly misted with a spray bottle and plastic sheeting placed over the top of the pots to minimise evaporation. Pots were checked daily for germination for 21 days, germination being determined by the emergence of cotyledons. Six replicate pots for each burial treatment were used.

To check for the effect of different substrate type on germination, additional pots were established using three different substrates: i) clay (taken from a freshwater swamp containing *M. ericifolia* at Cades Road, Whittlesea, Victoria); ii) washed river sand; and iii) peat moss. The protocol followed that given above, except that only surface-sown seed was used.

5.2.6 Statistical analysis

Data were analysed with Analysis of Variance (ANOVA) with the SPSS (version 12; http://www.spss.com/, verified September 2006) and Systat (version 5; http://www.systat.com/, verified September 2006) computer packages. Percentage data were arc-sine transformed before analysis. One-way and three-way fully orthogonal ANOVA designs were used for analysis. Since all factors considered as "fixed", treatment effects were calculated with reference to the MS residual (error) term (Zar 1999). Post-hoc tests used Bonferonni-corrected probability values.

5.3 Results

5.3.1 Viability

The seed viability of *M. ericifolia* was 6 %. Each milligram of seed contained about 560 seeds, indicating that the average seed weight was 0.0018 mg.

5.3.2 Interactive effects of salinity, light and temperature on germination

Individually, light, salinity and temperature all exerted highly significant ($P < 0.001$) effects on germination (Table 5.1). Since all interaction factors were highly significant ($P<0.001$), it is impossible to generalise about individual main effects without reference to the qualifying effects of other main effects. Nevertheless, some trends can be detected. For example, at 30^{o}C there was a consistent and rapid decrease in percentage germination with increasing salinity (Figure 5.1). No germination was observed at a salinity of 16 g L^{-1}, and only about 5 % of seeds germinated at 8 g L^{-1} at this temperature. In comparison, nearly 50 % germination was observed for seeds at 30^{o}C in fresh water. Seeds at 30 ^{o}C had consistently higher germination at all salinities in constant darkness than they did in a 12:12 hour light:dark cycle.

At 20^{o}C, seeds showed maximum germination success (40-50 %, depending on light conditions) at a salinity of 1 g L^{-1} and germination fell at a roughly constant rate with increasing salinities. Although about 10 % still germinated at a salt concentration of 16 g L^{-1}, no seeds germinated at a salinity of 32 g L^{-1}. Unlike the case at the highest

incubation temperature, seeds at 20^{o}C showed statistically significantly higher percentage germination in the light:dark cycle than in complete darkness.

The percentage of seeds germinating at 10^{o}C was about one half of that at 20^{o}C at a given salinity. The maximum germination at 10^{o}C (about 20 %) was observed with seed kept in the light:dark treatment over the three lowest salinities (i.e., 0 to 2 g L^{-1}). Germination success fell regularly, but slowly, at higher salinities and, as before, no germination was observed at a salinity of 32 g L^{-1}. Nevertheless, about 5 % of seeds still germinated at 16 g L^{-1} at 10^{o}C, a result in strong contrast to that found for seeds at 30^{o}C. There was a very strong effect of light:dark on seeds incubated at 10^{o}C, with seeds in the dark treatment showing about one-half the germination success of those kept in alternating light:dark cycles.

Table 5.1 Results of three-way ANOVA of primary and interactive effects of salinity, light and temperature on the germination of *M. ericifolia* seeds.

Source	**SS**	**df**	**MS**	**F-ratio**	***P***
Main effects					
Temperature	0.424	2	0.212	658.658	< 0.001
Salinity	2.450	5	0.490	1521.19	< 0.001
Light regime	0.025	1	0.025	76.477	< 0.001
Interaction terms					
Temp*salinity	0.491	10	0.049	152.520	< 0.001
Temp*light	0.049	2	0.024	75.543	< 0.001
Salinity*light	0.021	5	0.004	12.886	< 0.001
Temperature*salinity *light	0.082	10	0.008	25.317	< 0.001
Residual (error)	0.035	108	0.00032		

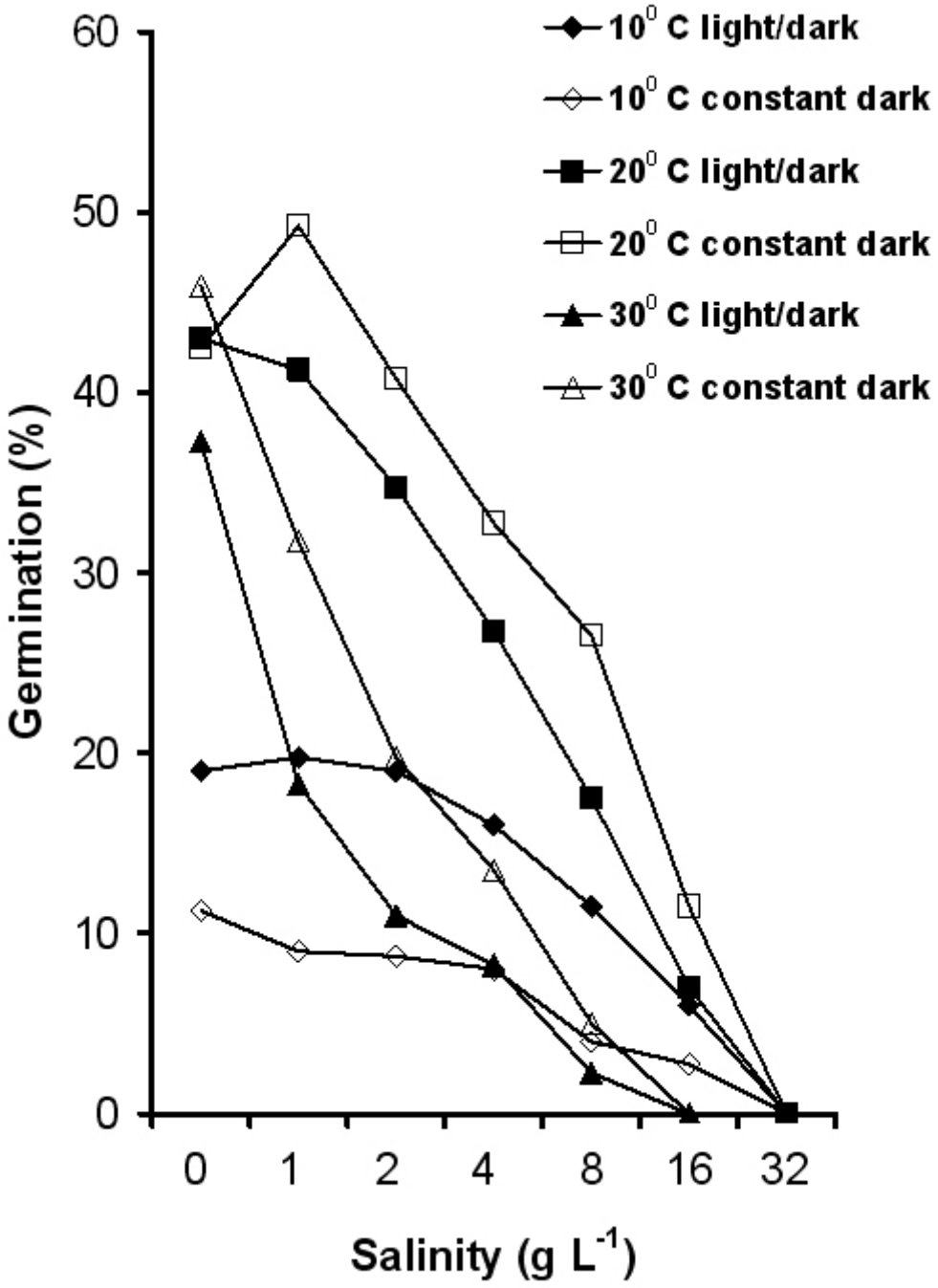


Figure 5.1 Effects of temperature, salinity and light regime on the germination of *M. ericifolia* seeds. Means are shown (n = 4): standard errors are smaller than the symbols used.

5.3.3 Effects of preliminary exposure to salt on germination

Figure 5.2 shows the effects of preliminary exposure to salt for periods of up to 16 days, followed by exposure to freshwater conditions, on germination success across six initial salinities, starting with freshwater conditions (Figure 5.2 a) and finishing with soaking in a saline solution of 16 g L^{-1} (Figure 5.2 f). Four main results are evident from this experiment.

First, seeds could germinate in highly saline solutions, a result confirming the observations in the previous experiment where seed was exposed to the saline solution from the beginning of the experiment and not subsequently exposed to freshwater conditions (Figure 5.1). In all treatments, seeds started to germinate even when they were soaking in the saline treatment solution, and germination continued after they were washed, transferred to paper filter discs and exposed to freshwater conditions. For example, 41 % of seeds had germinated in the saline treatment solution after 8 days of exposure to 4 g L^{-1}. Transfer of the seeds to freshwater conditions on filter discs resulted in an additional ~20 % of seeds germinating within 1 day, and eventually 80 % of seeds germinated despite their earlier exposure to salt at 4 g L^{-1} for over one week (Figure 5.2 d). Similarly, 17 % of seeds had germinated in the 8 g L^{-1} treatment solution after 8 days, and germination increased after transfer to freshwater conditions such that nearly 75 % of seeds had germinated the end of the experiment (Figure 5.2 e). Even seeds exposed to the highest salt concentration (16 g L^{-1}) showed some germination in the treatment solution (5 %) and ultimately about 58 % of seeds germinated after transfer to freshwater conditions despite this severe earlier salt exposure.

Second, and a corollary of the first conclusion, seeds continued to germinate in subsequent freshwater conditions even after lengthy earlier exposure to the highest salinity. Nearly 15 % of seeds had germinated in the 16 g L^{-1} treatment solution after 16 days, and this rate increased after their transfer to freshwater conditions such that nearly 50 % of seeds had germinated after 6 days in fresh water (Figure 5.2 f).

Third, despite the ability of seeds to germinate in saline solutions and increase their germination rate after transfer to freshwater conditions, salt exerted a strong inhibitory effect on the ultimate rate of germination. All seeds germinated in freshwater conditions and at a salinity of 1 g L^{-1} (Figure 5.2 a, b); this progressively fell to about 95 % germination at 2 g L^{-1} (Figure 5.2 c), 80 % at both 4 g L^{-1} and 8 g L^{-1} (Figure 5.2 d, e), and 50-75 % at 16 g L^{-1} (Figure 5.2 f).

Fourth, there was some evidence that soaking seeds in freshwater increased the germination speed (Figure 5.2 a). Seeds soaked in fresh waters for 1 day prior to transfer to filter papers showed slower germination (initiated on Day 3) than did seeds soaked for 2 days (initiated on Day 2). However, overall germination rates under both treatments on Day 3 were similar.

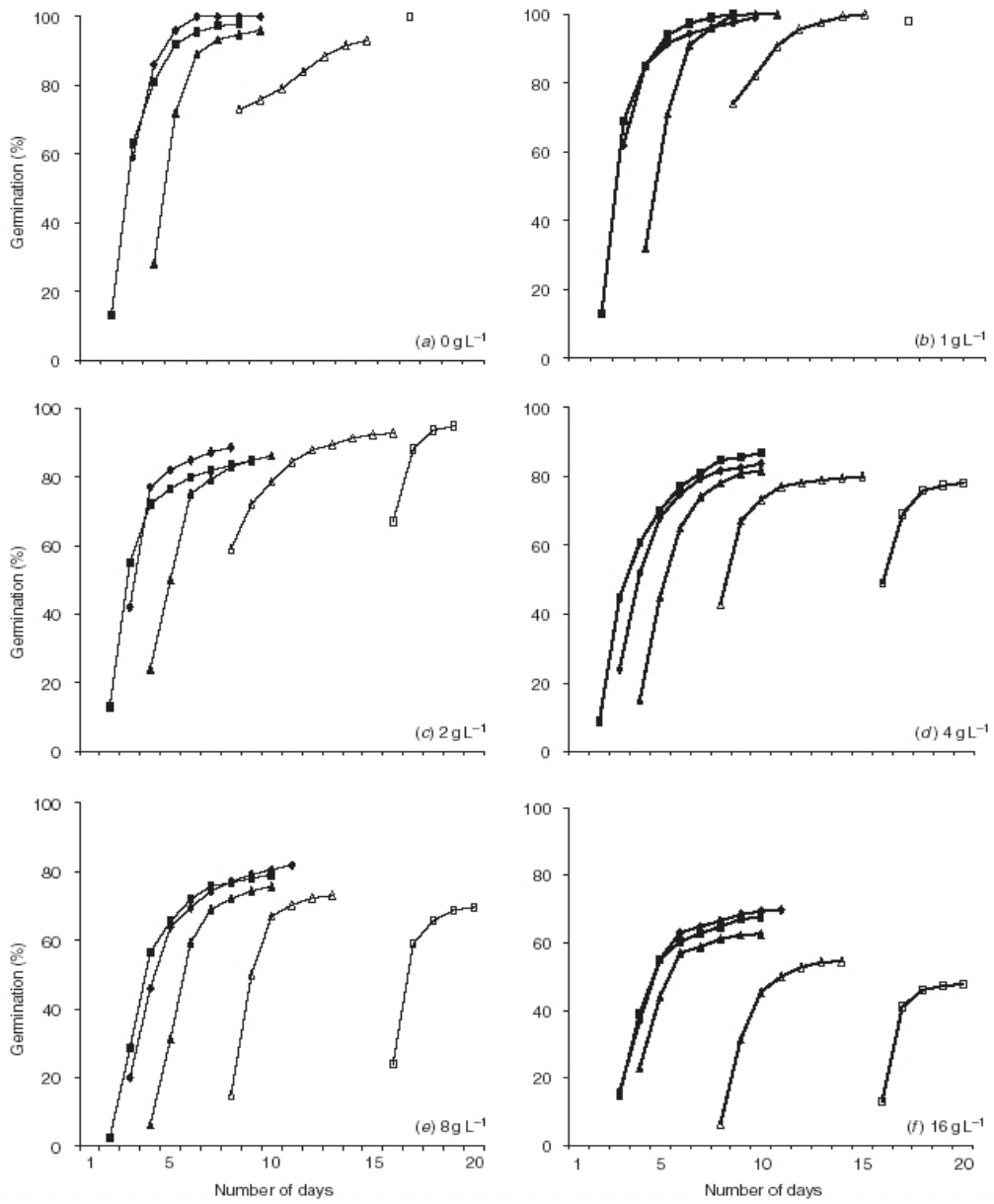


Figure 5.2 Effects of prior exposure to saline conditions for up to 16 days, followed by exposure to freshwater conditions, on the germination of *M. ericifolia* seeds. The six initial salinities used for prior exposure were: a) fresh water (0 g L^{-1}); b) 1 g L^{-1}; c) 2 g L^{-1}; d) 4 g L^{-1}; e) 8 g L^{-1}; and f) 16 g L^{-1}. The six curves shown in each graph a) to f) indicate germination after seeds had been transferred from the saline treatments to fresh water. Soaking periods are represented by (♦) = 1 day, (■) = 2 days, (▲) = 4 days, (△) = 8 days, (□) = 16 days.

5.3.4 Effects of seed burial and substrate type

There was a strong effect of deep burial in soil on the germination of *M. ericifolia* seeds (data not shown). Seeds failed to germinate at all if buried to 6 mm, and germination was decreased by nearly two orders of magnitude compared with surface-sown condition if seeds were buried to 3 mm with peat moss (1.6 % germination). In contrast, there was no significant difference ($P > 0.05$) in germination between surface-sown seeds (97 % $\pm$ 10 %) and those buried by 1 mm of peat moss (83 % $\pm$ 9 %).

Although burial had a highly significant effect on germination, substrate-type had no effect ($P>0.05$) on germination success. Regardless of soil type used for the trials, the percentage germination rate was about 95 % in each of clay, sand and peat moss.

5.4 Discussion

The results obtained in these experiments have major implications for understanding historical patterns in the natural recruitment of *M. ericifolia* into coastal wetlands and for attempts to rehabilitate these environments by manipulating their salt and water regimes. The key findings are that *M. ericifolia* shows low seed viability and that germination is affected strongly by salinity. Salinity effects, however, are moderated in a complex set of interactions with light and temperature. Seeds can germinate when exposed to high salt concentrations, even if there is a very marked depression in the ultimate germination rate of seeds exposed to a salinity of about one-half seawater. The inhibitory effects of short-term exposure to saline water, however, can be overcome by subsequent exposure to fresh water. Finally, it seems that germination is little affected by the type of sediment that the seeds finds themselves deposited onto, but is influenced greatly by burial by even a few millimetres of soil.

5.4.1 Poor seed viability and its causes

The viability of seed from the Dowd Morass population of *M. ericifolia* was low, at only 6 %. Low viability has been found in other populations of this species and other species of *Melaleuca*. Ladiges *et al*. (1981), for example, reported viability rates of 23 % to 28 % (of potentially germinable seed) for various populations of *M. ericifolia* across the eastern part of its range in Victoria. Meskimen (1962) found rates of between 3 % and 28 % viability for *Melaleuca quinquenervia* in northern NSW and southern Queensland. Recent viability testing of *M. ericifolia* (R. Robinson unpublished data) indicated total sterility for two isolated populations in western

Victoria, and between 0.25 % and 32 % viability for a range of populations throughout central and eastern Victoria.

Limited genetic diversity of the parent populations is widely recognised as a major contributing factor to low viability (e.g., Young *et al*. 1999; Cunningham 2000; Tretyakova and Bazhina 2000; Cochrane *et al*. 2001). Environmental factors, particularly high salinity, may interfere with effective seed set and significantly reduce viability (Redondo *et al*. 2004; Boscaiu *et al*. 2005). Human-induced alteration of environmental variables or management of populations, particularly via altered water and salinity regimes, changed vegetation structure, soil degradation and habitat fragmentation, have marked impacts on seed set and viability (Kingsford 2000; Tretyakova and Bazhina 2000; Renison *et al*. 2004). Major changes to environmental conditions at Dowd Morass over the past quarter of a century, especially altered water regime, increased salinity and increased nutrient loadings (Grayson 2003), are likely to have had considerable impacts on the overall health of the swamp paperbark populations, potentially limiting flowering ability and seed set.

The strongly clonal growth form of *M. ericifolia* may induce an inverse trade-off effect on seed set and viability. This response has been observed in other species (e.g., *Mimulus*: Sutherland and Vickery 1988). The extensive clonally-derived population of *M. ericifolia* presently found at Dowd Morass may have established from a much smaller founder population, which developed under vastly different salinity conditions, possibly even a largely freshwater environment (Grayson 2003). There is a strong indication that the present population is many times larger than the original population found in the area in 1957 (aerial photographs Run 14-1258 photos

85-91 and Run 15-1258 photos 98-104 (Department of Primary Industries, Government of Victoria, Aerial Photograph Library, Werribee). Recent rapid population expansion, coupled with low viability rates, is suggestive of limited genetic diversity in the founding population. It seems likely that a combination of the above factors has had, and continues to have, a limiting influence on seed set and viability on the Dowd Morass population of *M. ericifolia*.

5.4.2 Effects of chronic and acute exposure to salt

Periodic saltwater intrusions of various durations and flushing by fresh water are well documented in the Gippsland Lakes (Grayson 2003) and the resultant fluctuations in salinity are likely to have significant impacts on vegetation dynamics and recruitment in the fringing wetlands. There is some evidence that *M. ericifolia* seedlings are tolerant of high salinity (e.g., Ladiges *et al*. 1981; J. Salter et al. 2006). Furthermore, Ladiges *et al*. (1981) indicated that seed soaked at salinity of 20 g L^{-1} for 28 days recovered when placed in fresh water. Nevertheless, intrusions of water of a lower salinity and for shorter durations than those investigated by Ladiges *et al*. (1981) are frequent in the Gippsland Lakes (Grayson 2003) and are likely to have a direct impact on the seed bank and germination. The various salinity concentrations and durations of inundation used in this study (0 - 32 g L^{-1} and 1 - 16 days: Figures 5.1 and 5.2) show that there is indeed a direct effect on the overall germination of *M. ericifolia* seed at salinity levels and for durations well below those investigated by Ladiges *et al*. (1981).

The toxic effect of salinity on *M. ericifolia* seed reported in this study clearly indicates that the seed is largely intolerant of high levels of salinity even over relatively short durations. Ladiges *et al*. (1981) reported variation in salinity tolerance of individual provenances of *M. ericifolia*, and similar responses are known to occur in a wide range of species at provenance and individual-plant level in brackish wetland taxa (Marcar *et al*. 2003). However, the general intolerance of salinity by *M. ericifolia*, at least in the initial stages of germination, would indicate that sexual reproduction in this species has not evolved the same level of salt tolerance as shown by established adult plants of the same species. Indeed, many halophytes exhibit germination patterns restricted to periods of decreased salinity in the water column or substrate (Churchill 1983; Krauss *et al*. 1998; Barrett-Lennard 2003).

The ability of germinable seed to recover from saltwater intrusions by subsequent flushing with fresh water indicates a preference for low salinity environments for the successful germination of *M. ericifolia* seed. Regular freshwater flushing of the wetlands of the Gippsland Lakes area, caused by flooding from the lowland river systems that feed the lakes complex, is likely to be less frequent than was the case in the past (Grayson 2003). Such flooding would moderate the salinity regime in the wetlands to what would be considered ostensibly fresh or near fresh conditions of < 2g L^{-1}. Periodic drying of the wetlands and lowering of the water table in a drought year, or as part of the natural wetting and drying cycle, followed by moderate rainfall may also create minor flushing of the surface sediments, in particular of the hummocks that are a frequent feature of the wetlands surrounding the Gippsland Lakes. This in turn may provide the ideal germination conditions through the creation of a limited number of 'safe sites', which allow germination to occur in what would

otherwise be a hostile germination environment. Conversely, however, water-level drawdown could well result in markedly increased salinities over much of the remainder of the wetland, due to evaporative processes, and in these areas germination would be precluded.

5.4.3 Effects of environmental variables on germination

The successful recruitment of *M. ericifolia*, like other salt-tolerant species, is directly related to the ability of the seeds to respond to key environmental cues, particularly light and temperatures coupled with salinity levels (Ladiges *et al*. 1981; Khan and Ungar 2001). Progressively lower germination rates at increasing salinity supports suggestions made by Ladiges *et al*. (1981) and Clarke and Hannon (1970) that osmotic factors are critical in the inhibition of germination. The most direct osmotic interference is through reduction in the osmotic potential of the aqueous environment, reducing water availability and water absorption by the seeds (Khan and Ungar 2001). The likely role of osmotic factors in germination is further supported by the finding that there was a rapid decrease of germination at higher temperatures and in the light: both light and higher temperatures increase osmotic stress on seed and reduce the ability of seed to imbibe water (Khan and Ungar 1984) and may have a direct toxic effect on the embryo (Zekri 1993).

A wide range of halophytes shows an inhibition of germination through the interaction of light, temperature and salinity, including grasses (Khan and Gulzar 2003), chenopods (Gul and Weber 1999; Khan and Unger 2001) and even the ostensibly marine seagrass *Zostera marina* (Churchill 1983). Although the interaction of these factors on woody plants is poorly studied, the tolerance of several woody

plants to various levels of salinity alone is well documented (Zekri 1993; Barrett-Lennard 2003; Krauss *et al*. 1998; Marcar *et al*. 2003). The interaction of light, temperature and salinity, and their inhibitory effect on germination, ensures that species germinate under conditions that are optimal for recruitment. Many halophytes, including *M. ericifolia*, show a higher tolerance of salinity and temperatures as adult plants than they do as juveniles (Rozema 1995; Ladiges *et al*. 1981; J. Salter unpublished data).

5.4.4 Effects of seed burial and substrate type

The clear preference for germination on or just under the surface confirms germination-niche studies carried out by de Jong (2000) and Nicol and Ganf (2000). That germination rates are strongly related to depth of burial has been reported in a range of plants (McIntyre *et al*. 1995; Nicol and Ganf 2000); surface or shallow burial is often a requirement for successful germination of small seeds (Rotundo and Aguiar 2004; Eckstein and Donath 2005; Kostel-Hughes *et al*. 2005; Rotundo and Aguiar 2005). Seeds of *M. ericifolia* are extremely small (~0.0018 mg) and deep burial would probably overwhelm the seed's resources to raise cotyledons to the soil surface. It is interesting that deep burial has been shown to inhibit germination and reduce viability over time in the related paperbark species, *M. quinquenervia* (Van *et al*. 1998).

5.4.5 Implications for rehabilitation of coastal wetlands

Episodic recruitment of *M. ericifolia* at Dowd Morass and at other wetlands along the eastern and southern coasts of Australia is likely to be directly related to the spatial and temporal prevalence of suboptimal germination conditions and corresponding lack of 'safe sites'. Periodic flooding with fresh water or large rainfall events flushing salt from salinised, but otherwise potential, germination sites may provide the conditions required for successful germination. This study has shown that *M. ericifolia* seeds are tolerant of salinity at the germination stage. Optimal germination however, occurs at 20^{o}C with a salinity of < 2 g L^{-1} and with higher overall germination in darkness. If the successful regeneration of *M. ericifolia* is to be achieved in brackish-water wetlands, the sowing of seed must coincide with periods and conditions of optimal germination potential. In south-eastern Australia, the optimal temperature regimes (~20^{0}C day and ~10-12^{0}C night) take place in autumn and spring (Bureau of Meteorology 2005). The effect of darkness on germination success indicates that the best germination sites are those protected from sunlight, such as the base of other wetland plants or substrates with sufficiently rough surfaces to allow for the lodgement of seed in soil pores near the surface.

The conditions for the successful germination of *M. ericifolia* are relatively narrow, with the ideal being recently shed seed sown on or very near the surface, germination temperatures around 20^{0}C, fresh or near freshwater conditions, and in dark, or at least well shaded, conditions. Re-establishment of this species, through the use of seed, would need specific manipulations of the environment or intervention at times of ideal

natural conditions to achieve success. Even then, the low overall viability of the seed would present difficulties to large-scale rehabilitation.

Chapter 6

Effects of environmental conditions on the production of hypocotyl hairs in seedlings of *Melaleuca ericifolia* (Swamp Paperbark) Sm.

Abstract

The production of hypocotyl hairs in the early stages of seedling development can strongly influence the success with which plants recruit sexually in harsh environments. Although wetlands are one type of environment in which seedlings might be expected to develop hypocotyl hairs, there have been few studies of these structures in the woody aquatic plants. We investigated the production of hypocotyl hairs in *Melaleuca ericifolia* Sm., a small wetland tree widely distributed across swampy coastal areas of south-eastern Australia, in relation to water availability, salinity, temperature and light regime. Hypocotyl hairs were about 20 mm long x 30 μm wide; in contrast, root hairs were generally less than 5 mm long and 15 μm wide. Hypocotyl hairs were produced only under a narrow range of environmental conditions – low salinity, low water availability, moderate temperature, and darkness – and seedlings that failed to produce hypocotyl hairs did not survive. Since the conditions under which hypocotyl hairs were produced were at least as, and possibly even more, restricted than those required for successful seed germination, it is likely that the successful sexual recruitment of *M. ericifolia* would be rare and episodic under conditions existing in most coastal wetlands in south-eastern Australia.

6.1 Introduction

Seedlings have many strategies for improving their survival, particularly in habitats that are hostile to the germination of seeds and the establishment of juvenile plants (Aronne and De Micco 2004; Nishihiro *et al*. 2004). Hypocotyl hairs, single cell outgrowths from the base of the hypocotyl not associated with the true root system of the plant, are one means by which many plants can increase seedling survival in difficult environments. Despite their likely importance in the sexual recruitment of a number of taxa of plants, hypocotyl hairs have been comparatively little studied; they are rarely reported in other than a cursory manner in the literature (Hofer 1992; Kuo and Kirkman 1992; Kuo 1993) or are not recognized at all as unique entities (Mora *et al*. 2001). Additional confusion arises from the different names given to these single-celled structures: coleorhiza (Baranov 1957), hypocotyl epidermal cells (Grierson and Schifelbein (2002), or simply hair-like cells or cellular outgrowths on the hypocotyl (Kuo and Kirkman 1992; Kuo 1993).

Hypocotyl hairs occur in widely divergent families of both monocotyledons and dicotyledons across a wide range of habitats. The main families of wetland plants that produce hypocotyl hairs are the Podostemaceae (Rutishauser *et al*. 1999), Zosteraceae (Churchill 1983; Kuo 1993), Alismataceae and Hydrocharitaceae (Kaul 1978; Matsuo and Shibayama 2002), Salicaceae (Polya 1961) and Myrtaceae (Baranov 1957). Only two genera, *Myrtus* (Myrtaceae) and *Artemisia* (Asteraceae), have been identified as possessing hypocotyl hairs in Mediterranean-type ecosystems, despite the general harshness of these environments (Aronne and De Micco 2004;

Young and Martens 1991). Hypocotyl hairs have been found in several grassland plant families, most notably Asteraceae and Caryophyllaceae (Morita *et al*. 1995).

There is some commonality in the function of hypocotyl hairs across these various families and habitats, although differences are apparent as well, usually in accord with variations in prevailing environmental conditions. The role of hypocotyl hairs in the submerged aquatic genera *Marathrum*, *Ottelia*, *Vallisneria*, *Vanroyenella* and *Zostera* is believed to be primarily physical, and include anchoring juvenile plants to the substratum and facilitating the development of geotropism (Rutishauser *et al*. 1999; Churchill 1983; Kaul 1978). Hypocotyl hairs in emergent aquatic or amphibious species of *Alisma*, *Callistemon*, *Echinodorus*, *Limnocharis* and *Lophotocarpus* are believed to serve a similar role to those in the submerged aquatic taxa, but also facilitate the uptake of water, thereby guarding against desiccation in the early stages of seedling development (Kaul 1978; Baranov 1957). In purely terrestrial species, such as *Artemisia* and *Myrtus*, hypocotyl hairs also have been shown to anchor seedlings to the substratum soon after germination and to assist in the development of geotropism (Aronne and De Micco 2004; Young and Martens 1991). In terrestrial species, however, hypocotyl hairs may have additional functions in protecting the seedling against desiccation and herbivory. The mucilage produced on the hypocotyl hairs of *Artemisia* and *Myrtus*, for example, may provide additional protection against desiccation (Aronne and De Micco 2004; Young and Martens 1991) and the accumulation of phenolics by hypocotyl hairs in *Myrtus*, as reported by Aronne and De Micco (2004), may deter herbivory.

What information is available indicates that hypocotyl hairs are produced in a similar developmental sequence across the few species that have been examined in detail (Aronne and De Micco 2004; Young and Martens 1991; Kaul 1978). They are not only produced before emergence of the radical, secondary or adventitious roots, or root hairs, but the production of hypocotyl hairs is a prerequisite to the formation of these other structures, as they provide the necessary anchorage for radicles to penetrate the substrata and for the seedling to establish clear anisotropic growth. Matsuo and Shibayama (2002), for example, reported that *Monochoria vaginalis* seedlings that failed to produce hypocotyl hairs also usually failed to establish roots and, if they did develop roots, they failed to penetrate the substrata and the seedlings died. These studies have concentrated largely on non-woody taxa, and the factors influencing the production of hypocotyl hairs in woody wetland species are very poorly understood. Indeed, studies on only three woody wetland genera, *Callistemon* (Baranov 1957) and *Populus* and *Salix* (Polya 1961), have been reported; both the Baranov (1957) and Polya (1961) studies were limited to either simple documentation or to an examination of the effects of rapid moisture uptake on hypocotyl hair formation.

Hypocotyl hairs have been identified recently, by the authors, in Swamp Paperbark (*Melaleuca ericifolia* Sm.: Myrtaceae), a small wetland tree commonly found in freshwater and brackish-water swamps in south-eastern Australia. *Melaleuca ericifolia* is the dominant woody plant in many coastal and near-coastal swamps, where it forms a vegetation community, commonly known as swamp scrub, which is critically important as nesting and roosting habitat for colonially breeding water birds such as ibis (Bird 1962; Corrick and Norman 1980; Cowling and Lowe

1981). European settlement has caused such major alterations to the extent of *M. ericifolia* communities and their wetland habitats that Bowkett and Kirkpatrick (2003) predicted only the largest and most ecologically intact populations in north-east Tasmania and the Bass Strait Islands would be likely to survive in the long-term. Population of *M. ericifolia* in Victoria are similar in size to the Tasmanian and Bass Strait Island populations, but possibly face an even wider and more intense range of disturbances, arising from the greater intensity of human settlement in mainland Australia. Many of the coastal wetlands that formerly accommodated *M. ericifolia* have been subject to severe hydrological modifications, having either been completely drained or (less frequently) or inundated almost permanently following the construction of levees and other structures (e.g., see East 1935 for an early report on the scale of reclamation of Swamp Paperbark wetlands). The paperbark-dominated wetlands that remain are often subject to secondary salinization, or to contamination with nutrients and other catchment-derived chemical, including pesticides and other toxicants.

Because of the widespread loss of paperbark-dominated wetlands and the roles that the remaining ones play in providing a range of vital ecosystem services, there is a critical need to understand the environmental factors that control sexual recruitment in *M. ericifolia* (Jeanes 1996; de Jong 1997). A number of studies have examined the effect of environmental conditions on germination of *M. ericifolia* seeds; these studies have shown that effective recruitment from seed is episodic, strongly controlled by inundation, salinity, temperature and light, and limited to particular habitats within wetland ecosystems having the right combination of environmental conditions (e.g., see Ladiges *et al*. 1981; Jeanes 1996; de Jong 2000; Robinson *et al*. 2006). The fate

of young seedlings and the factors that control the establishment of young plants are, by contrast, far less well understood. Given the critical role played by hypocotyl hairs in herbaceous taxa and in non-woody aquatic species such as seagrasses (Kuo 1993), it would appear likely that these structures have important functions also in woody wetland taxa such as *M. ericifolia*. Accordingly, the aims of this study were two-fold: a) to describe the effects of a range of environmental variables on the production of hypocotyl hairs in *M. ericifolia*; and b) to use this information to infer whether the environmental conditions that currently exist in coastal, brackish-water wetlands would facilitate or inhibit the development of hypocotyl hairs and thus would be likely to have an impact on the sexual recruitment of this important species of wetland plant.

6.2 Methods

6.2.1 Field site

Dowd Morass is a 1,500 ha brackish-water wetland on the south-western shore of Lake Wellington near Sale, Victoria, southern Australia ($38^0$07'S $147^0$10'E). Vegetation in Dowd Morass is primarily composed of large areas of *M. ericifolia*-dominated swamp scrub (~ 500 ha) and extensive beds of the Common Reed, *Phragmites australis* (Cav.) Trin. ex Streud (~ 350 ha); the remaining areas are either open water or expanses of bare mudflats, depending on water levels. Dowd Morass is an important and sizable component of the Gippsland Lakes Ramsar site and a regionally important site for the breeding of Sacred and Straw-necked Ibis (*Threskiornis aethiopica* and *T. spinicollis*). The swamp scrub communities in the

wetland provide these birds with their main roosting habitat (e.g., see Cowling and Lowe 1981) and a perceived degradation in the extent and condition of *M. ericifolia* is a major concern of the agency responsible for managing the wetland and the larger Gippsland Lakes Ramsar site (Parks Victoria 1997). Dowd Morass is similar to many of the brackish-water wetlands that fringe the Gippsland Lakes; a range of previous papers have described the hydrology, salinity and vegetation of these areas (Bird 1962; Ducker *et al*. 1977; Corrick and Norman 1980; Parks Victoria 1997; Roache *et al*. 2006; Robinson *et al*. 2006; Salter *et al*. 2007; Raulings *et al*. 2007).

The large size and environmental heterogeneity of the Dowd Morass site, complicated by variation at the microtopographic scale in soil moisture, salinity, pH, organic matter content and elevation, has resulted in the juxtaposition of highly contrasting environmental conditions, sometimes within centimetres of each other. For example, large areas of the morass are permanently inundated whereas other areas, especially around the perimeter, experience alternating wet and dry periods; salinities vary from near freshwater (< 1-2 g L^{-1}) to over one-half seawater (i.e., > 16 g L^{-1}) according to inundation history and the periodicity of saline intrusions from Lake Wellington; light intensities vary widely according to canopy density and crown cover. The composition of potential seedbeds for *M. ericifolia* seeds varies from fine, tight clays, which seeds cannot penetrate, to highly porous hummocks of organic matter in which seeds may lodge deeply.

6.2.2 Life history of *M. ericifolia*

Melaleuca ericifolia Sm. is a small tree in the family Myrtaceae. It occurs, often as the dominant woody species, widely in coastal and near-coastal freshwater and brackish-water wetlands across south-eastern Australia. Most species of the genus *Melaleuca* are reliant on seed alone for reproduction, but *M. ericifolia* can also form extensive clonal stands through the production of ramets that can become physically independent of the parent genet once sexual recruitment has taken place (Bird 1962; Ladiges *et al*. 1981).

Seeds of *M. ericifolia* are held on the plant in hard woody capsules for many years (serotiny) and usually are released on the death of the attached stem. Seed, once released from the capsule, germinates within a few days if conditions are suitable (Robinson *et al*. 2006). There seem to be no particular triggers (e.g., dormancy requirements) for germination, but the conditions that maximise germination success are fairly specific: a temperature around 20^{o}C, salinity of 2 g L^{-1} or less, and darkness (Robinson *et al*. 2006). Germination is inhibited by inundation, but once seed has germinated seedling are able to survive in water for several weeks by floating on the surface (Ladiges *et al*. 1981). Hypocotyls emerge from the seed first, usually within 3-5 days, followed a few days later by the cotyledons. The first true leaves emerge after approximately 14 days under ideal conditions. Root hairs and secondary roots normally emerge after the production of hypocotyl hairs and production of the first true leaves, but may emerge earlier if hypocotyl hairs are not produced.

6.2.3 Seed collection

Seed capsules were collected in April 2004 from 25 genetically distinct plants in a large population of *M. ericifolia* at Dowd Morass. Seeds were collected from several generations of capsules, ranging from 1 to 5 years of age. Capsules were stored in paper bags at 20^0C for one week. The bags were lightly shaken to release seed from the capsules, and the contents of the bag sieved (mesh size: 1mm) to remove the empty capsules and other detritus. The cleaned seed was placed in paper bags for a further three days to remove any excess moisture, then transferred to sealed glass containers and stored at 20^0C in darkness until used. Experiments began in February 2005.

6.2.4 Effects of surface sterilisation

Surface-sterilised seeds were soaked in 1 % w/v sodium hypochlorite solution for set times (0.5, 1, 2, 5, 10 or 30 min) then rinsed three times in sterile de-ionized water. Excess fluid was removed between each step by pipetting seeds onto Whatmans #1 filter paper under a gentle vacuum. From each treatment, 100 seeds were plated onto 0.8 % w/v water agar in four replicate Petri dishes (i.e., 25 seeds per Petri dish). Control treatments were subject to the same procedure but soaked in sterile de-ionized water only, for either 5 or 30 minutes.

Seeds were incubated with a 20^0C:10^0C 16 hr:8 hr day:night cycle and shuffled periodically to randomise the possible impact of minor within-cabinet variations in environmental conditions. Following incubation, seedlings were

classified into three development classes: i) those with fully developed hypocotyl hairs; ii) hypocotyl hairs showing partial or impaired development; and iii) complete absence of hypocotyl hairs. Hypocotyl hairs were classified as being only partially developed or having impaired development if they failed to elongate or if only few of the hypocotyl hair cells elongated. Plates were observed for a total of 60 days.

6.2.5 Effects of water availability and flooding

To determine the effects of relative water availability on the production of hypocotyl hairs, 25 surface-sterilised seeds were placed onto each of four replicate Petri dishes (i.e., 100 seeds per treatment) containing bacteriological-quality agar at concentrations of 0.2 %, 0.5 %, 1 % or 10 % w/v. The Petri dishes were covered with laboratory film and incubated as described above, and the development of hypocotyl hairs was recorded for 21 days although Petri dishes were observed for 60 days. As before, seedlings were classified into one of three development classes: fully developed; partial or impaired development and absence of hypocotyl hairs. The development of hypocotyl hairs was recorded also in relation to seedling development; rates of germination, growth and geotropism (defined as radicle touching the agar and the apical hypocotyl becoming vertical). To determine whether seedlings that had not developed hypocotyl hairs could do so if they were exposed to conditions of lower water availability, some seedlings were transferred after 14 days of incubation from the 0.2 % to the 10 % w/v agar concentration.

Compound microscopy was used to document the zone of production of hypocotyl hairs. Seedlings were taken from the 0.2 % and 10 % water-agar dishes

each day for 7 days, beginning one week after germination. Seedlings were cut from agar and fixed in 2.5 % w/v glutaraldehyde (in pH 7.4 phosphate buffer), cleared in 10 % w/v KOH at 90^0 C for 3 hours and then stored in 70 % w/v ethanol until the last samples were processed. Five seedlings were sampled from each treatment on each day. Seedlings were then embedded in paraffin wax. Samples were arranged so that longitudinal sections could be obtained. Sections were cut to 8 μm thickness and stained with Mallory's Triple Stain. Stained sections were photographed using a Zeiss compound microscope with a Nikon Coolpix 4500 digital camera. Magnifications were 16X, 25 X, 32 X and 45 X depending on parts to be illustrated (scale is included on photographs (Fig. 2 a-d) to overcome alterations to magnification upon printing).

To determine the effects of flooding, surface-sterilized seed was soaked in sterilised distilled water for 2, 4, 8, 16, 32, 64, 128 or 256 hrs. From each treatment, 100 seeds were placed onto Whatmans #3 filter paper discs in four replicate Petri dishes (i.e., 25 seeds per Petri dish) and covered with 8 mL of de-ionized water. Seeds were incubated at 20^0C under constant darkness; these conditions were chosen because earlier trials had shown that hypocotyl hairs developed readily under this temperature-light-salinity regime.

6.2.6 Effects of temperature, light and salinity

A factorial design, using a total of 36 treatments, was used to quantify the prime and interactive effects of temperature, light and salinity on the development of hypocotyl hairs. Because the seedlings developed also root hairs and secondary roots, this

experiment allowed us to examine also the chronology of hypocotyl hair development in relation to the development of these other organs. The general protocol using seeds germinated on filter discs (i.e., that used previously to examine the effects of flooding on hypocotyl hair development) was re-used for these trials, except that two contrasting light regimes, three temperatures and six salinities were used in a fully factorial experimental design. Seeds were incubated under all the possible combinations of these different sets of environmental variables, in order to differentiate between prime effects and interactions among individual variables.

Surface-sterilised seed were incubated on Whatmans #3 filter paper discs in Petri dishes (as before, 100 seeds per treatment, four Petri dishes and 25 seeds per dish), covered with 8 mL of solution with the appropriate salinity. The light treatment consisted of incubation under a) constant darkness or b) 12hr:12hr light:dark cycle, with a light intensity of 40 μmol $m^{-2}s^{-1}$ at the level of the dishes. Incubation at a constant 10^{o}C, 20^{o}C or 30^{o}C comprised the three temperature treatments. The six salinities used to assess salinity effects spanned the range of surface-water salinities reported for Dowd Morass over the past ~ 5 years; salinities used were 0, 1, 2, 4, 8 or 16 g L^{-1}. Saline solutions were made up with a commercially available sea-salt mixture (Red Sea brand, Heidelberg Aquarium Supplies).

Following the random distribution of seeds among the various treatments and periodic shuffling of Petri dishes to randomise any within-cabinet variations in environmental conditions, seedlings were checked for development of hypocotyl hairs, root hairs and secondary roots every seven days. Seedlings were assigned to one of the three development classes of hypocotyl hairs outlined previously, as well as

two development classes (i.e., present or absent) for root hairs and secondary roots. The width and length of hypocotyl hairs were measured 6 hypocotyl hairs from five randomly selected seedlings from each salinity treatment. These seedlings were photographed using a Moticam 2000 2.0 pixel Digital Camera mounted on a Zeiss compound microscope set at 40 X magnification and processed using a Motic Images image-processing program. A representative seedling from each of the treatments is presented in Figure 6.

During the course of the above studies casual observations were made regarding the developmental progression of hypocotyl hairs and other aspects of the post-germination processes of *M. ericifolia* seedlings. It was beyond the scope of this paper to deal directly with the physiological aspects of hypocotyl hair development.

6.2.7 Data analysis

Data were analysed with Analysis of Variance (ANOVA) with the SPSS (version 12) and Systat (version 11.5) computer packages. Where appropriate, percentage data were arc-sine transformed before analysis. One-way and three-way orthogonal ANOVA designs were used for analysis (Zar 1999). Post-hoc tests used Bonferonni-corrected probability values. Estimates of variance were calculated using Microsoft Office Excel (2003).

6.3 Results

6.3.1 Effects of surface sterilisation

Between 45 % and 65 % of seeds that were surface sterilized for less than 2 minutes germinated. Soaking for periods longer than 2 minutes, however, markedly decreased germination rates, regardless of whether seeds were soaked in sodium hypochlorite or de-ionized water (Fig. 1). There was little difference in germination rates between seeds that were surface sterilized and those treated with de-ionized water for 5 minutes, but surface sterilization for 30 minutes reduced germination rates to less that 10 %. Hypocotyl hairs were produced by those seeds that had been surface-sterilized for 2 minutes, and about 30 % of these seedlings possessed hypocotyl hairs after 21 days of incubation with no additional hairs being produced after this time. Hypocotyl hairs were not produced from seeds that had been surface-sterilized for 5 or 30 minutes, but were produced from a small proportion of seeds (< 10 %) that were soaked in de-ionized water for these periods.

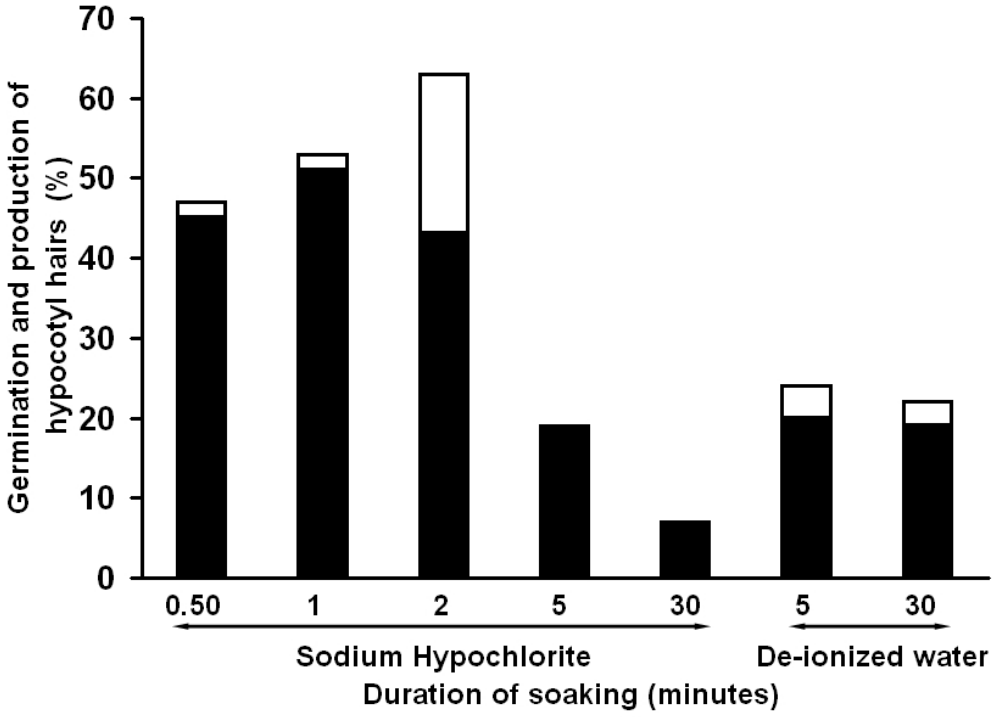


Figure 6.1 Percentage germination of *M. ericifolia* seeds and percentage of seedlings that produced hypocotyl hairs after soaking seeds with sodium hypochlorite (0.5 to 30 minutes) and de-ionized water (5 or 30 minutes). The height of each bar indicates the mean percentage germination and the black portion indicates the mean percentage of seedlings that showed the presence of hypocotyl hairs.

6.3.2 Origin and characteristics of hypocotyl hairs

The seedlings produced in the surface-sterilisation treatment were examined with microscopy for 60 days. Seedlings without developed hypocotyl hairs showed enlarged hypocotyl cell cells in the region where hypocotyl hairs would normally emerge (Fig. 2 a, b, c). The distinct zone where hypocotyl hairs develop is clearly distinguishable from the radicle and the rest of the hypocotyl, and forms an enlarged

area at the base of the hypocotyl above the join with the radicle (Fig. 2b). No other epidermal cells on the hypocotyl were observed to produce hairs. Hypocotyl hair cells, while present on the embryo pre-germination, did not elongate until germination took place. Elongation was synchronous and apparent within two to three days of sowing. The single-cell nature of the hypocotyl hairs was evident from the stained paraffin sections viewed under high-powered microscopy (Fig. 2 c, d). Fully formed hypocotyl hairs in *M. ericifolia* were about 20 mm long x 30 μm wide. By contrast, root hairs were generally less than 5 mm long and 15 μm wide.

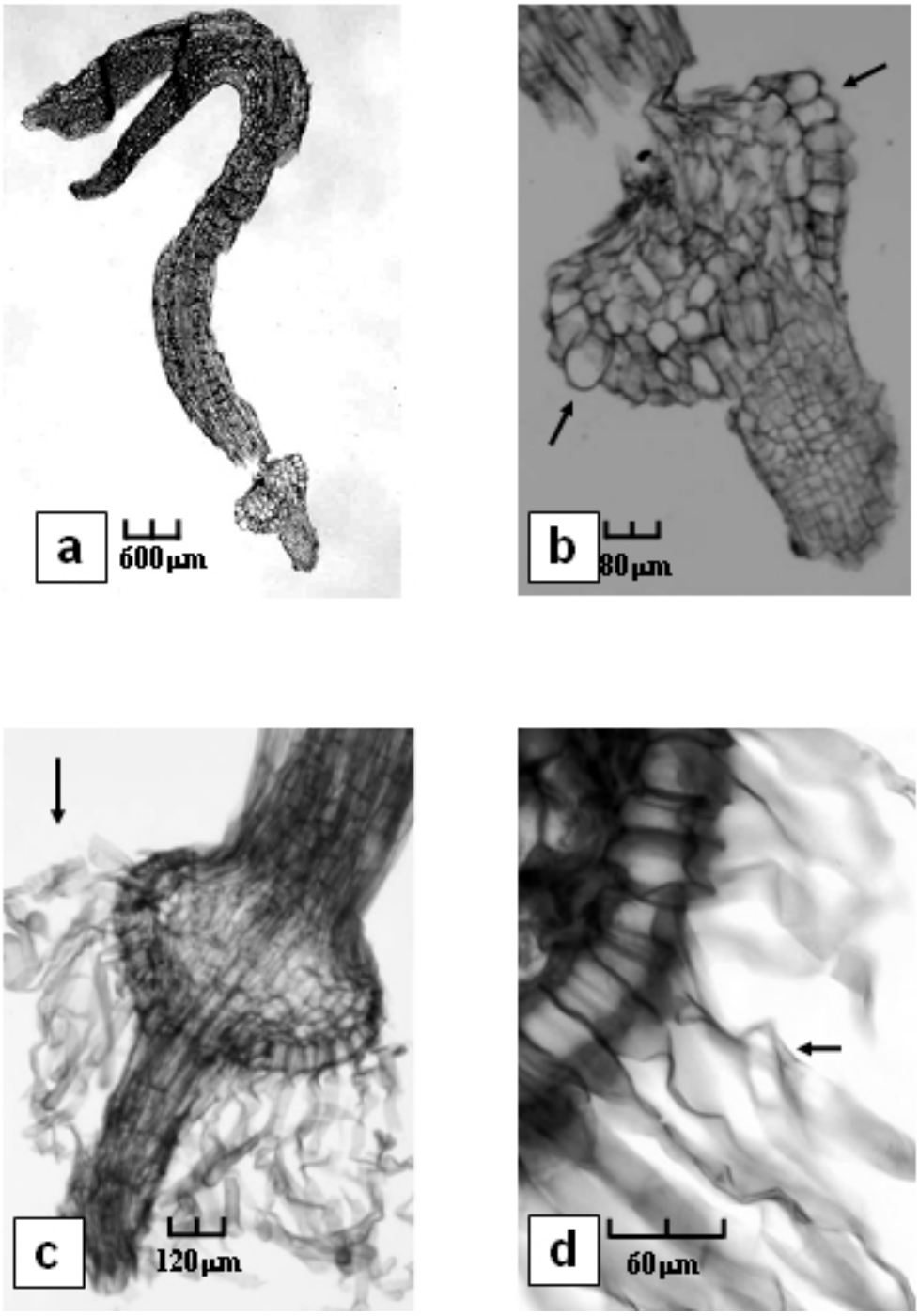


Figure 6.2 Microscopy images of *M. ericifolia* seedling without (a, b) and with (c, d) hypocotyl hairs. Unexpanded hypocotyl hair cells (image b) and hypocotyl hairs (image d) are evident as enlarged or elongated cells surrounding the base of hypocotyls. Unexpanded hypocotyl hair cells and formed hypocotyl hairs are marked with arrows.

6.3.3 Hypocotyl hairs and seedling development

A large proportion of seedlings (65 %) emerged from the seed coat with hypocotyl hairs that were impaired to well-developed. In other seedlings, however, hypocotyl hair growth was delayed by several days. Radicle elongation began shortly after germination in seedlings without hypocotyl hairs, and occurred sooner and more rapidly than in those with hypocotyl hairs. Those seedlings with hypocotyl hairs were more likely to produce root hairs on the radicle than those without. After 6-8 weeks the hypocotyl hairs began to change in appearance, becoming wrinkled and had most likely ceased to function. In general this event coincided with establishment of root hairs.

6.3.4 Effects of water availability and flooding

A range of experiments showed that the relative dryness of the environment had a strong effect on the development of hypocotyl hairs. Variations in relative water availability, produced by incubating seeds on media made up with various concentrations of agar, did not significantly affect germination rate, which remained relatively constant around 20 % (data not shown). Water availability, however, did influence strongly the development of hypocotyl hairs in those seeds that did germinate (Fig. 3). Over 98 % of seedlings on the driest substrate (10 % w/v agar) developed complete hypocotyl hairs and the 2 % of remaining seedlings showed partially developed or impaired hypocotyl hairs. In this driest treatment, hypocotyl hairs developed rapidly and reached full elongation with three days of emergence of the hypocotyl from the testa. About 80 % of seedlings developed hypocotyl hairs on

the 1 % agar treatment, consisting of about 50 % with fully developed hairs and the remaining 30 % showing impaired hair development. Seedlings on the substrates with highest water availability (0.2 % and 0.5 % agar) did not develop any hypocotyl hairs at all. No systematic data were collected on survival of seedling without hypocotyl hairs but it was noted that most of these seedlings had perished at the end of the 60-day observation period compared with almost complete survival of seedlings that had produced hypocotyl hairs for the same period.

To determine whether seedlings that had not developed hypocotyl hairs in the wetter environments could do so if exposed to conditions of lower water availability, we moved some seedlings from the 0.2 % to the 10 % w/v agar-concentration treatment. Seedlings that were moved to the drier conditions still failed to produce hypocotyl hairs. Within days of transfer, the stem and to a lesser extent the radicle of the surviving seedlings became highly pigmented (pink), possibly indicating a stress response, and produced a mass of short root hairs but no hypocotyl hairs. Most of the transferred seedlings died within three weeks.

To determine the effects of flooding, surface-sterilized seed was soaked in sterilised distilled water for 2, 4, 8, 16, 32, 64, 128 or 256 hrs. Flooding had a strongly negative effect on hair development, and hypocotyl hairs were not produced under any of the flooding treatments (data not shown). This result is consistent with the results shown in Fig. 1, which showed that surface sterilization for periods of more than 2 minutes resulted in the failure of hypocotyl hairs to develop and that only few seeds that were soaked in de-ionized water for 5 or 30 minutes developed hairs.

From these experiments we conclude that seeds soaked for more than about one hour are unlikely to develop hypocotyl hairs.

Water availability had a strong effect also on the development of geotropism in *M. ericifolia* seedlings (Fig. 4). Over 80 % of seedlings growing on the driest media (10 % w/v agar) showed positive geotropism, and this percentage fell uniformly until only about 20 % of seedlings that were sown on 0.05 % w/v agar showed a positive response. Seedlings growing on 0.02 % w/v agar did not show any positive geotropism at all.

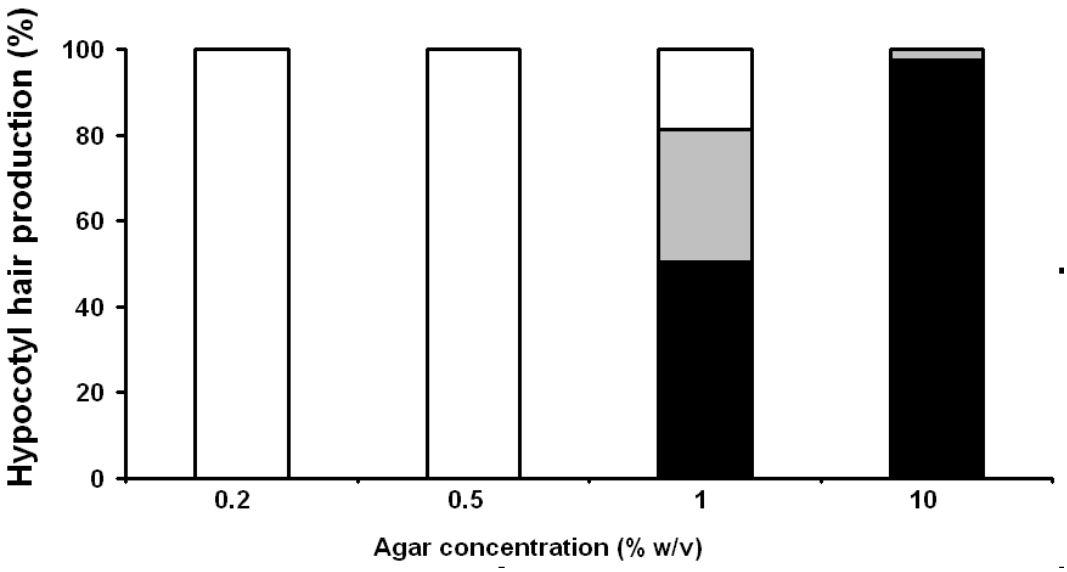


Figure 6.3 Percentage of *M. ericifolia* seedlings that produced hypocotyl hairs after germination and growth on substrata of different agar concentrations ranging from 0.2 % to 10 % w/v. The black portion of each bar indicates the mean percentage of seedlings showing full hypocotyl hair development; the grey portion indicates the mean percentage of seedlings with impaired development of hypocotyl hairs; the white portion indicates the mean percentage of seedlings showing no development of hypocotyl hairs.

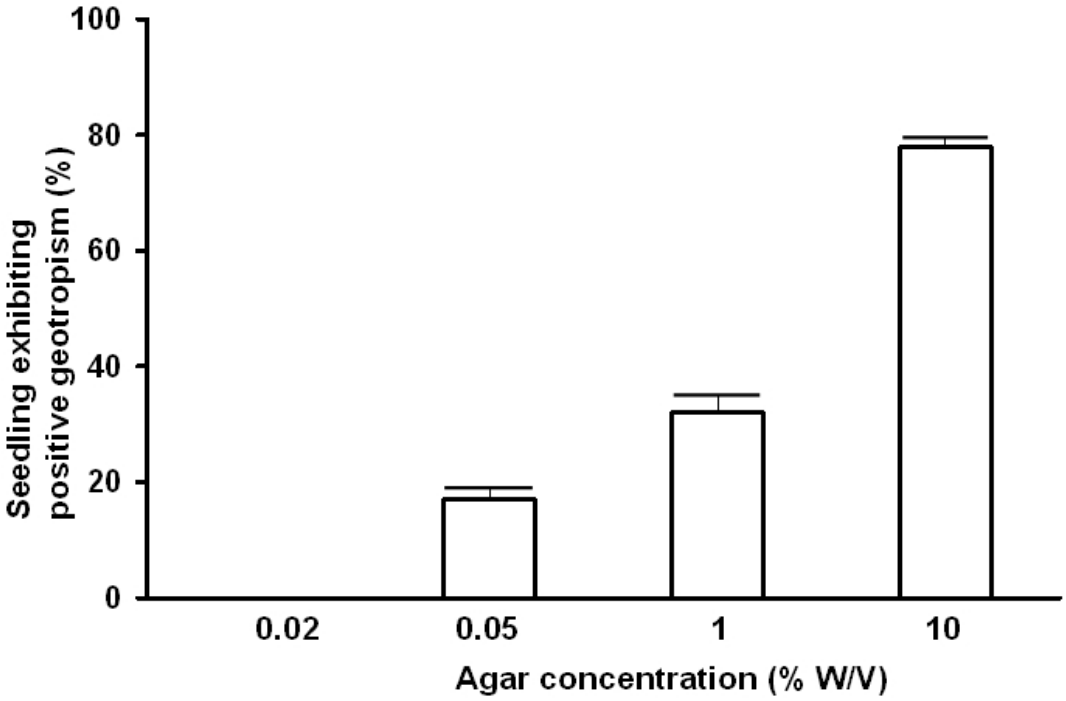


Figure 6.4 Percentage of seedlings exhibiting positive geotropism after germination and growth on substrata of different agar concentrations ranging from 0.2 % to 10 % w/v. Error bars show standard deviations. N = 4

6.3.5 Effects of temperature, light and salinity

The individual effects of salinity, light and temperature were highly significant ($P < 0.001$) terms of both the percentage of seeds germinating and the formation of hypocotyl hairs on those seeds that did germinate (Fig. 5). In addition to the effects of these three prime variables, there were also significant interaction ($P < 0.05$) terms in the ANOVA and these precluded making many generalizations as to any overall impacts of temperature, light or salinity. Nevertheless, the strongest individual inhibitory effect on hypocotyl hair formation was salinity, which accounted for 65 % of the variance in response. Across all temperature and light-regime treatments, there was a consistent decrease with increasing salinity of the proportion of seedlings

having fully developed hypocotyl hairs. Although there was an important interaction between salinity and temperature (see below), seedlings showed fully developed hypocotyl hairs only at the lowest salinities of 0 and 1 g L^{-1}; at salinities of greater than about 4 g L^{-1} there was either no, or very poor, development of hypocotyl hairs. This pattern occurred regardless of temperature or light regime.

Temperature exerted a smaller individual influence than salinity (10 % of variance) but still returned a highly significant prime effect ($P< 0.001$) on hypocotyl hair formation (Fig. 5). Germination was better at 20^{o}C than at 10^{o}C regardless of light regime, but differences in responses between 20^{o}C and 30^{o}C were conditional on whether seedlings were incubated under constant darkness or under an alternating light:dark cycle. Temperature also modulated the effect of salinity on hypocotyl hair formation, and higher temperatures exacerbated the inhibitory effects of salinity on the formation of these structures. For example, almost all seedlings developed fully functional hypocotyl hairs at a salinity of 1 g L^{-1} at 10^{o}C under alternating light:dark conditions, but the proportion fell to around 10-15 % at 20^{o}C and none produced fully functional hypocotyl hairs at this salinity if the temperature were increased to 30^{o}C.

The alternating light:dark treatment, although having the smallest inhibitory influence on hypocotyl hair production (<0.2 % of variance), nevertheless also returned a highly significant prime effect ($P < 0.001$). The formation of hypocotyl hairs was generally better in the dark than under the alternating light:dark cycle. As noted before, however, it is difficult to draw general conclusions because of the significance of the many interaction terms. Moreover, the interactive effect of all three factors - salinity, light and temperature - was also highly significant ($P< 0.001$).

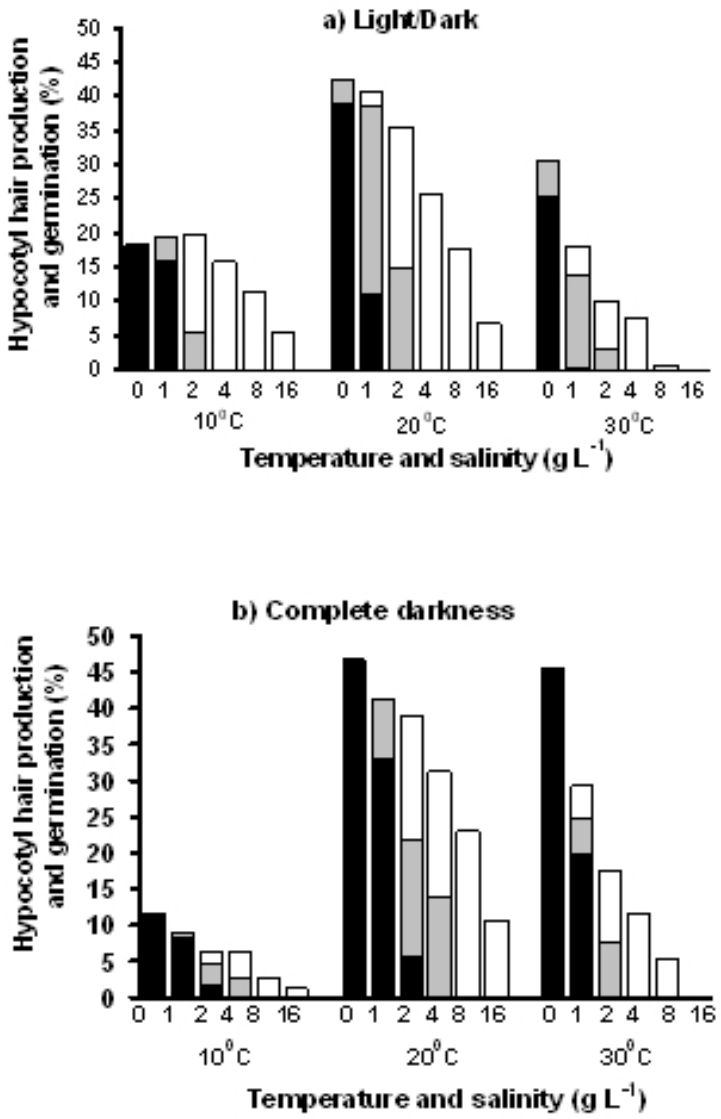


Figure 6.5 Percentage of seedlings showing hypocotyl hair development at six salinities (0, 1, 2, 4, 8 or 16 g L^{-1}), three temperatures (10°C, 20°C, 30°C) and under contrasting light regimes of a) alternating 12hr:12hr light:dark cycle or b) complete darkness. Means are shown; error bars have been excluded for clarity. The black portion of each bar indicates the mean percentage of seedlings showing full hypocotyl hair development; the grey portion indicates the man percentage of seedlings with

impaired development of hypocotyl hairs; the white portion indicates the mean percentage of seedlings showing no development of hypocotyl hairs.

The environmental conditions that encouraged the formation of hypocotyl hairs also encouraged the formation of root hairs: increasing salinity, temperature and exposure to light inhibited the production of root hairs (data not shown). Conversely, the production of secondary roots, with concurrent inhibition of primary radicles, was stimulated by increases in salinity and temperature and with exposure to light. Complete suppression or premature death of both the primary radicles and secondary roots took place at the highest salinities, as well as at 30^{o}C and with exposure to light. Thus there was an inverse relationship between the formation of hypocotyl hairs/root hairs and the formation of secondary roots. Indeed, secondary roots were initiated only at salinities above 2 g L^{-1} under all treatments except for 30^{0}C in dark conditions. A complete suppression of secondary roots occurred at 16 g L^{-1} under all treatments except 20^{0}C under constant darkness.

Fig. 6 shows the development of hypocotyl hairs on seedlings incubated under contrasting salinity regimes. At the lowest salinity of 0 g L^{-1}, the hypocotyl hairs were fully developed, as was the primary root and root hairs (Fig. 6a). Hypocotyl hair development was visibly impaired at a salinity as low as 1 g L^{-1} (Fig. 6 b) and there was a complete absence of hypocotyl hairs on seedlings grown at a salinity of 4 g L^{-1}: (Fig 6 d). At a salinity of 8 g L^{-1}, there were no hypocotyl hairs, no root hairs, only a stunted primary root and a stunted secondary root. The highest salinity of 16 g L^{-1} generated seedlings with no hypocotyl hairs, no root hairs, the primary root prematurely desiccated, no secondary roots, and the cotyledons cupped and noticeably thickened (Fig 6 f).

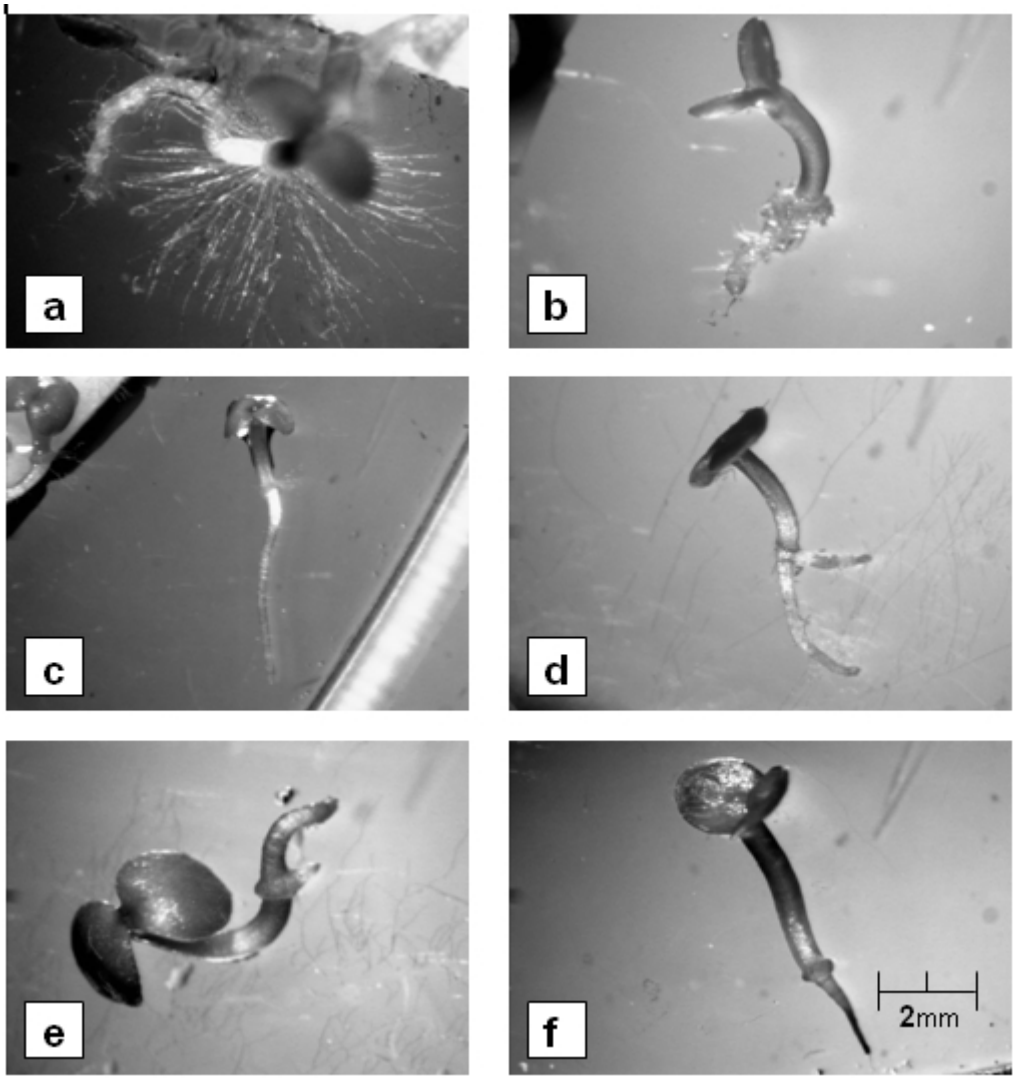


Figure 6.6 Microscopy images of the development of hypocotyl hairs and root hairs in *M. ericifolia* seedling grown under six contrasting salinity regimes. Photographed at day 14. a) salinity = 0 g L^{-1}: fully developed hypocotyl hairs, fully developed primary root with root hairs; b) salinity = 1 g L^{-1}: impaired development of hypocotyl hairs primary root with root hairs; c) salinity = 2 g L^{-1}: impaired development of hypocotyl hairs no root hairs; d) salinity = 4 g L^{-1}: no hypocotyl hairs, no root hairs moderate development of primary root, moderate development of secondary root; e) salinity = 8 g L^{-1}: no hypocotyl hairs, no root hairs, stunted primary root, stunted secondary root; f) salinity = 16 g L^{-1}: no hypocotyl hairs, no root hairs, primary root prematurely desiccated, no secondary roots, cotyledons cupped and noticeably thickened.

6.4 Discussion

The location of hypocotyl hairs on the base of the hypocotyl of *M. ericifolia* seedlings, and not on the radical, clearly indicates that these structures are not part of the normal root system of young Swamp Paperbark plants. They are transitory in nature and, although produced soon after germination, begin to desist within a few weeks. In most cases, hypocotyl hairs were present as the seedling emerged from the seed coat or else they developed very shortly afterwards. A similar developmental pattern has been reported for other woody plant species by Young and Martens (1991), Arrone and de Micco (2004) and Matsuo and Shibayama (2002) for the genera *Artemisia*, *Myrtus* and *Monochoria*.

Arrone and de Micco (2004) and Young and Martens (1991) proposed that hypocotyl hairs, which radiate out along the substrata, were important in providing anchorage and in facilitating the development of geotropism in young seedlings. Certainly these results show a strong correlation between the development of hypocotyl hairs and the development of positive geotropism (*cf.* Figs. 3 and 4). The ability of seedlings to produce hypocotyl hairs is likely to be particularly important for wetland plants such as *M. ericifolia*, which occurs in habitats where inundation is common, making it critical for young plants to be able to orientate themselves appropriately when establishing in mobile, possibly disturbed, substrata.

6.4.1 Effects of environmental variables

Water availability had strong effects on the production of hypocotyl hairs on *M. ericifolia* seedlings. Not only did it influence the development of geotropism, but water availability controlled the proportion of seedlings that developed fully functional hypocotyl hairs (e.g., see Fig. 3). Furthermore, soaking seeds for periods of more than about one hour seemed to be highly inhibitory to the formation of hypocotyl hairs, even though the seeds of *M. ericifolia* are highly likely to be deposited into wet environments. These results are consistent with those obtained by Polya (1961), who reported that soaking seed, even for very short periods of time, inhibited hypocotyl hair formation in *Populus* seedlings. As the process of soaking simulates flood conditions, these results suggest that the longer the wetting period, the less likely *M. ericifolia* seedlings will produce functional hypocotyl hairs. Even short periods of wetting, such as those experienced after heavy rainfall where seed may fall into puddles, may have a similar effect to long-term flooding in terms of hypocotyl hair production and presumably the successful establishment of young plants

The results we obtained on the effects of water availability on hypocotyl hair formation are similar also to those of Aronne and de Micco (2004), who observed the development and average length of hypocotyl hairs was greater in *Myrtus* seedlings that germinated under dry conditions than those that germinated under moist or saturated conditions. In combination, these findings suggest that hypocotyl hairs are either inhibited by exposure to flood-like conditions, as suggested by Polya (1961), or are primed by desiccation, as postulated by Aronne and de Micco (2004). Polya (1961) suggested a causal mechanism for this response was that rapid swelling of the

embryo in saturated conditions caused sudden distention of the cell membranes, resulting in tears in the protoplasm.

When *M. ericifolia* seedlings devoid of hypocotyl hairs were transferred from an environment with high water availability (0.2 % w/v agar) to one with low water availability (10 % w/v agar), they produced a mass of short root hairs but none formed hypocotyl hairs and most died within a few days. These results indicate that the ability of *M. ericifolia* seedlings to produce hypocotyl hairs is limited to the first few days of development, and perhaps is even stimulated prior to splitting of the seed coat. The implication is that timing of the wet and dry events may have significant impacts on the production of hypocotyl hairs and potentially on seedling survival under field conditions.

As well as duration of immersion, temperature, salinity and light showed very strong effects on the development of hypocotyl hairs in *M. ericifolia*. Hypocotyl hair development was inhibited strongly at salinities of more than about 1-2 g L^{-1} and, by 4-8 g L^{-1} depending on light regime, hypocotyl hairs did not develop at all (e.g., see Figs. 5 and 6). Such an extreme sensitivity to salt is somewhat surprising, given the salinity regime that exists in Dowd Morass (< 1-2 g L^{-1} to > 16 g L^{-1}; commonly > 8 g L^{-1}) and the well-reported salt tolerance of juvenile and adult *M. ericifolia* to salinity (Bird 1962; Ladiges *et al*. 1981; Robinson *et al*. 2006; Salter *et al*. 2007). This sensitivity to salt could be a function of osmotic stress on unexpanded hypocotyl hair cells or on the developing hypocotyl hairs, or a direct consequence of toxic ion effects (e.g., see Kozlowski 1997; Barett-Lennard 2003). The microscopy observations showed that hypocotyl hairs in *M. ericifolia* are single-celled outgrowths, about 20

mm long x 30 μm wide, with a consequently high surface-area-to-volume ratio. The large contact area with the external environment suggests a high degree of sensitivity to osmotic stress and/or exposure to toxic ions, a factor that was identified by Young and Martens (1991) as important for the response to low moisture levels of hypocotyl hair development in the dryland plant *Artemisia*.

6.4.2 Implications for seedling establishment and plant recruitment

This investigation indicated that *M. ericifolia* seeds that become soaked for more than about one hour or so by falling onto open water, especially if the salinity exceeds a few grams per litre, would be unlikely to form hypocotyl hairs. Hair formation is decreased also by low (10^{o}C) or high (30^{o}C) temperatures and by exposure to light, although these two environmental variables exerted weaker effects that either soaking or salinity. All species that have been reported to produce hypocotyl hairs (with the exception of *Zostera marina*, a submerged angiosperm growing in coastal waters) germinate on the sediment or soil surface. Buried seed or disturbed seed died in studies of some species in the families Alismataceae and Hydrocharitaceae (Kaul 1978), and substantial growth abnormalities were recorded when the connection of hypocotyl hairs and the substratum was disrupted in *Artemisia* (Young and Martens 1991). While seed of *M. ericifolia* and other species can germinate when flooded or after exposure to saline conditions (Ladiges *et al*. 1981; Robinson *et al*. 2006; Salter *et al*. 2007), the failure to produce hypocotyl hairs under these conditions would reduce markedly the subsequent ability of young plants to establish effectively into a wetland.

This study thus considerably extends what is known about the environmental conditions that are required for successful establishment of *M. ericifolia* seedlings: it is not a simple matter of the conditions required for successful germination of the seeds alone, but also the rather limited conditions that are required for hypocotyl hairs to develop fully and allow the young seedling to establish successfully. For germination to be successful, seed must find itself on moist, but not saturated, substrata of low salinity (Ladiges *et al*. 1981; Robinson *et al*. 2006). Seed of *M. ericifolia* can germinate on the sediment surface, but requires reduced light levels, possibly in the shade that is provided at the base of other plants or in the pores of coarse- to medium-textured organic or mineral soils. These conclusions are consistent with those of Polya (1961), who showed that the specific conditions for successful germination and establishment of the wetland tree species *Salix* and *Populus* were very narrow and broadly similar to those outlined above for *M. ericifolia*.

For successful establishment of the young seedling once seeds have germinated, environmental conditions must be suitable for hypocotyl hairs to develop; in this regard the sensitivity of hypocotyl-hair formation to even brief soaking and to salinities of more than about 1-2 g L^{-1} would further limit the capacity of *M. ericifolia* seedlings to establish in coastal, brackish-water wetlands. Such a strict combination of requirements, over diverse environmental variables including temperature, salinity and light, is even narrower than that required for seed germination and seedling establishment considered separately and would indicate that recruitment success would be limited to all but ideal conditions. This conclusion is consistent with preliminary studies carried out by one of the authors (RWR) on the establishment and

growth of *M. ericifolia*, which indicates that recruitment events are rare (less than one year in ten) under field conditions at Dowd Morass.

Conditions for successful seed germination and hypocotyl hair formation are likely to have been even further limited by the human-induced modifications that affect many coastal, brackish-water wetlands that are vegetated with *M. ericifolia*. Increasing salinisation, for example following changes to water regimes after the construction of levees and other structures or to more frequent saline intrusions following sea-level rise and the artificial opening of previously intermittently open coastal lagoons, would further reduce opportunities for germination and seedling establishment. Hydrological alterations that involve the replacement of alternating wet-and-dry cycles with permanently ponded water would similarly restrict the likelihood of young seedlings forming functional hypocotyl hairs, and thus further limit the chances of successful sexual recruitment.

Chapter 7

Historical recruitment events of *Melaleuca ericifolia* at Dowd Morass

Abstract

Determining specific safe site conditions in short-term studies can be difficult as recruitment in clonal species may take place only every few decades. This component of the project investigated how the combination of aerial photography, germination studies and historical rainfall and temperature data could be used to determine likely recruitment events for *M. ericifolia* over a period of ~ 60 years. Recruitment was found to be limited to specific events directly related to rainfall (flood events) and temperature. Recruitment occurred in just four or possibly five years over 60 years (1950, 1951, 1969, 1974 and 1993). Suitable climatic conditions in these years were related to average rainfall (> 800 mm, median 606 mm) and to magnitude of flooding (> 100 mm) at specific times (spring and autumn) with appropriate average rainfall during the intervening summer months. It is predicted that flooding or heavy precipitation would temporarily reduce salinity within the wetland bringing conditions within the range required for germination and establishment. Average rainfall during the putative period of establishment would reduce inundation and potential death of early-stage recruited seedlings.

7.1 Introduction

Predicting prior recruitment events is difficult due to the inherent speculation involved, lack of independent evidence for recruitment and the time lag needed to determine if predictions are correct. *Melaleuca* recruitment events in the field have been recorded only rarely (Di Stefano and Fisher 1983; de Jong 2000; Griffith *et al.* 2004) and the specific convergence of germination and establishment conditions even less so (Griffith *et al*. 2004). Studies of the way wetland species recruit have relied heavily on laboratory or field-based observational studies of microtopographical relief or responses to biotic or abiotic conditions (Ladiges *et al*. 1981; Budelsky and Galatowitsch 1999; Cornett *et al*. 2000; Nicol and Ganf 2000; Barsoum 2001; Robinson *et al*. 2006). Although predictive studies have used previously conducted laboratory-based studies to model potential recruitment sites (Green and Johnson 1999; Gourlet-Fleury *et al*. 2005; Ordonez *et al.* 2006), these studies have not, on the whole, confirmed if predictions were correct through follow-up investigations.

Aerial photography has been employed widely to map changes and landscape scale responses of vegetation (Williams and Lyon 1997; Kadmon and Harari-Kremer 1999; Herwitz *et al.* 2000; Fensham and Fairfax 2002; Fensham *et al.* 2003). The combination of spatial resolution and extent, and long-term temporal coverage allows for tracking of precise changes over periods of many years or decades. In large clonal species, such as *M. ericifolia,* identification and tracking of individual plants may be possible, allowing genet/ramet dynamics and potential recruitment events to be identified (Hudon *et al*. 2005; Kyncl *et al*. 2006). Studies of genet or ramet-level

population dynamics of clonal shrubs is particularly uncommon due to difficulties of aging genets and the difficulty of tracking ramet growth using common demographic techniques (Pysek 1991; Schenk 1999; Lantz and Antos 2002; Kyncl *et al*. 2006). While aerial photography is commonly used for tracking overall vegetation change it is rarely used at the genet level even though it may be very useful (Hudon 2005; Kyncl *et al*. 2006).

Historical climatic data has similar benefits to historical aerial photography in that it allows interrogation of information for specific combinations of abiotic conditions that may be suitable for germination and establishment. When climate data are combined with historical series of aerial photographs likely recruitment events and their potential climatic triggers may be ascertained. Germination responses and tolerances determined in the laboratory can be correlated to climatic data to more clearly elucidate relationships; the necessary background information on seed viability, germination conditions and seedling establishment has been reported in chapters 4-7 of this thesis.

Based on findings of Chapters 6 and 7, there was a strong indication that climatic variables and their influence on site conditions played a major role in creating spatial safe sites and temporal windows of opportunity for the recruitment of *M. ericifolia* in Dowd Morass. It was proposed that analysis of existing data, coupled with on-ground observations, would identify the possible climatic conditions that would permit successful recruitment.

This component of the thesis aimed to use a combination of historical aerial photographs spanning 46 years, historical climatic information spanning 63 years and previously established laboratory-based germination and recruitment data (Ladiges *et al*. 1981; Robinson *et al*. 2006; Chapters 4-7) to predict when *M. ericifolia* recruited in Dowd Morass over the ~ past half century.

7.2 Methods

7.2.1 Historical aerial photographs

The historical series of aerial photographs used in Chapter 4 was re-examined to determine evidence of a limited number of successful recruitment events over this ~ 50-year period. Recruitment events were determined by examining each photograph for cohorts of plants: these were then classified into either major or minor events depending on the number and distribution of young plants on each photograph. Major events represented a large number of plants scattered throughout the area represented in each air photograph. Minor events were limited to small numbers of plants restricted to small areas on the air photographs. The area represented in the aerial photographs is approximately 1.5 x 1.2 km.

7.2.2 Climatic and salinity data

Climatic data (rainfall, air temperatures) covering the years 1943 to 2005 were obtained from the Bureau of Meteorology (Government of Australia). These data contained daily rainfall and max/min air temperature readings for the East Sale weather station (38^{o} 12' S 147^{o} 13' E) approximately 1 km north of Dowd Morass. To determine whether there were potential patterns to climatic events, data were analysed using a single linkage method (nearest neighbour) hierarchical cluster analysis (hca) (SPSS vers. 15, SPSS Inc, Chicago, Illinois, USA), using Euclidean distance as the distance metric. Each data set (rainfall, mean maximum temperature, mean minimum temperature), was analysed separately in two runs; the first with daily

and then with monthly data. Dendrograms were produced for mean monthly rainfall data.

Salinity modelling data for Lake Wellington, immediately adjacent to the study site, was obtained from Grayson (2003). These data were used, where possible, to further elucidate the patterns established using climate data and recruitment events observed on the aerial photographs.

7.3 Results

7.3.1 Recruitment events determined using aerial photographs

Recruitment events were evident on four photographs in the historical series of aerial photographs; 1964, 1978 and 1991 and 2003 (Figure 7.1). The largest and most widespread recruitment was evident in the 1964 aerial photograph, with obvious young plants scattered across the whole area of the 1.8 km^2 image. As seen in Figure 7.1, *M. ericifolia* plants appear as dark points amongst the lighter coloured stands of *Phragmites australis*. Minor recruitment appears on the 1978 and 1991 aerial photographs. A final major recruitment event was evident on the 2003 aerial photograph, with small plants appearing amongst the much older established clones.

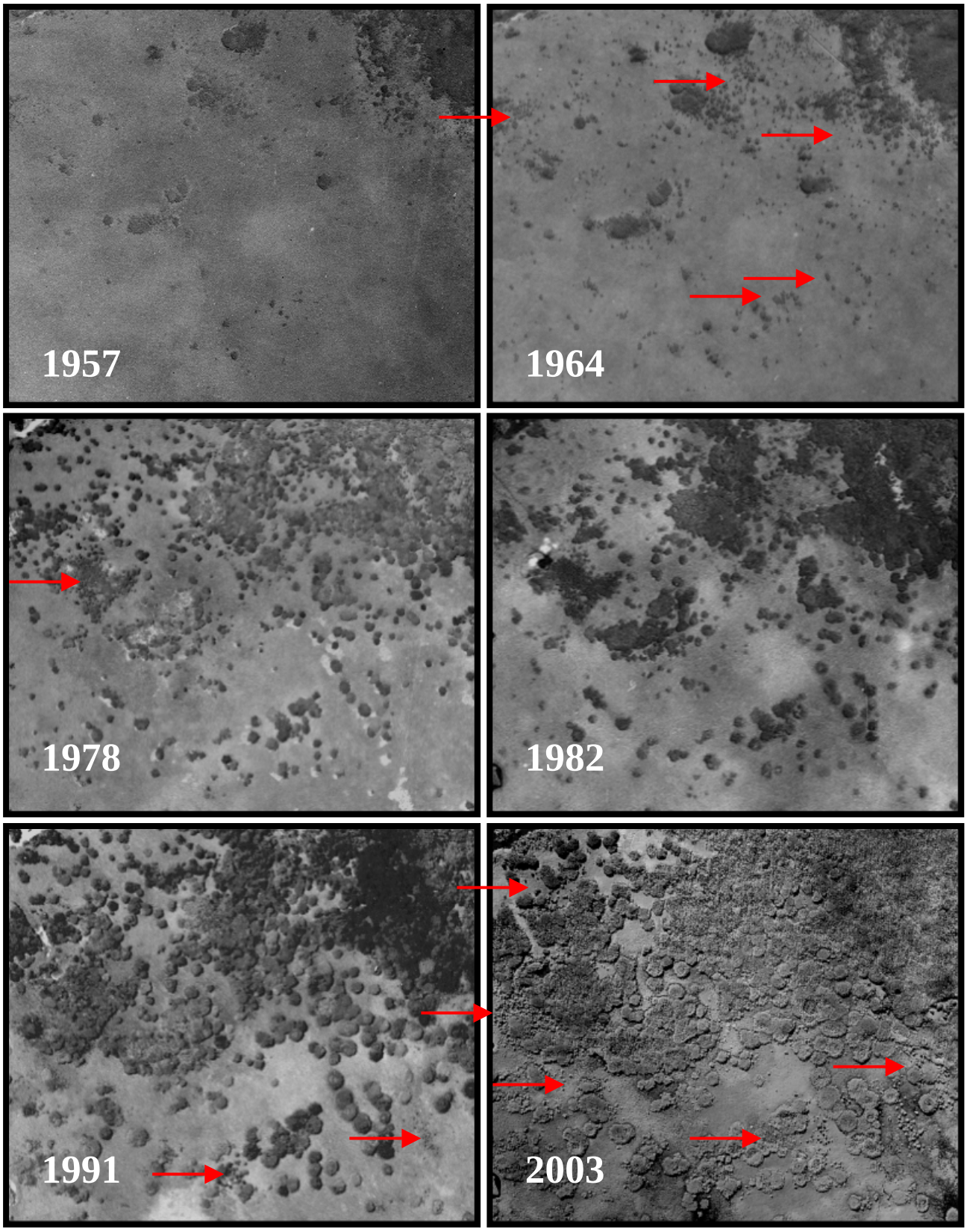


Figure 7.1 Photos of a single section of Dowd Morass at six time periods from 1957 to 2003. Light coloured areas are *Phragmites australis*, dark areas *Melaleuca ericifolia*. Circular patches are individual clones of *M. ericifolia*. Scale 1:150. Red arrows indicate areas of recruitment.

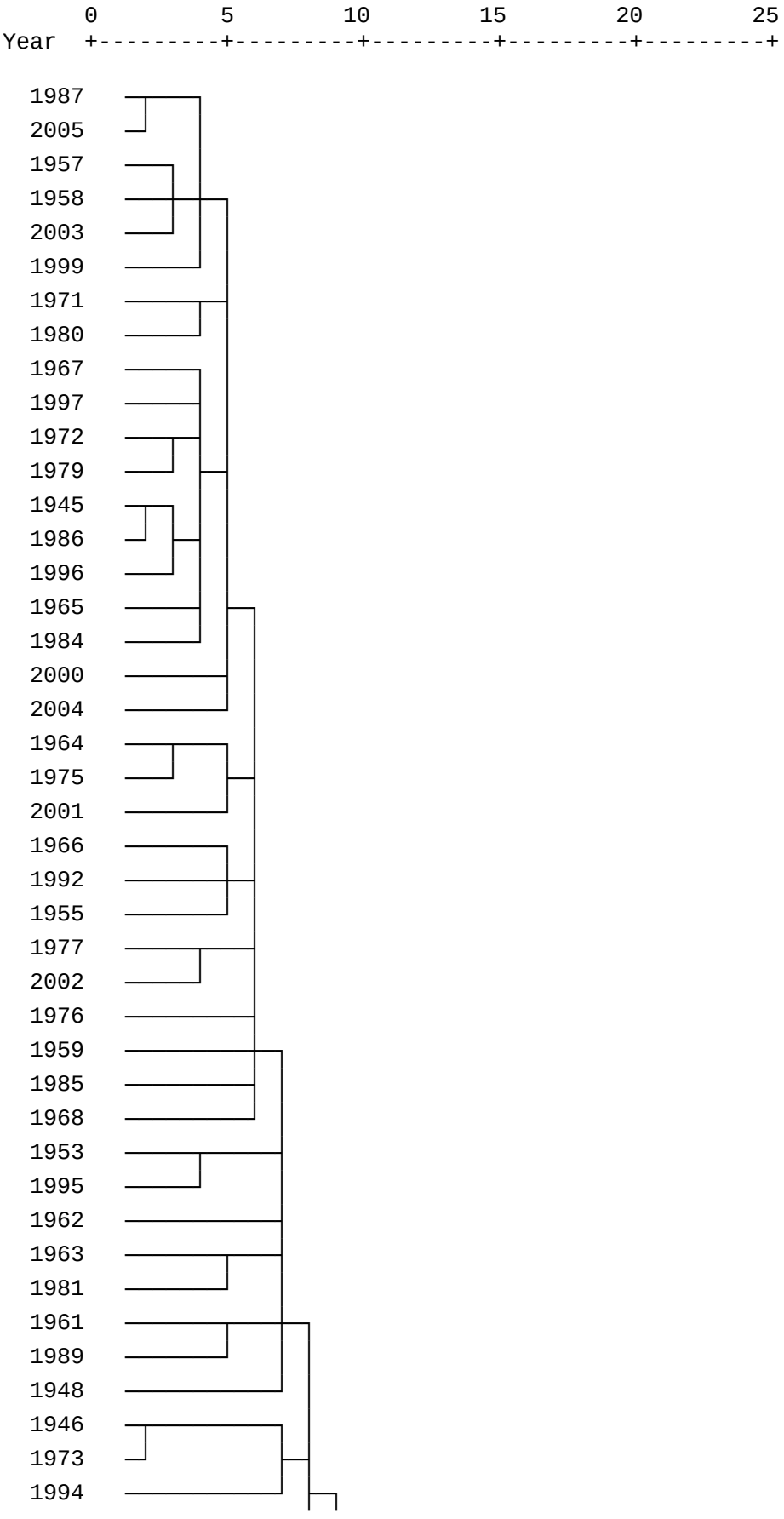
7.3.2 Climate data

Rainfall

The use of a hierarchical cluster analysis was, in many respects, an exploratory process to see if there were any patterns in the data that might help to explain the four recruitment events identified on the historical aerial photographs. It was assumed that the normal pattern of climatic events was not conducive to recruitment and that unusual climatic events would account for the four periods that supported recruitment.

Hierarchical cluster analysis of the 61 years of climate data revealed 4 main clusters (Figure 7.2). Forty-nine of the years clustered very tightly, occurring at a Euclidean Distance (ED) of 4-11. The remaining 12 years (outliers) formed three discreet clusters: seven years ranged from a ED of 13-16, three years at a ED of 19-21 and two years at a ED of 25.

Average rainfall over the entire period from July 1950 to June 2003 was 613 mm per annum (mean 606 mm), although individual years ranged from a low of 285 mm in 2002 to a high of 971 mm in 1951. Of the 12 outliers identified by the hierarchical cluster analysis the five years with a ED of greater than 19 had yearly rainfall averages well above the norm (> 705 mm) indicating a generally ‘wet’ year. The remaining 7 years, with an ED of 13-16 had below average to above average yearly rainfall (505-704 mm).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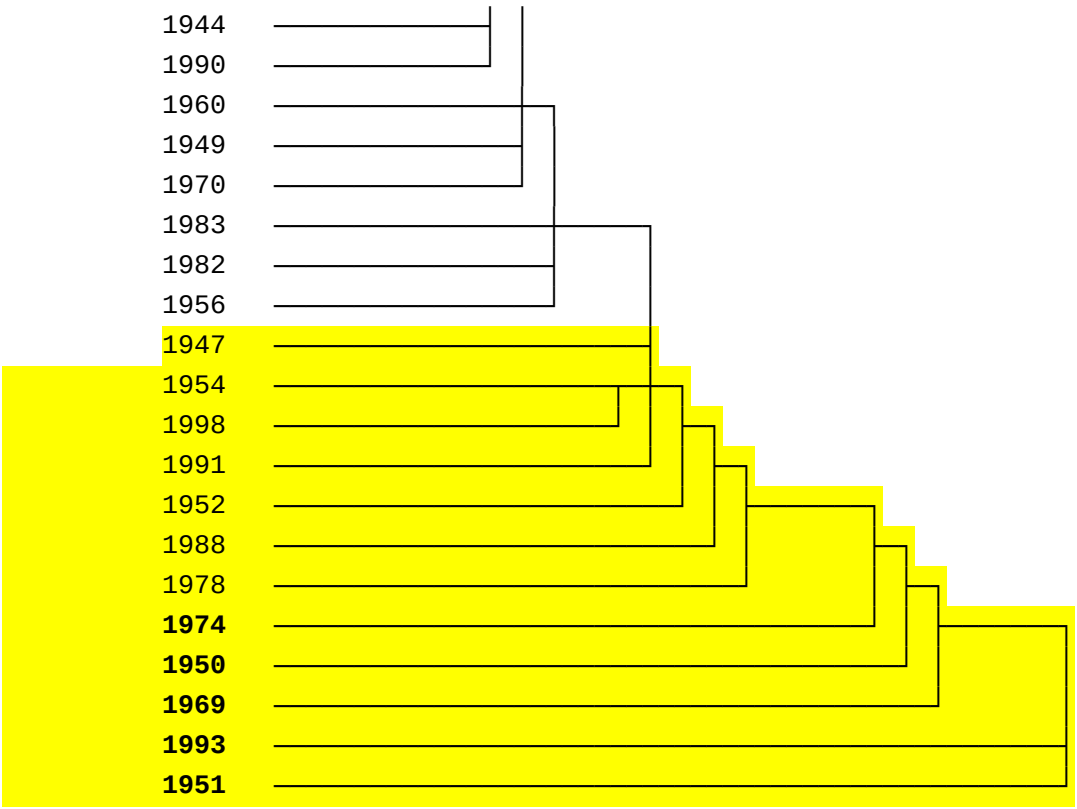

Figure 7.2 Dendrogram using Single Linkage derived from the hierarchical cluster analysis. Distance metric is Euclidian Distance. Years highlighted in yellow represent years with unusual rainfall patterns. The five years with bolded dates represent years with well above-average rainfall (> 705 mm) with well above-average rainfall in spring and/or autumn.

The five years with a Euclidean distance of greater than 19: 1974, 1950, 1969, 1993 and 1951 (Figure 7.2) were distinguished from the seven other outlier years based on pattern of rainfall within these years. Each of these five years shared the common feature of heavy rainfall in spring. Four of the years 1950, 1969, 1993 and 1951 had an additional period of heavy rainfall in autumn. Heavy spring and/or autumn rainfall in these five outlier years falls outside of the ideal temperature range for germination

and early establishment of *M. ericifolia*, namely October to January, although this is offset slightly for the year 1974. Spring and autumn rainfall in the five outlier years was significantly higher than the mean for these given seasons and ranged from 157 – 205mm (Figure 7.4). Mean monthly rainfall in spring and autumn for the 61 years varied from 42 – 63 mm. The timing and amount of average and above-average rainfall for Dowd Morass is shown in Figure 7.3.

Two potential recruitment events in spring-summer 1950 and 1993 had near identical climatic patterns: flood events in September/early October, average rainfall for mid October through January, followed by flood events in February (Figure 7.3). These two periods correspond to large recruitment events identified on the 1964 and 2003 aerial photographs (Figure 7.1). Spring and autumn of 1951 exhibited a similar rainfall pattern to 1950 and 1993, with heavy rainfall occurring in August and March with average rainfall in the intervening months. It was not possible to distinguish recruitment events that occurred in 1950 and 1951 on the aerial photograph, as plants established in these years would have been of comparable size on the 1964 aerial photograph. The potential for two consecutive recruitment years in 1950 and 1951 may account for the magnitude of recruitment evident on the 1964 aerial photograph.

The potential recruitment event identified from climatic data for 1969 corresponds with minor recruitment on the 1978 aerial photograph. The pattern of rainfall for 1969, while similar to 1950, 1951 and 1993 shows peak rainfall in November and March, slightly later than these three other years.

The final recruitment year, 1974, deviates from the pattern of the four years in that heavy spring rainfall is not followed by heavy autumn rainfall. There is however, a minor recruitment event evident on the 1991 aerial photograph that would correspond to potential recruitment in 1974.

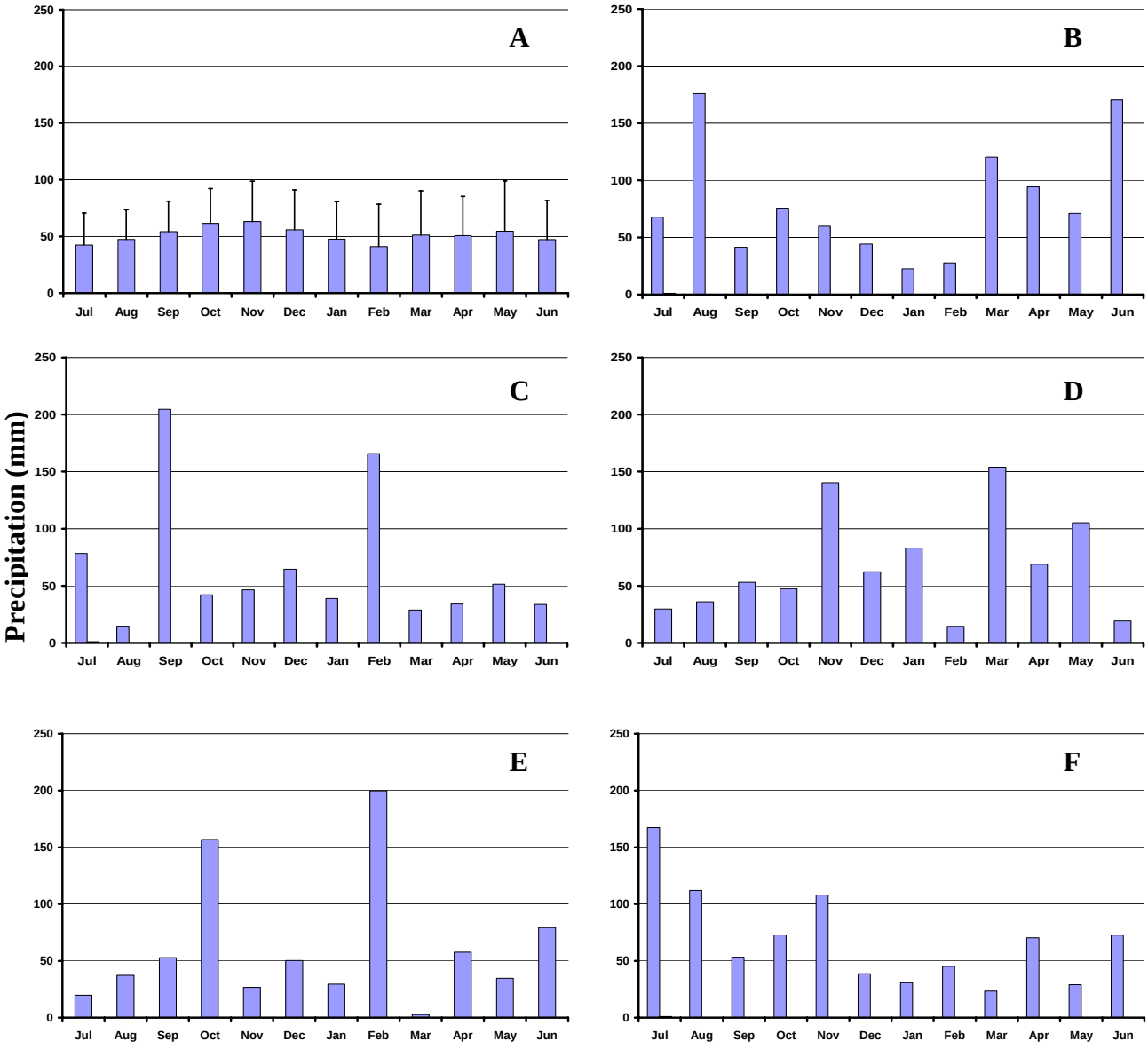

Figure 7.3 Monthly rainfall data from the 5 outlier years from the hierarchical cluster analysis and mean monthly rainfall for the 61 years from 1943 - 2004. A - Mean monthly rainfall July 1943 – June 2004, B - July 1951 – June 1952, C - July 1993 – June 1994, D - July 1974 – June 1975, E - July 1950 – June 1951, F - July 1969 – June 1970.

Temperature

Ideal day:night temperature range for germination (as identified in Chapter 5) occurs in two periods: October (18.5:8^{o} C) through November (21.5:9.5^{o} C) and again in April (20.5:8.5^{o} C) (BOM climate database, Commonwealth of Australia). Variation in mean maximum and mean minimum monthly temperatures was not significant over the 63 years for which data exists; standard deviation for mean maximum temperatures 1.6^{0} C, mean minimum temperature standard deviation 1.4^{0} C.

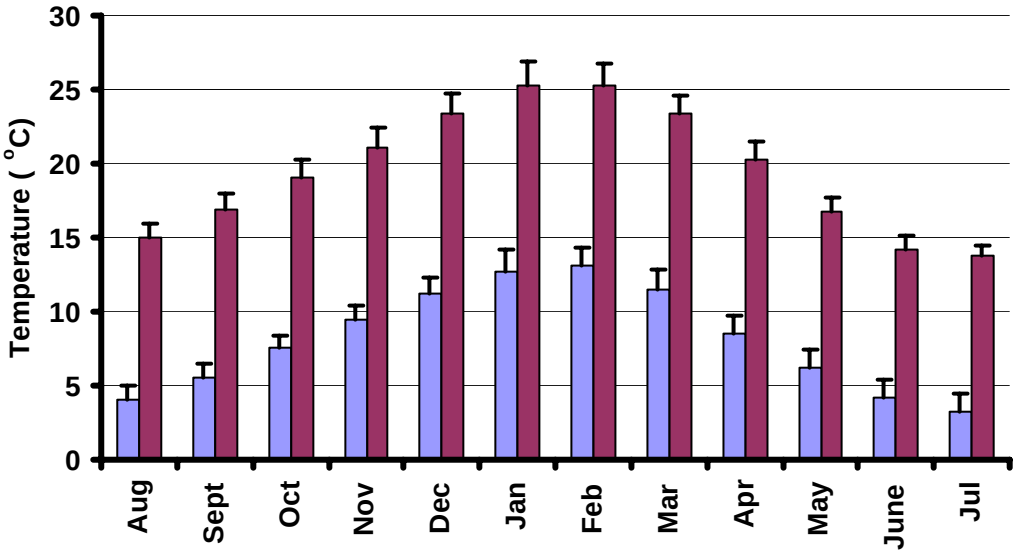

Figure 7.4 Mean monthly temperature levels from East Sale weather station, Sale, Victoria from July 1943 to June 2005 (BOM climate database, Commonwealth of Australia). Blue – mean monthly minimum temperature. Red – mean monthly maximum temperature. Error bars = standard error.

7.3.3 Salinity data

Surface-water salinity data for the adjacent Lake Wellington (Grayson 2003), within 0.85 km of the centre point of the area covered by the aerial photographs, exists for at least two of the periods identified in the climate data: July 1969 – June 1970 and July 1974 – June 1975. There is an annual rise and fall in the salinity of the surface water of Lake Wellington, with peak salinity during winter and a minimum in spring-summer. The magnitude and duration of maximum and minimum salinity varies widely not only from year to year but also within years, ranging from less than 1 g L^{-1} to just under 20 g L^{-1} in the period studies by Grayson (2003), namely January 1965 – December 1991.

The period of September 1969 through February 1970 represents a six month period when surface water salinity levels in Lake Wellington were 0.66 - 1.59 g L^{-1}, well within the tolerance range for both germination and hypocotyl hair production in *M. ericifolia* (Chapter 6 & 7). After a short period when salinity ranged from 3 - 4 g L^{-1} (March 1970 to May 1970), surface-water salinity of Lake Wellington fell to below 1.0 g L^{-1}, until April 1971. This represents a period of nearly 20 months of suitable conditions for recruitment and establishment of *M. ericifolia* seedlings.

There was a 6-month period between August 1974 and January 1975 where salinity was below 2 g L^{-1}. This period would be suitable for recruitment and establishment of *M. ericifolia* seedlings.

Both of these periods (September 1969 - February 1970 and August 1974 - January 1975) correspond with rainfall patterns identified in the hierarchical cluster analysis and which fall within the suitable temperature range for recruitment and establishment of *M. ericifolia* (Chapters 6 and 7). Surface waters in Lake Wellington during winter and outside of those listed times listed above were well above 4 g L^{-1} or dropped below 2 g L^{-1} for shorter periods of time, conditions that are unsuitable for recruitment and establishment of *M. ericifolia*.

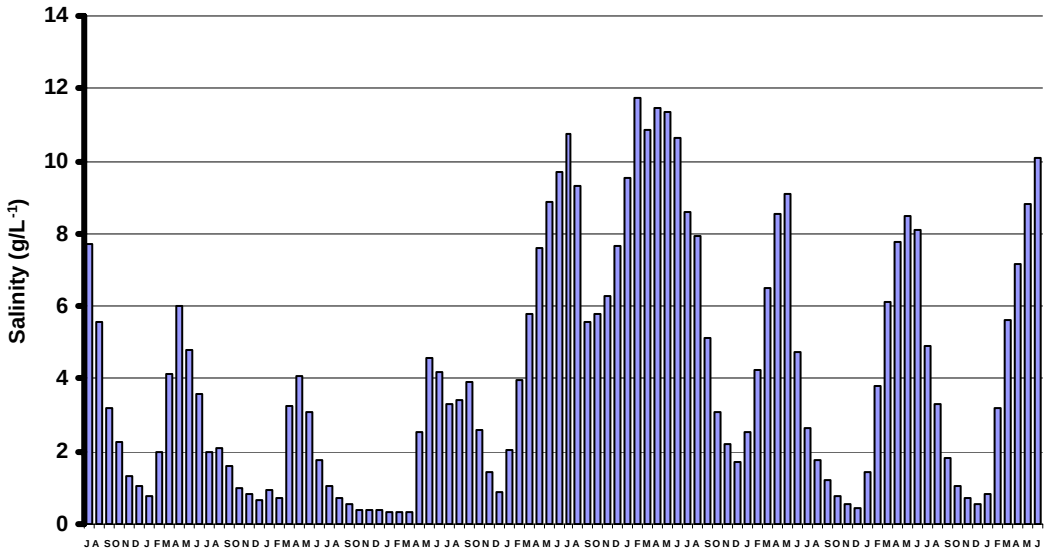

Figure 7.5 Mean monthly salinity of surface waters in Lake Wellington, Sale, Victoria from July 1968 to June 1975. Data derived from Grayson (2003)

7.4 Discussion

7.4.1 Aerial photographs

The analysis of the aerial photographs indicated that there were only five recruitment events over the period of 46 years covered by the available aerial photography. Episodic recruitment is widely reported for many plants but is especially prominent in clonal wetland species where specific combinations of spatially and temporally suitable environmental conditions are rare (Eriksson and Froborg 1996; Nicol and Ganf 2000; Barsoum 2002; Griffith *et al.* 2004; Stokes and Cunningham 2006). Hydrology is generally assumed to be the major driver in the germination and establishment of wetland species, and a major influence on the persistence of adult plants and structuring of the vegetation (Keddy 1984; Keddy and Ellis 1985; Keddy and Constable 1986; Coops and Van der Velde 1995; Coops *et al*. 1996; Leck and Brock 2000). For example, a comparative study of four helophyte species (*Phragmites australis, Phalaris arundinacea, Scripus maritimus* and *S. lacustris*) in the Netherlands reported a strong differential growth response to a water-depth gradient (Coops *et al.* 1996). In a later study investigating these same species, germination response and seedling growth were determined by the same moisture gradients favoured by the adult plants (Coops and van der Veld 1995).

Recruitment opportunities for species in the genus *Melaleuca* are very specific and related primarily to site conditions, particularly moisture and salinity (Browder and Schroeder 1981; Ladiges *et al.* 1981; de Jong 2000; Griffith *et al*. 2004). Germination of *M. ericifolia* in laboratory situations has been shown to be limited to a range of

conditions; ~20^{oC}, < 2 g L^{-1} salt and darkness (Chapter 6). A further potential impediment to seedling establishment in *M. ericifolia* is the sensitivity of hypocotyl hairs that form in the initial days after germination (Chapter 7). These structures are thought to be critical in successful establishment as they play a major role in geotropism and root formation. Hypocotyl hair formation however is favoured only by the conditions required for germination (Chapter 7).

7.4.2 Climate

Climate, particularly rainfall, and its effect on the hydrological regime of wetlands critically alter site conditions influencing the spatial and temporal wetting and drying cycle (Bedford 1996). The five years identified in the climate analysis of this study (1950, 1951, 1977, 1969 and 1993), exhibited rainfall events of greater than 150 mm each year in spring, events that periodically flood the wetland (Figure 7.4). These rainfall events were, on average, three times greater than the mean rainfall for the months in which they occur. Floods of this magnitude flush brackish-water wetlands, remove or dilute salt and provide a short period of altered site conditions that may be suitable for germination (Pezeshki 2001; Middleton 2002; Riis and Hawes 2002; Warwick and Brock 2003). This essentially converts brackish-water wetlands to freshwater wetlands for short but variable lengths of time. In the case of Dowd Morass in the key recruitment years, near-freshwater conditions existed for about 6 months, sufficient time for seedlings of *M. ericifolia* to establish. A period of release from hostile conditions (e.g. salinity, dryness) of 12-16 weeks has been found to be the most ideal for a range of herbaceous wetland species in swamps in New South Wales (Warwick and Brock 2003).

It is events such as flooding, flushing or drawdown that provide the window of opportunity for recruitment of many clonal wetland plants for which the normal prevailing conditions are unsuitable for the highly sensitive seedling stages but suitable for growth of adult plants (Eriksson and Froborg 1996; Rand 2000; Noe and Zedler 2001; Peterson and Baldwin 2004). It is well established that some wetland plants are reliant on drawdown or periods of drying for establishment (Middleton 1999; Nicol and Ganf 2000). However, a variety of otherwise freshwater species that occur in saline conditions are reliant on freshwater pulses to remove the osmotic stresses created by salt and allow germination and establishment (Churchill 1983; Gul and Weber 1999; Khan and Ungar 2001; Robinson *et al*. 2006). *Melaleuca ericifolia* and its responses to salinity and moisture suggest strongly that it is reliant on freshwater conditions for successful germination and early-stage establishment (Ladiges *et al*. 1981; Robinson *et al*. 2006).

This study confirmed that recruitment of *M. ericifolia* is episodic and tied closely to climatic conditions. However, further investigation to actual on-ground conditions needs to be carried out to determine exactly what effect rainfall is having on recruitment sites. The following chapter investigates safe sites for germination at Dowd Morass.

Chapter 8

Safe sites for recruitment of *Melaleuca ericifolia* in Dowd Morass

Abstract

Safe sites are critical for the successful recruitment of many plants. For long-lived clonal plants, safe sites, or windows of opportunity for recruitment, may be rare and limited in both time and space. At Dowd Morass recruitment safe sites were limited to the tops of hummocks, where hydrologic and edaphic conditions were suitable. Periodic flooding/flushing of the wetland is posited to dilute potentially toxic salinity, and reduced the influence of low pH levels within hummocks, while at the same time maintain suitable moisture for seedling recruitment. The physical structure and composition of the hummocks coupled with flood pulsing, triggered by abnormally high rainfall and flooding, are proposed as the major influences on successful recruitment in *M. ericifolia* in brackish-water wetland.

8.1 Introduction

Seedling establishment is dependent on the convergence of a series of precise events and conditions including temperature, moisture, light, substratum configuration, substratum chemistry and seed burial. These factors trigger germination and, if followed by favourable establishment conditions, recruitment of young plants into the established population is possible. Safe sites are the specific on-ground conditions, both spatial and temporal, that are conducive to the successful recruitment of plant species (Harper 1977).

The co-occurrence of the abovementioned events and conditions in both space and time, particularly in heterogenous environments such as wetlands, is likely to be rare. The lack of convergence of these abiotic factors may explain the episodic nature of recruitment events in a wide range of plant species (Grubb 1977; Eriksson and Froborg 1996; Kellogg *et al.* 2003; Bell and Clarke 2004, Chapter 8). In coastal wetlands, these factors are further complicated by other influences, such as salinity, acid sulphate soils and human-altered water regimes (Noe and Zedler 2001). These additional influences may further restrict the already limited safe sites or windows of opportunity for germination and establishment (Harper 1977).

The establishment of woody species of plants in wetlands has been shown to to occur only rarely, sometimes only once every few decades, due to the specific requirements of the seeds and seedlings in genera such as *Chamaecyparis*, *Nyssa*, *Salix*, *Taxodium* and *Vaccinium* (Eriksson and Froborg 1996; Conner 2002; Gengerelly and Lee 2005;

Stokes and Cunnigham 2006). Climatic change may shift microsite characteristics beyond the range that could be considered safe, particularly species that are persisting at the edge of their distributional or tolerance limits, such as *Juniperus sabina* in Mongolia. In this case, complete failure of recruitment for many decades was reported by Wesche *et al.* (2005). The loss or reduction of sexual reproduction, through the lack of safe sites for recruitment, is reported as being a factor in the evolution of long-lived clonal species such as *Melaleuca ericifolia* (Eckert 2001).

Safe site provision in heterogenous wetlands, although highly restricted, nevertheless does occur in both space and time. Hummocks, a common feature of wetlands, greatly alter the substratum topography providing a range of conditions, and commonly function as safe sites for wetland plants (Vivian-Smith 1997; Roy *et al*. 1999; Nungesser 2003; Peach and Zedler 2006; Raulings *et al.* 2007). Several authors have found that the majority of plants in wetlands were reliant on hummocks for recruitment and to maintain structural and floristic diversity in the broader vegetation community (Rheinhardt and Hershner 1992; Jones et al 1994; Crain and Berness 2005)

Hummocks in Dowd Morass are composed primarily of living and dead plant material. Hummock height varies greatly depending on the species that form hummocks and the subsidiary species that colonise them. Over time, through deposition of organic matter and consolidation by plant roots, hummocks can attain heights of a metre or more, raising the upper parts of the hummock above the prevailing water level of a wetland (Bertness *et al*. 1992; Fogel *et al*. 2004)

At Dowd Morass a range of species form hummocks, primarily: *Juncus* spp., *Melaleuca ericifolia*, *Paspalum distichum* and *Phragmites australis*. The hummocks formed by each of these species have different configurations and heights depending on the growth habits of the plants. *Paspalum distichum* forms low, broad hummock on the very edge of the wetland in periodically inundated sites. *Juncus* spp occupies a similar zone to *P. distichum* but forms more narrow upright hummocks. Both *Phragmites* and *Melaleuca* form tall hummocks (> 1 m high) that may be several metres across. Hummocks of both *Phragmites* and *Melaleuca* occur in areas that are permanently or near-permanently flooded and on the edges of wetlands that have less permanent standing water (Boon *et al*. 2007). Although *Melaleuca* and *Phragmites* hummocks occur in flooded conditions, the tops of the hummocks are elevated above the prevailing water level and rarely, if ever, become submerged. A wide range of herbaceous species is known to occupy the upper levels of the hummocks throughout Dowd Morass (Raulings *et al*. 2006).

Woody plant species are known to colonise hummocks in wetlands due to their favourable germination conditions: moist, well aerated and with generally higher temperatures than the surrounding substrates (Titus 1990; Eriksson and Froborg 1996; Gengerelly and Lee 2005). While hummocks provide relief from flooding, anaerobic stress and generally provide favourable temperatures, high transpiration rates coupled with saline conditions may create toxic conditions for seedlings. The specific microsite conditions created by individual species may influence the availability of safe sites by altering soil chemistry, moisture availability and degree of competition (Hatton 2004).

This part of the study carries on from Chapter 8, which attempted to identify the temporal occurrence of safe sites for recruitment of *M. ericifolia* at Dowd Morass. The specific aims are to determine the spatial occurrence of safe site, in particular to:

- Characterise the environmental conditions where potential safe sites may occur, and
- Determine potential safe sites for germination and establishment on-the ground.

It was predicted that recruitment would be limited in space, specifically in relation to suitable safe sites that provided salinity below 2 g/L^{-1}, day:night temperatures ~20^{o} C:12^{o} C, moderate moisture (neither dry or saturated/inundated) and darkness.

8.2 Methods

8.2.1 Recruitment sites in Dowd Morass

Potential recruitment events that may have occurred in more recent times would not have been evident from the 2003 aerial photographs utilised in Chapter 8, since there is a variable time delay between recruitment and the plants being large enough to distinguish on an aerial photograph. This delay is likely to be approximately 10 years - based on known growth rates of young plants and aerial photograph resolution. As a potential recruitment year (1993) identified in the hierarchical cluster analysis used in section 8.2.2 was able to be determined from the aerial photographs there was an opportunity for on-ground confirmation of recruitment. Aging juvenile plants presumed to have recruited recently could identify safe sites.

To determine if there had been more recent recruitment events at Dowd Morass in the past 10 years or so, 50 x 50 m quadrats were established in the field in February 2006 in three vegetation communities with potential recruitment conditions. These areas were based on previous vegetation classification carried out at Dowd Morass by Elisa Raulings (pers. comm.): Swamp Scrub, Reeds and Open Water/Bare Sediment. By definition, Reed communities encompass a wide range of herbaceous tussock-forming species including *Phragmites, Juncus, Paspalum* and *Baumea*. Three quadrats were placed in each vegetation community in three widely separated areas of Dowd Morass (Figure 8.1).

Each quadrat was searched for seedling or juvenile plants and the numbers and position (hummock/hollow) of these plants were recorded in a contingency table. Seedlings were identified by the characteristic of *M. ericifolia* to have an alternate pattern of the first few leaves and the rounded tips to these leaves; no other plant species in the wetland has these characteristics. Annual growth counts were carried out on all identified juveniles to determine approximate age; annual growth is readily identifiable on most *Melaleuca* species as new growth emerges from dormant buds leaving the basal section of the new stem covered in bracts.

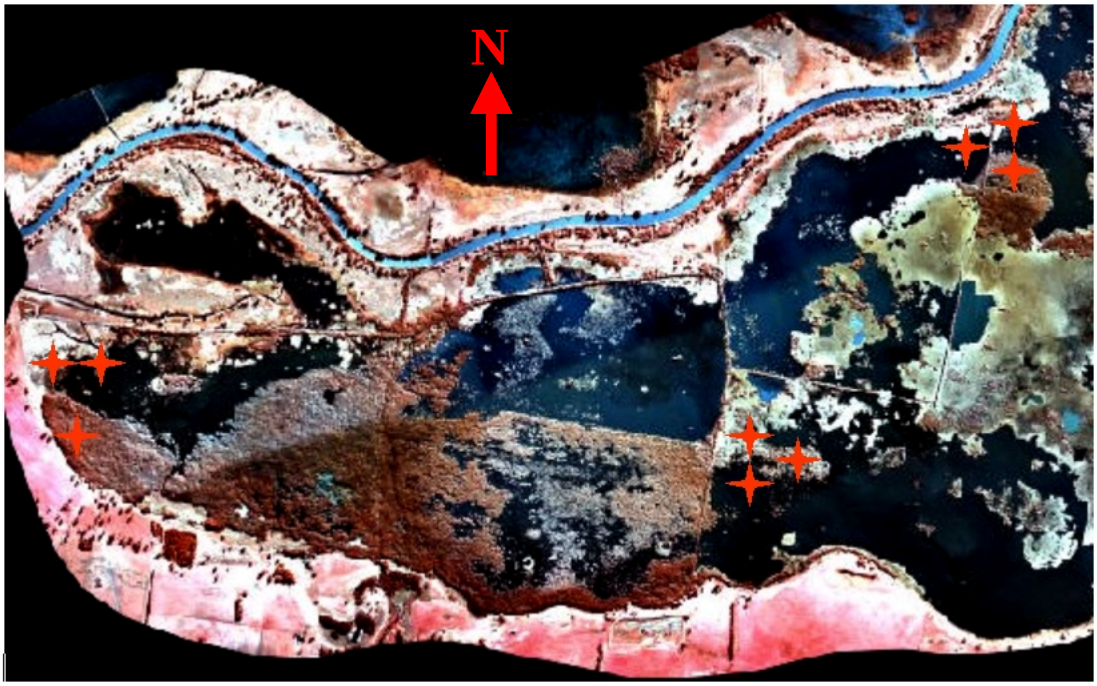

Figure 8.1 Sample sites for identification of possible recent recruitment of *Melaleuca ericifolia* in Dowd Morass. Sample sites are marked with a red cross. (Source – Wetlands Ecology Unit, Monash University)

Environmental data (salinity, pH, moisture content and organic matter content) were obtained for soil in the hummocks and in hollows of the reed communities. Soil cores, (5 cm wide x 10 cm deep) were taken laterally into the top, middle and base of 6 hummocks and from the surrounding substrate adjacent to each hummock in each 50 x 50 m quadrat. Samples were taken from all three of the quadrats in the reed communities; in all, 72 soil cores were taken. Soil cores were processed within 48 hours of collection. Samples were processed using Australian standard methods for pH (Department of Sustainable Natural Resources, n.d. - A), salinity (Department of Sustainable Natural Resources, n.d - B) and water content (Standards Association of Australia, 1977). To determine pH, approximately 50 g of each sample was air dried in tins. A 1:5 soil:water suspension was prepared with deionised water and the container mechanically shaken for 1 hour at 15 rpm. A hand-held pH/EC/TDS meter (Hanna Instruments, Westlab Laboratory Supplies, Ballarat, Victoria) was used to measure pH. To obtain water content approximately 80 g of each sample were weighed prior to drying and then oven-dried at 110^{o} C for 16 hours. All samples were weighed after drying to determine moisture content, expressed as percentage dry soil weight (MC%). Electrical conductivity (EC) followed the methods of Hatton (2004) and was measured using a TPS-LC81 salinity meter, with a k = 1 conductivity cell and a 1:5 soil:water suspension. The suspension comprised 10 g of powdered dry soil and 50 mL of deionised water. Reference solution consisted of 0.01 M KCl, which has a known EC of 1.41 μS $cm^{-}1$ was used to calibrate the salinity meter. Final EC readings (μS cm) were converted to salinity values of mg/l^{-1} using a conversion factor of 0.6 (MDBC, 1990). Organic matter content was determined using the Weight Loss on Ignition method, temperature 500^{o} C for 15 hours (Mullins and Heckendorn 2005)

8.2.2 Statistical analysis

Data were analysed with Analysis of Variance (ANOVA) with the SPSS (version 12; http://www.spss.com/, verified September 2006) and Systat (version 5; http://www.systat.com/, verified September 2006) computer packages. Percentage data were arc-sine transformed before analysis. One-way and three-way fully orthogonal ANOVA designs were used for analysis. Since all factors were considered as "fixed", treatment effects were calculated with reference to the MS residual (error) term (Zar 1999). Post-hoc tests used Bonferonni-corrected probability values.

The contingency table generally followed Zar (1999). However, due to values of zero appearing in all but one of the categories all values were increased by a value of 1.

8.3 Results

8.3.1 Recruitment sites determined in field inspections

The field inspection showed that juveniles of *M. ericifolia* were identified only in reed areas (Table 8.1, Figure 8.2). This classification is, by necessity, fairly wide and covers a range of herbaceous plant genera including *Phragmites*, *Juncus*, *Paspalum* and *Baumea* all of which form hummocks. Within these areas further discrimination could be made between hummocks and sediments, with juveniles only found on herbaceous plant hummocks.

Figure 8.2 View of reed community at the western end of Dowd Morass (see Figure 8.1). Hummocks in the foreground are composed of *Paspalum distichum* (Water Couch), those in the distance *Phragmites australis* (Common Reed). Arrows point to juvenile *M. ericifolia*.

Figure 8.3 Close up of juvenile *M. ericifolia* recruit on a hummock composed of *Juncus krausii* (Sea Rush) at Dowd Morass.

Juvenile *M. ericifolia* plants occurring on hummocks varied in height from 80 cm to 140 cm with only several of the larger plants exhibiting ramet production. Age was determined to be between 12 – 13 years based on averaged annual growth counts (data not shown).

Characterisation of hummocks and surrounding sediments identified significant differences between moisture, salinity and pH levels within the various levels of the hummocks and the surrounding sediments (Figures 8.4 –8.6). Soil moisture, salt and pH in the lower portions of hummocks and immediately adjacent sediment samples were not significantly different (N= 18, P = 0.88. 0.16 and 0.12, respectively). There were significant differences in all three variables between the upper and middle levels of the hummocks (N = 18, P < 0.001) and the middle levels and the hummock bases/sediments (N = 18, P < 0.001).

Salinity concentrations in hummocks overall were below 11 g L^{-1}, but decreased to 8 g L^{-1} in the middle levels of the hummock and averaged about 5 g L^{-1} at the base of the hummocks and in adjacent non-hummock sediments. The middle and lower level of the hummocks were at or below the water surface, and therefore salinity in these levels would have been partially or totally diluted, whereas raised salinity levels of the upper hummock level could have been influenced by capillary transport through evaporation.

There were significant differences in pH between the upper and lower portions of the individual hummocks. The upper portions of hummocks were the only positions that had pH above 5.0; middle portions of the hummocks had a mean pH of 4.0, but

ranged from 3.2 – 5.0. Lower portions of the hummock and sediments had a mean pH of just over 3.0, ranging from 2.3 – 4.0.

The mean moisture content in the two upper portions of the hummocks was 325 – 450% of dry weight. Mean moisture contents were generally less than 100% of dry weight in the hummock bases and in adjacent, non-hummock sediments (Figure 8.4). The very high moisture contents in the middle and upper portions of the hummocks may have been related to the relatively high organic matter content and capillary action of the organic matter. The lower portions of the hummocks and the surrounding substrates had relatively higher levels of mineral earths and consequently lower soil-moisture content.

Table 8.1 Contingency table of potential recruitment sites for *Melaleuca ericifolia* at selected locations at Dowd Morass.

Data	Swamp Scrub	Reeds	Open water/sediment	Total
Hummock	1	44	1	46
Hollow	1	1	1	3
Total	2	45	2	49
Expected				
Hummock	1.88	42.2	1.88	
Hollow	0.122	2.76	0.122	
Chi-square = 14.6				
Degrees of freedom = 2				
Probability = 0.001				

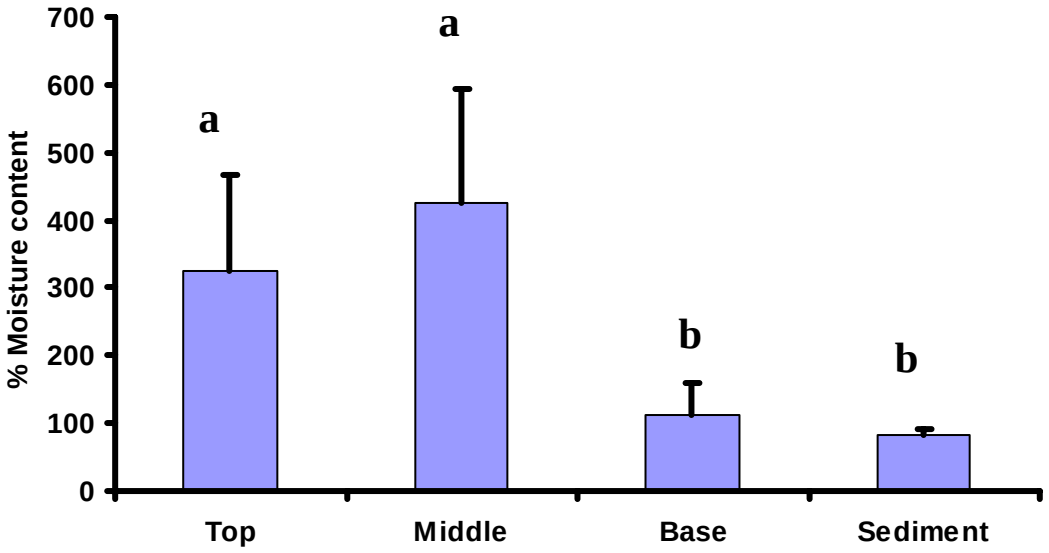

Figure 8.4 Moisture content of substrata at three contrasting positions on hummocks and surrounding sediments in reed beds at Dowd Morass (error bars = Standard Error, n = 18).

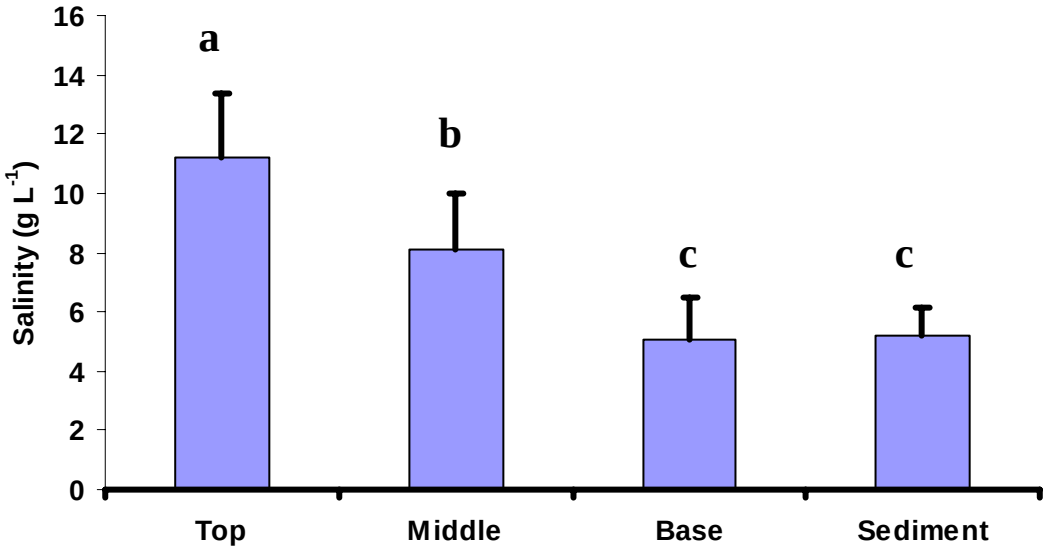

Figure 8.5 Salinity of substrata at three contrasting positions on hummocks and surrounding sediments in reed beds at Dowd Morass (error bars = Standard Error, n = 18).

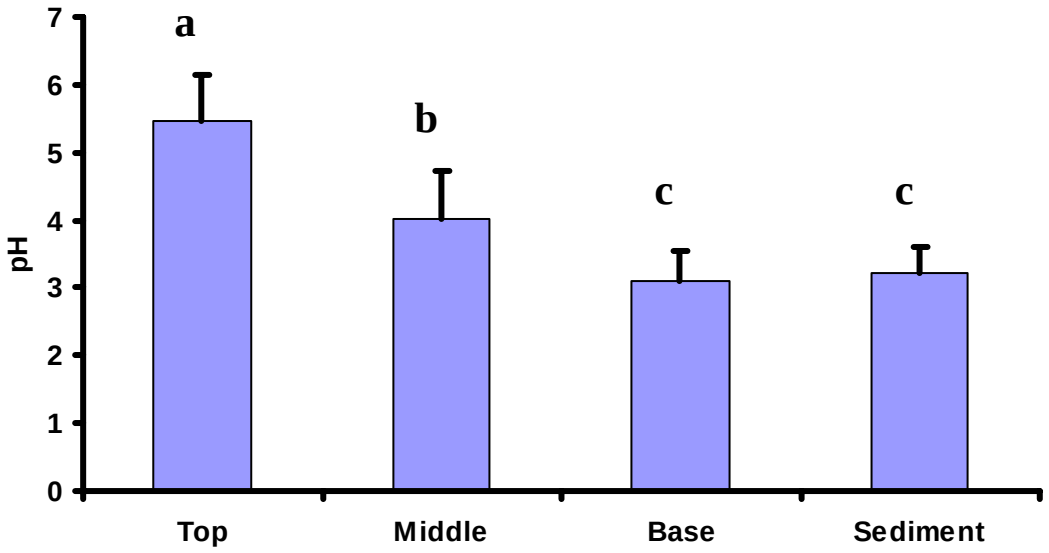

Figure 8.6 pH of substrata at various positions on hummocks and surrounding sediments in reed beds at Dowd Morass (error bars = Standard Error, n = 18).

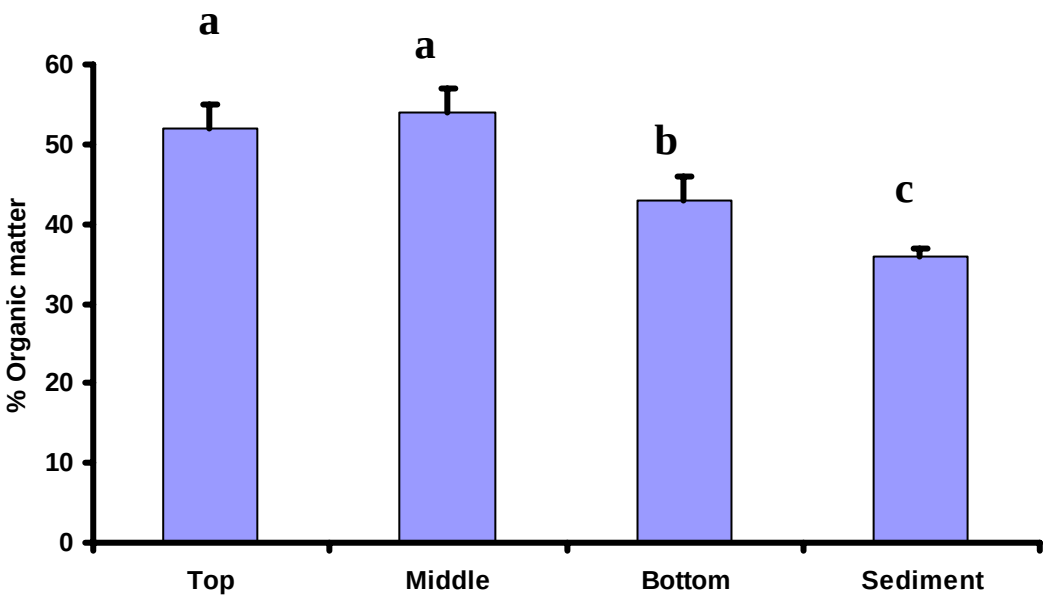

Figure 8.7 Organic matter content of various positions on hummocks and in adjacent sediments in reed beds at Dowd Morass (error bars = Standard Error, n = 18).

8.4 Discussion

8.4.1 Recruitment sites

The distribution of successfully recruited seedlings and juvenile plants of *M. ericifolia* across Dowd Morass was limited to specific vegetation types and specific microtopographic positions on the vegetated hummocks. Spatial and temporal variation in the abiotic conditions of safe sites, such as is found in wetlands that contain hummocks, can lead to habitat segregation based on the germination requirements of different species (Bell and Clarke 2004). Moisture, duration of inundation, salinity and temperature all play a major role in recruitment success as has been shown for several wetland species including *Melaleuca halmaturorum* (Mensforth and Walker 1996; Mineke 2002; Kellogg 2003) and others. The recruitment requirements of *M. ericifolia* are moderate moisture availability and low salinity levels (Chapter 6 and 7). Other species found at Dowd Morass presumably have different recruitment requirements, and therefore can utilize other safe site conditions, leading to strong patterning in the vegetation (Bell and Clarke 2004; Hatton 2004, Salter *et al.* 2007). It is presumed that edaphic conditions and possibly competition with mature *M. ericifolia* precludes establishment of seedlings under existing *M. ericifolia* plants.

Suitable safe sites for *M. ericifolia*, identified in this study, occurred on hummocks formed by the herbaceous plants in the reed community. The present conditions found on these hummocks were not within the range of tolerance for germination and hypocotyl hair formation for *M. ericifolia*. However, established juveniles of *M.*

ericifolia, when age counts were carried out, corresponded to a period in the wetland when conditions were temporarily favourable to recruitment, a situation induced by dilution or flushing of hummock substrates created by above-average rainfall and flooding with fresh water (Chapter 8). This implies that *M. ericifolia*, like many species that occur in brackish-water situations, needs periodic freshwater conditions to recruit.

Hummocks are widely identified as suitable safe sites for wetland plants as they moderate the extreme environmental conditions found in lower strata of wetlands, particularly highly saline conditions, permanent water-logging and acid sulfate soils (Ehrenfeld 1995; Karofeld 1998; Roy *et al*. 1999; Nungesser 2003). Soil pH values identified in this study would most probably be toxic to most plant recruits and adults (Sammut *et al*. 1995). Higher elevations of the hummocks identified in this study, moderated pH values to within a range that is conducive to seedling growth. Higher elevations of hummocks in this study at Dowd Morass, while not appreciably different in salinity levels at the time of sampling, have been shown to have significantly reduced salinity levels when subjected to higher than normal precipitation and/or flooding (Coppolino 2007).

The upper layers of hummocks, which are generally raised above the prevailing water level, provide advantages to seedlings as they are better aerated and provide warmer rooting zones (Roy *et al*. 1999). Both may be critical factors in rapid establishment before unsuitable condition return (Cousens *et al.* 1988; Cornett *et al.* 2000). The suitability of the upper portions of the hummocks for plant growth was clearly demonstrated by Raulings *et al.* (2006) at Dowd Morass, with 90% of *M. ericifolia*

seedlings surviving when planting into the tops of hummocks, while only 10 % of seedlings survived after 3 months in the less favourable lower elevations of the surrounding substrata.

The importance of hummocks to the restoration of wetlands is recognised in several large-scale projects being carried out at Dowd Morass and in wetlands of coastal Louisiana (Conner 2002; Bruland and Richardson 2005; Boon *et al*. 2007). In the case of Dowd Morass, artificial hummocks have been created to determine their potential use in rehabilitation where biotic conditions have altered considerably over time: from freshwater to brackish (Boon *et al*. 2007). In other situations such as at the Hemet/San Jacinto Wetlands in southern California and coastal wetlands in Louisiana, artificial hummocks have been installed in created wetlands to increase the patterning of vegetation in a wastewater treatment system (Bruland and Richardson 2005;Thullen *et al.* 2005).

Thullen *et al*. (2005) recognised a wide range of benefits provided by hummocks, apart from assisting plant recruitment, including: water quality enhancement, mosquito management, enhanced plant decomposition, hydraulic control and the meeting of wildlife management goals. However, the cost of construction of artificial hummocks at Hemet/San Jacinto Wetlands was high and maintaining integrity of created hummocks was problematic. This is likely to be the case with all artificially created hummocks. An additional problem in coastal wetlands is the disturbance of acid sulfate soils and exposure of these sediments to the air, which releases toxic compounds negating the benefits of hummock construction (Boon *et al* 2007).

The benefits associated with the creation of artificial hummocks, particularly microtopographic patterning, may overcome any short-term deleterious effects such as acid sulfate soils by creating suitable safe sites for the colonisation of wetland plants. Re-establishment of hummocks with their attendant influences on substrate conditions may accelerate restoration in artificially created or rehabilitated wetlands (Bruland and Richardson 2005).

Safe sites identified in this study, when combined with data from previous chapters (6, 7 and 8) provide a strong basis for future management of wetlands of the Gippsland Lakes. These data elucidate the specific conditions under which germination and recruitment of *M. ericifolia* is possible. When combined with historical aerial photography and climatic data, predictions can be made of when and where recruitment will take place. A combination of all of this data can, at least in theory, allow land managers to create the conditions under which recruitment and establishment is most likely to succeed.

Chapter 9

General Discussion

The six experimental chapters (Chapters 3-8) in this thesis have investigated various aspects of the growth and germination characteristics of *Melaleuca ericifolia* and the implications of these characteristics for the management of this species in coastal wetlands in southeast Australia.

Genetic studies described in Chapter 3 demonstrated unequivocally the clonal nature of *M. ericifolia* and how clonal growth influences interactions between individual clones (genets) of this species. Genetic studies were combined with historical aerial photography to determine growth rate and longevity of individual genets. Mean lateral growth was comparatively rapid at approximately 0.5 m per year although this rate increased with the age of the plants. Clones occupying areas up to 45 m in diameter were estimated to be approximately 46 - 52 years old. Clonality studies provided background information on the reliance or lack of reliance on seed recruitment in *M. ericifolia*.

Viability, or more precisely germinability, studies were described in Chapter 4. Germinability in the clonal *M. ericifolia* was contrasted with germinability in the non-clonal *M. parvistaminea* to determine whether there was a trade-off between seed germinability and clonality in two sympatric species in the same genus. Germinability was also compared within *M. ericifolia* across most of its range in south-eastern

Australia in relation to population size and human-induced alterations of habitat quality, namely fragmentation and secondary salinisation.

The influence of salinity, temperature and light on germination of the seed of *M. ericifolia* was described in Chapter 5. Hypocotyl hairs, a little investigated feature of newly germinated seedlings, were discovered serendipitously during germination trials in Chapter 5 and further investigated in Chapter 6, particularly in relation to their sensitivity to salinity, temperature and light.

Potential and actual safe sites for germination and recruitment events were investigated in Chapters 7 and 8 using a range of techniques including historical aerial photography, historical climate data, on-ground investigation of identified germination sites and comparison of this data with germination and hypocotyl hair data determined in previous chapters.

A more detailed description of the key findings for each of these components is provided in the following sections.

9.1 Clonality in M. ericifolia

It was critical to identify the extent of clonality and the interaction of individual genets of this species to fully understand the population structure and dynamics of *M. ericifolia*. No previous studies had identified the extent of individual clones of *M. ericifolia* but had, in some instances, made limited comment that the species was clonal (e.g. Jeans 1996; Craven and Lepschi 1999; Holliday 2004). Several other wetland species in south-eastern Australia, most notably *Casuarina*/*Allocasuarina* species and other *Melaleuca* species have been identified as being clonal but the extent of their clonality has not been investigated (Carter et al. 2006a; Carter et al. 2006b). A mixture of laboratory-based molecular biology approaches was complemented by an analysis of aerial photographs to demonstrate the extent of clonality in *M. ericifolia*.

The use of the genetic analysis technique, inter-simple sequence repeats was particularly useful. Inter-simple sequence repeats can be used when there is little available information on the genome of the species (Zietkiewicz et al. 1994) and allow the investigator to access variation in numerous microsatellite regions dispersed throughout the plant genome.

Individual clumps identified visually in the field by their characteristic dome shape (Chapter 3, Figure 3.2) were confirmed to be individual genets (plants) on the basis of the molecular analysis. Of particular note was the lack of intermingling of individual genets. Lack of intermingling is a characteristic of the phalanx mode of clonal growth (Lovett Doust 1981), in which the plant produces short and frequently branched

connections between ramets. The phalanx mode of growth is thought to be an adaptation to low-nutrient, high-light habitats with a high degree of spatial and temporal heterogeneity for other environmental conditions such as water regime and salinity (Van Groenendael et al. 1997; Kleign and van Groenendael 1999).

The interconnectedness of the individual ramets, clearly illustrated in Chapter 3 (Figure 3.1), allows for the sharing of water and nutrients allowing the genet as a whole to have wider ecological amplitude than plants that lack the clonal ability. Extensive clonality, as identified in a range of other wetland species and in *M. ericifolia*, presumably allows the individual genet to effectively capture limited resources and increase competitive ability (Rea and Ganf 1994; Barsoum 2002; Peltzer 2002).

The use of aerial photographs, coupled with genetic studies as utilised in Chapter 3, allowed ageing of individual clones and the determination of their extent and growth rates. The eighteen clones identified in Chapter 4 were approximately 46 years old but could have been as old as 52 years old based on the data shown in Chapter 7. Individual genets of *M. ericifolia* ranged in size from 1,174 to 3,274 m^2 which is considerably larger than has been recorded in other Australian and overseas Myrtaceae, (19 m^2 - 530 m^2) but smaller than some clonal species from other families: *Lomatia tasmanica* (1.2 km wide, Lynch *et al.* 1998), *Pteridium aquilinum* (1.2 km wide, Parks and Werth 1993), *Zostera marina* (6,400 m^2, Reusch *et al.* 1999) and *Populus tremuloides* (43.3 ha, Kemperman and Barnes 1976). Additional plants of *M. ericifolia* identified from locations close to Dowd Morass, but not specifically studied, were nearly twice as large (40 – 60 m wide) and were estimated to be between 80 and

100 years old. Growth rates of the various clones studied varied between 3.5 and 9 m^2 yr^{-1}, an approximate lateral spread of ~0.5 m yr^{-1}.

9.2 Trade-off between sexual and asexual recruitment and impacts on germinability

Plants can be loosely categorised into two broad groups according to regeneration strategy: obligate seed regenerators and rootstock regenerators (Lamont and Wiens 2003). There is commonly a trade-off between sexual reproduction and vegetative reproduction because of the high energenic costs of seed production (Lovett Doust 1989). The diversion of resources into vegetative growth would by necessity reduce sexual output; in contrast a limited opportunity for sexual reproduction could be offset by greater probability of reproduction by vegetative means.

There is a complete range of regeneration strategies within the genus *Melaleuca*, ranging from fully obligate seed regenerators (*M. parvistaminea*), rootstock regenerating species (*M. halmaturorum, M. quinquenervia*) to strongly regenerating clonal species (*M. cajaputi, M. ericifolia*). *Melaleuca parvistaminea* and *M. ericifolia* are largely sympatric but segregated within individual wetlands: *M. parvistaminea* occurs on rarely inundated areas whereas *M. ericifolia* more in regularly inundated areas. The co-occurrence of these two species in several wetlands in southern Australia provided a unique opportunity to determine if there was in fact reduced viability in the rootstock regenerating species *M. ericifolia*.

Viability of *M. ericifolia* seed was markedly lower, ranging from 0 – 38% across the species range in Victoria and Tasmania, whereas the viability of *M. parvistaminea* seed was much higher and ranged between 70 – 80%. The finding of reduced viability

in *M. ericifolia* is consistent with other studies of species pairs that found reduced viability in the clonal species. There were significant differences in seed weight with *M. ericifolia* ranging between 15-29 µg per seed while *M. parvistaminea* produced larger seeds, ranging between 31-33 µg per seed.

Viability within *M. ericifolia* was related also to population size, distance to nearest population and disturbance. Populations bigger than about than 50 ha generally had the greatest seed viability (23 – 38 %), whereas those below 5 ha generally showed very low viability (< 10 %). Exceptionally, the largest population at Dowd Morass (> 1,000 ha) had very low viability (6 %). Cades Road (Victoria) and The Apsley Marshes (Tasmania), both about 50 ha, exhibited viabilities of 13 and 14%, respectively. On further investigation of these three sites it was found that major alterations to water regime and accompanying secondary salinisation had occurred in all, and these environmental factors/disturbances were probably responsible for this lowered viability. Prior to European settlement all three of the above sites were freshwater swamps, but due to human interference have salinities that range up to approximately 20 g L^{-1} or more.

9.3 Environmental requirements for seedling establishment

9.3.1 Germination

The germination requirements of *M. ericifolia* are poorly understood, despite the fact that this species is widespread and abundant in southern Australia and its priority listing for rehabilitation. *Melaleuca ericifolia* occur in a range of freshwater swamps and estuarine situations, many of which have been altered by human settlement (Bowkett and Kirkpatrick 2003). Several key environmental parameters, which may act individually or synergistically, were investigated to better understand germination.

Seed of *M. ericifolia* is held in woody capsules on the plant and released after death of the branch from a range of factors, particularly fire, wind throw and damage by roosting colonies of birds, particularly ibis. Small quantities of seed are released throughout the year. Once seed is released from the seed capsules viability rapidly decreases with little or no germination after one year (Salter *pers. comm.*).

The ideal conditions for germination in *M. ericifolia* are on the surface of the substratum but in darkness with temperatures of ~20^{o}C and salinity of < 2 g L^{-1}. Salinity was found to be the strongest influence on germination, its effect becoming more pronounced under temperatures of 30^{o}C. The adverse effect of salinity was greatly reduced if seed was subsequently flushed by fresh water.

9.3.2 Hypocotyl hairs

A second aspect of the successful recruitment of *M. ericifolia* plants is seedling survival. Many studies have examined the germination of wetland plant seed, but very few the factors that influence the subsequent establishment of the young seedlings. Hypocotyl hairs were shown to be a critical element in successful seedling establishment. Hypocotyl hairs (single celled outgrowth from the base of the hypocotyl not associated with the true root system) occur in a range of plant species, especially aquatic monocotyledons. The identification of hypocotyl hairs in *M. ericifolia* in this study is the first time such structures have been identified in this genus. Hypocotyl hairs develop in very young seedlings, usually before the true root system, and help to anchor seedlings to the substratum. Hypocotyl hairs are essential in the recruitment process of the few species in which they have been identified; those seedlings not producing them failing to recruit (Aronne and De Micco, 20004), and it is likely that a similar stricture holds for *M. ericifolia*.

The sensitivity of hypocotyl hairs to a range of environmental conditions both before and after germination dictates their formation and subsequently the success of recruitment. Soaking of seeds inhibited the formation of hypocotyl hairs whereas more moderate levels of moisture availability in the substrate allowed for full development of hypocotyl hairs. Rapid imbibition of seed was found to rip the testa in the seed of other woody wetland species particularly *Populus* (Polya 1961).

Salinity, light and temperature all had significant individual effects on the formation of hypocotyl hairs. Salinity exhibited the strongest effect, with salinities greater than

1 g L^{-1} strongly inhibiting hypocotyl hair formation. Exposure to light inhibited hypocotyl hair development, as did higher temperatures. These last two factors increased moisture stress, quickly desiccating the single-celled hypocotyl hairs. The synergistic effect of combining salinity temperature and light was pronounced and highly detrimental to the formation of hypocotyl hairs.

Successful recruitment of *M. ericifolia* from seed is strongly dependent on the formation of hypocotyl hairs, which allow the seedlings to anchor to the substratum and establish positive geotropism. Seedlings of *M. ericifolia* that did not form hypocotyl hairs, or those which the connection of the hypocotyl hairs to the substratum was broken, failed to establish. The environmental sieves created by salinity, light and temperature exhibit strong influence on germination and hypocotyl hair formation in *M. ericifolia*. For *M. ericifolia* to successfully recruit, a specific combination of environmental factors is needed, namely surface germination in dark situations, approximately 20^{o}C, salinity levels below 1 – 2 g L^{-1} and moderate moisture levels in the substratum. These conditions are not normally found in the wetlands in which *M. ericifolia* grows, leading to the conclusion that recruitment events must be rare and episodic.

9.4 Safe sites for germination in M. ericifolia

Three main characteristics have been identified in *M ericifolia*; a strongly clonal growth form, low fecundity, and specific recruitment requirements of seed and seedling. Low fecundity, coupled with specific recruitment requirements and limited availability of safe sites, suggests strongly that these factors are the main evolutionary driver of clonal growth in this species. Limited recruitment opportunities, sometimes only occurring only every few decades, is well known in woody wetland species throughout the world (Eriksson and Froborg 1996; Conner 2002; Gengerelly and Lee 2005; Stokes and Cunningham 2006).

Despite the fragility of conditions required for sexual recruitment, the adults of *M. ericifolia* are tolerant of a wide range of environmental conditions, a trait that is strengthened by the extensive clonal growth form. From the studies carried out in Chapters 5 and 6, it is evident that germination and particularly the formation of hypocotyl hairs occurs within a relatively narrow set of temporal and spatial conditions.

9.4.1 Temporal requirements: climatic conditions

Historical aerial photographs are commonly used to map changes to vegetation cover over time (Williams and Lyon 1997; Kadmon and Harari-Kremer 1999; Herwitz et al. 2000; Fensham et al. 2003; Fensham and Fairfax 2002), and it proved especially useful for detecting cohorts of recruits at Dowd Morass. Using aerial photographs,

four successful recruitment events were tentatively detected in the 1950's, 1970's and 1990's. No other years in this 63 year-long period of analysis seemed suitable for *M. ericifolia* recruitment.

Analysis of climatic data (rainfall and temperature) over the period of 63 years indicated that four years experienced markedly different weather from the norm (1950, 1951, 1974 and 1993). While monthly temperatures varied little over the period studied yearly and monthly rainfall data varied considerably. Yearly rainfall totals varied between 285 and 970 mm, monthly totals between 5 and 200 mm. For the years in which recruitment putatively occurred, monthly rainfall totals in the critical months of August to October and March to April were significantly higher than average. In general they were four times the long-term monthly average. As well as this period of high rainfall, average or below average rainfall for November to February were the norms for these four years. In effect these two rainfall patterns suggest a flood/flushing event followed by a period of stability; followed by another flood/flushing event is required for successful recruitment of *M. ericifolia* from seed. This convergence of rainfall events is critical to understanding recruitment in the salinised wetlands in southeast Australia, such as those of the Gippsland Lakes.

9.4.2 Spatial requirements: the importance of hummocks

Recording on-ground recruitment via field inspections, coupled with growth data of juvenile *M. ericifolia*, suggested strongly that the juveniles had recruited during one of the few events predicted from the analysis of the climate data. Juveniles of *M. ericifolia* were recorded only on the tops of hummocks composed of herbaceous

plants such as *Phragmites*, *Juncus*, *Paspalum* and *Baumea*. The tops of these hummocks are raised above the normal water level of the swamp and are composed almost entirely of organic matter. They provide the suitably moist, dark, low-salinity conditions need for germination and recruitment. These hummocks, however, are not always favourable to germination and recruitment as salinity in the upper portions of the hummock can become very high (e.g. > 20 g L^{-1}). The flooding that occurs in critical years, especially during months of particularly heavy rain, presumably flush the salts out of the upper levels and dilute salinity in the surrounding waters, bringing the conditions within the range for successful recruitment. Work carried out on the hummocks at Dowd Morass by Coppolino (2007) strongly indicated that elevated precipitation and/or flooding modified salinity and moisture conditions within the upper portions of the hummocks to within a range suitable for germination and establishment of *M. ericifolia*.

9.5 Implications of plant and germination characteristics for management of brackish wetlands

The material summarised in earlier chapters shows clearly that natural recruitment of *M. ericifolia* is closely linked to specific climatic conditions: flood in periods outside ideal recruitment periods, average rainfall for 12-16 weeks during ideal temperature periods (day:night, 20:12^{o} C) and low salinity (< 2 g L^{-1}) within the preferred recruitment sites (dark, shaded by standing organic matter on tops of hummocks).

Manipulating the environment to provide ideal on-ground recruitment conditions is likely to be beyond the abilities of land managers. Alterations of stream flows, particularly to the LaTrobe River and water regimes in the wetlands of the Gippsland lakes (Dowd Morass, Heart Morass) has seen an overall decrease of potential flushing events and an inexorable increase in overall salinity due to the artificial opening to the sea at Lakes Entrance (Bird 1962; Grayson 2003). Mean and peak flows in these same wetlands and the rivers that feed them have been modified by the various impoundments (Boon *et al.* 2007). To achieve periodic flushing of the wetlands surrounding the Gippsland lakes, at least during peak flow periods, alteration to present environmental flow would need to be made. Such flows are largely over-bank flows, and thus beyond the aegis of day-to-day catchment management.

There are future plans by the DSE to increase environmental flows in the Thompson, Latrobe and Avon Rivers that feed into the Gippsland Lakes: the first two supplying

fresh water to Dowd Morass (WGCMA 2006). One of the concerns is to ensure the flushing and subsequent dilution of salinity in the various wetlands of this region (West Gippsland CMA 2006) to restore more natural hydrological regimes and restore biodiversity values. Re-instating environmental flows, especially during peak flow periods, is likely to approximate the natural flushing regimes and overall water regimes of the wetlands of the Gippsland Lakes prior to water impoundments and manipulation measures installed in the past century.

Landscape-scale recruitment of *M. ericifolia* from seed is reliant on natural flood pulsing to create the specific on-ground conditions for germination, hypocotyl hair formation and early-stage seedling establishment. Increased environmental flows and their effect on the hydrologic and edaphic conditions in the wetlands would have widespread benefits to both *M. ericifolia* and a range of other species dependent on these processes for recruitment and regeneration.

Large-scale restoration of wetlands is generally based on the "self-design" method. Self-design assumes that restoration of key abiotic factors, such as hydrological regime, will lead to restoration of the plant and animal communities that formerly occurred in the restored wetland (Mitsch *et al.* 1998). Other restoration ecologists propose that many wetlands have become so damaged that there is the need to actually 'design' restoration, including plantings and reintroduction of animals (Montalvo *et al*. 2002). There are many examples of highly damaged wetlands that lack vital elements, including keystone plant species and have suffered from a range of non-hydrological changes such as exotic flora and fauna (Pettit and Froend 2001; De Steven et al 2006). These damaged wetlands need some intervention other than

just re-instatement of water regime to achieve successful outcomes. Dowd Morass is a wetland that has changed significantly from its original configuration and there is little hope of fully restoring a hydrological regime that is similar to the pre-European pattern. Intervention in the form of planting or direct seeding of the keystone species may need to be carried out to achieve or maintain wetland function at Dowd Morass.

Small to medium size rehabilitation of Dowd Morass and other similar wetlands throughout south-eastern Australia can be informed by the safe sites for recruitment identified in this study. Other species of *Melaleuca*, e.g. *M. quinquinervia*, *M. halmaturorum*, have broadly similar requirements to *M. ericifolia* extending the possible relevance of this study to most of Australia and the introduced range of these species overseas (Florida, Hawaii). Replanting trials carried out by the author and others (Raulings *et al.* 2006) clearly indicate that planting on sediments was not successful, with most planted stock not surviving for more than 4 months. A small preliminary trial carried out by Raulings *et al*. (2006) where seedlings were planted on hummocks and the adjacent surrounding sediments showed that nearly all seedlings planted on hummocks lived while the seedlings planted in the surrounding sediments died. Ninety percent of seedlings planted on hummocks were still alive as of January 2007, over two years after initial planting, with many putting on over 1 m of growth in that time.

The planting of brackish wetland species on natural and artificially created hummocks is widely used in the coastal wetlands of the USA for *Taxodium distichum* (Bald Cypress) and a range of other species (e.g., Clewell and Lea 1990; Titus 1990; Barry *et al.* 1996; Anon 2004; Bruland 2005) The specific hydrologic and edaphic

conditions found on hummocks are conducive to the establishment of a wide range of species and contribute to overall diversity of wetlands by providing microtopographic diversity (Vivian-Smith 1997; Bruland and Richardson 2005).

The unique combination of factors investigated in this study provides a framework for future work on clonal plants in a wide range of ecosystems, especially wetlands. Two such ecosystems in Australia where this thesis is of particular relevance are *Melaleuca* forests and woodlands (90,513 km^2) and Hummock Grasslands (1,756,104 km^2) (National Land and Water Resources Audit 2001). Of particular note was the ability in this thesis to identify the specific edaphic conditions for germination and linking these with microtopography and climatic influences. The identification of extensive clonality, a feature common to many plants in a wide range of ecosystems in Australia, contributes significantly to our understanding of the vegetation dynamics of these systems. The lack of reliance of clonal plants on sexual reproduction reduces the imperative to ensure short-term sexual recruitment particularly in modified ecosystems. Clonality allows a land manager to adjust or modify on-ground conditions (e.g. water regime, salinity, topographical relief) relatively infrequently to ensure sexual recruitment and therefore the maintenance of genetic diversity within a population and sustainability of the species into the future.

Of particular note is the need to adopt a holistic approach to the study of the life cycle of species to achieve effective management. Wide-ranging and primary research into the recruitment dynamics of a species, as outlined in this project, can uncover previously unobserved factors that may be critical to management of a species (e.g. hypocotyl hairs). Partial or assumed knowledge of a species life attributes or

behaviours in relation to various edaphic conditions can lead to flawed management and lack of successful management outcome.

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