The Dispersal and Establishment of *Ammophila arenaria* from Rhizomes

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Abstract

This thesis investigates the potential for a sand-dune plant, *Ammophila arenaria* (L.) Link (marram grass, European beach grass) to disperse and establish new colonies from pieces of its rhizome system. Such dispersal threatens the natural character of temperate dune-systems globally. Four questions were examined: 1) how readily does *A. arenaria* regenerate from rhizome fragments; 2) how many propagules does the rhizome system of *A. arenaria* produce; 3) how far can rhizomes be dispersed; and 4) how does the stranding environment affect establishment? These questions were addressed through a series of comparative glasshouse-based experiments and empirical field-based measurements.

The regenerative potential of *A. arenaria* rhizomes was assessed by measuring the ability of fragments of rhizome to produce tillers under ideal growing conditions, and following exposure to abiotic stress (salinity, desiccation, burial). The density of dormant meristems (the bud bank) of *A. arenaria* was sampled at three dune-systems in southern New Zealand to determine propagule density. The distance that *A. arenaria* can disperse was inferred by determining the time that a fragment of rhizome could remain both buoyant and viable when in seawater. To examine the effect of the stranding environment on establishment, the effect of increasing levels of abiotic stress on tiller survival was measured. The focus in all components of this thesis was on identifying those biological aspects relating to the reproduction of *A. arenaria* from rhizome that may limit establishment, or make establishment more successful in one environment compared to another.

*A. arenaria* regenerates readily from fragments of rhizome. Between 45 – 100% of fragments were viable. A decrease in fragment length, and obtaining fragments from the horizontal rhizome system, from low-vigour populations, and during spring/summer months was correlated with a reduction in fragment viability. The bud bank of *A. arenaria* is large — of the order of 10² to 10³ buds m⁻³ depending on annual rates of sand accretion, the population vigour, and the height of the foredune. The rhizomes of *A. arenaria* are capable of being dispersed over long periods of time— they remain
buoyant and viable for at least 70 days. An increase in water temperature and a decrease in node number were correlated with a decrease in dispersability. Finally, *A. arenaria* possesses a high tolerance to most of the stresses it would experience when establishing from stranded rhizome on the back-beach. Of those examined, only desiccation and wave activity are likely to regularly limit establishment.

The principal factors limiting dispersal appear to be extrinsic variables relating to the processes involved in the transportation and stranding of rhizome stranding of rhizomes in a suitable location for growth, rather than the inability of fragments to regenerate. It was concluded that *A. arenaria* produces large numbers of propagules capable of dispersing over long distances. The dispersal of rhizomes in the sea allows *A. arenaria* to invade remote dune-systems considerable distances from existing populations. Few dune-systems are likely to be sufficiently remote that *A. arenaria* cannot arrive. Managing the spread of *A. arenaria* from marine-dispersed rhizomes is now critical for dune conservation in many regions.
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Table of Contents

Abstract i
Acknowledgements iii
Table of Contents iv
List of Figures viii
List of Tables xi

Chapter 1: General introduction 1

1.1 The vegetative regeneration of coastal plants 1
1.2 Impetus for the present study 2
1.3 Research questions 6
1.4 Thesis structure 8

Chapter 2: Intrinsic regenerative potential of Ammophila arenaria rhizomes 10

2.1 Introduction 10
2.2 Methods 12
   2.2.1 Sampling site 12
   2.2.2 Rhizome sampling and preparation 13
   2.2.3 Regeneration test 14
   2.2.4 Data analysis 15
2.3 Results 16
   2.3.1 Rhizome type — horizontal vs. vertical rhizomes 17
   2.3.2 Extraction depth 21
   2.3.3 Fragment length 21
   2.3.4 Multiple nodes 22
   2.3.5 Sampling month and population vigour 23
2.4 Discussion 24
Chapter 3: The *Ammophila arenaria* bud bank

3.1 Introduction

3.2 Methods

3.2.1 Sampling sites

3.2.2 Sampling procedure

3.2.3 Data analysis

3.3 Results

3.3.1 Bud bank comparisons between sites

3.3.2 Relationship between rhizome density, bud-site density and the proportion of bud-sites with a bud, and bud density

3.3.3 Vertical distribution of the bud bank

3.3.4 Relationship between rhizome density and sand deposition

3.3.5 Relationship between node density and sand deposition

3.4 Discussion

3.4.1 Rhizome density

3.4.2 The density of bud-sites

3.4.3 The proportion of buds to nodes

3.5 Conclusions

Chapter 4: The hydrochoric potential of *Ammophila arenaria* rhizomes

4.1 Introduction

4.2 Methods

4.2.1 Sampling site

4.2.2 Regeneration test

4.2.3 Buoyancy test

4.2.4 Data analysis

4.3 Results

4.3.1 Results of the preliminary regeneration test
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.2</td>
<td>Relationship between population vigour and the season of extraction, and regeneration</td>
<td>68</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Relationship between seawater temperature and regeneration</td>
<td>72</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Rhizome buoyancy</td>
<td>73</td>
</tr>
<tr>
<td>4.3.5</td>
<td>The dispersal time of <em>A. arenaria</em></td>
<td>74</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion</td>
<td>79</td>
</tr>
<tr>
<td>4.4.1</td>
<td>The effect of immersion on the regeneration potential of rhizomes</td>
<td>80</td>
</tr>
<tr>
<td>4.4.2</td>
<td>The buoyancy of <em>A. arenaria</em> in seawater</td>
<td>82</td>
</tr>
<tr>
<td>4.5</td>
<td>Concluding remarks</td>
<td>84</td>
</tr>
</tbody>
</table>

**Chapter 5: The tolerance of *Ammophila arenaria* to the sandy back-beach environment**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>85</td>
</tr>
<tr>
<td>5.2</td>
<td>Methods</td>
<td>86</td>
</tr>
<tr>
<td>5.2.1</td>
<td>The effect of environmental stress on fragment regeneration</td>
<td>86</td>
</tr>
<tr>
<td>5.2.2</td>
<td>The effect of environmental stress on tiller survival — glasshouse experiments</td>
<td>90</td>
</tr>
<tr>
<td>5.2.3</td>
<td>The effect of environmental stress on tiller survival — field observations</td>
<td>94</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Data analysis</td>
<td>95</td>
</tr>
<tr>
<td>5.3</td>
<td>Results</td>
<td>96</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Fragment regeneration</td>
<td>96</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Tiller survival in the glasshouse</td>
<td>103</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Tiller survival in the field</td>
<td>107</td>
</tr>
<tr>
<td>5.4</td>
<td>Discussion</td>
<td>111</td>
</tr>
<tr>
<td>5.4.1</td>
<td>The effect of environmental stress on fragment regeneration</td>
<td>111</td>
</tr>
<tr>
<td>5.4.2</td>
<td>The effect of environmental stress on tiller survival</td>
<td>113</td>
</tr>
<tr>
<td>5.4.3</td>
<td>Other observations</td>
<td>116</td>
</tr>
<tr>
<td>5.5</td>
<td>Concluding remarks</td>
<td>117</td>
</tr>
</tbody>
</table>
Chapter 6: General Discussion and Conclusions

6.1 Introduction

6.2 Research questions

6.2.1 How readily does *A. arenaria* regenerate from rhizomes?

6.2.2 How many propagules does the rhizome system of *A. arenaria* produce?

6.2.3 How far can the rhizomes of *A. arenaria* be dispersed?

6.2.4 How does the stranding environment affect establishment?

6.3 Applicability of the present study to other populations

6.4 The establishment of *A. arenaria* from rhizome fragments

6.5 Implications of the present study for the management and control of *A. arenaria*

6.6 Concluding remarks

References

Appendices

Appendix 1. Detailed ANOVA analysis

Appendix 2. Detailed Mann-whitney analysis

Appendix 3. Detailed binary logistic regression analysis
# List of Figures

## Chapter 1

| Figure 1.1 | Distribution of *A. arenaria* in New Zealand | 4 |
| Figure 1.2 | Comparison of dunes vegetated with and without *A. arenaria*. | 5 |

## Chapter 2

| Figure 2.1 | Morphology of an *A. arenaria* rhizome fragment | 11 |
| Figure 2.2 | Location of the sites of rhizome collection. | 12 |
| Figure 2.3 | The cumulative percentage emergence of *A. arenaria* from rhizome. | 18 |
| Figure 2.4 | Comparison of tiller emergence times. | 19 |
| Figure 2.5 | Comparison of tiller growth rates. | 20 |
| Figure 2.6 | Relationship between fragment length and weight | 22 |

## Chapter 3

| Figure 3.1 | Location of sampling sites. | 34 |
| Figure 3.2 | Cross section of the sampling sites showing dune morphology. | 35 |
| Figure 3.3 | Relationship between bud density and rhizome weight. | 44 |
| Figure 3.4 | Vertical distribution of the bud bank and rhizome mass at Allans Beach. | 46 |
| Figure 3.5 | Vertical distribution of the bud bank and rhizome mass at Masons Bay. | 47 |
| Figure 3.6 | Vertical distribution of the bud bank and rhizome mass at Chrystalls Beach. | 48 |
| Figure 3.7 | Relationship between rhizome density and sand accumulation. | 49 |
| Figure 3.8 | Relationship between foredune height and rhizome density. | 50 |
| Figure 3.9 | Relationship between rhizome density and dune age. | 50 |
| Figure 3.10 | Relationship between node density and sand deposition. | 51 |
Figure 3.11  Rhizome density of *A. baltica* as measured by Wallen (1980).  55

Chapter 4

**Figure 4.1**  The effect of sampling month on the viability of *A. arenaria* following immersion in seawater.  67

**Figure 4.2**  The effect of population vigour and season of extraction on viability following immersion.  68

**Figure 4.3**  The effect of seawater temperature on viability following immersion.  72

**Figure 4.4**  The effect of node number on fragment buoyancy.  74

**Figure 4.5**  Comparison between the predicted values obtained via binary regression and the measured values.  77

**Figure 4.6**  The probability that a fragment remains both buoyant and viable when in seawater.  78

Chapter 5

Location of the sites of rhizome collection and field study sites.  87

Photo showing rhizome deposited during a July 2007 stranding event.  95

The cumulative emergence of tillers from rhizome buried at increasing depth.  98

Tiller growth rates from rhizome buried at increasing depth.  98

Relationship between rhizome weight and tiller emergence from depth.  99

The effect of rhizome desiccation on fragment viability and moisture content.  101

The effect of rhizome desiccation on tiller emergence times.  102

The effect of rhizome desiccation on tiller growth rates.  102

Tiller survival following burial.  103

Tiller survival following desiccation.  104
Tiller survival following a simulated wave overwash event. 106

Photos showing the distribution of tillers from stranded rhizome at Allans beach 109

Terrain models showing tillers survival at two study sites from February 2007 to November 2010. 110
List of Tables

Chapter 2
Table 2.1 Comparison of total bud viability between multi-noded fragments. 23

Chapter 3
Table 3.1 The number of buds, nodes and rhizome obtained between sites. 42
Table 3.2 Comparison of node density and proportion of nodes with buds between sites 44
Table 3.3 Relationship between node density and the proportion of nodes with buds with depth. 47

Chapter 4
Table 4.1 Previous studies on the hydrochoric potential of A. arenaria 61
Table 4.2 The effect of sampling month and the vigour of the source population on tiller emergence time and growth rates. 70
Table 4.3 The effect of seawater temperature on tiller emergence time and growth rates. 71

Chapter 5
Table 5.1 The effect of fragment weight on the emergence ability of tillers from rhizome buried at increasing depth. 99
Chapter 1

General introduction

1.1 The vegetative regeneration of coastal plants

The dispersal of vegetative propagules, typically pieces of rhizome or stolon possessing dormant meristems plays an important role in the colonisation of sandy beaches by perennial foredune plants (Maun, 2009). Extensive rhizome and stolon systems are characteristic of the sand-binding plants of the foredune environment. These systems allow these plants to tolerate large amounts of burial through either the vertical extension of rhizomes or activation of dormant buds when buried, facilitate population expansion through vegetative spread, and serve as reservoir and transportation conduit for stored resources aiding population persistence and recovery post disturbance (Suzuki and Stuefer, 1999; Maun, 2009). These systems also have the potential to act as independent propagules when disconnected from the parent plant allowing for the establishment of new colonies. This potential for the rhizomes and stolons of foredune plants to act as independent propagules is the focus of the present study.

Colonisation from vegetative rather than sexual propagules is advantageous for several reasons. Plants produced from vegetative fragments grow faster, display higher survival rates, and reach maturity faster than those from seeds (Fenner, 1985; Maun, 2009). Vegetative reproduction offers a simple means of spread and reproduction without the complicated processes involving flowering and seed production (Duke, 1985). In addition, reproduction from vegetative propagules allows plants to emerge from greater depths compared with seeds (Harris and Davy, 1983a; Duke, 1985). The ability to emerge from depth is essential for coastal plants. Deep burial is a primary cause of seed mortality in the foredune environment (Maun, 2009). There are disadvantages associated with vegetative propagules. The genetic make-up of the progeny is identical to that of the parent increasing the consequences of disease and insect infestation, they lack the dispersability of seeds, and are vulnerable to desiccation (Duke, 1985; Maun, 2009). Overall, however, regeneration and establishment is more likely to be successful from vegetative fragments than from seeds (Maun, 2009).
In general, the colonisation of sandy coasts by the vegetative propagules of foredune plants is well understood. Their rhizome systems are fragmented during episodes of dune erosion by storm waves, are transported by waves and surface currents, and are then stranded by wave activity on the back-beach. If conditions are favourable and the buds remain viable following dispersal, the fragments may regenerate. However, little is known about the ability of individual species to disperse and establish from vegetative fragments. The role of vegetative fragments in the reproductive ecology of dune plants has been explicitly recognised and examined for only a handful of species (e.g., *Ammophila breviligulata* (Maun, 1984; Maun, 1985); *Calamovilfa longifolia* (Maun, 1985); *Thinopyrum junceiforme* (Harris and Davy, 1986a and b); *Panicum racemosum* (Cordazzo and Davy, 1999); *Blutaparon portulacoides* (Cordazzo and Seeliger, 2003)), although several other studies have examined traits relevant to vegetative reproduction as part of a wider focus (e.g., dispersal potential (Aptekar and Rejmánek, 2000; Knevel, 2001)). It is apparent from the existing literature that the establishment potential of vegetative fragments varies from species to species, even though certain processes (fragmentation by wave activity; dispersal in water) are shared. Colonisation of the sandy backshore is highly species specific. This thesis, therefore, focuses on understanding the establishment of one species, *Ammophila arenaria* (L.) Link (marram grass, European beach grass), from fragments of rhizome, following dispersal by waves and surface currents.

1.2 Impetus for the present study

*A. arenaria* is a perennial, rhizomatous grass associated with the foredune and mobile dune habitats (Huiskes, 1979; Doing, 1984). It requires a temperate climate and sandy substrate for growth. The vigour of *A. arenaria* is dependent on ongoing burial by sand (Huiskes, 1979). Burial promotes flowering, increases tiller density and enhances clonal growth (Marshall, 1965; Huiskes, 1979; Baye, 1990). *A. arenaria* can persist on sites with minimal sand accretion but vigour is greatly reduced (Marshall, 1965; Huiskes, 1979). Other growth requirements of *A. arenaria* include well aerated soils containing low amounts of organic matter at temperatures from 10 – 40°C, a persistent substrate with less than 1% salinity, a pH from 4.5 – 9.0, and a climate with moderate rainfall and
without long drought periods (Ranwell, 1958; Huiskes, 1979). These conditions, however, are typical of foredune and mobile dunes on temperate coasts. Overall A. arenaria has few specialist growth requirements. Provided a coastline lies within the latitudinal limits of A. arenaria (30 – 63° Lat) and experiences some sand mobility, A. arenaria has the potential to establish.

A. arenaria is a ubiquitous component of the temperate dune flora globally (Huiskes, 1979; Wiedemann and Pickart, 2004). Native to the west coast of Europe, between Norway and the Mediterranean Sea, it was deliberately introduced to a number of countries, including the west coast of North America, South Africa, Chile, south-east Australia and New Zealand, for the purpose of dune stabilisation (Hertling and Lubke, 1999; Wiedemann and Pickart, 2004; Hilton et al., 2006; Hilton, 2006). A. arenaria possesses several attributes which make it more effective at stabilising mobile dune-systems than the indigenous vegetation: principally a unique ability to produce virtually unlimited quantities of both vertical and horizontally spreading rhizomes and an exceptional tolerance to sand burial. Because of these attributes A. arenaria is able to form large colonies rapidly in environments where excessive sand mobility would prevent the growth of most other sand-binding species. As a result, A. arenaria has been systematically planted on many temperate coasts.

Since its introduction A. arenaria has naturalised and is now a principal component of the dune flora in most regions. For example, a national survey conducted during the 1980s of the New Zealand sandy coast found that A. arenaria was present in at least 61% of New Zealand dune-systems (Johnson, 1992; Partridge, 1992). A. arenaria was the dominant foredune plant in several regions (Figure 1.1). Recent surveys have shown that A. arenaria has continued to spread since the 1980s (Hilton, 2006). A. arenaria is now the dominant species on most New Zealand dunes (Figure 1.1). A. arenaria similarly dominates dunes along the south-eastern coast of Australia (Kirkpatrick, 1993), and the Pacific coast of North America between California and Washington State (Johnson, 1993; Wiedemann, 1993). A. arenaria is widespread in South Africa but does not appear to be invasive (Hertling and Lubke, 1999). It occurs at most sites here because of prior planting.
Figure 1.1 Distribution and dominance of *A. arenaria* (marram grass) and the native dune plants *Ficinia spiralis* (pingao) and *Spinifex sericeus* (spinifex) on the New Zealand coast as surveyed by Johnson (1992) and Partridge (1992) in 1985 and by Hilton (2006) in 2005. The distribution and density of *A. arenaria* has increased considerably since 1985, especially on the western coast of the North Island south of Auckland, on the eastern coast south of East Cape, and along the western coast of the South Island. (Adapted from Hilton, 2006).

The prevalence of *A. arenaria* on temperate coasts globally is of concern as *A. arenaria* results in significant changes to the character of the native dune-systems (Hilton, 2006; Hilton *et al*., 2006; Hacker *et al*., 2012). In general, the introduction of *A. arenaria* is associated with the modification of the geomorphic processes and the displacement of the indigenous flora. In New Zealand dunes, for example, *A. arenaria* forms foredunes that are higher, steeper and potentially less stable than foredunes formed by the indigenous dune binding plants (Esler, 1970). It outcompetes and displaces the indigenous flora in most circumstances (Partridge, 1995; Hilton *et al*., 2005; Hilton, 2006). It stabilises transgressive dune-systems, promoting succession and facilitates the invasion of other, less burial-tolerant, weedy species (Hilton, 2006; Hilton *et al*., 2006). It can result in the progradation of foredunes so that dune erosion is more frequent and habitat for shore birds is reduced (Moore and Davis, 2004; Hilton *et al*., 2006). It has
also altered the aesthetics and landscape qualities of New Zealand’s dune-systems (Figure 1.2). *A. arenaria* forms a dense grassland in contrast to the sparsely vegetated character of the unmodified dune-systems. Community mosaics are usually replaced by a monotonous topography and species cover. A similar effect on dune biota and geomorphology has been noted in most regions where *A. arenaria* has been introduced (e.g., Barbour and Johnson, 1977; Wiedemann and Pickart, 1996; Lubke, 2004). In contrast to the deliberate planting during the 18th and early 19th centuries, dune management today is principally focussed on minimising the spread of *A. arenaria*, and eradicating *A. arenaria* from dune-systems of high conservation value (Wiedemann and Pickart, 1996; Martínez et al., 2004; Hilton and Konlechner, 2010).

![Figure 1.2 Comparison of two sections of the dune-system at Mason Bay, New Zealand (46.9°S, 167.7°E) dominated by: a) the indigenous dune-binder *F. spiralis*; and b) *A. arenaria*. Note the large amount of un-vegetated sand where dunes are dominated by native species (a) compared to that dominated by *A. arenaria* (b). The arrow identifies the same point in both photos.](image)

The ability of *A. arenaria* to disperse and establish from fragments of rhizome presents a substantial management challenge. *A. arenaria* establishes only infrequently from seed, however, the dispersal of vegetative propagules provides an alternative mechanism by which *A. arenaria* can reproduce (Huiskes, 1979). Regeneration from rhizome fragments eroded during dune erosion by wave activity increases both population persistence by facilitating re-colonisation following disturbance, and provides for population spread and the colonisation of new territory through the transport of rhizomes by waves and currents. Such dispersal of rhizome fragments
allows *A. arenaria* to form large colonies rapidly. Rates of spread of up to 7 ha yr\(^{-1}\) have been recorded for *A. arenaria* growing in both New Zealand and California (Buell *et al.*, 1995; Hilton *et al.*, 2006). Much of this spread has followed clonal growth and the establishment of satellite populations from seed, however, in both cases the new colonies originated from rhizomes stranded on beaches above the level of spring high tides. Spread occurred rapidly due to the establishment of multiple colonies within embayments through the distribution and redistribution of rhizomes. These colonies act as foci for the subsequent spread of *A. arenaria*.

### 1.3 Research questions

It is timely to examine the dispersal and establishment of *A. arenaria* from pieces of rhizome, given the threat that this species poses to temperate dune-systems throughout the world. Several studies have previously recognised the potential for *Ammophila* spp. to establish from fragments of rhizome (e.g., Wallen, 1980; Buell *et al.*, 1995; Hilton *et al.*, 2006), however, little directly is known about the vegetative regeneration of *A. arenaria*. This study will provide the first systematic examination of the regenerative potential of *A. arenaria* rhizomes.

The attributes of *A. arenaria* that determine its reproduction from rhizomes will be examined through empirical laboratory- and field-based studies. The focus will be on identifying those biological aspects relating to the reproduction of *A. arenaria* that may limit establishment, or make establishment more successful in one environment compared to another. This study does not address all aspects of the vegetative regeneration of *A. arenaria*, but examines key aspects relating to the regeneration of this species from rhizome fragments.

The establishment process of *A. arenaria* can be conceptualised as a series of barriers that must be overcome before it can successfully form a new colony (Richardson *et al.*, 2000). For example, a viable propagule must be produced, it must remain viable while undergoing dispersal, and it must regenerate and be able to emerge from the soil surface. The end point of establishment is seldom strictly defined (Fenner and
Thompson, 2005). In some studies, establishment has occurred when independent survival is possible (i.e., when the establishing shoot is no longer dependant on the energy reserves of the propagule) (Fenner and Thompson, 2005). As the current study is primarily concerned with the potential of A. arenaria to found new colonies from stranded rhizomes, establishment will be defined as when a plant resulting from a rhizome fragment survives until maturity (i.e., a second generation of propagules is formed).

The following research questions are addressed:

1) **How readily does A. arenaria regenerate from rhizome fragments?**

The mortality of immature plants following regeneration is a primary factor limiting establishment (Fenner, 1985; Maun, 2009). However, for a plant to establish from any propagule, the propagule must first be capable of regeneration. It must possess the potential to establish at the time of detachment from the parent plant (i.e., be viable), retain this viability during dispersal and be stranded in an environment suitable for regeneration (Maun, 2009). The potential for A. arenaria to regenerate from fragments of rhizome is a fundamental question which has not yet been fully examined.

2) **How many propagules does the rhizome system of A. arenaria produce?**

It has long been recognised that large numbers of propagules need to be dispersed so that a few establish successfully (Ridley, 1930; Carlquist, 1967). There are obstacles at each stage of the establishment process that need to be overcome for dispersal to be effective. The greater the reproductive capacity of the rhizome system of A. arenaria, the larger the number of propagules involved in any one dispersal event, and consequently, the greater the probability that at least one propagule will establish successfully.

3) **How far can the rhizomes of A. arenaria be dispersed?**

The distance that a propagule of A. arenaria can be transported in a single dispersal event is of fundamental practical importance for coastal and conservation managers. Dispersal over only short distances principally serves to aid population persistence with
a limited role in population spread. The transportation of propagules well beyond the boundaries of the existing population increases the effective population size, allows rapid spread, and increases gene flow (Nilsson et al., 2010).

Fragments of *A. arenaria* rhizomes are dispersed by hydrochory (the dispersal of disseminules by water; after van der Pijl, 1982). This potential for *A. arenaria* to disperse through marine transportation has long been recognised (e.g., Ridley, 1930), and there have been several studies which recognise the role hydrochory plays in the invasion of dune-systems by *A. arenaria* (e.g., Buell et al., 1995; Hilton et al., 2005). However, currently the distances over which *A. arenaria* can be dispersed remains unclear.

4) **How does the stranding environment influence the establishment of *A. arenaria* from rhizomes?**

The suitability of the receiving environment for growth has been identified as the most important determinant of successful establishment (Carlquist, 1967). Wave activity will typically strand propagules along the back-beach between the high-tide line and the toe of the foredune. Certain combinations of events, such as storm events, tidal surge and redistribution by biotic agents may result in stranding further inland. These events are relatively rare, and although they may occasionally result in the successful establishment of a species which is not tolerant to the coastal environment, they are unlikely to facilitate widespread establishment. For the most part, the establishment of *A. arenaria* will depend on this species’ ability to regenerate and survive in the back-beach environment.

1.4 **Thesis structure**

The research questions outlined in Section 1.3 are addressed through a series of empirical investigations in Chapters 2 – 5. Each chapter examines a different aspect of the vegetative regeneration of *A. arenaria*. The relevant literature and methodology of each investigation is reviewed, outlined, and the results presented and discussed in each individual chapter.
The potential for *A. arenaria* to regenerate under ideal conditions for growth is examined in Chapter 2 through a series of comparative growth experiments. Comparisons are made between rhizome fragments under equivalent conditions for growth to assess the effect of intrinsic properties of rhizome fragments on the regenerative ability of *A. arenaria* (Research Question 1.). The density of dormant meristematic buds (the bud bank; after Harper, 1977) at three dune-systems in southern New Zealand is measured in Chapter 3. Comparisons within and between these dune-systems are made to identify factors that influence the size of the *A. arenaria* bud bank. These results, combined with those of Chapter 2, allow the reproductive capacity of *A. arenaria* to be quantified (Research Question 2). Chapter 4 examines the dispersal potential of *A. arenaria* through a series of laboratory-based experiments (Research Question 3). The only limit on how far a marine dispersed species can be transported is the ability of the propagule to remain buoyant and viable while in seawater (Ridley, 1930). The effect of immersion in seawater on rhizome buoyancy and viability is examined with the aim of determining the potential dispersal time of *A. arenaria* (i.e., the time during which a propagule can be transported and still retain the ability to regenerate after stranding). The effect of seawater on the regenerative potential of *A. arenaria* from rhizome fragments is also considered in Chapter 4 (Research Question 1). The effect of the stranding environment on the regeneration of *A. arenaria* from rhizome fragments and the survival of juvenile plants is examined in Chapter 5 (Research Questions 1 and 4). The tolerance of *A. arenaria* to key stresses of the sandy back-beach is examined through a combination of comparative growth-experiments and field-observations.

Chapter 6 synthesises the findings of Chapters 2–5 with respect to the overall aim of this thesis — to understand the ability of *A. arenaria* to disperse and establish from fragments of rhizomes. Throughout Chapters 2 – 5 experiments and sampling strategies were devised to identify key factors that make establishment more successful in one environment or from one population compared to another. Key themes that have emerged from these examinations are discussed in Chapter 6 with reference to each research question. Finally the implications of this research for the establishment of *A. arenaria* following dispersal will be considered.
Chapter 2

Intrinsic regenerative potential of *Ammophila arenaria* rhizomes

2.1 Introduction

For *Ammophila arenaria* to establish from rhizome fragments, the dispersed propagule must possess the ability to regenerate when detached from the parent plant, retain this ability when immersed in the sea, and strand in an environment suitable for regeneration. The regenerative ability of *A. arenaria* following immersion in seawater and the effect of the abiotic environment on regeneration is examined in Chapters 3 and 4 of the present study respectively. This chapter examines the intrinsic regenerative potential of *A. arenaria*; that is, the potential for *A. arenaria* to regenerate from fragments under ideal conditions for growth.

The regeneration of *A. arenaria* depends on its ability to produce tillers from the meristematic buds borne on its rhizome system following the disconnection of rhizomes from the parent plant. The rhizomes of *A. arenaria* consist of two types; those spreading orthotropically (vertical rhizomes) and those spreading plagiotropically (horizontal rhizomes) (Huiskes, 1979). Both forms of rhizomes are morphologically similar. They both consist of slender, woody internodes of varying lengths separated by nodes. The internodes are hollow and each node bears a single meristematic bud (Figure 2.1). Some of these buds regenerate while still attached to the parent plant; however, most remain dormant (Greig-Smith *et al.*, 1947). It is the dispersal of these dormant buds which facilitates the establishment of *A. arenaria* from rhizomes.

Little is known about the intrinsic potential of *A. arenaria* to regenerate from rhizomes. The literature recognises that *A. arenaria* establishes from fragmented rhizomes. This process has been observed in the field (e.g., Gemmell *et al.*, 1953), and the regenerative ability of *A. arenaria* from rhizomes in response to specific environmental conditions, mostly increasing levels of salinity, has been investigated (e.g., Baye, 1990). These identify the environmental conditions that are required for regeneration, but provide
little information on the regenerative potential of the fragments themselves. The potential of \textit{A. arenaria} to regenerate from rhizomes has been shown to vary by at least 30\% over the course of a year — viability was close to 80\% when tested during winter but decreased to 50\% during summer (Pavlik, 1983a). It is clear that the ability of \textit{A. arenaria} to regenerate from rhizomes is at times high, but is also variable.

This chapter aims to: 1) determine the intrinsic potential of \textit{A. arenaria} to regenerate from rhizome fragments; and 2) identify the variability of this potential. To address these aims the regenerative ability of rhizome fragments is examined in relation to fragment length, node number, the depth from which rhizomes are obtained, the rhizome type (vertical vs. horizontal), the time of year that fragmentation occurs, and the vigour of the source plant in a series of controlled growth experiments. These factors have been correlated with the reproductive ability of rhizomes in at least one other species (e.g., Leakey \textit{et al.}, 1977; Maun, 1984; Ayeni and Duke, 1985; Harris and Davy, 1986a; Woitke \textit{et al.}, 1997; Cordazzo and Davy, 1999)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2_1.png}
\caption{Morphology of an \textit{A. arenaria} rhizome fragment. Inset depicts a cross section through the hollow internode.}
\end{figure}
2.2 Methods

2.2.1 Sampling site

As this chapter aims to assess variability in the intrinsic viability of *A. arenaria*, rather than variation between populations, rhizomes were obtained from only one dune-system — Allans Beach, Otago Peninsula, New Zealand (45.9°S, 170.7°E). Allans Beach is a complex Holocene sand barrier located on the south-east coast of New Zealand. *A. arenaria* was deliberately introduced to this dune-system sometime between 1960 and 1980; hence the genetic variation within *A. arenaria* at this site is likely to be minimal. Rhizomes were obtained from the foredune at two sites (Figure 2.2). Site 1 is located within the mouth of an inlet where shelter from the prevailing south-east swell and winds, and a steady sediment supply have resulted in the formation of a progradation sequence of four dune ridges. Samples were obtained from the second dune ridge. In contrast, there is no foredune proper at Site 2, an open coast site. Samples were obtained from the face of stable dunes which are periodically scarped.

**Figure 2.2** Location of sites of rhizome collection at Allans Beach, Otago Peninsula
These sites provide an opportunity to compare two populations of different vigour growing within the same dune-system. The community associated with Site 1 is typical of a vigorous A. arenaria population — tillers are densely and uniformly distributed, plants are large, green and fresh in appearance, and flowering occurs freely (after Marshall, 1965). The community associated with Site 2 is typical of a population of low vigour. Plants are smaller, they are associated with more dead leaf matter, flowers are sparse and tillers exhibit a marked tussock growth form (Marshall, 1965).

2.2.2 Rhizome sampling and preparation

Rhizomes were sampled from both sites at three monthly intervals between March 2008 and December 2009, allowing the interaction between population vigour and the time of sampling to be examined over two years. Only vertical rhizomes were sampled and all samples were collected from a depth of 100 – 140 cm. To establish the relationship between rhizome age and rhizome viability, regeneration time or the growth rate of the resulting tillers, additional samples were collected from Site 2 in June 2009 from depths of 20 – 60 cm at the same site and compared with those collected from depths of 100 – 140 cm. The horizontal rhizome system was also sampled from Site 1 in June 2009 from depths of 100 – 140 cm and compared with vertical rhizomes collected from the same depth.

To examine the significance of fragment length, rhizomes were cut into fragments of one of four lengths; 10 cm, 4 cm, 2 cm and 1 cm. This range of fragment sizes would be sufficient for any relationship between length and regenerative ability to be apparent. Lengths shorter than 1 cm would result in damage to the meristematic bud and a loss of viability, and the reproductive ability of A. arenaria has been shown to not differ when fragments exceed 20 cm in length (Aptekar and Rejmánek, 2000). Each fragment had one node and the internodes were of equal length. The weight of each fragment was recorded and correlated with fragment length.

To control for the effect of fragment length in all other experiments, rhizomes were cut into equal lengths. To investigate the effect of node number independently of fragment length, rhizomes were cut into fragments 10 cm in length containing 1, 2, 3 or 4 nodes.
Rhizomes were cut into fragments 10 cm in length with one node for all other treatments.

Thirty fragments for each treatment level were cut in total. Each fragment was visually inspected to confirm the presence of intact buds and to ensure they showed no evidence of decay or regeneration. Those that were decayed or had regenerated were discarded. Following washing, all root and sheath material was removed as this material might restrict shoot emergence (after Baye, 1990). Each treatment level was then randomly divided into three replicates of ten fragments and assayed for regeneration as detailed in Section 2.2.3.

### 2.2.3 Regeneration test

Fragment viability was recorded as a measure of regenerative potential for all fragments. The time taken for tillers to be produced as well as the growth rate of the resulting tiller were recorded as additional measures of regeneration for fragments obtained between March 2009 and December 2009. These factors were tested by propagating fragments under conditions suitable for growth as determined from previous growth experiments and preliminary trials (Pavlik, 1983a; Baye, 1990; Aptekar and Rejmánek, 2000). Fragments were propagated under three centimetres of beach sand. Propagation depth is critical as excessive burial can prevent tiller emergence leading to an inaccurately low measure of viability (Maun, 2009). Preliminary trials found that regenerating fragments buried at three centimetres of depth emerged readily from the sand surface. Fragments were planted horizontally in trays under 3 cm of sand within five to six hours of collection with ten fragments in each tray. Fragments were propagated in a glasshouse with supplementary light and heat. Day-length and temperature varied by 8 hours and an average of 5 ± 3°C during the year. Trays were watered as required. All trays were watered at the same time with an equal amount of water.

Viability was measured as the ability of a fragment to produce at least one tiller which emerged above the sand surface during 120 days of growth. Testing viability by means of a growth test has been criticised, primarily because it does not distinguish between
dormant and non-viable propagules (Gibson, 2002; Elias, 2006). To identify dormant buds, the buds of all fragments were inspected after 120 days of propagation. No single-noded fragments retained a bud after 120 days — all buds had either formed a tiller or decayed. A portion of buds on many multi-noded fragments remained intact. It is not known whether these buds were dormant or, alternatively, unable to regenerate. However, these buds were located only on fragments where at least one bud had produced a tiller. All buds on non-regenerating multi-noded fragments had decayed during the 120 days period. The total percentage emergence during this period is, therefore, an accurate measure of viability for all treatments in the current study.

The day of regeneration was recorded as the day when a tiller emerged from the sand surface. Trays were checked daily for emergent tillers. Each new tiller was marked by a ring labelled with the date and the number of days from propagation calculated.

The growth rate of each tiller was calculated by:

\[
\frac{(x_{20} - x_0)}{20}
\]

where \(x_{20}\) = the length of the tiller at age 20 days; and \(x_0\) = tiller length at the day of emergence.

The length of each tiller was measured after 20 days of growth as measured from the day of emergence to control for any systematic change in growth with age. Tiller length was measured in millimetres from the sand surface to the tip of the central blade. Dead tillers were excluded from the calculation.

### 2.2.4 Data analysis

Analysis of variance was performed to identify significant treatment effects. A three-way ANOVA with interaction was used to test for the effect of sampling year, sampling month and population vigour on viability. The effect of fragment length, extraction depth, rhizome type, node number and sampling month on viability, time until tillers are produced and the growth rate of the resulting tiller were analysed using one-way
ANOVA followed by a Tukey comparison of means test (Zar, 1999). Mann-Whitney tests were performed for each sampling month to test the effect of population vigour on viability, the time until tillers are produced and the growth rate of the resulting tiller.

All independent variables were tested for normality and homoscedasticity prior to analysis using the Kolmogorov-Smirnov and Levene’s test respectively. Where these assumptions failed, appropriate data transformations were performed or non-parametric tests used. The viability data were arcsine transformed, and where necessary, the regeneration time and growth rate data were square-root transformed \( \left( \sqrt{n} + 0.5 \right) \) or log transformed \( \log_{10}(x + 1) \) respectively prior to analysis (Zar, 1999). All tables and figures present untransformed values. Results were accepted as significant if \( P<0.05 \). The degrees of freedom, F-values and P-values of all ANOVAs are presented in Appendix 1. The results of all Mann-Whitney tests are presented in Appendix 2.

### 2.3 Results

The effect of the sampling year on regeneration was not significant, nor was there any significant interaction between the year of sampling and either sampling month or vigour (Appendix 1). Fragment regeneration in the current study followed a consistent annual pattern, at least over the years sampled. For additional analyses, the years were grouped. The month of sampling and the population vigour both had a significant effect on regeneration; however, significant interaction was also detected between these covariates (Appendix 1). This indicates that any annual cyclic fluctuation in viability depends on the population from which the rhizomes originate. As a result the effect of sampling month on fragment regeneration was examined separately for each population.

Fragment viability, the time until regeneration and tiller growth rates varied between treatments (Figures 2.3, 2.4 and 2.5). However, some consistent trends regarding the regeneration ability of *A. arenaria* are apparent regardless of the treatment.

The ability of fragments to produce tillers varied considerably between treatments — by 55% (Figure 2.3). However, a substantial proportion of fragments in all treatments were able to produce tillers — at least 45%. In addition, none of the observed factors (length,
node number, vigour, sampling month, depth and type) prevented rhizomes from regenerating. At least one fragment in all treatments was viable.

There was a lag between planting and regeneration for all treatments (Figure 2.3). For most treatments, tillers did not emerge until 12 – 20 days after planting, although in some treatments tiller emergence was delayed until 37 – 40 days after planting. Once tiller emergence had commenced, the remaining tillers emerged at a relatively constant rate. In general tillers emerged rapidly. With the exception of fragments from the high-vigour population, all tillers had emerged within 20 days of the first tiller, and all viable fragments in all treatments had regenerated within 83 days of propagation (Figure 2.3).

As with the fragment viability, growth rates of tillers varied between treatments — by 0.6 cm day\(^{-1}\) (Figure 2.5). In general, the variation in growth rates between treatments followed similar patterns to that of fragment viability. Tillers from treatments with higher final percentage viabilities also grew faster.

### 2.3.1 Rhizome type — horizontal vs. vertical rhizomes

Regeneration differed with rhizome type. Fragments from the vertical rhizome system produced both more emergent tillers and produced tillers faster than fragments taken from the horizontal system (Figure 2.3a and 2.4a). The slower production of tillers by horizontal rhizomes arose from a delay in the initiation of regeneration rather than from a slower overall emergence rate. The first tiller emerged from horizontal rhizomes 22 days later than the first tiller from vertical rhizomes. However, once emergence had commenced, all tillers from both horizontal and vertical rhizomes emerged within 14 days of the first tiller (Figure 2.3a).

The average growth rate of tillers originating from vertical rhizomes was higher than those from horizontal rhizomes (Figure 2.5a). However, this increase was slight (less than 0.1 cm day\(^{-1}\)), and was not statistically significant.
Figure 2.3 The cumulative percentage emergence from rhizome fragments with: (a) rhizome type; (b) extraction depth; (c) fragment length; (d) node-number; and (e and f) population vigour and season of extraction. Values at 120 days indicate the final percentage emergence per treatment. Vertical bars indicate ± 1 std. error. Different letters indicate significant differences at P<0.05 within treatments according to the Tukey procedure following analysis by ANOVA on arc-transformed data. * indicate significant differences P<0.05 between high and low vigour populations within each sampling month as obtained by Mann-Whitney tests.
Figure 2.4 Comparison of the average time until tillers emerged with (a) rhizome type; (b) extraction depth; (c) fragment length; (d) node-number; and (e and f) population vigour and season of extraction. Vertical bars indicate ± 1 std. error. Different letters indicate significant differences at P<0.05 within treatments according to the Tukey procedure following analysis by ANOVA on square-root transformed data. * indicate significant differences P<0.05 between high and low vigour populations within each sampling month as obtained by Mann-Whitney tests.
Figure 2.5 Comparison of the average growth rate (cm day⁻¹) with: (a) rhizome type; (b) extraction depth; (c) fragment length; (d) node-number; and (e and f) population vigour and season of extraction. Vertical bars indicate ± 1 std. error. Different letters indicate significant differences at P<0.05 within treatments and * indicate significant differences P<0.05 between high and low vigour populations within each sampling month according to the Tukey procedure following analysis by ANOVA on log-transformed data.
2.3.2 Extraction depth

The depth from which rhizomes were obtained had little effect on the regeneration ability of fragments. No significant difference in the number of regenerating fragments or the tiller growth rate was detected between depths (Figures 2.3b and 2.4b). Although the emergence time of tillers from fragments obtained from 20 – 60 cm depth was consistently 2 – 4 days slower than those from 100 – 140 cm depth, this difference was not statistically significant (Figure 2.5b).

2.3.3 Fragment length

There is a significant correlation between the regenerative ability of a fragment and its length. The ability of a fragment to produce a tiller and the growth rate of the resulting tiller increased with fragment length (Figures 2.3c and 2.5c), although fragment length affected viability only when fragments were less than 2 cm in length. No difference in fragment viability was detected when fragments were between 2 and 10 cm in length, whereas the viability of fragments 1 cm in length was significantly lower than all other lengths tested. The emergence time of tillers was consistent between length treatments — regardless of length, the first tillers emerged after 14 – 15 days of growth, and all tillers emerged within 20 days of the first tiller (Figure 2.3c).

There is a significant correlation between fragment weight and length (Figure 2.6). Fragment weight increases by one gram for every additional 7.9 centimetres of fragment length. Rhizome weight varies considerably within each length category despite the strong correlation between weight and length — up to ± 2.9 g within each length category (Figure 2.6). Fragment weight increases in variability as length increases. There is substantial overlap in the range of weights between each length category. However, despite this overlap all fragment weights differed significantly between all length categories (Figure 2.6).
2.3.4 Multiple nodes

There was no correlation between the number of nodes on a fragment and a fragment’s ability to regenerate. Although the ability to produce tillers varied by 25% depending on node number, this difference was not significant nor did it vary systematically (Figure 2.3d). There was a slight positive correlation between node number and the time until emergence, and a slight negative correlation between node number and growth rates (Figures 2.4d and 2.5d). However, neither correlation was significant.

Although the number of nodes on a fragment did not affect the proportion of viable fragments (i.e., the number of fragments that produced at least one tiller), node number did affect the proportion of viable buds per treatment (i.e., the total number of buds that produced at least one tiller per treatment). The total bud viability was highest when fragments possessed only one node (Table 2.1). This indicates that more tillers would arise from buds borne on single-noded fragments than would from a comparative number of buds on multi-noded fragments. There was a positive correlation between

![Figure 2.6 Relationship between rhizome length and rhizome weight. The best fit line using least squares linear regression accounts for 86.8% of the variation in rhizome weight between lengths. The relationship is significant at 5%. Different letters indicate significant differences at P<0.05 within treatments according to the Tukey procedure following analysis by ANOVA.](image)
total bud viability and node-number when fragments possessed more than one node (Table 2.1).

Not all buds on viable fragments produced tillers when fragments possessed multiple nodes. The majority of multi-noded fragments possessed at least one bud that had not regenerated during 120 days of growth (Table 2.1). The number of non-regenerating buds per fragment was consistent between treatments. On average, only one bud failed to regenerate regardless of total node number (Table 2.1). On inspection, few of these buds showed signs of decay and most did not differ in appearance from their pre-propagation state. It is not known whether these buds were viable or not, or dormant.

<table>
<thead>
<tr>
<th>Number of nodes</th>
<th>Total bud viability (%)</th>
<th>Viable fragments with at least one non-regenerating bud (%)</th>
<th>Average number of non-regenerating buds per viable fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90.00</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>50.00</td>
<td>75</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>61.90</td>
<td>60</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>75.00</td>
<td>62</td>
<td>1.00</td>
</tr>
</tbody>
</table>

### 2.3.5 Sampling month and population vigour

The regenerative potential of *A. arenaria* rhizomes is seasonal, but only when fragments were obtained from populations of low vigour. Fragments obtained in September and December from the low vigour population showed a significant decrease in the ability of rhizomes to produce tillers, and in the growth rates of the resulting tillers (Figures 2.3f and 2.5f). This result is consistent with those of Pavlik (1983a) who showed that the viability of *A. arenaria* rhizomes was at a maximum during winter months and at a minimum during summer months. In contrast, the ability of fragments obtained from the high vigour population to produce tillers and the resultant tiller growth rates did not differ throughout the year (Figures 2.3e and 2.5f).
Population vigour had a significant effect on fragment regeneration, but, for the most, only when fragments were sampled in certain months. Both the ability of fragments from the low vigour population to produce tillers and the growth rates of the resultant tillers did not differ from that of fragments from the high-vigour population when rhizomes were obtained in March or June, but was significantly lower when fragments were obtained in September or July (Figures 2.3e and 2.3f).

The time taken to produce tillers did not differ seasonally, but the time was affected by population vigour (Figure 2.4e). Tillers were significantly slower to emerge from fragments obtained from the high vigour population. The emergence difference between these populations is due to both a delayed commencement of growth and from a slower rate of overall tiller emergence. Tiller emergence commenced between 22 – 24 days later when fragments were obtained from the high vigour population than from the low (Figure 2.3e and 2.3f). Total tiller emergence took 19 – 30 days longer.

2.4 Discussion

The present study was designed to determine the intrinsic potential of *A. arenaria* to regenerate from rhizome fragments, and to identify the variability of this potential. The regenerative potential of *A. arenaria* was found to be high. At least one fragment from each treatment was viable, and although the regenerative potential of *A. arenaria* decreased with fragment length, the viability of the smallest fragments (1 cm in length) was still high (74%). It is also evident that the regenerative potential of *A. arenaria* rhizomes is not constant. Viability in the present study varied by 55% depending on the length of the fragment, the type of rhizome, the vigour of the source population, and the time of year that fragments were obtained — from a maximum of 100% to a minimum of 45%. In contrast, the number of nodes and the depth of extraction had no effect on fragment regeneration. These results are consistent with those of similar studies on *A. arenaria* as well as other rhizomatous species (Pavlik, 1983a; Maun, 1984; Ayeni and Duke, 1985; Harris and Davy, 1986a; Cordazzo and Davy, 1999; Cordazzo and Seeliger, 2003). It appears that considerable variation in regenerative potential may be universal in species that reproduce vegetatively from rhizomes.
The mechanism underlying the regenerative potential of a fragment must be understood in order to interpret the results of the present study. The evidence from the literature indicates that the regenerative potential of vegetative fragments depends primarily on the stored reserves within the rhizomes at the time of fragmentation. Carbohydrates and/or nutrients, principally starch and nitrogen respectively, are stored in rhizomes and stolons as a precaution against variability in the growing conditions of plants (Chapin et al., 1990; Suzuki and Stuefer, 1999). In general, reserves are stored within the rhizomes of clonal plants to serve as a source of energy that can be drawn upon to improve plant survival and growth (Chapin et al., 1990). Reserves are frequently transported from one part of the plant to the other as they are mobilised in times of photosynthetic deficit. Similarly, reserves may be stored preferentially in parts of the rhizome system over others; depending on several factors such as the rhizome age and function (Chapin et al., 1990; Woitke et al., 1997). Due to this constant flux and/or preferential storage the amount of reserves within otherwise comparative fragments of rhizome can differ considerably. A positive correlation between at least one of these stored reserves within the rhizomes at the time of fragmentation and a fragment’s ability to regenerate is consistently identified by those studies that have examined this relationship (e.g., Harris and Davy, 1986a; Cordazzo and Davy, 1999).

Storage in the rhizomes of *A. arenaria* was not examined in the present study, however the results indicate that regenerative potential of *A. arenaria* depends on the reserves within the fragment. The seasonal flux in the viability of *A. arenaria* rhizomes from a low-vigour population is consistent with a positive relationship between stored reserves and regeneration. In environments with strong seasonality, storage in rhizomes follows a regular and predictable pattern related to seasonal growth (Chapin et al., 1990). Reserves decline when growth is most rapid or with the onset of sexual reproduction, and increase when growth rates slow and/or when senescence recycles leaf nutrients back to storage organs. The decrease in the viability of *A. arenaria* during late spring/early summer months, observed in the present study and by Pavlik (1983a), corresponds to periods of vigorous growth following limited growth in autumn and winter, the development of flowers and seed in spring months (Huiskes, 1979), and the corresponding depletion of stored reserves within the rhizomes. It is surprising that a seasonal flux in regeneration was identified only in rhizomes obtained from a low-vigour population and not in rhizomes obtained from the vigorous population. As
populations of *A. arenaria* display similar patterns of seasonal growth regardless of their vigour it could be expected that rhizomes from both populations would show a consistent annual regenerative cycle (Wallen, 1980).

Additional evidence that the regeneration of *A. arenaria* from rhizomes depends on the reserves stored within a fragment is provided by the positive correlation between tiller growth rates and rhizome viability. The initial growth rates of most establishing plants are determined by the energy reserves within the propagule (Westoby *et al.*, 1992; Fenner and Thompson, 2005). Further work, is required to confirm this relationship in *A. arenaria*; however, the correlation between fragment viability and the growth rate of the resulting tiller is consistent with a positive relationship between the storage of energy reserves prior to fragmentation and the rate of tiller growth.

It can be expected that due to the relationship between stored reserves and regeneration, the regenerative potential of *A. arenaria* will decrease below the levels indicated in the present study. Any factor that caused these reserves to decrease below levels tested in the present study would have a cumulative effect on regeneration. For example, the effect of length, rhizome type, extraction depth, and node number on regeneration was examined only in July (winter) when the reserves of the rhizomes are likely to be highest (Chapin *et al.*, 1990). The regenerative potential of these fragments is likely to be substantially lower when rhizomes are obtained in September/December, due to the lowered reserves stored in the rhizomes at this time. Nonetheless, the gross patterns of regenerative ability with season, vigour, rhizome type and length observed in the present study are robust. Overall, the rhizomes of *A. arenaria* can be expected to be less viable as the vigour of the source population decreases, the fragment length decreases, if it consists of horizontal rhizomes and if it is obtained during spring/early summer.

The relationship between regeneration and the reserves of a rhizome fragment is not linear. The viability of any propagule remains relatively constant as reserves decrease to a threshold value (Murthy and Sun, 2000; Powell, 2006). Viability is lost rapidly at an exponentially increasing rate once reserves decline beyond this threshold. This requirement for the energy reserves of the fragment to decrease below a threshold value before regeneration is affected explains why fragment length has no effect on viability until fragments were shorter than 1 – 2 cm in the present study. Although the reserves
decrease linearly as fragments become shorter, it appears that the differences between fragments of *A. arenaria* rhizomes greater then 1 – 2 cm in length are not sufficient to affect viability, i.e., they are greater than the critical threshold required for regeneration. A non-linear correlation between regeneration ability and the reserves of a rhizome fragment also provides a possible explanation for the observed relationship between population vigour and regeneration. The results of the present study indicate that the stored reserves in the vigorous population remained above the critical threshold throughout the year, and so regeneration was not affected despite a probable decline in reserves when sampled late spring/early summer. In contrast, the reserves of the low vigour population decreased below the threshold in late spring/early summer, hence the reduction in viability. Finally, this correlation suggests that though the number of nodes and the depth of extraction had no discernible effect on fragment regeneration. Repeating the present study when reserves are likely to be lower may detect a significant effect. The results of the present study are not sufficient in themselves to say conclusively than node number or depth has no effect on the regenerative potential of *A. arenaria*, only that when the seasonal flux of reserves is likely to be at a maximum these factors do not influence viability.

The present study has shown that a rhizome fragment of *A. arenaria* is less likely to regenerate if it originates from the horizontal rhizome system than from the vertical system. It is possible that these results are due to storage occurring preferentially in the vertical rhizomes. Such partitioning with rhizome type has been identified in other clonal species. Woitke *et al.* (1997) found that the horizontal rhizomes of *Phragmites australis* contained greater amounts of nitrogen and free amino acids than did the vertical rhizomes. It was suggested this partitioning is indicative of a difference in physiological state between rhizome types, and is likely to reflect the different roles that vertical and horizontal rhizomes play in the growth of *P. australis*. It is likely that the relative viabilities of the vertical and horizontal rhizomes reflect a similar relationship in *A. arenaria*. However, although the regenerative ability of *A. arenaria* differs with rhizome type, this result is unlikely to have a significant effect on the regeneration of *A. arenaria* in the field. Horizontal rhizomes accounts for only a small proportion (less than 3%) of the total *A. arenaria* rhizome system (Greig-Smith, 1961; Pavlik, 1983b; Baye, 1990). Most rhizome fragments can be expected to be from the more viable and vigorous vertical system.
On average 88% of fragments produced tillers, provided a fragment consisted of vertical rhizome, was not obtained from a low vigour population in early-spring/summer and exceeded 1 cm in length. This value is probably close to the maximum viability of *A. arenaria* regardless of the amount of reserves within the fragment. It was measured from rhizomes obtained when the seasonal flux of viability is likely to be at a maximum (Chapin *et al.*, 1990), in rhizomes obtained from a population displaying maximum vigour, and although the fragment lengths tested were relatively short, the findings of the current study indicate that viability is unlikely to increase if length exceeds 10 cm. The results of existing studies provide some support for the hypothesis that the maximum viability of *A. arenaria* is between 80 and 90% with growth experiments on *A. arenaria* rhizomes consistently recording maximum viabilities of less than 80% (e.g., Pavlik, 1983a; Aptekar and Rejmánek, 2000). It is not clear why 12% of fragments in the present study failed to regenerate. These fragments did not differ in appearance from viable fragments, and were obtained from populations where the majority of similar fragments could regenerate.

Buds on rhizomes remain dormant due to apical dominance (Klimešová and Klimeš, 2007). Fragmentation breaks this dormancy, but regeneration is delayed as it takes some time for the inhibitory compounds to be leached from the fragments (Bell, 1974; Fenner, 1985). *A. arenaria* rhizomes regenerate relatively rapidly when fragmented with rhizomes taking between 12 – 83 days to produce tillers in the present study. For the most there was little difference in the time tillers took to emerge between treatments. Of the factors investigated, only rhizome type and the vigour of the source population had a significant effect on the rate that tillers emerged. It is not clear why these tillers in these treatments took significantly more time to emerge. The increased lag between propagation and regeneration when fragments are obtained from the horizontal rhizome system or from a vigorous population imply that these systems contain more inhibitory compounds than do the vertical rhizome system or the rhizome system associated with less vigorous populations. There was, however, a positive correlation between regeneration time and fragment length. If the length of time between propagation and regeneration is related to the concentration of inhibitory compounds within a fragment, then an increase in the regeneration time with increasing length would be expected.
Finally, the present study found no relationship between rhizome viability and the number of nodes on a fragment. However, the regenerative potential of *A. arenaria* may still increase with increasing node number because of the ability of fragments with multiple nodes to prevent regeneration in some buds. At least one bud on most multi-noded fragments in the current study did not regenerate, and when inspected after 120 days showed no signs of decay. This response is likely to be the result of apical dominance imposed by the regenerating buds. It is not known whether these non-regenerating buds in *A. arenaria* were unable to regenerate or, alternatively, they were just dormant. However, a similar response has been observed in several species, and the non-regenerating buds shown to be viable (Duke, 1985). Maintaining a proportion of buds dormant on each fragment provides the opportunity for regeneration in the event of a catastrophic disturbance.

### 2.5 Concluding remarks

For *A. arenaria* to establish following dispersal, the dispersed propagule must be able to regenerate when detached from the parent plant. The present chapter has shown that *A. arenaria* regenerates readily from rhizome fragments, however several factors were identified that resulted in a considerable decrease in the regenerative ability of *A. arenaria*. The regenerative potential of *A. arenaria* can be expected to decrease as the vigour of the source population decreases, as the fragment length decreases and if it is obtained during spring/early summer. The morphology of the fragment as well as processes relating to the growth of the rhizomes prior to disconnection will influence on the probability that regeneration is successful.

The intrinsic regenerative ability of *A. arenaria* can be expected to vary between dispersal events. The evidence from the literature indicates that this variation is due to the storage of reserves prior to dispersal. As such any factor that influences the storage of resources in the rhizomes of *A. arenaria* has the potential to affect its ability to regenerate and it can be expected that the viability of *A. arenaria* rhizomes will at times be lower than that indicates in the present study. However, the results of the present study, particularly the still high viability of very small lengths of rhizome, indicate that
the reserves in *A. arenaria* rhizomes are typically in surplus to requirements for regeneration. Although overall viability may be decreased, it seems reasonable to conclude that at least some rhizomes from any given population of *A. arenaria* will be capable of regeneration.
Chapter 3
The *Ammophila arenaria* bud bank

3.1 Introduction

The hydrochoric dispersal of *Ammophila arenaria* depends on the fragmentation of the rhizome system, usually during episodes of dune erosion by wave activity, into pieces possessing a viable bud. The number of propagules that are dispersed in an event, therefore, depends on: (i) the density of the below-ground population of meristems (buds) associated with the supporting rhizome system (the “bud bank” after Harper, 1977); (ii) the intrinsic viability of the rhizome fragments; and (iii) the magnitude of the erosion event. The viability of *A. arenaria* rhizomes following fragmentation was documented in Chapter 2. This chapter will examine the ability of *A. arenaria* to form a bud bank. The magnitude of erosion events will not be examined in the current study, since it depends on the interrelated factors of exposure to storm surge and beach/dune geomorphology, and as such, varies considerably both between and within bays. By determining the reproductive capacity of *A. arenaria* (the density of viable buds available for dispersal), the maximum number of propagules undergoing dispersal will be estimated for any dispersal event, provided the volume of foredune eroded is known.

The size of the *A. arenaria* bud bank is currently unknown. A number of studies have examined aspects of the clonal growth of *A. arenaria*; however, few have addressed the formation of a bud bank (e.g., Greig-Smith *et al.*, 1947; Gemmell *et al.*, 1953; Pavlik, 1983a; Pavlik, 1983b; Baye, 1990). Studies that have examined the bud bank of *A. arenaria* only measured a proportion of the total bud bank (Baye, 1990); expressed bud numbers in terms the ratio of buds per plant rather than bud density (Pavlik, 1983a); measured bud production only in young plants (<3 years of age) (Pavlik, 1983a; Baye, 1990); measured bud production only in garden- or laboratory-based growth experiments (Pavlik, 1983a; Baye, 1990); or failed to differentiate between dormant buds and active buds (Baye, 1990). Only a proportion of the total buds produced by *A. arenaria* remain dormant, the remainder differentiate into either tillers or rhizomes (active buds) (Greig-Smith *et al.*, 1947; Huiskes, 1979). These will be termed ‘dormant
buds’ and ‘active buds’ respectively. Only dormant buds retain regenerative potential and can be considered “the bud bank”. Measuring the total number of bud sites without differentiating between dormant and active buds overestimates the bud bank.

Most plant species maintain a reserve of dormant buds, however the size of the bud bank varies considerably between species (Vesk and Westoby, 2004; Klimešová and Klimeš, 2007). Bud banks serve an important role in population persistence by providing for rapid growth and recovery after a disturbance event (Klimešová and Klimeš, 2007). There are, however, nutrient and metabolic costs associated with maintaining a bud bank (Vesk and Westoby, 2004). The size of a species’ bud bank, therefore, reflects a trade-off between the need for rapid regeneration and persistence, versus the potential for short-term growth and expansion. All other things being equal, a species can be expected to be associated with a large bud bank if there is a high probability of bud use (i.e., frequent disturbance, long bud lifespan), if conditions post-disturbance are unfavourable for seedling recruitment, or if clonal expansion is favoured over seeds (Vesk and Westoby, 2004).

_A. arenaria_ is likely to form a sizable bud bank. It meets most of the criteria listed by Vesk and Westoby (2004). _A. arenaria_ is likely to experience regular disturbance from burial and wave activity; it produces buds that remain dormant for months to years; it establishes from seed only infrequently; and reproduction occurs primarily from clonal growth (Ranwell, 1972; Huiskes, 1979). However, the size of the _A. arenaria_ bud bank is likely to vary considerably between populations. The size of a bud bank is closely linked to the architecture of the organs with which the meristems are associated (Klimešová and Klimeš, 2007). This association between the bud bank density and the growth morphology of the supporting organs indicates that the bud bank density of any species is determined by the density of supporting organs, the density of bud-sites per supporting organ and on the proportion of bud-sites which possess a bud. As the rhizome system of _A. arenaria_ exhibits considerable phenotypic plasticity depending on the environment in which it is growing, for example, the rhizome weight of individuals from the same clonal stock differed by 18-fold when grown under different environments (Gray, 1985), it can be anticipated that such plasticity of the rhizome system of _A. arenaria_ will manifest in substantial levels of variation in the density of this species bud bank.
The primary aim of this chapter is to quantify the size and variability of the *A. arenaria* bud bank. To address this aim, the bud bank of *A. arenaria* is measured from three dune-systems in southern New Zealand. It is hypothesised that the bud bank of *A. arenaria* will be large, but that differences in rhizome architecture are likely to manifest in considerable differences in the bud bank between populations. Variation in the bud bank between sites is examined in relation to rhizome architecture (i.e., fluctuating rhizome densities, bud-site to rhizome ratios or proportions of dormant buds to bud-sites).

### 3.2 Methods

#### 3.2.1 Sampling sites

The bud bank of *A. arenaria* was sampled from three dune-systems in southern New Zealand: Allans Beach, Chrystalls Beach and Mason Bay (Figure 3.1). *A. arenaria* is the dominant foredune vegetation in all three systems. These systems are located within the same climatic zone, but differ in their geomorphic regimes (e.g., sand supply, wind regime, beach type). Comparisons between these systems offer an opportunity to gain an understanding of the relationship between sand deposition and the bud bank of *A. arenaria*. 
Allans Beach

Allans Beach is a complex Holocene sand barrier located on the south-east coast of New Zealand. The bud bank of *A. arenaria* was sampled within an inlet at the rear of the barrier where the relative shelter from the prevailing south-east swell and winds and ample sediment from the inlet at low tide has resulted in a progradation sequence of four foredune ridges (labelled F1 – F4, Figures 3.1a and 3.2a). This site provides an opportunity to examine the effect of increasing age and stability on the bud bank of *A. arenaria*.

The age of each foredune at the time of sampling ranged from 4 – 26 years with a new foredune ridge forming every 5 – 10 years. Ages were determined by analysis of aerial photography combined with field observations since 2007. A variable photographic record meant that only the approximate age of F2, F3 and F4 could be established. The initiation of F1 from stranded rhizome was observed in 2007.
Figure 3.2 Cross-sections across the rhizome sampling sites at: a) Allans Beach, as surveyed along the shore-normal transect in June 2011; b) Chrystalls Beach, as surveyed along the shore-normal transect in February 2010; and c) at Mason Bay, as surveyed along the shore-normal transect in February 2010. The arrows indicate the location of the shore-parallel profiles; hence the elevation and distance inland from which the samples were obtained. The dune height at Allans Beach and Mason Bay were measured from the spring high tide where a change in slope differentiates dune and berm landforms. The dune height at Chrystalls Beach was measured from the beach facies.
Accretion rates across the sequence of foredunes were determined by repeated topographic surveys across permanent transects on F1 and F2. F1 accreted by 1.1 m between February 2008 and November 2010; a rate of about 0.36 m year$^{-1}$. Over the same time period F2 accreted by only 0.08 m; a rate of about 0.03 m year$^{-1}$. Given the limited rates of sand accretion on F2, it can be assumed that the current accretion rates over F3 and F4 are negligible. F2, F3 and F4 must have experienced greater rates of sand accretion, historically. At the time of sampling the maximum height of F2 was 4.12 m as measured from the toe of F1 (which approximates to the elevation of spring high tide). Given the maximum age of F2 of 10 years, this height requires sand accretion rates of at least 0.41 m year$^{-1}$.

The vegetation community associated with each foredune is consistent with descriptions of *Ammophila* spp. with increasing dune age and stability (see Greig-Smith *et al.*, 1947; Krajnyk and Maun, 1981). *A. arenaria* is growing vigorously on F1 in response to ongoing sand deposition. The vegetation cover is dense, but heterogeneous. Tillers are distributed in patches of 30 – 150 cm in diameter, reflecting the distribution of rhizome at the time it was stranded. Within each patch, tillers are clustered in groups of 20 – 40, with each group separated by 5 – 20 cm. Vigorous *A. arenaria* accounts for most of the plant cover on F2. Scattered individuals of *Senecio elegans* and *Lupinus arboreus* also occur on F2. Tillers are uniformly distributed — groups of 20 – 40 tillers separated by 5 – 20 cm form a dense, homogenous cover. *A. arenaria* remains the dominant species on F3 but other species represent a much greater proportion of the biomass. Only scattered individuals of *A. arenaria* occur on F4. The *A. arenaria* present on F3 and F4 displays a different distribution pattern to F1 and F2. Tiller distribution is highly heterogeneous with tillers aggregated in dense tussocks of 50 – 200 tillers. Tussocks are distributed fairly uniformly on F3 separated by of 10 – 70 cm. Tussock distribution becomes highly heterogeneous on F4 with sparse *A. arenaria* present in individual or small groups of tussocks.

**Chrystalls Beach**

Chrystalls Beach is located on the south-east coast of the South Island of New Zealand, ~ 60 km south of Allans Beach. The dune-system comprises of a narrow Holocene...
barrier with a continuous established foredune, fronted by a low incipient foredune (Figure 3.1b and 3.2b). The bud bank of *A. arenaria* was sampled from the incipient foredune. The development of similar dunes, observed elsewhere by the author, suggests that this foredune was at least 1 year old when first visited in 2008. The incipient foredune is absent from Chrystalls Beach in 2004 aerial imagery, although small coppice dunes are widespread along the upper beach. These probably developed from stranded rhizomes. Therefore, at the time of sampling the incipient foredune sampled at Chrystalls Beach was between three and six years in age.

Annual rates of sand accretion can be estimated by dividing the height of the foredune by its age. A beach facies, a coarse wave-deposited sand layer, was observed approximately 55 cm below the dune surface. The sediment above this layer was primarily aeolian in origin. The elevation of the beach facies corresponded to the upper limit of the intertidal at the time of sampling (Figure 3.2b). No rhizomes were found below this facies. This facies probably corresponds to the original beach level at the time of dune formation from stranded rhizomes. A dune height of 55 cm gives an annual accretion rate of 9 – 18 cm.

The vegetation community on the foredune at Chrystalls Beach is similar to that associated with F1 at Allans beach. Tiller growth was vigorous. Tillers were distributed in groups of 20 – 40, separated by 5 – 20 cm of bare sand. The vegetation cover was dense but patchy, reflecting the distribution of stranded rhizomes at the time of formation.

**Mason Bay**

Mason Bay is located on the west coast of Stewart Island. Here, exposure to the prevailing westerly winds, coupled with large amounts of available sediment, has resulted in the formation of a large foredune complex (after Doing, 1985) backed by a sparsely vegetated transgressive dune hinterland (Figures 3.1c and 3.2c).

The bud bank was sampled on the foredune complex at a distance ~ 20 m from the toe of the foredune (Figure 3.1c). The progradation and accretion of this foredune has been documented by Hilton *et al.*, (2005) using aerial photography from 1958, 1978, and
1998. *A. arenaria*, when the foredune comprised a scatter of shadow dunes associated with both *A. arenaria* and *Ficinia spiralis*. By 1978 these patches had expanded to form a continuous foredune and *F. spiralis* had been largely displaced by *A. arenaria*.

Repeated topographic surveys across permanent transects since 1999 have allowed recent sand accretion rates to be determined. The foredune close to where the bud bank was sampled had accreted by 1.07 m between Feb 04 and Dec 2010; an annual rate of 0.16 m. Although the age of the foredune complex was between 32 and 52 years old at the time of sampling, the portion of the foredune sampled was much younger. Samples were obtained only from the upper 1 m. The samples obtained from Mason Bay were between 0 – 6 years in age at the time of sampling based on an annual accretion rate of 0.16 m per year.

The vegetation community on the foredune at Mason bay was consistent with that on F2 at Allans beach. Tiller growth appeared to be vigorous and homogenously distributed in groups of 20 – 40 tillers separated by 5 – 20 cm.

**Primary versus secondary dunes**

The development of the *A. arenaria* rhizome system is closely coupled with sand deposition and the vertical growth of foredunes. Sand burial around tillers stimulates the elongation of stem internodes in order to raise the apical meristem to a non-lethal depth, resulting in the formation of vertical rhizomes (Gemmell *et al*., 1953; Huiskes, 1979). Consequently, the foredunes sampled in the current study can be classified into two types based on their exposure to sand deposition and associated rhizome growth. The Allans Beach F1, Chrystalls Beach, and Mason Bay foredunes, are characterised by ongoing sand accumulation (‘primary dunes’ after Masselink and Hughes, 2003). The rhizome system at these sites reflects the ongoing production of vertical rhizomes in response to continued foredune accretion. In contrast, the Allans Beach (F2, F3 and F4) foredunes are characterised by stability (‘secondary dunes’ after Masselink and Hughes, 2003). They experience no or little sand accumulation, due to the presence of an incipient foredune which prevents landward sand transport. Only a small amount of rhizome is likely to have formed at these sites since they became stable. The rhizome system at these sites reflects the density of the rhizome system at the time of stability.
less any subsequent breakdown of the rhizomes. Strictly speaking, the Allans Beach F2 foredune is intermediate between these two categories. It is still accreting, albeit at a low rate, even though the development of F1 has greatly reduced the supply of sand. It can be expected that accretion will eventually cease in response to the continued vertical growth of F1.

3.2.2 Sampling procedure

Samples were obtained from each of the four foredune ridges at Allans Beach. At Mason Bay samples were obtained from the established foredune and at Chrystalls Beach from the incipient foredune. Sampling locations were determined by establishing a shore-normal transect across each site. Shore-parallel transects were established perpendicular to the shore-normal transect and six points along each shore-parallel transect within 20 m as measured from the shore-normal transect were randomly identified for sampling. Figures 3.1 and 3.2 illustrate the location of the shore-normal and shore-parallel profiles at each site.

The bud bank was sampled using a soil corer with a 20 cm diameter. Sampling occurred at 20 cm intervals to a depth of 100 cm at Allans Beach and Mason Bay and to a depth of 60 cm at Chrystalls Beach. Each sample was passed through a 2 mm sieve to separate the rhizome fragments. Each sample at each depth interval was bagged separately to allow examination of inter-sample variability and the relationship of bud density with depth. In total, six cores were sampled at each site and each core consisted of five samples.

Estimates of propagule density are likely to be inaccurate when propagules are heterogeneously distributed within the soil, if the sample size is too small, or too few samples taken (Warr et al., 1993). Further, the corer may destroy a proportion of the buds. To compare sampling using the corer with other sampling techniques, a 1 m² pit was excavated at both Allans Beach (F2) and Chrystalls Beach. The pits were located to ensure tiller density was consistent between sampling methods. The bud bank was sampled at 20 cm intervals to a depth of 100 cm at Allans Beach and 60 cm at Chrystalls Beach; the same sampling interval as used with the corer. Sampling using
either method obtained similar quantities of rhizome and nodes (two sample t-tests, P>0.05). Bud numbers when sampled by digging pits were constantly 15% larger than when sampled with the corer. To compensate, 15% was added to measurements of the bud bank in the current study prior to analysis.

### 3.2.3 Data analysis

The number of buds, bud sites and the rhizome weight of each sample were measured. A bud was measured as an intact and apparently dormant meristem associated with a node. Those which displayed signs of growth (i.e., activation), or decay were not recorded as buds. As every node on A. arenaria rhizomes possesses either an active or dormant bud (Greig-Smith et al., 1947), nodes were counted as a measure of the bud-sites on A. arenaria rhizomes. To measure rhizome weight, samples were washed to remove any sand and the root and sheath material separated from the rhizomes. Samples were then dried to constant moisture content at 80°C for 24 hours. All dry weights were recorded in grams to 2 decimal places.

To compare sites, the number of buds, the number of nodes and the rhizome weight per sample were summed to obtain totals for each core. Significant differences in bud density between sites were identified using one-way ANOVA followed by a Tukey comparison of means test (Zar, 1999). To examine the vertical distribution of the bud bank, a one-way ANOVA followed by a Tukey comparison of means test was performed for each site to identify significant differences in the numbers of buds per sample with sampling depth. Where bud numbers differed between sites, additional analyses were performed to identify whether the difference is due to differences in the density of supporting organs (the weight of rhizomes), the density of bud-sites per supporting organ (the number of nodes) and the proportion of bud-sites which possess a bud. The number of nodes per gram of rhizome and the proportion of nodes with buds were calculated for each core. Least squares linear regression was used to examine the relationship between bud density and rhizome weight, the number of nodes per gram of rhizome and the proportion of nodes with buds.
A positive correlation between sand deposition and rhizome density has been established for *Ammophila breviligulata*, a species closely related to *A. arenaria* (Disraeli, 1984). Regression analysis was used to examine this relationship in the current study of *A. arenaria*. The relationship between environmental stability and rhizome density was examined separately for primary and secondary foredunes. Least squares linear regression was used to examine the relationship between annual rates of sand deposition and the average rhizome density per primary foredune (Allans Beach – F1, Allans Beach – F2, Mason Bay and Chrystalls Beach). Foredune age was used as a proxy for substrate stability for all secondary foredunes (Allans Beach – F2, Allans – F3, Allans Beach – F4). Least squares linear regression was used to examine the relationship between foredune age and the average rhizome density per secondary foredune. The evidence from the literature indicates that the node density of *A. arenaria* is likely to depend on the rates of sand accretion experienced during the growth of the vertical rhizome system (e.g., Disraeli, 1984; Maun and Lapeirre, 1984; Baye, 1990, Maun, 2009). Least squares linear regression was used to examine the relationship between sand accretion and node density per gram of rhizome for those sampling sites with robust measures of sand accretion.

All independent variables were tested for normality and homoscedasticity prior to analysis using the Kolmogorov-Smirnov and Levene’s test respectively. Where these assumptions failed, data were square-root transformed ($\sqrt{(n+0.5)}$) (Zar, 1999). All tables and figures present untransformed values. Results were accepted as significant if $P<0.05$. Unless given in the text, the degrees of freedom, F-values and P-values of all ANOVA are presented in Appendix 1.

### 3.3 Results

#### 3.3.1 Bud bank comparisons between sites

The number of buds, nodes and the weight of rhizomes obtained from each site are presented in Table 3.1. Large numbers of buds were obtained from all sites — at least 360 ± 333 buds m$^{-3}$. On average, bud density measured 1618 ± 1671 buds m$^{-3}$. Bud
density differed considerably between all sampled foredunes, although due to large inter-sample variability within sites, few significant differences were detected (Table 3.1). Bud density varied by a similar amount within dune-systems as it did between dune-systems. At Allans Beach, bud density varied by 1232 buds m\(^{-3}\) between dune-ridges, similar to the difference between the incipient foredunes at Allans Beach (F2) and Chrystalls Beach (935 buds m\(^{-3}\)). No significant difference was detected in bud numbers between the foredunes at Allans Beach (ANOVA: d.f. = 3, F =1.24, P = 0.321), although samples obtained from F4 contained fewer buds than samples from the other three ridges. Bud numbers at F1, F2 and F3 were approximately three – five times greater than that of F4. There was little difference in bud density between F1 – F3 (396 buds m\(^{-3}\)).

**Table 3.1** The number of buds, nodes and rhizome weight per m\(^3\) obtained from all sites. Values are averages ± 1 std. dev per site. Different letters indicate significant differences in bud numbers at P<0.05 between sites according to the Tukey procedure following analysis by ANOVA on square root-transformed data.

<table>
<thead>
<tr>
<th></th>
<th>Allans Beach</th>
<th>Chrystalls Beach</th>
<th>Masons Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
</tr>
<tr>
<td>Buds</td>
<td>1592 ± 2035</td>
<td>1196 ± 922 A</td>
<td>1373 ± 706 A</td>
</tr>
<tr>
<td>Nodes</td>
<td>2117 ± 2634</td>
<td>2462 ± 1869</td>
<td>2578 ± 1534</td>
</tr>
<tr>
<td>Rhizome weight (g)</td>
<td>336 ± 467</td>
<td>476 ± 343</td>
<td>325 ± 156</td>
</tr>
</tbody>
</table>

### 3.3.2 Relationship between rhizome density, bud site density, and the proportion of bud-sites with a bud, and bud density

Regression analysis revealed a significant relationship between average bud density and rhizome weight between sites (Figure 3.3). The inter-site variation in bud density is, at least in part, the consequence of differences in the volume of rhizomes between sites.
Based on the results of the regression analysis, rhizome density over all sites increased by 5 – 6 buds m$^{-3}$ per additional gram of rhizome.

Rhizome density explains only 62% of the variability in bud density between sites (Figure 3.3). Including the number of nodes per unit of rhizome improved the regression fit. Together rhizome and node density explains 90.4% of the variability in the bud density between sites (Least squares linear regression; $F = 141.61$, $P = 0.000$). The proportion of buds to nodes has little effect on bud density. Including the proportion of nodes with buds explains only 3.3% more of the variation between sites than does rhizome and node density ($R^2 = 93.3\%$ (least squares linear regression; $F = 134.22$, $P = 0.000$) vs. 90.4%). The bud density of *A. arenaria*, therefore, depends on both the amount of rhizome per site and the density of nodes per volume of rhizomes.

Node density measured an average of 8.4 ± 3.5 nodes per gram of rhizome over all sites. Samples obtained from Mason Bay contained significantly more nodes per rhizome than did samples from all other sites (Table 3.2). The larger bud bank at Masons Bay compared to Allans Beach and Chrystalls Beach is due to both increased rhizome densities, and also an increased numbers of nodes per volume of rhizome. Node density did not differ significantly between the foredunes at Allans Beach (ANOVA: d.f. = 3, $F = 2.56$, $P = 0.089$), although F2 possessed fewer nodes per volume of rhizome than the other sites (Table 3.2). The bud density of F2 is lower than expected if bud number is determined only by the density at which rhizomes are produced. The node density as obtained from F4 does not differ from F1 and F3. The lower bud numbers at this site compared to the other sites at Allans beach are due to lowered amounts of rhizomes, rather than differences in the formation of nodes with rhizome weight.

The proportion of nodes with dormant buds differed by 17% between sites, although this difference was not significant (Table 3.2). Overall, 62 ± 0.18% of all nodes possessed a dormant bud.
Figure 3.3 Relationship between bud density and rhizome weight. Symbols indicate average bud density and average rhizome weight m$^{-3}$ per site. The best fit line using least squares linear regression on square-root transformed data accounts for 62% of the variation in bud bank density within sites. The relationship is significant at 5%.

Table 3.2 Comparison of the node density and proportion of nodes with buds obtained between sites. Values are averages ± 1 std. dev per site. Different letters indicate significant differences in bud numbers at P<0.05 between sites according to the Tukey procedure following analysis by ANOVA on square root-transformed data.

<table>
<thead>
<tr>
<th></th>
<th>Allans Beach</th>
<th>Chrystalls Beach</th>
<th>Masons Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
</tr>
<tr>
<td>Node density</td>
<td>7.4 ± 1.6 AB</td>
<td>5.0 ± 1.9 A</td>
<td>7.9 ± 2.3 AB</td>
</tr>
<tr>
<td>Nodes with buds (%)</td>
<td>73 ± 6 A</td>
<td>56 ± 30 A</td>
<td>58 ± 13 A</td>
</tr>
</tbody>
</table>

x = 181 + 4.59y  
$R^2 = 0.62$, $P = 0.000$, $F = 54.88$
3.3.3 Vertical distribution of the bud bank

Allans Beach

No significant change in bud numbers with depth was detected in samples obtained from F1 and F2 at Allans Beach (Figure 3.4a). Bud numbers in samples obtained from F3 and F4 decreased significantly with increasing depth. The effect of sampling depth on bud distribution is more pronounced when sampled from F4 than from F3. Over 80% of the bud bank is located in the upper 40 cm of F4 compared to only 67% of F3.

The vertical distribution of rhizomes followed a similar distribution to the buds (Figure 3.4). Rhizome weight did not differ with depth when sampled from F1 and F2, whereas the majority of the rhizomes obtained from F3 and F4 was located within the upper 40 cm. No significant relationship between node density and the proportion of nodes with buds with depth was detected (Table 3.3). The vertical distribution of buds on all foredunes at Allans Beach is determined primarily by the volume of rhizomes.

Mason Bay

No significant relationship between bud or rhizome density with depth was detected at Mason Bay, although bud and rhizome densities in samples obtained from depths of 0 – 20 cm were significantly lower than those obtained from other depths (Figure 3.5). No difference in bud and rhizome density was detected between samples obtained from depths greater than 20 cm. Node density decreased with depth although this increase was slight — node density differed by only 3 nodes per gram of rhizome between the minimum and maximum sampling depths. This relationship was not statistically significant (Table 3.3). No significant relationship between the proportion of nodes with buds and depth was detected (Table 3.3). Overall the vertical distribution of buds at Mason Bay is determined primarily by the volume of rhizomes at each sampling depth.
Figure 3.4 Vertical distribution of a) the bud bank and b) rhizome at Allans Beach. The labels F1 – F4 refer to the foredune ridges at Allans Beach labelled sequentially by age with F1 the youngest ridge and F4 the oldest. Values given are the average bud density (± 1 std. error) per 20cm sampling interval. Different letters indicate significant differences at P<0.05 between depths according to the Tukey procedure following analysis by one-way ANOVA on square root-transformed data.
Table 3.3 Results of general linear regression analysis to test for a relationship between node density and the proportion of nodes with buds with depth. $P$ is significant at an $\alpha$ level of 0.05.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Node density</th>
<th></th>
<th>Nodes with buds</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td>$R^2$</td>
<td>$F$</td>
</tr>
<tr>
<td>Allans Beach – F1</td>
<td>4.72</td>
<td>0.118</td>
<td>0.61</td>
<td>2.27</td>
</tr>
<tr>
<td>Allans Beach – F2</td>
<td>2.48</td>
<td>0.214</td>
<td>0.46</td>
<td>1.33</td>
</tr>
<tr>
<td>Allans Beach – F3</td>
<td>2.56</td>
<td>0.206</td>
<td>0.46</td>
<td>0.11</td>
</tr>
<tr>
<td>Allans Beach – F4</td>
<td>0.25</td>
<td>0.665</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>Mason Bay</td>
<td>4.52</td>
<td>0.053</td>
<td>0.25</td>
<td>2.55</td>
</tr>
<tr>
<td>Chrystalls Beach</td>
<td>0.63</td>
<td>0.250</td>
<td>0.03</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Figure 3.5 Vertical distribution of a) the bud bank and b) rhizome at Mason Bay. Values given are the average bud density ($\pm$ 1 std. error) per 20cm sampling interval. Different letters indicate significant differences between depths at $P<0.05$ according to the Tukey procedure following analysis by ANOVA on square root-transformed data.

Chrystalls Beach

Bud numbers decreased as the sampling depth increased although this decrease was not significant at depths greater than 20 cm (Figure 3.6a). Rhizome weight also decreased
with depth (Figure 3.6b). No significant relationship between node density and the proportion of nodes with buds with depth was detected (Table 3.3). As at Allans Beach and Mason Bay, the vertical distribution of buds at Chrystsalls Beach is determined primarily by the volume of rhizomes at each sampling depth.

![Figure 3.6](image.png)  
**Figure 3.6** Vertical distribution of a) the bud bank and b) rhizome at Chrystsalls Beach. Values given are the average bud density (± 1 std. error) per 20cm sampling interval. Different letters indicate significant differences between depths at P<0.05 according to the Tukey procedure following analysis by ANOVA on square root-transformed data.

### 3.3.4 Relationship between rhizome density and sand deposition

**Primary dunes**

Regression analysis detected no significant relationship between rhizome density and sand deposition for the primary foredunes sampled in the present study (Figure 3.7). This result was somewhat unexpected as a positive correlation between sand accumulation and rhizome density has been identified for *A. breviligulata* (Disraeli, 1984)). Regression analysis did, however, indicate a significant positive relationship
between rhizome density and dune height (Figure 3.8). The rhizome density of the primary foredunes sampled in the present study was greatest on the highest dunes. This relationship was not linear. Rhizome density increased by an average of 217 g m\(^{-3}\) between the lowest two primary dune ridges (Chrystalls beach and Allans Beach – F1), but increased by an average of only 47 g m\(^{-3}\) between the highest two ridges (Mason Bay and Allans Beach – F2) (Figure 3.8).

### Secondary dunes

Regression analysis indicated a significant inverse linear relationship between rhizome density and environmental stability (Figure 3.9). The average rhizome density of each foredune ridge at Allans Beach decreased with increasing time since stability at a constant rate of 14 g m\(^{-3}\) yr\(^{-1}\).

**Figure 3.7** Relationship between rhizome density and sand accumulation for primary foredunes. Symbols indicate the average rhizome density per site. Vertical bars indicate 1 standard error from the mean. The best line fit was obtained by linear regression and accounts for 13.6% of the variation in the average rhizome density between sites. The relationship is not significant at 5%.

\[ y = 464.8x + 237.9 \]

\[ R^2 = 0.136, P = 0.632, F = 0.31 \]
Figure 3.8 The development of the rhizome system with increasing dune height for primary foredunes. Symbols indicate the average rhizome density per site. Vertical bars indicate 1 standard error from the mean. The best line fit was obtained by logistic regression and accounts for 98.6% of the variation in the average rhizome density between sites. The relationship is significant at 5%.

Figure 3.9 The relationship between rhizome density and substrate stability across a progradational dune sequence at Allans Beach. Symbols indicate the average rhizome density per secondary dune ridge. Vertical bars indicate 1 std. error from the mean. The best line fit was obtained by general linear regression and accounts for 99.9% of the variation in the average rhizome density between sites. The relationship is significant at 5%.
3.3.5 Relationship between node density and sand deposition

Robust measures of sand accretion during foredune development exist for four of the sampled foredune ridges — the Allans Beach – F1, Allans Beach – F2, Chrystalls Beach and Mason Bay foredunes. Regression analysis shows a negative linear relationship between sand accretion and node density at these sites. Those ridges receiving the lowest rates of annual sand deposition had the greatest number of nodes per length of rhizome. This relationship, however, was not significant at P<0.05.

![Figure 3.10](attachment:image.png)

**Figure 3.10** The relationship between the average node density per site and annual rates of sand accretion. Vertical bars indicate 1 std. error from the mean. The best line fit was obtained by linear regression and accounts for 51.4% of the variation in the average node density between sites. The relationship is not significant at 5%.

3.4 Discussion

This study has established *A. arenaria* has the potential to form a sizable bud bank. Bud density measured in the present study varied from 360 ± 333 to 4529 ± 507 buds m⁻³. On average, *A. arenaria* produced 1618 ± 1671 buds m⁻³ over all sites sampled. As
expected, however, the size of the bud bank of *A. arenaria* varies considerably between populations — the maximum bud-density recorded in the current study was 14-fold larger than that of the smallest. Studies in a range of habitats have identified similar variation in the size of the bud bank between populations. For example, the bud bank of several grass species varied by 12-fold when compared between several grasslands in the US (Dalgleish and Hartnett, 2006).

The bud bank of any species is closely linked to the architecture of the organs with which the meristems are associated (Klimešová and Klimeš, 2007). Plasticity in the growth morphology of individual plants in response to, for example, habitat productivity, competition and the frequency and intensity of disturbance, can manifest in considerable differences in the size of the bud bank between populations of a species. Three architectural traits were identified as potentially having an effect on the size of the *A. arenaria* bud bank — the density of rhizome, the density of bud-sites per supporting organ, and the proportion of bud-sites which possess a bud. The current study has established that the density of rhizome and the density of bud-sites have a significant effect on the size of the *A. arenaria* bud bank. In contrast, the proportion of nodes with dormant buds was remarkably consistent between sites; it varied by only 17% between all sampling locations and no significant differences were detected.

A substantial body of literature exists on the clonal growth of *A. arenaria*. This, in combination with the results of the present study, allows some initial findings regarding rhizome density, node density and the differentiation of dormant buds under different environments.

### 3.4.1 Rhizome density

**Primary foredunes**

Rhizome density varied between the primary foredunes sampled in the current study. Such variation may reflect differences in the growth of rhizomes under different environmental conditions (Klimešová and Klimeš, 2007). However, gross differences in the growing environment cannot easily explain the inter-site variation in rhizome
density observed in the present study. No significant relationship between annual rates of sand deposition and average rhizome density per primary foredunes was detected in the present study. Further, the foredune ridges F1 and F2 at Allans Beach formed under similar environmental conditions, yet the average rhizome density of these foredunes differed by over 100 grams.

A positive relationship between rhizome density and dune height, due to a cumulative increase in the density of the rhizome system with foredune growth, provides an alternative explanation for this result. The vertical rhizome system of *A. arenaria* is characterised by profuse branches (Grieg-Smith, 1961). As branching is initiated by burial (Gemmell *et al*., 1953), and foredunes accrete in response to sand accretion, an increase in rhizome density with increasing dune height can be expected. The results of the present study support this hypothesis. There was a strong correlation between the height of the primary foredune and rhizome density regardless of the sampling site. Further, due to differences in dune height, samples obtained from depths of 0 – 20 cm at Chrystsalls Beach, and 80 – 100 cm from F1 at Allans Beach, were obtained from similar depths as measured relative to spring high tide (60 – 80 cm). The density of rhizome at these depths at each site was remarkably consistent (43 g m$^{-3}$ vs. 52 g m$^{-3}$). The results indicate, therefore, that the differences in rhizome density between the primary foredunes in the current study stems from differences in dune height. They are the consequence of increased branching with dune development.

The rate of increase in rhizome density in the present study slowed with increasing dune height, indicating that there is a maximum limit to the density of *A. arenaria* rhizomes. It implies that branches form primarily during the initial stages of dune development, after which they occur infrequently. This result is consistent with the observations of Greig-Smith (1961) that usually only one tiller per apical meristem elongates in response to burial — consequently branches are relatively rare. The vertical distribution of rhizomes as measured in the present study provides further evidence that branches form less frequently with increasing dune-height. Rhizome density was sampled throughout the entire height of the dune only at Chrystsalls Beach, and only at this site was a significant correlation between dune height and rhizome density detected. In contrast, the rhizome density of F1, F2 and Mason Bay did not differ substantially with depth. When sampled from Mason Bay, the density of rhizomes in the uppermost 20 cm
of the dune profile was significantly lower than that at other depths; however, rhizome density did not differ at depths in excess of 20 cm. This probably reflects the depth of the apical meristem at this site. It may have been that sampling occurred shortly following a burial event and tillers had not yet elongated, or alternatively, that the apical meristem is located at greater depths at Mason Bay than at the other sampling sites. It appears that any increase in rhizome density with increasing height at F1, F2 and Mason Bay occurs sufficiently slowly that the sampling strategy of the present study was not sufficient to detect a significant relationship. Samples would need to be obtained deeper than 1 m to detect a correlation between density and height.

A maximum limit to the density of *A. arenaria* rhizomes is consistent with the results of Wallen (1980). When sampled across a progradational dune sequence the rhizome density of *Ammophila baltica* increased rapidly with distance inland until the third foredune ridge after which it remained constant (Figure 3.11). The dune sequence sampled by Wallen (1980) is similar to that sampled at Allans Beach in the present study; the formation of each successive foredune reduces sand supply to those behind. In contrast to the present study, however, the rhizome of *A. baltica* continues to form even on stable secondary dunes. Horizontal rhizomes accounts for a much greater proportion of the rhizome system of *A. baltica* than *A. arenaria* (Rihan and Gray, 1985). In contrast to vertical rhizomes, the development of horizontal rhizomes is not dependant on burial so, theoretically, the density of the rhizome system of *A. baltica* could increase indefinitely.

Wallen (1980) suggested that the steady rhizome density after 10 years stems from a changing balance between above-ground and below-ground biomass. The increasing respiratory burden of the rhizome mass with increasing age, combined with the decline in photosynthetic ability resulting from a loss of vigour under stable conditions, results in the formation of a steady-state where no net increase in biomass occurs. The respiratory cost of maintaining a large rhizome system does not easily explain the relationship between rhizome density and dune height observed in the present study. Even though rhizome density remained relatively constant once dune height exceeded 60 – 80 cm, the respiratory burden of the rhizome system would still increase linearly with height. Further, the literature indicates that the maintenance cost of a rhizome system and its associated bud bank is not large (e.g., Baye, 1990; Čižková and Bauer,
An alternative explanation is simply that a lack of available space limits rhizome branching once height exceeds 60 – 80 cm.

The rhizome density at Mason Bay is probably close to the maximum density of *A. arenaria* rhizomes. Despite more than 3 m difference in dune height, the average rhizome density of Mason Bay and F2 differed by less than 50 g m\(^{-3}\). A foredune would have to be considerably higher than the foredune at Mason Bay to result in any substantial increase in rhizome density. Further, there was relatively little variation in rhizome density between samples obtained from Mason Bay (280 g m\(^{-3}\)) compared to samples obtained from F1 (6271 g) and F2 (4011 g), indicating that the rhizome system of *A. arenaria* is distributed relatively homogenously at Mason Bay. There appears to be little potential for any further increase in density at Mason Bay, even with ongoing accretion.

![Figure 3.11](image-url) Figure 3.11 Development of rhizome biomass of *A. baltica* as measured across four prograded dune-ridges (Wallen, 1980). Error bars represent ± 95% confidence limits.
Secondary foredunes

The chronosequence of secondary dune ridges at Allans Beach (F2, F3 and F4) provided an opportunity to examine the *A. arenaria* rhizome system following stabilisation. These foredunes were of similar height, formed (prograded and accreted) at a reasonably constant rate, and currently experience little or no sand deposition. As the rhizome system of *A. arenaria* reflects the processes involved in foredune growth it can be assumed that the density of rhizome was similar for each ridge at the time of stabilisation. The rhizome density of F2 is probably close to the density associated with F3 and F4 at the time of stabilisation. Hence, any differences in rhizome density between the secondary foredunes at Allans Beach reflect the decay of the rhizome system with time.

The present study found a significant decrease in rhizome density with dune age (and time) since the cessation of accretion (stability). The cause of this decay is not clear. Even though the metabolic cost of the rhizome system is likely to be low, the decrease in the vigour *A. arenaria* in the absence of sand accretion may mean that it is unable to support the full system developed when vigour was high. Alternatively, the observed decrease in rhizome density in rhizome density may reflect the lifespan of *A. arenaria* rhizomes. Regardless, the increasingly shallow distribution of rhizomes with dune age at Allans Beach indicates that older, hence deeper, rhizome branches decay preferentially.

3.4.2 The density of bud-sites

The present study has shown that the node density of *A. arenaria* varies considerably between populations. The ratio of nodes to rhizome varied from five nodes per gram of rhizome to over 14 nodes per gram of rhizome. This variation in the node density of *A. arenaria* can have a substantial effect on the size of the bud bank of *A. arenaria*. For example, although the rhizome density of Mason Bay and F2 differed by less than 9%, the higher node density associated with Mason Bay meant that the bud bank at Mason Bay was over 74% larger than that associated with F2. This equates to a difference of over 3000 buds m$^{-3}$ between these dune-systems.
A. arenaria survives burial through the elongation of internodes (Gemmell et al., 1953; Huiskes, 1979). Several studies have reported an increase in the internode length of A. arenaria spp. with burial due to the need for greater elongation to raise the apical meristem to a non-lethal depth (Disraeli, 1984; Maun and Lapeirre, 1984; Baye, 1990). An increase in the internode length when plants experience increased burial implies a negative relationship between the rate of sand deposition and node density. Increasing internode length would result in widely spaced nodes, and hence a corresponding decrease in node density.

It was difficult to examine this relationship in the current study due to the uncertainty surrounding the date of foredune formation and hence rates of sand accretion for some sites, however robust measures of accretion rates did exist for the Allans Beach–F1 and F2, Chrystalls Beach and Mason Bay foredunes. The present study showed some evidence for a negative correlation between node density and the rates of sand accretion during foredune formation. In general, sites with the lowest node densities were those with the highest annual rates of sand accretion, although this relationship was not statistically significant. Taken with the weight of evidence from existing literature, there is strong support for the hypothesis that the node densities of A. arenaria are greatest when rhizomes are formed under low rates of sand accumulation.

There is a limit to the node density of A. arenaria. The length of individual buds in the present study varied between 0.1 and 1 cm. The length of each node, however, was fairly consistent — about 0.4 cm. There can be no more than 2 – 3 nodes cm\(^{-1}\) of rhizome. Rhizome weight was measured in the present study rather than rhizome length, however, a close correlation between rhizome length and weight has been established (Chapter 2, Section 2.3.3). Rhizome weight increases by 1 gram per every 7.9 cm increase in length. The maximum node density of A. arenaria can be calculated, therefore, at about 20 nodes g\(^{-1}\) of rhizome.

### 3.4.3 The proportion of buds to nodes

The results of the present study indicate that A. arenaria maintains a consistent ratio of dormant to active buds regardless of the growth environment. The proportion of buds to
nodes varied by no more than 17% between dune-systems. Further, these results are consistent with measurements of bud activation of *A. arenaria* in the Hebrides by Greig-Smith *et al.* (1947). They found 65% of nodes possessed a dormant bud, compared with 62% in the current study. This may indicate that the proportion of buds to nodes is not only consistent between populations in southern New Zealand, but is also consistent over a wider geographic range. It appears that bud activation in *A. arenaria* is not environmentally determined, but is rather an innate characteristic of this species. Further work, however, is required to establish this relationship.

It is surprising that the proportion of dormant buds to nodes did not vary between sampling sites or with depth, particularly when sampled across the progradational sequence at Allans Beach. The evidence from the literature indicates that fewer nodes will possess a bud on older dune-ridges and on older, hence deeper, rhizomes. Similar studies on *Carex arenaria* identified considerable variation in the bud/node ratio between what was termed “mature” populations (equivalent to that of *A. arenaria* associated with F2) and “slack” populations (equivalent to that of *A. arenaria* associated with F4) (Nobel *et al.*, 1979). The proportion of nodes with buds associated with the “slack” population was consistently 54 – 60% lower than that associated with the “mature” population. Further, field and glasshouse studies have noted that fewer nodes on older rhizomes possess buds, due to increased decay and/or the differentiation of buds into new rhizome with time (Greig-Smith *et al.*, 1947; Pavik 1983b). There is no clear explanation for this result in the present study, except that the buds of *A. arenaria* are held dormant by apical dominance which requires some injury to break dormancy. It may be that the gradual dieback of the rhizome system of *A. arenaria* with decreasing sand deposition and/or age is not sufficient to trigger regeneration. Alternatively, below-ground bud banks serve as reserves to protect against catastrophic disturbance; hence, the maintenance of a constant proportion of buds could imply a trade-off between long term population persistence versus a short-term increase in density.

Nobel *et al.* (1979) identified a seasonal pattern in the size of the *C. arenaria* bud bank. The proportion of nodes with dormant buds decreased by 40 to 60%, from the start to the end of summer. Several studies have identified similar patterns in the size of a species below-ground bud bank with season (e.g., Benson *et al.*, 2004; Dalgleish and Hartnett, 2006). The size of the bud bank typically decreases at the start of the growing
season as buds are utilised to support regeneration. As the growing season continues, these are replaced by the production of new rhizomes. The present study did not investigate the potential for seasonal variation in the size of the bud bank associated with A. arenaria. All samples were obtained in late summer. However, although the growth of A. arenaria is seasonal, it is unlikely that the bud bank displays any substantial seasonal flux. A. arenaria tillers do not die-back completely over winter, and any increase in tiller density during spring occurs through the elongation of more than one tiller rather than regeneration from its bud bank (Gemmell et al., 1953; Huiskes, 1979).

3.5 Conclusions

The current study has determined that A. arenaria has the potential to form a sizable bud bank, but this bud bank may vary considerably between populations, depending on rhizome and node density. There is a maximum limit to the density of the A. arenaria bud bank. The present study has shown that rhizome density is unlikely to exceed 500 g m$^{-3}$ and that node density cannot exceed more than 20 nodes g$^{-1}$ of rhizome. The maximum size of the A. arenaria bud bank can be calculated therefore at about 10,000 buds m$^{-3}$.

The size of the A. arenaria bud bank is dependent on the depositional regime of the growth environment. Sand deposition facilitates the formation of the rhizome system, hence the development of a bud bank. Sand deposition also maintains a vigorous population that can support an extensive rhizome system, which in turn determines node density and increases rhizome density by increasing dune height. To maintain the vigour of A. arenaria and increase rhizome density burial will need to be ongoing. Too much burial, however, increases internode lengths and reduces the volume of the bud bank. All other things being equal, the largest bud banks would be associated with high foredunes in an accretionary environment but which receives only small amounts of sand on an annual basis.
Chapter 4

The hydrochoric potential of *Ammophila arenaria* rhizomes

4.1 Introduction

This chapter examines the hydrochoric potential of *Ammophila arenaria* rhizomes in the sea — the ability of a fragment to remain buoyant and viable in a salt-water environment. Fragments of *A. arenaria* rhizome are dispersed by waves and the movements of the surface waters. The longer a fragment can remain buoyant and viable while in seawater, the greater the distances it can be transported.

The hydrochoric potential of *A. arenaria* remains unclear. Individual plants of *A. arenaria* that can only have arisen from marine-dispersed rhizomes have been recorded hundreds of kilometres from the nearest source of rhizome (e.g., Johnson, 1979). Although these populations may be the result of a series of short distance dispersal events, the frequency with which they are observed indicates that *A. arenaria* is capable of dispersal over periods of weeks to months. In contrast, the results of laboratory-based experiments indicate that the hydrochoric potential of *A. arenaria* is low. The buoyancy and/or viability of *Ammophila* spp. when immersed in seawater has been examined in a number of studies (Table 4.1). These concluded that the rhizomes of *Ammophila* can float for up to 6 days when in seawater (Maun, 1985), and remain viable for between 13 and 71 days (Baye, 1990; Aptekar and Rejmánek, 2000). As the hydrochoric dispersal of most species can only occur while a propagule remains both buoyant and viable (Ridley, 1930), these studies indicate that the dispersal period of *A. arenaria* is between 3 – 6 days.
Table 4.1 The length of time that *Ammophila* spp. (*A. arenaria* and its North American congener, *Ammophila breviligulata*) can remain buoyant or viable when in seawater.

<table>
<thead>
<tr>
<th>Variable tested</th>
<th>Species</th>
<th>Immersion periods tested (days)</th>
<th>Maximum longevity (days)</th>
<th>Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buoyancy</td>
<td><em>A. breviligulata</em></td>
<td>0 – until no fragments remained buoyant</td>
<td>5 – 6</td>
<td>Maun (1985)</td>
</tr>
<tr>
<td></td>
<td><em>A. arenaria</em></td>
<td>0 – 7</td>
<td>3 – 4</td>
<td>Knevel (2001)</td>
</tr>
<tr>
<td>Viability</td>
<td><em>A. arenaria</em></td>
<td>0 – 71</td>
<td>4 – 71</td>
<td>Baye (1990)</td>
</tr>
<tr>
<td></td>
<td><em>A. breviligulata</em></td>
<td></td>
<td>at least 71</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. arenaria</em></td>
<td>0 – 13</td>
<td>at least 13</td>
<td>Aptekar and Rejmánek, (2000)</td>
</tr>
<tr>
<td></td>
<td><em>A. arenaria</em></td>
<td>0 – 7</td>
<td>Unknown. Only one fragment regenerated. The immersion period of that fragment is not stated.</td>
<td>Knevel (2001)</td>
</tr>
</tbody>
</table>

There is reason to believe that the measurements of Maun (1985) and Knevel (2001), have underestimated the buoyancy of *Ammophila*. Several morphological features are known to enhance the buoyancy of drift seeds, for example: water resistant outer tissues, spongy mesophyll, and hollow pericarps (Maun, 2009). Such features aid buoyancy either by decreasing the weight to volume ratio of the propagule and/or by preventing water intrusion, allowing the retention of air pockets and the original weight to volume ratio to be maintained. Knevel (2001) recognised that the hollow internodes of *A. arenaria* rhizomes may serve the same purpose by ‘storing’ air inside the fragments, but failed to test the implications of this principle on the buoyancy of *A. arenaria*. Both Knevel (2001) and Maun (1985) examined the buoyancy of single-noded fragments, which lack a sealed air cavity. When a fragment possesses multiple nodes, the nodes seal the internal internodes, potentially resulting in a persistent air cavity. It is likely that substantially longer floatation times may have been reported had Knevel (2001) and Maun (1985) tested the buoyancy of multi-noded fragments.

If buoyancy has been underestimated, then the ability of *A. arenaria* to retain viability when in seawater may be crucial in determining the dispersability of this species. *A. arenaria* remains viable after 13 days in seawater (Aptekar and Rejmánek, 2000), but
cannot withstand immersion for more than 69 days (Baye, 1990). There is no data on
the regeneration of *A. arenaria* following immersion between 13 and 71 days. Further,
there is some evidence to suggest that the longevity of *A. arenaria* rhizomes when
immersed may not be constant between populations or dispersal events. The viability of
rhizomes following different periods of immersion was remarkably consistent when
comparing the results of Baye (1990) and Aptekar and Rejmanek (2000). However, that
determined by Knevel (2001) was substantially lower. Further work examining both the
buoyancy and regeneration ability of *A. arenaria* following seawater immersion is
warranted.

This chapter seeks to determine the time over which hydrochoric dispersal can occur.
Based on the preceding discussion, the following research questions were identified:

- How does immersion in seawater affect the regenerative potential of *A. arenaria*
rhizomes?
- How long can the rhizomes of *A. arenaria* remain buoyant while in seawater?
- How long can the rhizomes of *A. arenaria* remain both buoyant and viable while
in seawater (i.e., the period during which a propagule can be transported and still
retain the ability to regenerate on stranding).

To address these questions the regenerative potential and buoyancy of *A. arenaria*
rhizomes were examined after different immersion periods in a series of experimental
investigations. The effect of node number on fragment buoyancy is examined as this
factor is likely to affect the buoyancy of *A. arenaria* rhizomes. The regenerative ability
of *A. arenaria* from rhizomes differs depending on the vigour of the source population
and the time of year that fragmentation occurs (Chapter 2). Such variation may result in
quite different tolerances to immersion depending on when dispersal occurs or the
physiology of the source population. The relationship between these factors and ability
of *A. arenaria* to regenerate following immersion will be considered. Binary regression
was used to predict the probability that a fragment remains either buoyant or viable.
These probabilities were combined to determine the maximum dispersal time of *A.
arenaria* — when the probability of a fragment remaining both buoyant and viable is
zero.
4.2 Methods

4.2.1 Sampling site

Rhizomes were obtained from the foredune at two sites within Allans Beach, Otago Peninsula, New Zealand (45.9°S, 170.7°E) as described in Section 2.2.1. These sites provide an opportunity to compare two populations of different vigour growing within the same dune-system. Site 1 is associated with a vigorous population of A. arenaria. In contrast, the vigour of the population associated with Site 2 is relatively low.

4.2.2 Regeneration test

Rhizomes were obtained to ensure uniformity in the pre-immersion viability of fragments (as determined in Chapter 2). Only vertical rhizomes were sampled and all samples were collected from a depth of 100 – 140 cm. Rhizomes were cut into fragments 10 cm in length with each length containing one node. Each fragment was visually inspected to confirm the presence of intact buds and to ensure they showed no evidence of decay or regeneration. Those that had decayed or had regenerated were discarded. Following washing, all root and sheath material was removed as it might restrict shoot emergence (after Baye, 1990).

Preliminary test

A preliminary experiment was conducted to determine whether the time of year that rhizomes were separated from the parent plant and immersed in seawater influenced regeneration. Rhizomes were sampled from Site 2 in both March and June 2007, late-summer and mid-winter in southern New Zealand. On each occasion 225 fragments were cut and randomly allocated between three tanks with a continuous flow of seawater (n = 70 fragments per tank). Fragments were floated in seawater sourced from the outer sections of Otago Harbour (45.83°S, 170.64°E) for between 0 and 70 days. Fifteen control fragments were not immersed in seawater but assayed for regeneration within six hours of sampling. Regeneration was measured as the ability of a fragment to
produce a tiller when planted under 3 cm of sand in glasshouse during 120 days. Remaining rhizomes were floated in seawater within 8 hours of sampling. Five fragments per tank were removed every fifth day and assayed for regeneration.

The results of the preliminary experiment are presented in Section 4.3.1. These indicated that the season of extraction did have a significant effect on rhizome viability following immersion in seawater. It was not clear whether this effect was related to the physiology of the plant or simply a factor of the experimental conditions. Seawater temperature differed considerably between the two experiments. Seawater temperature was between 9.9 – 15.9°C during the March experiment and between 5.7 – 10.3°C during the June experiment. Seawater temperature decreased during the March experiment and increased during the June experiment. All other experimental conditions were constant between experiments. In consequence, experiments were designed to test the effect of population vigour, the time of fragmentation and seawater temperature on fragment regeneration. The results of these experiments are presented in Sections 4.3.2 and 4.3.3.

**Test for the effect of population vigour, the time of fragmentation and seawater temperature on regeneration**

Rhizomes were obtained from both Site 1 and Site 2 in July and December 2008 to test for the effect of population vigour and seasonality on the regeneration ability of rhizomes following immersion in seawater. On each occasion 160 fragments were cut from each site and floated in trays of filtered seawater in growth cabinets at 10°C. An additional 20 fragments were cut from each site on each sampling occasion and were not immersed in seawater but instead assayed for regeneration as described in Section 2.2.3 within six hours of sampling. Fragment viability, the time until tillers are produced and the growth rate of the resulting tillers was recorded as measures of a fragment’s regenerative ability. All remaining rhizomes were floated in seawater within 8 hours of extraction. A further 480 fragments were also obtained from Site 1 in June 2008 to test for the effect of seawater temperature on rhizome longevity. Fragments were floated in trays of filtered seawater in three growth cabinets at 5, 15 and 25°C respectively (n =
160 fragments per growth cabinet). These temperatures encompass the temperature range of New Zealand coastal waters (Chiswell, 1994).

Rhizomes were immersed for a maximum duration of 40 days. Seawater was replaced every 10 days to minimise the growth of microbes and to prevent increased salinity arising from evaporation. Twenty fragments from each temperature and age treatment were removed every fifth day and assayed for regeneration.

Limited availability of growth cabinets and space within the growth cabinet prevented more than one replicate per treatment.

4.2.3 Buoyancy test

To assess the ability of *A. arenaria* to float in seawater, rhizomes were obtained from Site 2 and cut into seventy-four fragments possessing one, two or three nodes (n = 23, 31 or 20 fragments per node category respectively). Fragments were then floated in seawater sourced from the outer sections of Otago Harbour (45.83°S, 170.64°E) until no fragments remained buoyant. The water was replaced every seven days. The water was not agitated. All sand and root and sheath material was removed from the fragments prior to floatation.

The number of fragments floating was recorded after 24 hours, every second day for 25 days, and then every seven days until no fragments remained buoyant. All fragments which had sunk were removed and the number of nodes on each fragment was recorded.

4.2.4 Data analysis

Where appropriate, the data from the regeneration and buoyancy tests were analysed using analysis of variance followed by a comparison of means test to identify significant covariates. The data from the preliminary regeneration test were analysed using a one-way ANOVA followed by a Dunnett’s procedure to test for the effect of increasing immersion time on viability (Zar, 1999). One-way ANOVAs followed by a Tukey
comparison of means tests were performed for all covariates to test for the effect of immersion time on the time for tillers to emerge and tiller growth rates (Zar, 1999). One-way ANOVAs followed by a Tukey comparison of means test were performed for each immersion period to examine the effect of seawater temperature on the time for tillers to emerge and tiller growth rates. All independent variables were tested for normality and homoscedasticity prior to analysis using the Kolmogorov-Smirnov and Levene’s test respectively. Where these assumptions failed, appropriate data transformations were performed. The viability data were arcsine transformed, and where necessary, the regeneration time and growth rate data were square-root transformed \((\sqrt{n} + 0.5)\) or log transformed \((\log_{10}(x + 1))\) respectively prior to analysis (Zar, 1999). Results were accepted if significant if \(P<0.05\). The degrees of freedom, F-values and P-values of all ANOVAs are presented in Appendix 1.

A binary logistic regression with logit link function was performed for all levels within covariates to determine the probability that a fragment remains both buoyant and viable (P(VB)) in seawater after different periods of immersion. Coefficients and constants derived from this regression were used to calculate the probability that a fragment remains either buoyant (P(B)) or viable (P(V)) when immersed in seawater. Calculations were made at sequential 24 hr periods. The predicted probabilities were extrapolated until P(B) or P(V) were less than 1% (P<1). P<1 was used to indicate a 100% loss of rhizome buoyancy or viability as at P<1 it is highly improbable that a fragment remains buoyant or viable.

For each regression the p-value of the estimated coefficients was examined. Where there was insufficient evidence that the buoyancy or viability correlated with increasing duration of immersion at a P-level of 0.05, the average value for that coefficient was calculated and used as a constant over all periods of immersion. The model fit of the data were examined using the Pearson, deviance, and Hosmer-Lemeshow goodness-of-fit tests. The P(B) and P(V) obtained via the regression analysis were multiplied together for each 24 hour time period to determine P(VB) of \(A. \ arenaria\) rhizomes. A separate joint probability curve was calculated for each level within factors.
4.3 Results

4.3.1 Results of the preliminary regeneration test

Viability fluctuated during the initial days of immersion, particularly during the June (winter) experiment; nonetheless, there is a clear trend of decreasing viability with increasing immersion periods in both experiments (Figure 4.1). Some fragments, however, remained viable after 70 days – the maximum period of immersion in the present study.

The regenerative potential of fragments differed between experiments — fragments immersed in March displayed a lower tolerance to seawater compared with those immersed in June. Fragments immersed in June remained viable for 35 days longer than those immersed in March (Figure 4.1). Further, fragments immersed in March lost viability at a faster rate than those immersed in June. Fragment viability in March was consistently less than the control for all immersions exceeding 5 days. In contrast, the viability of fragments immersed in June did not differ from the control until immersion exceeded 40 days.

![Figure 4.7](image-url)

Figure 4.7 The effect of the timing of sampling on rhizome viability with increasing immersion in seawater. * indicates that values differ significantly from the control (immersion = 0 days) at P<5% within treatments. Contrasts obtained via Dunnett’s procedure following one-way ANOVA. Vertical bars indicate ± 1 std. error from the mean.
4.3.2 Relationship between population vigour and the season of extraction, and regeneration

Fragment viability

The vigour of the population from which rhizomes were obtained and the month that rhizomes were sampled had little effect on the ability of rhizomes to retain viability when immersed in seawater. The viability of rhizomes obtained from the low vigour population in December was considerably lower than rhizomes obtained in July or from the vigorous population for all immersion periods less than 30 – 35 days (Figure 4.2). This was a consequence of the lowered intrinsic viability of rhizomes obtained from low-vigour populations over summer months, however, rather than a process related to immersion. Viability was lost at a similar rate regardless of the time of year that samples were obtained (July or December), or population vigour. It took the same period of time for fragment viability to decrease below 50% of pre-immersion levels in all treatments, regardless of treatment (20 – 25 days), and no fragments remained viable when immersed for 35 – 40 days. Viability was not lost at a linear rate. It remained close to pre-immersion levels until immersion exceeded 20 – 25 days after which it decreased rapidly (Figure 4.2).

![Figure 4.8](image)

**Figure 4.8** The effect of population vigour and seasonal of extraction on rhizome viability with increasing immersion in seawater.
Time until tiller emergence

All fragments that were immersed in seawater took significantly longer to produce tillers than those that were not immersed (Table 4.2). However, there was no relationship between the immersion period, nor the vigour of the source population, nor the month that rhizomes were obtained and the magnitude of the delay. Immersion in seawater delayed regeneration by about 18 days regardless of treatment.

All fragments obtained from the vigorous population, regardless of the month of obtainment or whether they experienced immersion or not, took significantly longer to produce tillers compared with those obtained from the low-vigour population (Table 4.2). Comparisons between fragments obtained in December and July for both the high and low vigour populations detected no significant difference in emergence times regardless of the immersion time. These results are consistent with the results of Chapter 2 that showed that the rhizomes obtained from high vigour populations take longer to produce tillers compared with those from low vigour populations, and that emergence times do not differ throughout the year. These results are, therefore, unrelated to immersion in seawater. Immersion in seawater did not alter the difference between high and low vigour populations with both immersed and non-immersed fragments from the vigorous population taking about 30 days longer than did fragments from the low vigour population.

Tiller growth rate

Growth rates did not vary between immersed and non-immersed fragments within treatments, regardless of the immersion time (Table 4.2). Tillers derived from fragments obtained from the low vigour population in December grew more slowly than all other fragments at all immersion periods. However, due to the high variability in growth rates within treatments, this difference was not significant. This pattern was the same, regardless of whether a fragment experienced immersion or not. These results are also consistent with the results of Chapter 2; tillers derived from rhizomes obtained from low vigour populations in December grow slower than those from high vigour populations or from those obtained in July. This result is, therefore, due to the intrinsic properties of the rhizomes and unrelated to immersion in seawater.
Table 4.2: The effect of the sampling month and the vigour of the source population on the time for tillers to emerge from the sand surface and the growth rates of the resulting tillers after 20 days of growth following immersion in seawater. Values are averages ± std. dev. Different letters in capitals indicate significant differences within treatments. Different letters in lower-case font indicate significant differences between treatments. All differences are significant at P<0.05. Differences were obtained by the Tukey procedure following analysis by ANOVA. Emergence data were square-root transformed prior to analysis. All dead tillers were excluded from the analysis of growth rates.

<table>
<thead>
<tr>
<th>Time in seawater (days)</th>
<th>Days until emergence</th>
<th>Growth rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>December</td>
</tr>
<tr>
<td></td>
<td>high-vigour</td>
<td>low-vigour</td>
</tr>
<tr>
<td>0</td>
<td>50 ± 2</td>
<td>17 ± 3</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>67± 5</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>10</td>
<td>68± 6</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>15</td>
<td>71± 10</td>
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<td>B</td>
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</tr>
<tr>
<td></td>
<td>a</td>
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</tr>
<tr>
<td>20</td>
<td>67± 7</td>
<td>41 ± 7</td>
</tr>
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<tr>
<td></td>
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<td>25</td>
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<td>65± 11</td>
<td>32 ± 5</td>
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Table 4.3: The effect of the seawater temperature and the vigour of the source population on the time for tillers to emerge from the sand surface and the growth rates of the resulting tillers after 20 days of growth following immersion in seawater. Values are averages ± std. dev. Different letters in bold indicate significant differences within treatments. Different letters in italics indicate significant differences between treatments. All differences are significant at P<0.05. Differences were obtained by the Tukey procedure following analysis by ANOVA. Emergence data were square-root transformed prior to analysis. All dead tillers were excluded from the analysis of growth rates.

<table>
<thead>
<tr>
<th>Time in seawater (days)</th>
<th>Days until emergence</th>
<th>Growth rates</th>
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<tr>
<td></td>
<td>5°C</td>
<td>10°C</td>
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<tr>
<td>0</td>
<td>17 ± 3</td>
<td>17 ± 3</td>
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<tr>
<td></td>
<td>A</td>
<td>A</td>
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<tr>
<td>5</td>
<td>34 ± 5</td>
<td>35 ± 10</td>
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<tr>
<td>10</td>
<td>35 ± 4</td>
<td>39 ± 8</td>
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<tr>
<td>15</td>
<td>33 ± 5</td>
<td>37 ± 3</td>
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<td>20</td>
<td>34 ± 11</td>
<td>41 ± 7</td>
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<td>a</td>
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<tr>
<td>25</td>
<td>31 ± 4</td>
<td>38 ± 4</td>
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<td>30</td>
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<tr>
<td>40</td>
<td>33 ± 5</td>
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4.3.3 Relationship between seawater temperature and regeneration

Viability

The temperature at which rhizomes are immersed affects their ability to remain viable, with fragments immersed at warmer temperatures displaying a reduced tolerance to immersion (Figure 4.3). When immersed in cooler water, rhizomes retained viability at levels close to pre-immersion levels for longer periods of immersion, and also remained viable for longer. For example, when immersed at 5°C, there was no apparent decrease in viability over the duration of the experiment and rhizomes remained viable after 40 days in seawater (Figure 4.3). In contrast, immersion at 25°C resulted in a rapid decline in viability when immersion exceeded 5 days, and no fragments regenerated when immersed for more than 20 days.

Figure 4.9 The percentage of fragments viable after varying periods in seawater at four temperatures: 5, 10, 15 and 25°C.
Time until tiller emergence and tiller growth rate

The effect of temperature on the time tillers took to emerge from the sand surface and growth rates following immersion in seawater were consistent with the effect of population vigour and the sampling month following immersion. Immersion significantly increased the time that tillers took to emerge, but there was no correlation between the immersion time or seawater temperature and the delay in emergence (Table 4.3). Similarly, no correlation between immersion time or temperature, and the growth rate of the tiller could be detected. Although significant differences were detected in the tiller growth rates within and between some treatments, these differences were not consistent.

4.3.4 Rhizome buoyancy

Rhizomes remained buoyant for up to 13 – 172 days depending on the number of nodes on a fragment (Figure 4.4). Overall, fragments with more nodes float better (Figure 4.4). Single-noded fragments are initially more buoyant than both double and triple-noded fragments. All single-noded fragments were buoyant at time 0, whereas 12.9% of double-noded fragments and 5% of triple-noded fragments sunk immediately on being placed in seawater (Figure 4.4). The buoyancy of single-noded fragments, however, exceeded that of multi-noded fragments for only the first 24 hours of immersion. Buoyancy is positively correlated with node number at all immersion periods greater than 24 hours.

The presence of at least two nodes was the most important factor influencing rhizomes buoyancy. Multi-noded fragments retained buoyancy for 144 – 165 days longer than did single-noded fragments, whereas triple-noded fragments only remained buoyant for 20 days longer than double-noded fragments (Figure 4.4). The rate that buoyancy was lost also differed between single and multi-noded fragments. The number of buoyant single-noded fragments declined rapidly at a reasonably linear rate, although it did slow slightly with time. In contrast, the buoyancy of multi-noded fragments declined rapidly
at first, and then reached a plateau at 80% after 5 days, and 52% after 15 days for triple and double-noded fragments respectively. Few fragments then lost buoyancy until 81 days in seawater after which the number of fragments floating decreased rapidly.

4.3.5 The dispersal time of *A. arenaria*

The number of nodes on the rhizome fragment and the temperature of the seawater into which a fragment is dispersed were identified in Sections 4.3.3 and 4.3.4 of the present study as affecting the time that *A. arenaria* rhizomes remain buoyant or viable when in seawater. A binary logistic regression was performed for each level of these covariates to obtain the probability that a fragment remains either buoyant or viable following immersion. The vigour of the population from which rhizomes are obtained and the month that immersion occurs had no detectable effect on fragment longevity in seawater (Sections 4.3.1 and 4.3.2). These covariates were excluded from all the regression
analysis as the evidence indicates that they will not affect the period during which dispersal can occur.

The detailed results of the regression analysis are presented in Appendix 3. Buoyancy or viability decreased significantly with increasing time in seawater for all covariates except when immersed at 5°C (P>0.05). No significant change in viability with increased immersion time was detected when immersed at 5°C. The average rhizome viability of 84.4%, as determined in Section 4.3.3, was used as a constant for all periods of immersion instead of the probabilities obtained by the regression. The regression models were a satisfactory fit for all buoyancy treatments and the remaining viability treatments (Appendix 3), although the model may over-predict buoyancy and viability during initial immersion periods and under-predict during mid-range immersion periods by 5 – 20% (Figure 4.5). The regression model did not adequately predict fragment viability prior to immersion. The probability that fragments remained viable at day 0 differed between treatments by 8.7%. As the viability of fragments prior to immersion should be constant for all treatments the results of the regression were standardised by:

\[
\frac{y(x \times x0)}{100}
\]

where \(x\) = the predicted value; \(x0\) = the predicted value at day 0; and \(y\) = the measured fragment viability at day 0 as determined in Chapter 2 (88%).

The predicted probabilities presented in Figure 4.5 were multiplied together for all possible combinations of covariates to obtain the probability that a fragment remains both viable and buoyant (P(VB)). These combined probabilities are presented in Figure 4.6.

P(VB) decreased with time spent immersed in seawater regardless of node number or water temperature (Figure 4.6). The rate that buoyancy and viability was lost slowed with time. It took between 2.6 and 9.7 times longer for the P(VB) of all treatments to decrease below 0.01 (when the probability that a fragment remains both buoyant and viable is effectively 0), than it did to decrease below 0.5 (when 50% of all fragments remain both buoyant and viable).
The time it took for P(VB) to decrease below 0.01 varied from 10 – 11 days (single-noded, 25°C) to 224 – 225 days (triple-noded, 5°C) (Figure 4.6). Although the regression model illustrates the potential for *A. arenaria* to tolerate immersion of up to 225 days, P(VB) values for immersion exceeding 40 days at 5°C are unlikely to be realistic. The use of a constant viability value means that the changes in P(VB) with increasing immersion for the 5°C treatment was determined solely by the rate at which fragments sink. It is unlikely, however, that the rhizomes of *A. arenaria* can remain viable for this period of time. The retention of viability at immersion exceeding 40 days has not been examined, but when immersed at an average temperature of 8.18 ± 1.03°C, viability declined significantly after 40 days (Figure 4.2). The predicted probabilities for all other temperatures are more robust.

Overall, fragments remained buoyant and viable longer when fragments possessed more nodes and were immersed in cooler temperatures (Figure 4.6). Temperature has little effect on the ability of a fragment to remain buoyant and viable when it has only one node (Figure 4.6). The P(VB) of single-noded fragments differed between temperature treatments by no more than 11% for all immersion periods and the time till P(VB) differed less than 1% (or 3 days). In contrast, the P(VB) differed by up to 53 and 68% for double and triple-noded fragments respectively, and the time till P(VB) was less than 1% by 38 and 42 days (excluding 5°C). The difference between double and triple-noded fragments was slight (Figure 4.6). For example, when immersed at 10°C, the time till P(VB) differed less than 1% (or only 2 days) between double and triple-noded fragments.
Figure 4.11 The probability that a fragment remains either buoyant or viability when in seawater as predicted by binary regression analysis compared to the measured probabilities.
Figure 4.12 The effect of node number and water temperature on the probability that a fragment will remain both viable and buoyant (P(VB)) with increasing time in seawater.
4.4 Discussion

The present chapter examines the period over which the dispersal of *A. arenaria* can occur. To do so, both the regenerative potential and the buoyancy of *A. arenaria* rhizomes were examined following immersion in seawater. Fragments of *A. arenaria* rhizome can remain buoyant for up to 172 days and they are able to regenerate after at least 70 days in seawater. Hence *A. arenaria* is capable of undergoing dispersal for periods of weeks to months. The implications of these results for the dispersal distance of *A. arenaria* will be discussed in Chapter 6.

It is possible that the dispersal potential of *A. arenaria* rhizomes is longer than the present study indicates. For example, this study has shown that the buoyancy of *A. arenaria* rhizome is positively correlated with the number of nodes on a fragment; hence it can be expected that fragments with more than three nodes will be capable of floating for longer than 172 days. However, the buds of *A. arenaria* begin to decay following immersion of about 71 days (Baye, 1990). The dispersal potential of *A. arenaria* is likely to be rapidly limited once immersion exceeds 70 days. The maximum dispersal time of *A. arenaria* is almost certainly less than 100 days, and is probably closer to 75 – 85 days.

The present study has shown that the hydrochoric potential of *A. arenaria* is not constant. The number of nodes a fragment possessed and the temperature of the water into which it is immersed both affected the time over which dispersal could occur. Multi-noded fragments floated longer than fragments with only one node, and a 1°C decrease in seawater temperature equates to about a two day increase in the dispersal time of *A. arenaria*. In contrast, the vigour of the source population and the month rhizomes were obtained had little to no effect on dispersal. The dispersal ability of *A. arenaria* in seawater is, therefore, likely to vary between different populations of *A. arenaria*, between dispersal events from the same population, and even between individual propagules originating from the same population — depending on the morphology of the rhizome fragment.
4.4.1 The effect of immersion on the regenerative potential of rhizomes

The potential for variation in the rate at which viability is lost when immersed in seawater is seldom examined. Most hydrochoric studies assume that the retention of viability is constant between propagules of species. Several studies which have examined the ability of vegetative propagules to remain viable in seawater found similar levels of variation to the present study, although the underlying causes differ. Hall et al. (2006) found that the longevity of the seagrasses, *Halodule wrightii* and *Halophila johnsonii*, varied seasonally. The ability of fragments of rhizome from both species differed two-fold between spring and summer months although the seasonal pattern differed between species. *H. wrightii* remained viable for longer when immersed in spring months compared to autumn months, while *H. johnsonii* remained viable longer when immersed in autumn. A similar response to immersion at different salinities has been reported for some mangrove species (Clarke et al., 2001). Substantial variation in the hydrochoric potential of individual species appears be relatively common, at least amongst vegetative propagules, yet remains poorly recognised within the hydrochoric literature.

How temperature affects the longevity of rhizomes when in seawater is not clear. Morphological features, such as the possession of an impervious coat, like that of some seeds, confer a tolerance to seawater immersion by delaying salt intrusion (Stephens, 1958), while physiological features, such as the ability to sequester salt away from meristematic tissues have also been linked to the retention of viability when in seawater (Guja et al., 2010). In consequence, the ability of a propagule to remain viable when immersed in seawater is generally assumed to be related to salt toxicity (e.g., Erickson and Young, 1995; Guja et al., 2010). Increased rates of toxicity in more saline waters probably explains the effect of salinity on the viability of some mangrove species as observed by Clarke et al. (2001). It is possible that warmer temperatures increase the rate at which buds of *A. arenaria* absorb seawater. Alternatively, the effect of temperature may be related to the reserves stored within the rhizomes when fragmented, and the relative rate of respiration during immersion. The seagrasses, *H. wrightii* and *H.*
both remained viable for longer when fragments were immersed when the amount of stored reserves was highest, implying that rhizome longevity in seawater is determined by the rate at which respiration depletes reserves in the rhizome fragment beyond that required to support shoot growth (Hall et al., 2006). Respiration increases with increasing temperature, providing a potential explanation for the observed relationship between viability and temperature (Öpik et al., 2005). Finally, toxic compounds accumulate as by-products of the respiratory process in low oxygen environments. An increase in the rapidity with which toxic compounds accumulate when rhizomes are immersed in warmer waters may explain the effect of temperature on viability. Further work is required to establish the processes underlying the longevity of A. arenaria rhizomes in seawater. It is likely that its viability in seawater is more variable than the present study indicates.

Under certain conditions, immersion for short periods of time does not affect the viability of A. arenaria rhizomes. Except at 25°C, fragments lost viability slowly during the initial days of immersion. This result is consistent with Baye (1990) and Aptekar and Rejmánek (2000) who found that viability did not decrease significantly until immersion exceeded 4 and 3 days respectively. The ability of A. arenaria to maintain viability close to pre-immersion levels for limited periods of time indicates that immersion does not affect viability until a critical threshold is reached. This may occur when salt penetrates the meristematic tissue, or when the by-products of respiration accumulate to toxic levels, or when the reserves within the rhizomes are insufficient to support regeneration.

The vigour of the source population and the time of year that immersion occurred had little effect on the longevity of A. arenaria rhizomes indicating that the ability of a rhizome fragment to withstand immersion is unrelated to its intrinsic viability. However, the intrinsic viability of a fragment will indicate the dispersal potential of a population by determining the proportion of fragments capable of withstanding immersion of any period. For example, when fragments were obtained from a low-vigour population and immersed in winter, the proportion of fragments that could tolerate any immersion was about 50% less than fragments obtained from vigorous
populations in summer. In contrast, node number caused the proportion of fragments capable of dispersal to vary by only 12.9%.

Baye (1990) found that immersion in water correlated with an increase in the regeneration of *A. arenaria* rhizomes. It was suggested that the apparent stimulation of regeneration in *A. arenaria* by immersion may indicate a possible adaption to hydrochory. However, the current study detected no such relationship. Overall, the ability of rhizomes to regenerate decreased with increasing periods of immersion, and viability was not substantially greater than that of the control following any immersion period. Examination of the results of similar studies on both *A. arenaria* and other rhizomatous species (Aptekar and Rejmánek, 2000; Knevel, 2001) indicates that the results of Baye (1990) are atypical. The ability to maintain viability at pre-immersion levels is not sufficient evidence of hydrochory adaptation.

This study has shown that immersion in seawater increases the time that tillers take to emerge. This lag in regeneration indicates that immersion in seawater induces dormancy in *A. arenaria*. Preventing regeneration when exposed to high levels of salinity is an important characteristic of terrestrial hydrochorous plants such as *A. arenaria* (Ridley, 1930; Lee and Ignaciuk, 1985). It enhances the chances of establishment by ensuring regeneration does not occur in environments of lethal salinity. This trait in *A. arenaria* may, *prima facie*, be indicative of an adaption to marine dispersal.

### 4.4.2 The buoyancy of *A. arenaria* rhizomes in seawater

The relationship between node-number and increased buoyancy is likely to be due to the formation of sealed air cavities within internodes, and the relative rate that they are flooded. The possession of sealed air-spaces is known to enhance buoyancy (Maun, 2009). As the internodes of single-noded fragments are not sealed they would be rapidly flooded when immersed in seawater resulting in a rapid loss of buoyancy. When a fragment possesses multiple nodes, the nodes seal the internal internodes, which may delay the intrusion of seawater. The rate at which multi-noded fragments lose buoyancy
will depend on the speed at which water is absorbed. The ability of multi-noded fragments to maintain high levels of buoyancy indicates that *A. arenaria* rhizomes can remain impervious to seawater for some time.

The inclusion of multi-noded fragments does not fully explain the difference in buoyancy as determined by the current study compared to previous investigations. Both Maun (1985) and Knevel (2001) found that no fragments floated when immersion exceeded 5 – 7 days. However, twenty-six percent of single-noded fragments still retained buoyancy after 7 days in the present study. It is possible that the inclusion of water agitation by both Maun (1985) and Knevel (2001) may have lead to accelerated sinking. By not including water agitation, this study may have overestimated the buoyancy of rhizome fragments, particularly that of single-noded fragments. Increased turbulence may have increased the rate at which water could flood the open internodes of the single-noded fragments. The rate at which multi-noded fragments sink is likely to reflect the rate at which water infiltrates the rhizome tissue. Therefore, water agitation can be expected to have only a limited effect on the buoyancy of multi-noded fragments. The buoyancy of multi-noded fragments in still water, as recorded in the current study, can be expected to be similar to that in turbulent water.

Rhizome buoyancy may vary with rhizome age. The buoyancy of a fragment appears to be strongly dependant on sealed air-spaces formed by hollow internodes. Young rhizomes lack hollow internodes — the interior of the internodes consists of a coherent pith (Huiskes, 1979). It is possible that the buoyancy of these rhizomes is limited regardless of how many nodes it possesses. It is not known when hollow internodes form; however, the appearance of rhizomes sampled in Chapter 3 did not differ with depth or age. This indicates that only very young rhizomes (less than 1 year) lack hollow internodes. The bulk of the rhizome system of *A. arenaria* rhizome system will have the potential to form sealed air-spaces.
4.5 **Concluding remarks**

This study has established that *A. arenaria* can withstand immersion in seawater for longer than previously indicated. In general, *A. arenaria* can withstand immersion for days to months with bud decay probably limiting dispersal when immersion exceeds 70 days. Significant variation in the tolerance of *A. arenaria* to immersion in seawater has been identified. Rhizome longevity is influenced by water temperature and buoyancy by the number of nodes on a fragment. It was also shown that the tolerance of *A. arenaria* to immersion decreases with immersion time. However, the potential exists for *A. arenaria* to be dispersed over considerable distances.

The dispersal period of *A. arenaria* depends primarily on the ability of a fragment to remain buoyant and viable when in seawater. Rhizome buoyancy is enhanced when fragments possess multiple nodes due to the formation of sealed air-spaces within the internode. A loss of viability with seawater immersion is typically assumed to relate to salt toxicity. The current study has identified an alternative mechanism related to the respiratory rates of a rhizome fragment. There is a need to understand the mechanism behind the retention of viability to understand the potential for variation in the dispersal ability of *A. arenaria*.

Immersion in seawater appears to induce secondary dormancy in the rhizomes of *A. arenaria*. This may be an indication that *A. arenaria* possesses specific adaptations related to the hydrochoric process.
Chapter 5

The tolerance of *Ammophila arenaria* to the sandy back-beach environment

5.1 Introduction

The back-beach environment is characterised by episodic substrate disturbance by wave action during storm surge conditions, high rates of burial, low nutrient and water availability, periods of extreme temperatures, exposure to salt aerosols and periods of high substrate salinity (Lee and Ignaciuk, 1985; Davy and Figueroa, 1993; Maun, 1994). As a result, few species are able to colonise and survive in such an environment. Only six species in Britain habitually colonise the strandline (Lee and Ignaciuk, 1985), while New Zealand has only one true indigenous strandline species (Hilton, 2006). Although not typically associated with the back-beach, *A. arenaria* is capable of establishing in this environment from stranded rhizomes. However, little is known about the tolerance of *A. arenaria* to the stresses of the back-beach. If this species can withstand only low levels of stress then the successful establishment of *A. arenaria* is likely to be an infrequent event, which is dependent on the coincidence of events leading to stranding in appropriate environment for growth. The current chapter presents the first systematic evaluation of the tolerance of *A. arenaria* to the abiotic back-beach environment while establishing from fragments of rhizome.

When established, *A. arenaria* can withstand high levels of most stresses associated with the back-beach. For example, it can withstand rates of burial up to 50% of the leaf height (Ranwell, 1958), high rates of salt aerosols (Sykes and Wilson, 1988), four weeks without water stress (Dixon *et al.*, 2004), and temperatures in excess of 50ºC (Huiskes, 1979). The tolerance of immature plants to stress, however, is typically lower than that of mature plants. For example, *A. arenaria* seedlings exhibit a lower tolerance to salt-spray compared with mature plants. Artificial application of salt spray increased shoot necrosis and decreased the growth rates of *A. arenaria* seedlings, while mature
plants were unaffected (Sykes and Wilson, 1988). The tolerance of juvenile plants of *A. arenaria* from rhizomes to stress can be expected to be reduced compared to that of mature plants.

Two questions were addressed in the present chapter: 1) how does environmental stress affect the regeneration ability of a rhizome fragment, and 2) how does environmental stress affect tiller survival while establishing? These questions were addressed though both glasshouse experiments and field observations. Glasshouse experiments were designed to examine either regeneration from rhizomes or the survival of immature plants subjected to key stresses. Regeneration was examined in relation to fragment burial and desiccation. Survival was examined in relation to tiller burial, water deficit, salt-spray, episodic high substrate salinities and extreme heat. The effect of nutrient supply, prolonged high substrate salinities, substrate erosion, and extreme cold were not examined as sufficient knowledge exists as to the effect of these stresses on establishment and/or they are unlikely to be significant in the New Zealand environment. Fragments and tillers were exposed to increasing levels of each stress with the maximum levels in excess of likely field conditions. Each stress was investigated individually. It was intended that this strategy would allow identification of those variables that are particularly important in limiting establishment which would allow the characterisation of beaches by such variables in terms of how suitable they are for establishment. This would allow beaches to be characterised in terms of their vulnerability to *A. arenaria* establishment.

## 5.2 Methods

### 5.2.1 The effect of environmental stress on fragment regeneration

Rhizomes were obtained from Allans Beach, New Zealand (45.9°S, 170.7°E) to test the effect of burial and desiccation on the regeneration ability of *A. arenaria* (Figure 5.1). To maximise the intrinsic fragment viability (as per Section 2.4.1 of the current study),
Rhizomes were obtained during winter from depths of 100 – 140 cm and from only the vertical rhizome system. Rhizomes were cut into fragments, each with one node. Each fragment was visually inspected to confirm the presence of intact buds and to ensure they showed no evidence of decay or regeneration. Those that were decayed or had regenerated were discarded. All root and sheath material was removed as it might restrict shoot emergence (after Baye, 1990).

Figure 5.1 Location of the site of rhizome collection and field study sites at Allans Beach, Otago Peninsula. The arrow indicates the location where rhizomes were obtained for the glasshouse studies. The labels ‘Site 1’ and ‘Site 2’ indicate the location of the field sites for the examination of tiller survival in the field.

**Rhizome burial**

To examine the effect of fragment weight on the regeneration ability of *A. arenaria* following burial, rhizomes were cut into fragments of three lengths; 4, 10 and 20 cm, and weighed to 2 decimal places. Nine fragments of each length were then randomly assigned to one of seven burial treatments: burial at 10, 15, 20, 25, 30, 35, and 40 cm
from the sand surface. Analyses of variance on log-transformed data were performed to ensure that the effect of burial on regeneration was independent of any differences in fragment weight between treatments. No significant difference in weight was detected between burial treatments at a 5% significance level (One-way ANOVA: d.f = 6, F = 0.44, P = 0.851). Fragment weight per burial treatment was 1.12 ± 0.09 g, on average.

Each fragment was labelled with its weight and planted horizontally in pots over 5 cm of beach sand. Dry sand was placed on top of fragments until each fragment was buried to the appropriate treatment level. Three fragments of each length were planted in each pot. There were three pots per burial treatment. All replicates were watered every three days.

All replicates were checked daily for emergent tillers. Each new tiller was marked by a ring labelled with the date and the number of days from propagation calculated. The length of each tiller was measured twenty days following emergence and the growth rate of each tiller calculated by:

\[
\frac{(x_{20} - x_0)}{20}
\]

where \(x_{20}\) = the length of the tiller at age 20 days, and \(x_0\) = tiller length at the day of emergence.

After 120 days the number of tillers per replicate was counted. Each tiller was excavated and the weight of the associated rhizome fragment recorded.

**Rhizome desiccation**

To examine the effect of desiccation on the regenerative ability of *A. arenaria*, rhizomes were cut into fragments 10 cm in length with one node. Fifteen fragments were immediately planted under 3 cm of sand as a control (Day ‘0’ exposure). The remaining fragments were randomly assigned to one of three treatments. One third of fragments were exposed on wire racks at ambient glasshouse conditions for one of ten exposure periods; from 1 to 10 days at 24 hour intervals (Treatment 1). Fifteen fragments were
randomly selected following each exposure period and assayed for regeneration. Similar studies have indicated that rehydration of the propagule following exposure, such as by rain or over-wash by waves, may increase the tolerance of a species to desiccation (Miller et al., 2003). To assess the effect of rehydration on the tolerance of *A. arenaria* rhizome to desiccation, fragments were exposed as in Treatment 1, but then immersed in freshwater for 24 hours before regeneration was assayed (Treatment 2). Finally, to differentiate the effect of exposure from desiccation, fragments were wrapped loosely in moistened cheesecloth to prevent moisture loss, and then exposed and analysed as in Treatment 1 (Treatment 3). The cheesecloth was kept moist throughout the duration of the experiment.

Regeneration following desiccation was tested as described in Section 2.2.3 of the current study. Fragments were planted in trays under 3 cm of sand with five fragments per treatment in each tray and tiller emergence observed for 120 days. Viability was measured as the ability of a fragment to produce at least one tiller. The day of regeneration was recorded as the day which a tiller emerges from the sand surface. Trays were checked daily for emergent tillers. Each new tiller was marked by a ring labelled with the date and the number of days from propagation calculated. The growth rate of each tiller was calculated by:

\[
\frac{(x_{20} - x_{0})}{20}
\]

where \(x_{20}\) = the length of the tiller at age 20 days; and \(x_{0}\) = tiller length at the day of emergence.

Tiller length was measured twenty days following emergence to control for any systematic change in growth with age. Tiller length was measured in millimetres from the sand surface to the tip of the central blade. Dead tillers were excluded from the calculation.
In order to relate the effects of exposure to moisture loss, all fragments were weighed immediately prior to and following exposure. The weight change of each fragment following exposure calculated and converted into moisture change by:

\[
\text{Change in the moisture content of a fragment (\%) = } \frac{(x_1 - x_0)}{y} \times 100
\]

where \( x_1 \) = fragment weight following exposure, \( x_0 \) = fragment weight before exposure; and \( y \) = the percentage moisture content of a rhizome fragment before exposure. \( y \) was determined by drying an additional rhizome sample at 80°C for 48 hours. On average, water accounts for 66% of fragment weight.

### 5.2.2 The effect of environmental stress on tiller survival — glasshouse experiments

To test the tolerance of *A. arenaria* to the back-beach environment, the survival of juvenile individuals grown from rhizomes in response to burial, desiccation, salt spray, seawater inundation, and heat was examined. These plants were grown under ambient glasshouse conditions from rhizome fragments ten centimetres in length with one node obtained from Allans Beach as per Section 5.2.1.

Fragments were planted horizontally in trays under 3 cm of beach sand with ten fragments to each tray with treatment commencing 50 days following propagation. Due to variation in the emergence rates of individual tillers, tiller length varied considerably within trays. To control for the effect of tiller length on survival, only tillers 10 – 20 cm in length were included for analysis. There were at least 5 tillers per tray with an average of 8 tillers per tray.

Tiller survival was measured by visual examination 120 days following treatment. A tiller was deemed not to have survived the treatment if all of the above-ground tissue was necrotic. A period of 120 days was sufficient for tillers to recover from any short-term stress imposed by the treatment, allowing the long-term effect of stress to be examined.
**Tiller burial**

Trays of tillers were randomly assigned to one of five burial treatments in proportion to the average shoot height above the sand surface of each tray; no burial (control), buried to 25% of average shoot height, buried to 50% of the average shoot height, buried to 100% of the average shoot height and buried to 150% of the average shoot height per tray. There were three replicates (trays) per burial treatment with at least five tillers per replicate.

Dry sand was placed in each tray until the desired burial level as measured from the original sand surface was reached. Care was taken not to crush any shoots or individual leaves. Once burial levels were reached, trays were watered. Any decrease in sand levels was measured and trays were topped up with dry sand to the required burial level. Tillers were then assayed for survival.

The variation in tiller height per tray at the time of burial meant that individual tillers experienced rates of burial between 29 and 151%. For analysis, tillers were sorted into categories based on the rate of burial: 0%, 20 – 39%, 40 – 59%, 60 – 79%, 80 – 99%, and greater than 100%. The number of tillers per category varied, but $n$ was at least 10 for each burial rate. Tiller length did not differ significantly between burial categories at a significance level of 0.05 (One-way ANOVA: d.f. = 5, $F = 1.52$, $P = 0.199$).

**Tiller desiccation**

To test the effect of drought stress on tiller survival, trays of tillers were exposed to one of five periods without water: 0 days, 7 days, 14 days, 21 days and 28 days. All trays were watered to saturation upon the commencement of the drought treatment. Following each period of exposure, three trays were randomly selected, watered to saturation, and then assayed for survival.

Soil moisture was initially measured 12 hours following commencement of the drought treatment and then after each exposure period. Soil moisture was effectively 0% within the first week of treatment and did not differ between treatments at any exposure period.
nor did soil moisture in the control differ significantly from pre-treatment levels for the duration of the experiment.

Analysis of variance was used to test for differences in tiller length between treatments. No significant differences in tiller length were detected at a 5% significance level (One-way ANOVA: d.f. = 5, F = 2.01, P = 0.087); although tillers in the 21 day treatment were on average 3 cm shorter than those in the other treatments. Tiller length in all other exposure periods was on average between 13.5 ± 4.2 and 17.6 ± 4.3 cm. All results can, therefore, be considered to be independent of differences in tiller length between treatments.

**Salt spray**

To test the effect of exposure of *A. arenaria* to salt aerosols tillers were treated with a fine spray of either seawater or freshwater (control). Water was applied to each shoot from all sides and above until droplets beaded on the aerial parts of the plants. Such beading typically occurs on the leaf surfaces of coastal plants (Boyce, 1954). The base of each plant was flooded with freshwater immediately after spray was applied to prevent an increase in substrate salinity without removing the salts deposited onto tillers during treatment. Substrate salinities were measured two days following each spray application. At no stage the substrate salinity did differ from pre-immersion levels. All plants were watered every two days by flooding the substrate of each replicate with freshwater.

Three trays were randomly selected per salt or freshwater treatment five days following the initial spray application and washed with freshwater to remove all salt from the tillers. These tillers received no further salinity treatment and were assayed for survival. Spray was applied to the remaining trays every 5 days for a total of 20 days. This spraying frequency recreates the most extreme conditions plants growing on the New Zealand coast will experience (Sykes and Wilson, 1988). All plants were washed with freshwater five days following the final spray application to remove all salt from the tillers and then assayed for survival.
Analysis of variance was used to test for differences in tiller length between treatments. No significant differences in tiller length was detected at a 5% significance level (One-way ANOVA: d.f. = 3, F = 0.76, P = 0.522). All results can therefore be considered to be independent of differences in tiller length between treatments.

**Seawater inundation**

Tillers were subjected to a simulated over-wash event to examine the effect of seawater on tiller survival. Each tray of tillers was placed in larger trays filled with either seawater or freshwater (as a control) so that all shoots were immersed in seawater to a depth of 1 cm for 3 hours. This immersion time is similar to the immersion period that a plant growing at the high-tide line would experience during a tidal cycle. Following immersion, one third of the seawater treated tillers were left to drain for 24 hours, after which they were watered with sufficient freshwater to flush any residual salinity from the substrate (Treatment 1). They were then assayed for survival. Another third of the seawater treated tillers were watered with sufficient freshwater to flush any residual salinity from the substrate immediately following immersion before being treated as in Treatment 1 (Treatment 2). This was to simulate an immersion event coinciding with a rain event. The final third of the seawater treated tillers were exposed to a second immersion event 12 hours following the first before being treated as in Treatment 1 (Treatment 3). This was to examine the effect of sequential high-tides on tiller survival.

There were three trays of tillers per treatment. Analysis of variance was used to test for significant differences in tiller length between treatments. Tiller length did not differ between treatments at a 5% significance level (One-way ANOVA: d.f. = 3, F = 1.20, P = 0.316).

**Heat**

Tillers were exposed to one of four temperatures, 30, 40, 50 and 60°C, for three hours. There were three trays of tillers per temperature and exposure period. Tillers were saturated with freshwater to prevent drought stress during treatment. To provide for the
potential damage arising from contact between a dry sand surface and the stem (Maun, 2009), three centimetres of dry sand was placed on top of the saturated sand layer. Tillers were placed in a drying oven at the appropriate temperature. A further fifteen tillers experienced no heat treatment but remained under ambient glasshouse conditions as a control. Following exposure, tillers were watered to saturation and assayed for survival.

Analysis of variance was used to test for significant differences in tiller length between treatments. Tiller length did not differ between treatments at a 5% significance level (One-way ANOVA: d.f. = 8, F = 1.07, P = 0.405).

5.2.3 The effect of environmental stress on tiller survival — field observations

The survival and maturation of plants from stranded rhizomes was observed at two locations within the Allans beach dune-system (Figure 5.1). These sites provide an opportunity to examine the effect of exposure to elevated sea-levels on plant survival. The location of Site 1 on the open coast means it is vulnerable to all three components of elevated sea-levels: tidal stage (astronomical tides), particularly the monthly cycle of spring and neap tides, the magnitude of storm surge (related to atmospheric pressure and wave set-up), and wave height and period. In comparison, Site 2 is protected from wave activity. Episodes of extreme elevated sea-level, during which the back-beach is inundated and/or the foredune scarped, are most likely to occur when spring high tides correspond with low atmospheric pressures. A storm surge in excess of 0.5 m may occur along the Otago Peninsula, and at Allans Beach, indicating there is potential for the rhizomes of *A. arenaria* to be deposited well above the usual level of spring high tides.

Large quantities of rhizomes were deposited at both sites in June 2007. Tangled clumps of rhizome were deposited across the back-beach within a zone approximately 20 m wide (Figure 5.2). Tiller establishment and survival was observed between February 2007 and 2010. Observations were made within a 45 × 60 m section of the foredune and
back-beach at Site 1 and a 50 × 15 m section at Site 2. From March 2008 each site was mapped using a Leica Total Station. The total station was used to produce digital terrain models and record vegetation cover, including the landward and seaward extent of the rhizome derived tillers and the seaward limit of the foredune vegetation.

Figure 5.2 Rhizomes deposited during June 2007 on the back-beach at Site 2. This image is representative of the stranding event at Site 1. The density and the deposition of rhizomes in large mats, which indicates the rhizomes originated locally. There was recent scarping of the foredune within 100 m of both study sites. The arrow identifies the north-east limit of the surveyed area.

### 5.2.4 Data Analysis

The total percentage fragment viability or tiller survival was calculated for each treatment. Where appropriate, analysis of variance was performed to identify significant treatment effects. All independent variables were tested for normality and homogeneity of the variance prior to analysis using the Kolmogorov-Smirnov and Levene’s test respectively. Where these assumptions failed, appropriate data transformations were
performed, or non-parametric tests used. The viability data were arcsine transformed, and where necessary, the regeneration time and growth rate data were square-root transformed \( \left( \sqrt{n + 0.5} \right) \) or log transformed \( (\log_{10}(x + 1)) \) respectively prior to analysis (Zar, 1999). Mann Whitney tests were used to test for differences between two samples. One-way ANOVAs were used to test for differences between more than two samples. Contrasts were obtained for any significant treatments by the Tukey comparison of means test following one-way ANOVA (Zar, 1999). Results were accepted as significant if \( P<0.05 \). The degrees of freedom, F-values and P-values of all ANOVAs are presented in Appendix 1. The W-values and P-values of all Mann Whitney tests are presented in Appendix 2. All tables and figures present untransformed values.

5.3 Results

5.3.1 Fragment regeneration

Burial

The ability of \( A. \ arenaria \) to regenerate from rhizome fragments decreased with increasing depth (Figure 5.3). No tillers emerged from the greatest depth of 40 cm; however, tiller emergence at this depth did not differ significantly from emergence from depths of 30 – 35 cm. Only one fragment per length treatment produced an emergent shoot at depths exceeding 30 cm. Burial also affected the emergence rate. Tillers took longer to emerge from greater depths (Figure 5.3). Overall, fragments buried at shallower depths produce more emergent shoots and produce them faster.

No relationship between the burial depth of a fragment and the growth rate of the associated tillers was detected. When measured after 20 days of growth, tiller growth rates did not differ significantly between depths (ANOVA on log transformed data: d.f = 5, F = 2.08, P = 0.089). The average tiller growth rate varied between depths by less than 0.3 cm day\(^{-1}\) (Figure 5.4).
Regression analysis indicates that the ability of a fragment to produce tillers from depth depends on the weight of the fragment (Figure 5.5). There is a significant relationship between the minimum weight of fragments producing emergent tillers and increasing burial. For each additional centimetre of burial, fragment weight must increase by 0.09 g in order to produce an emergent tiller. A fragment weight of 3.00 g was required to emerge at 40 cm depth — 0.67 g more than the heaviest fragment buried at this depth. Similarly, no fragments 5 cm in length were of sufficient weight to emerge from depths greater than 10 cm. Multiple Mann-Whitney tests provide further evidence that the ability of a tiller to emerge is related to the fragment weight. There was no significant difference between the weights of those fragments producing emergent tillers and those that did not when buried at 10 cm; however, fragments with emergent tillers were heavier than those without emergence tillers at all other depths (Table 5.1).

Not all fragments capable of emerging had necessarily emerged at the completion of the experiment. Tillers were still emerging within 13 days of the experiment being concluded (Figure 5.3), and observation of non-emergent fragments showed that several fragments in most length and depth treatments possessed tillers that had not yet reached the sand surface. The fate of these tillers is unknown. The weight of several fragments with non-emergent tillers was sufficient to produce emergent tillers. It can be expected that given sufficient time these tillers would have reached the sand surface. Therefore, the total percentage emergence from depth can be expected to be higher than the results of the current study indicate.

Upon excavation it was noted that all emergent tillers had formed vertical rhizomes. All new rhizome possessed nodes and each node had a dormant bud. There was no sign of regeneration from any new buds. One single-noded fragment 4 cm in length was cut from each tiller and assayed for viability. All buds produced tillers when planted under 3 cm of beach sand.
Figure 5.3 Cumulative emergence of tillers from rhizome fragments with increasing burial depth. Values at 120 days indicate the final percentage emergence per treatment. Different letters indicate significant differences in final percentage emergence values between depths at a P-level of 0.05 as obtained by the Tucky procedure following one-way ANOVA on arcsine transformed data.

Figure 5.4 The average tiller growth rates (± 1 std. error) from rhizome fragments buried at different depths. Vertical bars indicate one standard-error from the mean.
Figure 5.5 The relationship between rhizome weight and tiller emergence with increasing burial depth. Dashed lines identify the best-fit lines as obtained by regression analysis between the weights of the lightest fragments producing tillers at each depth. All relationships are significant at P<0.05.

Table 5.1 The effect of fragment weight on emergence ability. The values given are the average fragment weight per depth (g) ± 1 st. dev. * indicates a significant difference between emergent and non-emergent fragments within depths as obtained via multiple Mann Whitney tests. No analysis was preformed for depths exceeding 30 cm due to low rates of tiller emergence. All differences are significant at a P-level of 0.05.

<table>
<thead>
<tr>
<th>Burial depth (cm)</th>
<th>Tiller emergence</th>
<th>No tiller emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.03 ± 0.70</td>
<td>0.96 ± 0.59</td>
</tr>
<tr>
<td>15</td>
<td>1.98 ± 0.97</td>
<td>0.82 ± 0.58*</td>
</tr>
<tr>
<td>20</td>
<td>1.50 ± 0.22</td>
<td>0.90 ± 0.47*</td>
</tr>
<tr>
<td>25</td>
<td>2.06 ± 0.44</td>
<td>1.04 ± 0.56*</td>
</tr>
<tr>
<td>30</td>
<td>1.89 ± 0.32</td>
<td>1.06 ± 0.57</td>
</tr>
<tr>
<td>35</td>
<td>2.62 ± 0.18</td>
<td>1.16 ± 0.62</td>
</tr>
<tr>
<td>40</td>
<td>1.19 ± 0.62</td>
<td></td>
</tr>
</tbody>
</table>
Rhizome desiccation

The evidence indicates that the ability of *A. arenaria* to tolerate exposure depends primarily on the desiccation rate of the rhizome fragment. Independent of treatment, viability is high (at an average of 99.09 ± 1.29%) at moisture levels of at least 100% (Figure 5.6). Viability remains close to non-desiccated levels until the moisture content decreases below 55 – 65%, after which viability declines exponentially. No fragments in any treatments exhibited viability at moisture levels less than 18.25%.

Saturation in freshwater following exposure (Treatment 2) increases the tolerance of rhizomes to desiccation, although the effect is not large or persistent. The viability of fragments in Treatment 2 was at least 10% higher than rhizomes that received no re-hydration in freshwater following exposure (Treatment 1) for all exposure periods up to 5 days, although this difference is not significant at any exposure period (Figure 5.6a). Further, rhizomes in Treatment 2 remained viable for up to 24 hours longer than did those in Treatment 1 (Figure 5.6a). The moisture content of fragments in Treatments 1 and 2 differed significantly following exposure for 7 days (Figure 5.6b). There was no difference between these treatments at any other exposure periods. The differences between treatments 1 and 2 are not due to differences in the desiccation rates. These results indicate that the effects of desiccation are irreversible.

Desiccation results in a significant increase in regeneration time. Both Treatments 1 and 2 took significantly longer to produce tillers at longer exposure periods with both the average and minimum days until emergence increasing with increasing exposure (Figure 5.7). The emergence time of fragments exposed under non-desiccating conditions (Treatment 3) decreased significantly with increasing exposure time due to fragment regeneration during exposure. After 4 days of exposure, fragments in Treatment 3 were displaying signs of regeneration. By 10 days of exposure, fragments had elongated to a maximum length of 3 cm. No fragments in Treatments 1 and 2 displayed any indications of growth while exposed.

The effect of exposure and treatment on growth rates was not clear due to large variation in growth rates between and within treatments and exposure times (Figure
There is some evidence that exposure, regardless of desiccation, increased growth rates. The average growth rate of all exposed rhizomes were faster than that of the controls, regardless of exposure time or treatment. This increase was not significant for all exposure periods, nor was it large. On average, exposure increased growth rates by only 2 mm day$^{-1}$.

Figure 5.6 The effect of increasing exposure time on: a) the percentage viability of rhizome fragments and; b) the percentage moisture content of rhizomes. The total moisture content of *A. arenaria* rhizomes is 66 ± 8 % of its dry weight. Vertical bars indicate one standard-error from the mean between replicates. Different letters indicate significant differences between exposure times within treatments as obtained by the Tukey procedure following one-way ANOVA on arc-transformed data. * indicates a significant difference between treatments 1 and 2 within exposure times as obtained by Mann Whitney tests. All values are significant at a P-value of 0.05.
**Figure 5.7** The average number of days until tiller emergence (± 1 std. error) from rhizome fragments: a) exposed; b) exposed, but saturated prior to propagation; and c) exposed, but protected from desiccation with increasing exposure time. Vertical bars indicate one standard-error from the mean between replicates. Different letters indicate significant differences at a P-value of 0.05 between exposure times within treatments as obtained by the Tukey procedure following one-way ANOVA on square-root transformed data.

**Figure 5.8** The average tiller growth rates (± 1 std. error) from rhizome fragments: a) exposed; b) exposed, but saturated prior to propagation; and c) exposed, but protected from desiccation with increasing exposure time. Vertical bars indicate one standard-error from the mean between replicates. Different letters indicate significant differences at a P-value of 0.05 between exposure times within treatments as obtained by the Tukey procedure following one-way ANOVA on log transformed data.
5.3.2 Tiller survival in the glasshouse

Tiller burial

The effect of burial on tiller survival is shown in Figure 5.9. Survival is reduced with increased burial rates, but only when burial exceeded 60% of the tiller height. No tillers survived when burial exceeded 100% of the tiller height.

All surviving tillers that had experienced burial formed new rhizomes. Only vertical rhizomes were produced and it formed only along emergent shoots. All new rhizomes possessed nodes and each node had a dormant bud. There was no sign of regeneration from any new buds. One single-noded fragment 4 cm in length was cut from each tiller and assayed for viability. All buds produced tillers when planted under 3 cm of beach sand.

![Figure 5.9](image-url) Percentage tiller survival with increasing burial as a percentage of the total tiller height at the time of burial.
Tiller desiccation

Tillers were exposed to periods between 0 and 28 days without water to examine the drought tolerance of *A. arenaria*. When examined one week after treatment commenced tillers showed no differ in appearance from the control. In contrast, by week 2, tillers showed signs of wilt and the tips of most tillers were chlorotic. This effect, however, was not persistent. When returned to a regular watering regime tillers regained turgor rapidly, and after 120 days did not differ in appearance from that of the control. A small portion of tillers died when exposed to 1 and 2 weeks without water (Figure 5.10). This decrease in survival, however, was not significant when compared to the control. In contrast, no tillers survived when exposure exceed more than three weeks without water. Tillers continued to display increasing signs of stress even when returned to a regular watering regime, and all tillers were necrotic within 30 days of treatment.

![Figure 5.10](image-url) The survival of *A. arenaria* tillers when exposed to increasing desiccation. Vertical bars identify one standard-error from the mean between replicates.
Salt spray

Exposure to salt-spray had no effect on survival. All tillers remained alive 120 days following treatment regardless of the treatment: exposure to a spray of either a 3.5% saline solution or freshwater once (Treatment 1), or exposure four times over a 20 day period (Treatment 2).

There was some evidence that exposure to salt-spray had a non-lethal effect on the growth of tillers in Treatment 2. By the third spray application, when tillers had been exposed to salt for 10 days, the tips of most salt treated tillers where noticeably more chlorotic than tillers treated with freshwater. The majority of each tiller, however, remained green and by the end of the experiment the salt treated tillers did not differ in appearance from those treated with freshwater. Salt and freshwater treated tillers in Treatment 1 did not differ noticeably in appearance at any stage of the present experiment.

Episodic high substrate salinity

Immersion in seawater significantly reduced tiller survival compared to the control in all Treatments (Figure 5.11). Within seven days of immersion all tillers immersed in seawater (Treatments 1, 2 and 3) were notably more chlorotic than those that were immersed in freshwater (Control), and by the end of the experiment no more than 17% of tillers in each seawater treatment remained alive. There is some evidence that an application of freshwater immediately following immersion increased survival. A portion of tillers that were washed in freshwater immediately following exposure to seawater (Treatment 2) remained alive 120 days following immersion, while no tillers in Treatments 1 and 3 survived immersion (Figure 5.11). The number of surviving tillers in Treatments, 1, 2, and 3, however, did not differ significantly indicating that a freshwater treatment has little effect on the survival of A. arenaria following over-wash.
**Figure 5.11** Tiller survival following exposure to seawater. Vertical bars identify one standard-error from the mean between replicates. Different letters indicate significant differences at P-level of 0.05 between treatments as obtained by the Tukey procedure following one-way ANOVA on arc-transformed data. No statistical analysis was performed to test the treatment effect when Type 2 tillers experienced two immersions due to a lack of variation between replicates.

### Heat exposure

Exposure to heat had no effect on tiller survival. All tillers, regardless of the exposure temperature or duration remained alive 120 days following treatment. Temperatures exceeding 30°C did however have a substantial non-lethal effect. Some leaf necrosis was apparent within 2 days of treatment when exposed to temperatures of 40, 50 and 60°C and all the aboveground biomass died within 8 days of treatment when exposed to 50 and 60°C. This effect, however, was not persistent. When tillers were examined 120 days following treatment, vigorous re-growth was apparent at the base of all tillers. It is expected that given sufficient time, these heat-affected tillers would have made a full recovery.
5.3.3 Tiller survival in the field

The initial distribution of tillers was similar at both Site 1 and Site 2. When surveyed in February 2008, tillers had established from rhizomes stranded in June 2007 in a wide strip parallel to the established foredune at both sites (Figures 5.12 and 5.13). The distribution of tillers was dense but patchy, reflecting the distribution of rhizomes when stranded (as shown in Figure 5.2). The tillers at both sites were green and vigorous although those at Site 1 were smaller and appeared to be less developed than tillers at Site 2. There were no obvious signs of stress except for some chlorosis of the most seaward culms. This was probably the result of salt-stress from wave over-wash. The date of the over-wash event was unknown; however, a fresh wrack line within the tiller zone indicated that it had occurred recently (Figure 5.13). There was no evidence that this event had resulted in the physical removal of tillers.

When visited in June 2008 it was evident that both sites had experienced at least one over-wash event. A well defined wrack line was present within the tiller zone and there had been some beach erosion and scarping since February 2008 (Figure 5.13). The effect of these events on each tiller population was quite different. Beach erosion had resulted in the physical removal of 54% of the original population at Site 1. Of those tillers remaining, 96% were yellowed and appeared dead. A recently deposited wrack line at the base of the original foredune indicted that salt-stress resulting from wave over-wash was the cause. It was evident from recently deposited wrack at Site 2 that seawater had reached the toe of the incipient foredune. However, aside from some minor erosion and yellowing of the most seaward tillers, this event had no impact on the tiller population.

The increased exposure of Site 1 to elevated sea-levels relative to that of Site 2 provides a possible explanation for these differences. Differences in the potential for sand accretion between sites appear also to have contributed. Subtracting the difference between the June 2008 and February 2008 surfaces allowed rates of sand accretion to be calculated. The sand surface at Site 1 within the zone of surviving tillers remained largely unchanged from February 2008 levels when surveyed in June 2008. In contrast, the sand surface at Site 2 accreted by an average of 0.23 m between February and June.
2008, and sufficient sand had accumulated around the tillers that a distinct incipient foredune was forming in front of the existing foredune. It is likely that the increased elevation of the sand surface at Site 1 served to limit the inland extent of the over-wash, as evidenced by wrack found only along the toe of the incipient foredune, and protecting the majority of the tillers from exposure to seawater. The lack of sand accretion at Site 1 allowed seawater to penetrate to the toe of the established foredune, inundating all the tillers originating from the stranded rhizomes. The greater rates of sand deposition at Site 2 may also explain the increased vigour of the tillers at this site compared to those at Site 1.

Between June 2008 and November 2010, Site 1 cycled between moderate beach erosion and minor accretion. An erosion event between June 2008 and July 2008 lowered the beach to the toe of the established foredune creating a beach scarp 50 – 100 cm high, with the loss of all tillers surveyed in February 2008 (Figure 5.13). No new tillers were observed to establish from stranded rhizomes and when surveyed in November 2010, the beach had been eroded beyond the toe of the original foredune. In contrast, the incipient foredune at Site 2 continued to form with minimal interference from episodes of elevated sea level. *A. arenaria* associated with this developing foredune flowered for the first time in spring 2009. By November 2010, the incipient foredune had accreted by 0.7 m above the July 2008 surface and prograded by 6.7 m beyond the February 2008 seaward limit of tillers. Horizontal rhizomes, approximately 1 – 2 m from the original population, have extended along the seaward margin of the incipient foredune. *A. arenaria* is now attempting to colonise the narrow back-beach environment between the developing incipient foredune and the line of spring high tides. This ‘back-beach’ environment is now only a few metres wide.
Figure 5.12 The alongshore distribution of tillers originating from stranded rhizomes as observed during February 2008 at: a) Site 1 on the open coast; and b) Site 2 within the inlet.
Figure 5.13 Classified digital terrain models showing vegetation cover, the survival of tillers, and foredune development between February 2008 to November 2010 at: a) Site 1 on the open coast; and b) Site 2 within the inlet.
5.4 Discussion

5.4.1 The effect of environmental stress on fragment regeneration

The present chapter examined the effect of desiccation and burial on the ability of rhizomes stranded on the back-beach to regenerate. Both factors were found to significantly reduce regeneration, but only when the level of stress exceeded critical thresholds. Desiccation had no significant effect on the regeneration ability of *A. arenaria* until the moisture content of the fragment decreased below 55 – 65% of the total, and regeneration was prevented only at levels less than 18.25%. Burial had a significant effect on regeneration only when fragments were buried at depths greater than 15 cm, and no tillers in the current study emerged from rhizomes buried deeper than 35 cm.

The effect of desiccation on the regeneration ability of *A. arenaria* is consistent with similar studies. Most vegetative propagules are unable to remain viable once moisture levels decrease below 15 – 20% (Duke, 1985). In contrast, the ability of *A. arenaria* to emerge from buried rhizomes appears to exceed that of other coastal species. The ability of *Thinopyrum junciforme* to emerge from buried rhizomes at depths of up to 17.8 cm was examined by Harris and Davy (1983b), and that of *Panicum racemosum* from fragments buried at depths of up to 16 cm (Cordazzo and Davy, 1999). The length of rhizomes used and/or the month that rhizomes were obtained in these studies was not specified, making comparisons between the ability of *T. junciforme* and *P. racemosum* to emerge from depth with that of *A. arenaria* difficult. Nonetheless, regeneration from rhizome fragments of both species decreased with even small increases in burial depth, and no single-noded fragments of either *T. junciforme* or *P. racemosum* produced emergent tillers when buried at depths of 16 cm or 17.8 cm respectively. In contrast the regeneration ability of *A. arenaria* when buried at depths of 20 cm in the present study was still reasonably high at 27%.
Desiccation rates in the field would depend on several environmental factors including temperature, windiness, light intensity and the exposure of a propagule to these conditions. The desiccation rate of *A. arenaria* stranded on the back-beach, therefore, will vary between dispersal events and stranding environments. For example, it could be expected that a fragment stranded in summer months is more likely to desiccate to lethal levels more rapidly than those stranded in winter months. However, the back-beach environment is essentially a dry environment and desiccation is recognised as a primary factor limiting establishment in the coastal environment (Davy and Figueroa, 1993; Maun, 2009). Fragments of rhizome stranded on the sand surface can desiccate to lethal levels within days. For example, most fragments of *Uniola paniculata*, a coastal grass similar to *A. arenaria*, could not establish when exposed on the back-beach for more than 7 days (Miller *et al.*, 2003). The results of the present study indicate, therefore, that the desiccation of stranded rhizomes is likely to be a key factor limiting the regeneration of *A. arenaria*.

The ability of *A. arenaria* to emerge from buried rhizomes depends on the weight of the buried fragment. A similar relationship between propagule weight and emergence has been identified for seedlings (Barbour *et al.*, 1985; Maun and Lapierre, 1986; Weller, 1989). It reflects the amount of energy stored within the propagule available to support tiller establishment (Maun, 2009). It follows that the maximum depth from which *A. arenaria* can establish will fluctuate according to the flux of reserves within the rhizome system prior to erosion and dispersal. At times *A. arenaria* will be able to emerge from depths both substantially shallower and deeper than the results of the current study indicate.

Large amounts of sand can be deposited by storm waves or through the collapse of beach and dune scarps (Maun, 2009). Such events have the potential to bury *A. arenaria* to depths greater than its ability to emerge. These events, however, are relatively rare. More typically *A. arenaria* is likely to be covered by only a shallow layer of sand. Objects on the back-beach induce sand deposition by creating surface roughness and decelerating wind-flows (Masselink and Hughes, 2003); hence the burial of a fragment on the back-beach is self-facilitating. As sand accretion ceases once burial exceeds the height of the object (Masselink and Hughes, 2003), it can be expected that most
fragments will be covered by a layer of sand of only a few centimetres. The burial of stranded rhizomes is therefore unlikely to normally limit the ability of *A. arenaria* to regenerate on the back-beach.

**5.4.2 The effect of environmental stress on tiller survival**

The results of the current study indicate that juvenile plants of *A. arenaria* can tolerant high levels of most stresses associated with the back-beach environment. Burial did not affect survival until it exceeded 60% of the tiller height. Plants could withstand up to three weeks without water, could withstand temperatures of 60°C, and survival is not affected by exposure to salt-spray. At times, conditions in the field may exceed the tolerance limits of *A. arenaria*. For example, the collapse of a scarped dune-face would result in the abrupt, deep burial of plants growing at the base of the dune. The typical levels of burial, temperature, drought and aerial salinity conditions associated with the back-beach, however, are within the tolerance limits of *A. arenaria*. Most of the time, burial, temperature, desiccation and aerial salinity are unlikely to limit the survival of *A. arenaria* from stranded rhizomes.

It is surprising that desiccation and burial do not affect the survival of establishing plants of *A. arenaria*. Desiccation and burial have been identified as the primary cause of seedling mortality when plants establish on sandy coasts (McLeod and Murphy, 1977; Payne and Maun, 1984; Kollmann et al., 2007). Further, desiccation and burial have been identified as the main causes of death when *A. arenaria* is establishing from seed (Huiskes, 1977). The origin of plants from rhizomes rather than seed provides a possible explanation for the findings of the present study. Plants derived from rhizomes display a greater tolerance to stresses than do comparable plants from seed. For example, when the establishment and survival of *Ammophila breviligulata* was followed for one year of growth, only 4% of seedlings remained alive following one year of growth compared to 35% of rhizome-derived plants (Maun, 1985). The relative robustness of rhizome-derived plants compared to seedlings derives from the greater supply of growth reserves stored within rhizomes compared with seeds. These reserves
both allow for rapid growth as well as providing a reserve which can be drawn upon in the event of a decrease in photosynthetic ability (Duke, 1985).

The results of the current study should be interpreted with caution as the constraints inherent in the glasshouse experiments may have led to an over-estimation of the tolerance of *A. arenaria* to stress. Rhizome fragments were cut to equal lengths (10 cm), and only tillers within a restricted range of lengths (10 – 20 cm) were included in the analysis. Larger propagules have been linked to an increased tolerance to stress, primarily because they produce larger seedlings that grow more rapidly (Maun, 2009). Fragments less than 10 cm in length may be less tolerant of stress than the present study has indicated. Similarly, tillers shorter than those used in the present study may also be less tolerant to stress, and may be more likely to experience lethal levels of stress. For example, burial affects tiller survival when tillers were buried by more than 100%. In the field, smaller tillers are more likely to be buried to lethal levels than larger ones. The ability of *A. arenaria* to establish from stranded rhizomes may depend on the growth rate of the resulting tiller, especially during the initial days of growth, and/or the timing of stress in relation to tiller emergence. Finally, the present study examined the effect of a one-off exposure to stress on the survival of plants growing in an ideal growth environment. The tolerance of *A. arenaria* to stress can be expected to be reduced following repeated stress events and/or when tillers are stressed at the time of exposure. The reduced tolerance of plants to repeated stress events has been documented in several studies. For example, the tolerance of seedlings to burial has been shown to depend on the original depth of the propagule. When *Panicum virgatum* seedlings emerged from depths of 3 – 4 cm they could withstand burial of up to 6 cm, while seedlings emerging from 10 – 11 cm could withstand burial of only 1 – 2 cm (Zhang and Maun, 1991).

There was some evidence in the present study that exposure to stress has a substantial non-lethal effect on the growth of tillers of *A. arenaria*. Exposure to non-lethal levels of desiccation, salinity and heat exposure resulted in an increase in leaf chlorosis and a loss of turgor. Similar reductions in vigour have been recorded in several species in response to different stresses (e.g., Sykes and Wilson, 1988; Brown, 1997; Dixon *et al*., 2003). The non-lethal effect of stress is not likely to be permanent, nor will it necessarily be
long-lived. Further, vigour may be enhanced upon recovery from stress (Zhang and Maun, 1990). Nonetheless, any non-lethal effect of stress has the potential to indirectly affect the establishment of *A. arenaria* by, for example, rendering it less competitive when growing amongst other plants or increasing vulnerability to other stresses.

Of the stresses examined in the current study, only exposure to wave over-wash affected tiller survival with sufficient severity for it to be considered a primary factor limiting *A. arenaria* survival. Immersion equivalent to one high-tide event resulted in the mortality of all tillers. This indicates that exposure to a single immersion event is sufficient to affect the survival of *A. arenaria* in the field. Immersion coinciding with rain increased survival, but overall survival remained low. The results of the field study corroborate those of the glasshouse experiment. Over-wash events at both study sites resulted in the death of inundated tillers. The results of the field study also indicate that although inundation will severely affect the plant survival due to salt-stress, the primary effect of inundation on *A. arenaria* is due to beach erosion. Substrate erosion and over-wash related salt-stress accounted for similar numbers of dead tillers during the inundation event(s) at Site 1 between February and June 2008. Subsequent erosion events in the flowing months, however, would have resulted in the death of all tillers had they not previously died as a result of salinity stress.

The results of the present field study have illustrated that the vertical growth of *A. arenaria* in response to sand accretion provides a mechanism by which *A. arenaria* can reduce its vulnerability to wave action. The stranded tillers at Site 2 rapidly formed an incipient foredune limiting the inland extent of subsequent over-wash events to the seaward margin of the recently foredune. In contrast, tillers growing at Site 1 experienced little sand accretion and so remained vulnerable to over-wash. These findings indicate that the depositional regime of the stranding environment will strongly influence the vulnerability of *A. arenaria* to over-wash.
5.4.3 Other observations

The production of rhizomes in some of the glasshouse experiments and the results of the field study allow some initial observations regarding the rate at which rhizome-derived tillers reach maturity.

*A. arenaria* reaches sexual maturity slowly. No flowers formed in the field until the plants derived from stranded rhizomes were older than two years old. This result is surprising. Plants derived from rhizomes typically reach sexual maturity faster than those from seed (Maun, 2009). The results of the current study, however, are consistent with the maturation rates of *A. arenaria* from seed (Huiskes, 1979). This implies that the origin of *A. arenaria* has no effect on the maturation rate; plants from both seeds and rhizomes reach maturity after about 2 years of growth.

*A. arenaria* produces vegetative propagules far more rapidly than it produces flowers. Dormant buds were observed in the glasshouse after only 120 days of growth – more than six times faster than flowers were produced in the field. It can be expected that *A. arenaria* reproduces vegetatively faster than this study indicates. The formation of a bud bank appears to be related to sand accretion during tiller establishment. Dormant meristems were observed in the glasshouse only when rhizomes and tillers were treated with burial and non-emergent shoots in the current study lacked nodes and buds indicating that buds forms on rhizomes only after tillers have emerged above the surface of the sand. This probably occurs shortly after etiolation has raised the apical meristem to a non-lethal depth. It can be expected, therefore, that *A. arenaria* is capable of forming a bud bank within days to weeks of regeneration, provided that it experiences some sand accretion.

All planted buds were viable irrespective of the depth of the burial treatment. An increase in the ratio of above-ground to below-ground biomass in response to increasing burial has been measured in several species including *Ammophila* (e.g., Disraeli, 1984; Martínez and Moreno-Casasola, 1996), implying a corresponding decrease in the reserves stored within the rhizomes. The high viability of the new rhizomes formed in the present study indicates that *A. arenaria* begins to store resources within its rhizomes.
systems shortly after regenerating, and that when buried still retains sufficient resources in the rhizome system to support regeneration in the event of a catastrophic disturbance.

5.5 Concluding remarks

A. arenaria has been shown to possess a high tolerance to most of the stresses it would experience when establishing from stranded rhizomes. Of those examined, only desiccation and wave activity and associated erosion are likely to regularly limit regeneration and survival. In order for A. arenaria to establish following hydrochory, rhizomes must be stranded in an environment that provides protection from desiccation and wave activity.

The depositional regime of the environment in which a fragment strands will exert a strong influence on establishment success. Burial enhances regeneration by reducing exposure to lethal desiccation, and survival by facilitating dune formation that allows A. arenaria to avoid wave activity. When rhizomes is stranded in a non-accretionary environment, survival will be limited to relatively rare safe sites. Finally, the rapidity with which a bud bank is formed depends on burial. Burial is a ubiquitous component of sandy coasts, however, as the current study has illustrated, accretion rates differ substantially between environments. Thus, the hypothesis that establishment is limited primarily by the availability of sandy substrates has been proven to be only partly true. Establishment is dependent on stranding on a sandy coast, but the desiccation rate, elevation of stranding and the potential for sand accretion are also important.
Chapter 6
General Discussion and Conclusions

6.1 Introduction

*Ammophila arenaria* has been systematically introduced into temperate dune-systems throughout the world. It has been belatedly recognised that this species is associated with the modification of indigenous dune-systems — *A. arenaria* results in changes to foredune morphology, the displacement of the indigenous flora, and the stabilisation of transgressive dune-systems (e.g., Barbour and Johnson, 1977; Wiedemann and Pickart 1996; Lubke, 2004; Hilton, 2006; Hilton *et al.*, 2006). In consequence, dune management in many regions globally is focussed on eradicating this species from dune-systems with high conservation values, and minimising its further spread (Wiedemann and Pickart, 1996; Martínez *et al.*, 2004; Hilton and Konlecher, 2010).

This thesis examined the potential for *A. arenaria* to disperse and establish from fragments of rhizome. This aim stemmed from recognition that such dispersal allows *A. arenaria* to establish new colonies, threatening hitherto isolated dune-systems of high conservation value. Several studies had previously recognised the potential for *Ammophila* spp. to establish from fragments of rhizome, however, until the present study little directly was known about the vegetative regeneration of *A. arenaria* (e.g., Wallen, 1980; Buell *et al.*, 1995; Hilton *et al.*, 2006). This study has provided the first systematic examination of the regenerative potential of *A. arenaria* from marine-dispersed rhizomes.

Four key research questions were identified and investigated through a series of empirical studies. These were: 1) how readily does *A. arenaria* regenerate from rhizome fragments; 2) how many propagules does the rhizome system of *A. arenaria* produce; 3) how far can rhizomes be dispersed; and 4) how does the stranding environment affect establishment? Relevant findings from these chapters are discussed in Section 6.2 with
reference to each research question. The applicability of the present study to other populations of A. arenaria is considered in Section 6.3. The general implications of the present study for the dispersal and establishment of A. arenaria from fragments of rhizome will be considered in Section 6.4. Finally, suggestions for managing the spread of A. arenaria are discussed in Section 6.5.

6.2 Research Questions

6.2.1 How readily does A. arenaria regenerate from rhizomes?

The regenerative potential of A. arenaria from rhizomes is limited primarily by extrinsic variables (exposure to stress, the suitability of the stranding environment for growth), rather than by the intrinsic ability of rhizomes to regenerate. The regenerative potential of A. arenaria rhizomes is high, albeit variable. Most fragments in the present study produced robust tillers when planted in ideal conditions. Several intrinsic factors significantly decrease the viability of A. arenaria; decreasing fragment length, for example. However, at least some fragments of rhizome from any given population of A. arenaria will be capable of regeneration (Chapter 2). Exposure to stress (salinity, burial and desiccation) reduced the regenerative ability of A. arenaria rhizomes (Chapters 4 and 5).

Separation from the parent plant initiates regeneration in viable fragments of A. arenaria rhizome. A similar response has been observed for A. breviligulata (Maun, 1984), and Bell (1974) suggests that this response to fragmentation is universal in plants that reproduce vegetatively. There is some evidence that exposure to inhospitable growth conditions induces secondary dormancy in A. arenaria. No A. arenaria rhizomes regenerated while in seawater, for example. When exposed to ideal conditions for growth, rhizomes that had been immersed took an average of 18 days longer to produce tillers than rhizomes that were not exposed to seawater (Chapter 4). A similar response to burial has been observed in fragments of Panicum racemosum rhizome (Cordazzo and Davy, 1999).
Little is known about the potential for secondary dormancy in vegetative propagules including fragments of rhizome. Such dormancy is common in seeds, particularly those of species associated with disturbed environments (Harper, 1977; Fenner, 1985). Dormancy aids establishment by preventing regeneration under unfavourable circumstances (Fenner, 1985). The effect of such dormancy on the establishment of *A. arenaria* from rhizomes is unclear. It enhances establishment by preventing rhizomes from regenerating when exposed to lethal salinities. However, delaying regeneration also increases the exposure of a fragment to other potentially lethal stresses. For example, the regenerative potential of *A. arenaria* rhizomes decreased in proportion to the length of time they were exposed to desiccating conditions (Chapter 5). Any delay in regeneration time, therefore, increases the likelihood of rhizomes desiccating to lethal levels.

As with desiccation, the effect of immersion in seawater on the regenerative potential of *A. arenaria* depended primarily on the duration of exposure. Fragment viability in response to both immersion and desiccation decreased as the period of exposure to stress increased. The correlation between exposure time and viability loss was not linear for either stress. In particular, there was little change in the regeneration ability of rhizomes when exposed to stressful conditions for short periods of time. Viability did not decrease significantly until fragments were exposed to desiccating conditions for more than 24 hours and, for the most part, *A. arenaria* was able to retain viability at pre-immersion levels for at least 5 days when in seawater. It appears that brief periods of immersion or exposure to desiccating conditions will have little-to-no effect on the regeneration ability of *A. arenaria*.

The intrinsic viability as well as the tolerance to burial of vegetative propagules such as pieces of rhizome depends on the reserves stored at the time of fragmentation (Harris and Davy, 1986a; Cordazzo and Davy, 1999). The amount of stored reserves may also influence viability when rhizomes are immersed in seawater (Hall *et al*., 2006). Non-structural carbohydrates are found within the rhizome system of *A. breviligulata*, and the ability of *Ammophila* spp. to tolerate burial probably stems from the conversion of starch to sugars, which are then available to support vertical growth (Seliskar, 1994). Overall, little is known about the storage of reserves in the rhizome system of *A.*
arenaria. There is a need to further investigate reserve storage in the rhizomes of A. arenaria, given its implications for regeneration.

6.2.2 How many propagules does the rhizome system of A. arenaria produce?

The rhizome system of A. arenaria contains large numbers of dormant buds capable of acting as propagules when rhizomes are fragmented. The bud bank of A. arenaria, as measured in the present study contained at least 360 ± 333 buds m$^3$, and in some environments may reach densities as high as 10,000 buds m$^3$ (Chapter 3). Most of these buds are likely to be viable (Chapter 2). Similar results have been obtained for other rhizomatous dune plants (Nobel et al., 1979; Krajnyk and Maun, 1981). For example, the bud bank of Carex arenaria, a rhizomatous sedge also associated with mobile dunes, has been measured at 267 – 1,400 buds m$^3$ (Nobel et al., 1979). It appears that a large reserve of dormant, viable meristems is a common and useful trait in the dynamic sand dune environment.

Measurements of the viable bud bank will overestimate the number of propagules except when rhizomes are fragmented into lengths containing only one viable bud. The morphology of naturally occurring rhizome fragments was not examined in the present study. Studies on species similar to A. arenaria have shown that most rhizome fragments formed during foredune erosion contain more than one node (e.g., Cordazzo and Davy, 1999; Maun 1984). As most nodes of A. arenaria rhizomes contain a dormant viable bud, it can be expected that the majority of fragments of A. arenaria rhizome are likely to possess multiple buds. Nonetheless, the bud bank is still a useful measure of relative reproductive capacity.

Most populations of A. arenaria can be considered to be a potential source of propagules. However, the reproductive capacity of A. arenaria varies considerably depending on the size of the bud bank and the reproductive ability of rhizomes following fragmentation. For example, significantly fewer viable buds were recorded
when rhizomes were sampled from the less and more vigorous populations at Allans Beach (Chapter 3). There were at least 60% more buds on rhizomes obtained from high vigour populations. Buds obtained from the less vigorous populations were also less viable (Chapter 2). Except for the most moribund populations, or populations associated with very young plants which are yet to form extensive rhizome-systems, the bud density of *A. arenaria* will be of the order of at least $10^2$ viable buds m$^{-3}$.

The rhizomes of some species fragment naturally through abscission (van der Pijl, 1982). In contrast, the rhizome system of *A. arenaria* is dependent on fragmentation during episodes of foredune erosion by elevated sea-levels. The number of propagules associated with any population of *A. arenaria* will depend not only on the density of the viable bud bank as examined in the present study, but also the extent and frequency of erosion events. Given that most populations of *A. arenaria* contain large numbers of viable buds, the relative number of propagules associated with different populations probably depends more on processes relating to dune erosion (i.e., magnitude and frequency of erosion events), rather than the reproductive capacity of the rhizome system. For example, all other factors being equal (bud bank density, frequency of erosion), increasing dune height will correlate with more propagules, due to the greater volume of sand removed when higher dunes are scarped compared to lower dunes during comparative erosion events. The alongshore extent and uniformity of a population of *A. arenaria* will similarly correlate with propagule number.

*A. arenaria* has several traits that increase the magnitude of erosion events. For example foredune morphology is influenced by vegetation type. The height, width and overall form of these dunes depend on the growth form of the plant species (Hesp, 2002). The high, dense growth-form of the foliage of *A. arenaria* means that foredunes formed by this species are typically high (c. 5 – 10 m) with steep seaward faces (Doing, 1985; Hesp, 2002). Similarly, the ability of *A. arenaria* to displace the indigenous dune vegetation where it has been introduced outside of its natural range allows *A. arenaria* to form long continuous foredunes (e.g., Wiedemann and Pickart, 1996; Hilton *et al.*, 2006). The combination of these two factors, high, steep foredunes and extensive populations of *A. arenaria*, means that coastal erosion typically results in the formation of high dune scarps, the removal of large volumes of sand, and hence, the dispersal of
large numbers of propagules. *A. arenaria* may also increase the frequency of dune erosion. In parts of New Zealand, *A. arenaria* has caused the coast to prograde (e.g., Hilton *et al.*, 2006). Such progradation can result in a narrowing of the back-beach, and consequently, an increase in the frequency and magnitude of dune erosion due to the increased proximity of the foredune to the sea.

### 6.2.3 How far can the rhizomes of *A. arenaria* be dispersed?

*A. arenaria* has the potential to be dispersed over long periods of time. The dispersal potential of *A. arenaria* was determined by measuring the ability of its rhizomes to remain buoyant and viable when immersed in seawater. The longer a fragment of rhizome can remain both buoyant and viable, the greater the distances it can be transported by marine processes. The present study has shown that the rhizomes of *A. arenaria* can remain buoyant for up to 172 days and viable for at least 70 days in seawater. The maximum dispersal time was not determined in the present study, but the evidence indicates that the decay of buds will limit dispersal rapidly once immersion exceeds 70 days (Baye, 1990).

The distance that a fragment will be transported depends on the speed and direction of the surface drift and residual flows (tidal and oceanic circulation) during the period of dispersal. These are highly variable, both spatially and temporally, hence the distance over which *A. arenaria* can be transported will vary between individual dispersal events and dispersal regions. Further, as the movements of surface waters are driven by wind speed and direction, rhizomes are unlikely to be transported in a constant direction once dispersal exceeds a few days. Nonetheless, the dispersal of other drift plants indicates that *A. arenaria* is capable of dispersal over considerable distances — probably in the order of $10^2$ to $10^3$ km. For example, measurements between new and established populations of *Zostera marina* determined that the rhizomes of this species was transported 108 km within approximately 11 days (Harwell and Orth, 2002). These measurements were taken in a sheltered estuarine environment with relatively slow movement of the surface water, 0.1 - 1.1 m s$^{-1}$. Transportation speeds can be expected
to be faster on the open coast with oceanic currents reaching speeds of up to 1.3 m s\(^{-1}\) (Cousens et al., 2008).

The dispersal potential of *A. arenaria* is not constant — the ability of a fragment in this study to remain buoyant and viable varied by weeks to months depending on the number of nodes a fragment possessed and the temperature of the water into which it was immersed. Even if all other dispersal factors are equal (for example, the speed and direction of the surface drift and residual flows), the distance that *A. arenaria* can be transported will vary between different populations of *A. arenaria*, between dispersal events from the same population, and even between individual propagules involved in the same dispersal event.

The dispersal potential of most fragments of rhizome will be much shorter than 70 days. Only when fragments in the present study were immersed in water at temperatures below 10°C did they remain viable for 70 days. Dispersal was limited to periods of less than 48 days for multi-noded fragments at all other temperatures, and less than 14 days for all single-noded fragments. However, surface drift can be rapid, hence the potential still exists for rhizomes to be transported over reasonably long distances (10\(^2\) to 10\(^3\) m) in just a few days (e.g., Harwell and Orth, 2002; Shanks et al., 2003).

Overall, the dispersal potential of *A. arenaria* is high but not exceptional when compared to that of some other coastal plants. For example, when the propagules of 13 Australian dune plants were exposed to seawater, about a third remained both buoyant and viable after 70 days of immersion (Guja, 2010) — the maximum dispersal time of *A. arenaria*. The dispersal potential in the marine environment of some dune plants greatly exceeds that of *A. arenaria*. The seeds of *Euphorbia paralias*, for example, remain buoyant and viable when in seawater for up to six years (Heyligers, 2007). The superior dispersability of this species compared to *A. arenaria* probably stems from features peculiar to seeds, such as a water resistant seed coat. Such seeds are capable of crossing ocean-scale bodies of water. The tolerance of *A. arenaria*, however, is such that dispersal is only possible within coastal waters. The initial introduction of *A. arenaria* into regions outside its natural range could only have occurred through the transportation of this species by humans.
6.2.4 How does the stranding environment affect establishment?

The suitability of the receiving environment for growth has been identified as the most important determinant of successful establishment (Carlquist, 1967). For dispersal and establishment to be effective, propagules must be able to regenerate and the resulting plants establish as seedlings or ramets in the new environment (Maun, 2009). In general the growth requirements of *A. arenaria* are well understood — it requires a sandy substrate in a temperate climate between latitudes 32 – 60° (Huiskes, 1979; Wiedemann and Pickart, 2004). Simply, the establishment of this species from rhizomes depends on a fragment of rhizome being stranded on a sandy beach at an appropriate latitude in a temperate clime. The present study has identified additional criteria necessary for the establishment of *A. arenaria* from rhizomes.

The influence of the abiotic environment on the establishment of *A. arenaria* was examined in the present study (Chapter 5). Biotic factors, including competition, grazing, disease and human disturbance can also limit establishment (Fenner, 1985; Maun, 2009). Juvenile plants of *A. arenaria* are browsed by rabbits (Esler, 1969), and cattle have been observed by the author to graze on plants from stranded rhizomes in southern New Zealand. Nematodes and fungi have been associated with a significant reduction in the growth of *A. arenaria* (De Rooij-van der Goes, 1995). Although such biotic factors may be locally important, they are unlikely to limit the establishment of *A. arenaria* from stranded rhizomes. Propagule regeneration and tiller survival on sandy coasts is primarily limited by the abiotic environment (e.g., Maun, 1981; Payne and Maun, 1984).

Successful establishment may only occur rarely, dependant on a coincidence of events that deposits rhizomes in a suitable environment for regeneration and survival. The establishment of *A. arenaria* probably depends on rhizomes stranding in an environment that provides some protection from desiccation (Chapter 5). This may be, for example, an environment where rhizomes are rapidly buried once stranded, which experiences persistent rain in the period between stranding and regeneration, or one with existing vegetation (Miller *et al.*, 2003; Maun, 2009). Similarly, the establishment of *A. arenaria* is dependent on the juvenile plant avoiding exposure to seawater or
substrate erosion resulting from wave activity (Chapter 5). Successful marine dispersal concludes in the stranding of a propagule on a beach by wave action; hence all plants of \textit{A. arenaria} that derive from marine-dispersed rhizomes are vulnerable to wave induced mortality. The likelihood of inundation decreases as the stranding elevation increases. The establishment of \textit{A. arenaria} following hydrochory is probably limited to stranding events that coincide with elevated sea-levels, such as when storm surge coincides with a spring tide. \textit{A. arenaria} must then establish before the sea reaches similar levels again.

Burial of the rhizomes shortly following stranding is likely to be essential for the establishment of \textit{A. arenaria}. Small amounts of burial are necessary for successful establishment of most coastal plants (Adair et al., 1990; Maun, 1998). Burial enhances establishment by improving soil contact, providing protection from predation, helps to anchor young plants in the soil and, importantly for the establishment of \textit{A. arenaria}, burial provides considerable protection against desiccation (Maun, 2009). The vertical growth of \textit{A. arenaria} in response to sand accretion limits the exposure of \textit{A. arenaria} to wave action (Chapter 5). Finally, burial is important for reproduction. Prolific flowering of \textit{Ammophila} ssp. is correlated with sand accretion (Hope-Simpson and Jefferies, 1966; Eldred and Maun, 1982). This study has shown that burial is also important for the formation of a bud bank (Chapter 5). These findings indicate that the depositional regime of the stranding environment will strongly influence the establishment of \textit{A. arenaria}.

Establishment is more likely to be successful when plants originate from rhizomes rather than seeds (Fenner, 1985; Maun, 2009). Plant establishment in the back-beach environment, however, is probably always a rare event, even when plants originate from fragments of rhizome. For example, only a few fragments of \textit{A. breviligulata} rhizome stranded on the shores of Lake Huron produced tillers, and only 35\% of these tillers remained alive after 12 months of growth (Maun, 1984). Consistent with the present study, desiccation limited fragment regeneration while rearrangement of the substratum was the principle cause of plant mortality. This was probably the result of wave activity, although erosion and sand deposition by wind may have also played a role. A similar decrease in tiller survival due to erosion and burial by wind and waves was identified when the survival of \textit{Thinopyrum junceiforme} from stranded rhizomes was examined on
the Norfolk coast (Harris and Davy, 1986b). It is likely that stranded fragments of *A. arenaria* display similarly high rates of mortality. The vulnerability of *A. arenaria* to desiccation and wave over-wash probably limits the frequency of establishment of this species.

### 6.3 Applicability of the present study to other populations

There was no site replication in the present study making the ability to generalise the results of this study to other sites questionable. For example, tests examining the effect of population vigour on a rhizome fragment’s regenerative potential (Chapter 2) and tolerance to seawater (Chapter 4) used rhizomes obtained from only two sites — one site associated with a low vigour population of *A. arenaria* and one site associated with a high vigour population. Further, all rhizomes used in Chapters 2, 4 and 5 were obtained from one population growing in one dune-system in south-eastern New Zealand.

*A. arenaria* is a species which shows considerable phenotypic plasticity depending on climatic and geomorphic regime in which it is growing (Grey, 1985). Other studies have established a relatively high level of genetic variation between geographically separate populations growing in Europe (Rodríguez-Echeverría *et al.*, 2008). Without further testing it is impossible to say whether such phenotypic or genetic variability would mean that the viability, tolerance to seawater, and tolerance to strandline stress as determined in the present study would differ from rhizomes obtained from other populations. However, the results of the present study are broadly consistent with similar work across the geographic range of *A. arenaria*. For example, the maximum viability of *A. arenaria* rhizomes in the present study was consistently between 80 and 90%, similar to the maximum viabilities of comparative rhizomes obtained from populations in California (Aptekar and Rejmánek, 2000). Where differences do exist between the results of the present study and the existing literature (for example, the buoyancy of rhizomes in seawater), factors unrelated to the biology of *A. arenaria* have
been identified in the present study as the probable cause. It appears that certain biological conditions relating to regeneration from rhizomes may be consistent between different populations of *A. arenaria*. Hence the results of the present study are likely to be broadly applicable to at least other populations of *A. arenaria* within New Zealand, if not in other countries. Finally, it should be noted that the present study has shown that the potential of *A. arenaria* to disperse and establish from fragments of rhizome is not constant. The reproductive potential of *A. arenaria* rhizomes, its dispersal potential, and its ability to establish following stranding varies depending on several factors including, for example, the storage of reserves in rhizomes before dispersal and the temperature of the water in which it is dispersed. The absolute values as identified in the present study may differ between populations, however, those factors which cause variability in the reproductive and dispersal potential of *A. arenaria* are likely to be consistent across all populations of *A. arenaria*.

### 6.4 The establishment of *A. arenaria* from rhizome fragments

For *A. arenaria* to establish its rhizome system must first be fragmented into lengths with the potential to regenerate, and these fragments must be transported and strand in a suitable environment for growth before the fragment loses viability or sinks. Finally, the fragment must regenerate and the resulting plant survive long enough to form a self-sustaining population. This study has established that *A. arenaria* has the potential to regenerate readily when fragmented into lengths with a dormant bud, that it has the potential to produce large numbers of propagules, that it is capable of dispersing over long distances, and that the juvenile plants of *A. arenaria* derived from rhizome fragments possess a high tolerance to most stresses. The establishment of *A. arenaria* from rhizomes is probably limited primarily by the processes involved in the transportation and stranding of rhizomes in a suitable location for growth, rather than the inability of fragments to regenerate.
The establishment of *A. arenaria* from rhizomes on a sandy shore from marine-dispersal is probably a rare event. Experiments using drift cards to model surface flows in coastal waters have shown that once in the sea few objects re-strand on the coast, even when their buoyancy is sufficient to allow them to float for months to years (Heath and Shakespeare, 1977; Steinke and Ward, 2003). Despite the ability of *A. arenaria* rhizomes to float and remain viable in seawater for long periods of time, it is likely that a large portion of fragments sink or die before they re-strand. To establish, *A. arenaria* must strand on a sandy coastline, however, the coastal environment can consist of any number of types depending on substrate type and exposure. Shorelines range from the mud/silt environment of estuaries to sandy coastlines through to hard rocky coastlines. Sandy coasts comprise only a relatively small proportion of most coastlines — about 50% of the Australian coast (Short and Woodroffe, 2009), and less than a third of the New Zealand coast. The probability that a fragment will strand on an unsuitable coast is high. Finally, even if stranded in a suitable environment for growth, the evidence from this and similar studies indicates that few fragments will produce tillers that survive till maturity. This conclusion, that establishment from rhizomes is rare and is limited by external variables involved in transportation and stranding, is supported by observation of rhizomes in the field. Large scale erosion of *A. arenaria* foredunes dispersing large numbers of propagule is observed frequently, however few tillers are observed on sandy back-beaches from stranded rhizomes, and those that do rarely persist for long.

Although the establishment of *A. arenaria* from rhizomes is likely to occur rarely, it is important to note that establishment does not have to occur frequently for it to be effective (Carlquist, 1967). An individual of *A. arenaria* can expand horizontally through clonal growth, while the dispersal of seeds and redistribution of rhizomes may establish multiple satellite populations away from the parent colony. The rapidity with which this dual strategy of population expansion facilitates the spread of *A. arenaria* has been documented on several occasions (e.g., Buell *et al*., 1995; Hilton *et al*., 2006). Therefore, the establishment of only one fragment of rhizome is required for *A. arenaria* to rapidly establish a large and persistent colony.

The focus throughout this study has been on identifying those factors that may limit establishment, or make establishment more successful in one environment compared to
another. Few factors examined in the present study were found to be constant. Based on the results of this study, several conditions can be identified which influence the successful establishment of *A. arenaria*.

**The frequency of stranding events**

The persistence of a species in a new region is strongly correlated with increasing propagule pressure (the number of individuals arriving or released into a new region; after Lockwood *et al.*, 2005) (Alroth *et al.*, 2003; von Holle and Simberloff, 2005; Colautti *et al.*, 2006; Eschtruth and Battles, 2009). Although propagule pressure does not negate the role that the receiving environment plays in establishment, increasing the number of propagules does reduce the influence of environmental stochasticity and Allee effects on establishment (Lockward *et al.*, 2005). The ability of *A. arenaria* to establish a new colony is likely to be strongly related to the frequency of stranding events.

*A. arenaria* is dispersed by the movements of surface waters. The speed and direction of these are variable, but not random. Although surface waters can move in any direction, depending on the direction of the wind and residual flows, their movement tends to be predominately unidirectional at the seasonal scale. For example, near-surface currents off the west coast of the United States flow predominantly to the south from April to July, and predominately to the north from August to March (Aptekar and Rejmánek, 2000). Under such conditions the frequency with which rhizomes might strand in any one dune-system will be skewed towards the predominant direction of transport.

**Transportation distance**

The role of propagule pressure in explaining establishment success means that proximity to a source of propagules plays an important role in the establishment and spread of a species (Lockwood *et al.*, 2005). For example, the distribution and cover of three invasive plants in South Africa could be accurately predicted by their distance from the original founder population, particularly when environmental heterogeneity
was also incorporated into the model (Rouget and Richardson, 2003). Because of the spatial dynamics of local individual dispersal movements greater densities of all three species were found close to the founder population than environmental factors predicated alone.

A similar relationship between transportation distance and the establishment of *A. arenaria* can be expected. There is considerable empirical evidence that although *A. arenaria* has the potential to be transported over considerable distances, most dispersing fragments will strand close to the propagule source (e.g., Heath and Shakespeare, 1977; Steinke and Ward, 2003). For example, 82% of drift-cards released in the North Taranaki Bight, New Zealand (38.483°S, 174.416°E) during May – June 1975 stranded within 31 km of the release point (Heath and Shakespeare, 1977). The remaining 18% were transported over distances of at least 100 km. The frequency and number of propagules involved in long distance transport increases in proportion to the offshore distance of the release point (e.g., Heyligers, 2002). It appears that the processes governing the movement of inner coastal waters, particularly those of the surf-zone, act as an effective barrier to long-distance dispersal.

*A. arenaria* is more likely to establish a persistent population in dune-system located closer to an existing source of propagules than in an otherwise equitable dune-system located further away, because of an increased likelihood of successful stranding and an increase in propagule pressure when rhizomes are transported over shorter distances. The dispersal potential of *A. arenaria* (the buoyancy and viability of rhizomes) decreases significantly once immersion exceeds a few days. It is likely that most fragments of *A. arenaria* rhizomes which are dispersed over long distances sink or lose viability before they are re-stranded. The transportation of *A. arenaria* over short distances may also correlate to a decrease in vulnerability to some stresses post stranding. Rhizomes derived from local sources is typically stranded in dense wrack lines as observed at Allans Beach (Chapter 5). The regeneration of such rhizomes allows the rapid development of uniform foredunes which are continuous alongshore (Hilton and Konlechner, 2011). In contrast, longer dispersal typically results in stranding of individual fragments of rhizome and formation of isolated dunes. The present study suggests that such populations will be more vulnerable to wave overwash.
(Chapter 5). They are also likely to possess a smaller bud bank, thereby reducing the opportunity for re-colonisation following a disturbance event.

Short distance dispersal plays an important role in population recovery following disturbance, whereas long distance dispersal distances facilitates the establishment of new colonies considerable distances from the original source of the rhizomes (Maun, 2009; Nilsson et al., 2010). Although the rhizomes of A. arenaria can be dispersed over long distances, the evidence suggests that most plants from stranded rhizomes will be from rhizomes originating from local sources. This success of short distance dispersal event versus long distance dispersal suggests that the vegetative regeneration of A. arenaria from rhizomes plays an important role in population recovery following disturbance and persistence and a relatively limited role in spread and range expansion (Maun, 2009; Nilsson et al., 2010). However, as discussed, establishment does not need to be frequent to be effective. The long distance dispersal of only one fragment is sufficient for A. arenaria to establish a large colony.

Size of the propagule supply

The establishment of A. arenaria from rhizomes is more likely to be successful when large numbers of propagules are dispersed. Large numbers of propagules need to be dispersed in order that a few (or one) establishes successfully (Ridley, 1930; Carlquist, 1967). A viable propagule must enter the sea, it must be transported away from the parent plant, it must arrive at a sandy coastline while still viable, and it must regenerate, grow and survive until maturity. Most fragments of A. arenaria rhizome will sink or lose viability before stranding and a further proportion of fragments will not successfully establish because of stranding in an inappropriate environment. Finally, even if rhizomes are stranded in a suitable location for growth, few fragments are likely to produce plants that survive to maturity. An increase in the number of propagules entering the sea increases the likelihood that at least one propagule will successfully overcome these obstacles and ultimately form a self-sustaining population.
Several factors which result in large amounts of *A. arenaria* rhizomes being dispersed have been identified in Section 6.2.2. These relate to the density of the viable bud bank, the extent of the source population, and the magnitude and frequency of dune erosion events. In general, more propagules can be expected to be dispersed from extensive populations associated with high foredunes and a dense bud bank, growing on coastlines which experience frequent foredune scarping.

**Fragment morphology**

The ability of *A. arenaria* to form new plants from stranded rhizomes will be influenced by fragment morphology, particularly node number and fragment length. *A. arenaria* is more likely to establish from multi-noded fragments of rhizome than from single-noded fragments. The more nodes on a fragment, the greater the probability that at least one of these nodes will contain a dormant and viable bud. A portion of buds on multi-noded fragments remain dormant allowing for regeneration in the event of disturbance (as discussed in Section 6.2.1). Multi-noded fragments are also more buoyant than single-noded fragments (Chapter 4). As a result, multi-noded fragments are both more likely to reach a suitable site for establishment before sinking, and have the potential to be dispersed over longer distances than single-noded fragments. The effect of fragment length on establishment is not clear. Longer fragments are more viable, grow faster, and are likely to have a greater tolerance to burial than shorter but otherwise equivalent fragments of rhizome. Increased viability, faster growth and a high tolerance to burial have been correlated with successful establishment (Maun, 2009). However, more propagules will also be formed when rhizomes are fragmented into smaller lengths, thereby enhancing successful establishment. It remains to be seen which factor is more important for the establishment of *A. arenaria*; greater numbers of propagules or the ability of these propagules to reproduce and tolerate stress. Fragment length and node number are not unrelated. Longer fragments of rhizome usually contain more nodes (Maun, 1984; Harris and Davy, 1986b; Cordazzo and Davy, 1999).

Most fragments of *A. arenaria* rhizome are likely to consist of multiple nodes (as discussed in Section 6.2.2). Fragment length, however, is likely to be variable. Several
studies have examined the morphology of rhizome fragments resulting from dune erosion and demonstrated that fragment size varies considerably (Maun, 1984; Harris and Davy, 1986b; Cordazzo and Davy, 1999). For example, the length of naturally occurring fragments of A. breviligulata rhizomes was between 10 cm and 80 cm when examined on the shores of Lake Huron (Maun, 1984). Similar variation in the length of A. arenaria fragments stranded on New Zealand beaches has been observed.

The length of individual fragments of rhizome is probably related to wave energy (Maun and Baye, 1989). It can be anticipated, therefore, that the rhizomes of A. arenaria would be fragmented into smaller lengths when erosion occurs on high energy coasts or by storm waves. Observations of rhizomes stranded in the field indicate that dispersal distance, particularly local vs. non-local dispersal, also influences fragment length. The high, steep dunes formed by A. arenaria means that dune erosion during strong wave activity occurs primarily through undercutting by wave swash followed by slumping of the overlying sand. This results in the dispersal of large tangled clumps of rhizomes. Although some additional fragmentation would occur, examination of fragments that are derived from local sources shows that these clumps remain largely intact when dispersed over distances of c. <100m. In contrast, rhizome fragments which have been transported over longer distances typically strand as single fragments. The length of time rhizomes spend in the surf zone rather than wave energy may be critical in determining fragment length.

**Timing of dispersal**

The potential exists for viable rhizomes to be dispersed and strand at all time of the year. The rhizomes of A. arenaria is viable throughout the year, even though fragment viability was significantly decreased during certain months (late spring/summer) (Chapter 2). Similarly, the elevated sea levels and coastal erosion required for the fragmentation and dispersal of A. arenaria rhizomes can occur in any month, although increased cyclogenesis increases the magnitude and frequency of erosion events during winter months. However, the ability of A. arenaria to establish a self-sustaining
population is likely to be strongly influenced by the time of year that the rhizomes are dispersed and strands.

The rhizomes of *A. arenaria* will only regenerate under field conditions during late winter/spring months. Rhizomes stranded during winter months at Allans Beach did not produce tillers until the following spring — ca. five months after stranding. A similar pattern of stranding during winter months and regeneration the following spring has been reported for fragments of *A. breviligulata* (Maun, 1984). These results are supported by observations of stranded rhizomes throughout southern New Zealand by the author. Rhizomes have been observed to strand at all months of the year, but newly emerged tillers have been observed only during August – November. These results indicate that stranding during autumn and winter conditions induces dormancy in the rhizomes of *A. arenaria*. Such dormancy is common in the seeds of coastal plants (Maun, 2009), but as yet is poorly recognised in vegetative propagules. It is not known what happens to rhizomes stranded during summer months. They may experience a similar form of dormancy. Alternatively, fragments may be capable of regeneration, but exposure to desiccating conditions results in the mortality of most rhizome fragments and emerging tillers during summer months.

All other things being equal (e.g., size of the bud bank, transportation distance), *A. arenaria* is more likely to establish from rhizomes stranded during mid to late winter. The mortality of rhizomes stranded during summer and autumn months is likely to be high, because of the length of time that they must to remain viable before regeneration occurs in spring. More propagules will be dispersed during winter months compared to other months. The high viability of *A. arenaria* rhizomes during winter months coincides with an increase in the magnitude and frequency of erosion events. Because of the increased frequency and magnitude of elevated sea-levels during winter, the probability that rhizomes are stranded in an environment protected from further wave activity is increased. Finally, although the present study found no correlation between rhizome longevity and the month of dispersal, fragments dispersed during winter months can still be expected to be more tolerant of immersion in seawater than rhizome dispersed during other months, because of the positive correlation between seawater temperature and longevity. Water temperatures are typically warmer over summer
months and cooler over winter months. For example, surface seawater temperatures (SST) in New Zealand follow a seasonal cycle with temperatures peaking in February (late summer) and reaching a minimum in August (late winter) (Garner, 1969; Greig et al., 1988; Chiswell, 1994). In general, SST displays a seasonal variability of approximately 5°C (Chiswell, 1994). This correlates to a 10 day increase in dispersal time when entering the sea during winter, compared to summer.

**Interaction with other foredune vegetation**

The establishment of *A. arenaria* will be limited by the presence of some other sand-binding foredune species, particularly those capable of forming dunes seawards of *A. arenaria*. *A. arenaria* dominates foredunes within its native range (Doing, 1985). However, more salt-tolerant species, principally *T. junciforme*, may form continuous incipient foredunes seawards of the established *A. arenaria* foredune. This juxtaposition of incipient-established foredune also occurs in parts of Australia where *T. junciforme* has been introduced. In some countries the indigenous dune vegetation forms similar dunes to *A. arenaria* (e.g., *Spinifex sericeus* in south-eastern Australia and in northern New Zealand). Such foredunes are likely to limit the establishment of *A. arenaria* from rhizomes by both preventing the entry of rhizomes into the sea during coastal erosion and by forcing rhizomes to strand in a location unsuitable for growth. The establishment of *A. arenaria* from rhizomes in such regions will be rare, in comparison to dunes where *A. arenaria* strands within the indigenous vegetation where the vegetation is associated with hummocky foredunes (e.g., southern New Zealand (Hilton et al., 2005); north-western North America (Widemann, 1993)).
6.5 Implications of the present study for the management and control of *A. arenaria*

The present study indicates that managing the establishment and spread of *A. arenaria* from rhizomes will pose several challenges to management agencies.

1. *A. arenaria* establishes from stranded rhizomes relatively rarely, when the total amount of propagules capable of regeneration is considered. However, although most fragments of *A. arenaria* rhizome will not establish, the dense bud bank and frequent erosion of *A. arenaria* dunes facilitates the dispersal of large numbers of propagules. This frequent dispersal of many viable propagules allows large numbers of plants to form from the establishment of only a small proportion of the total propagules. Further, the ability of *A. arenaria* to reproduce both vegetatively and from seed allows large colonies to form rapidly from only one fragment.

2. Geographic isolation is unlikely to prevent invasion of *A. arenaria*. For *A. arenaria* to establish in any particular dune-system a connection between an existing population and the new site must be present (Carlton, 1996). This connection depends on the balance between three factors: the distance that needs to be travelled (the isolation between the receiving dune-system and a source of propagules), the distance that can be travelled (the ability of the propagule to remain buoyant and viable), and the speed and direction of the surface waters during dispersal. *A. arenaria* is widespread in most regions where it has been introduced (e.g., Hilton, 2005; Hertling and Lubke, 1999; Wiedemann and Pickart, 2004), hence most dunes will be close to a source of propagules — probably in the order of at least $10^1$ – $10^2$ km. Further, the high tolerance of *A. arenaria* to immersion in seawater, the buoyancy of its rhizomes, and the nature of the marine environment, results in a system where long-distance transport is relatively common (in comparison to terrestrial systems) (Carr *et al*., 2003). It is
likely that *A. arenaria* has the potential to disperse to all except the most remote dune-systems.

3. Identifying and managing the source of propagules will be difficult in most circumstances. Almost all populations of *A. arenaria* are a potential source of propagules, the rhizomes of *A. arenaria* is capable of dispersing over long distances, and several populations are likely to be eroded simultaneously. Rhizomes stranded on any particular beach, therefore, could have originated from multiple sources, some of which may be located many kilometres away.

4. Large volumes of propagules can be dispersed at any one time. This not only increases the chances that a fragment will disperse and establish successfully, but also that more than one fragment will give rise to a new plant. Fragments involved in any one dispersal event can establish multiple populations simultaneously. It is unlikely that any two propagules will follow exactly the same dispersal trajectory (Cousens *et al.*, 2008). These populations could be, therefore, located many kilometres apart.

5. *A. arenaria* forms a bud bank rapidly — within weeks when stranded in an appropriate depositional environment (Chapter 5), hence, sequential dispersal events can occur within weeks to months of each other. This allows *A. arenaria* to form large colony rapidly by the redistribution and local dispersal of these rhizomes (e.g., Buell, 1995; Hilton *et al.*, 2005). The dispersal of rhizomes from sequential populations formed from stranded rhizomes also allows *A. arenaria* to disperse in a “leap-frog” fashion over distances far exceeding the dispersal ability of a single rhizome fragment, and resulting in a rapid exponential increase in the total area occupied by *A. arenaria*.

*A. arenaria*, therefore, has the ability to disperse and establish large populations rapidly from the transportation and stranding of pieces of rhizomes. Such populations may occur unpredictably and in isolated regions. Few dune-systems are likely to be sufficiently isolated that *A. arenaria* is incapable of reaching them. Geographical
isolation, therefore, does not prevent invasion, yet isolation is likely to hinder early detection.

Managing the spread and invasion of A. arenaria from marine-dispersed rhizomes is aided by the current dominance of A. arenaria along most coast-lines. In most temperate regions A. arenaria is already extensively established (Kirkpatrick, 1993, Johnson, 1993, Wiedemann, 1993, Hertling and Lubke, 1999, Hilton, 2006). Dune-systems retain few conservation values where A. arenaria has been present for more than 25 years (Hilton, 2006), hence there is little to be gained by managing A. arenaria in these systems. Conservation efforts can now be focussed on relatively small, discrete sections of coast where A. arenaria is largely absent and/or still retain high conservation values and a high degree of naturalness. For example, less than 50 dune-systems in New Zealand remain in a near natural state — less than 5% of New Zealand’s dune-systems. For the most part, these systems do not contain A. arenaria or it has only recently established from stranded rhizomes (Hilton, 2006).

A. arenaria is most likely to establish from rhizomes eroded from local sources. The eradication of local populations of A. arenaria (i.e., within the same embayment) therefore must be a priority for managers. The eradication of A. arenaria is difficult (Hilton and Konlechner, 2010). The extensive rhizome system of A. arenaria combined with a persistent seed-bank means that new plants will continue to emerge for many years after the initial population has been removed (Konlechner and Hilton, 2010). Any eradication attempt needs to be systematic and ongoing to be successful. However, until such populations are removed, the regular and frequent establishment of A. arenaria from rhizomes must be anticipated.

Regular systematic surveillance is essential to effectively manage marine dispersed A. arenaria. For most dune-systems it is a case of ‘when’ and not ‘if’ invasion occurs. Early detection and eradication offers the best prospect of long-term effective long-term management. A. arenaria is relatively easy to eradicate before an extensive rhizome system and seed bank develops. Once these are present, however, the effective control of A. arenaria will take years at significant financial cost (Hilton and Konlechner, 2010).
Regular surveillance for new infestations is already undertaken in some regions. For example, the New Zealand’s Department of Conservation annually flies the coast line between Jacksons Bay and Sandhill Point in New Zealand, eradicating new infestations of *A. arenaria*. Infestations of *A. arenaria* remain small along this coastline because of this programme. Surveillance of *A. arenaria* coasts should occur annually to control all new colonies formed over the previous year. Surveillance during spring/early summer months offers the best opportunity to eradicate newly formed plants before substantial bud bank forms, and these rhizomes are further dispersed.

### 6.6 Concluding remarks

Many beach and dune plants are capable of reproducing from fragments of rhizome (Maun, 2009). This study investigated the potential for one of these, *A. arenaria*, to establish new colonies from the fragmentation and dispersal of its rhizome system. The potential for the vegetative reproduction of *A. arenaria* is very high. It produces a dense rhizome system containing large numbers of buds, most of which are capable of regenerating when fragmented. This fact, combined with the extensive, dense growth of *A. arenaria* on foredunes throughout temperate regions globally allows large numbers of propagules to be dispersed during coastal erosion.

The establishment of *A. arenaria* from stranded rhizomes is likely to be rare. Most dispersed fragments will fail to form a new colony. The principal factors limiting dispersal appear to be related to extrinsic variables relating to the processes involved in the transportation and stranding of rhizomes. Such processes remain poorly understood, in particular the role of the surf zone in limiting dispersal, the potential for offshore and onshore transport, and role of the stranding environment in influence successful establishment.

The present study has shown that *A. arenaria* has the potential to be dispersed over considerable distances. Many beach and dune plants disperse over short distances by the transportation of rhizomes in the sea, however relatively little is known about the role of
long distance dispersal in these species (Maun, 2009). Such dispersal in *A. arenaria* is probably rare, but plays an important role in the spread and invasion of this species outside its natural range. Understanding and managing this spread is now critical for dune conservation in many regions.
References


Appendix I

Detailed ANOVA results given by reference (Section, Figure or Table) with the degrees of freedom (DF), F- and P-value for each test.

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**Chapter 3**

| Table 3.1 | # of buds | Sampling site | 5 | 7.80 | 0.000 |
| Table 3.2 | node density | Sampling site | 5 | 13.17 | 0.000 |
| | Nodes with buds (%) | Sampling site | 5 | 0.38 | 0.858 |
| Figure 3.4 | # of buds with depth | Allans Beach – F1 | 4 | 0.14 | 0.966 |
| | | Allans Beach – F2 | 4 | 0.44 | 0.779 |
| | | Allans Beach – F3 | 4 | 3.36 | 0.025 |
| | | Allans Beach – F4 | 4 | 3.91 | 0.013 |
| | Rhizome weight with depth | Allans Beach – F1 | 4 | 0.27 | 0.892 |
| | | Allans Beach – F2 | 4 | 0.29 | 0.882 |
| | | Allans Beach – F3 | 4 | 3.41 | 0.025 |
| | | Allans Beach – F4 | 4 | 4.53 | 0.008 |
| Figure 3.5 | # of buds with depth | Mason Bay | 4 | 6.05 | 0.010 |
| | Rhizome weight with depth | Mason Bay | 4 | 6.16 | 0.009 |
| Figure 3.6 | # of buds with depth | Chrystalls Beach | 2 | 20.87 | 0.017 |
| | Rhizome weight with depth | Chrystalls Beach | 2 | 14.60 | 0.028 |

**Chapter 4**

| Figure 4.1 | Viability | June | 14 | 11.14 | 0.000 |
| | | December | 14 | 9.85 | 0.000 |
| Table 4.2 | Emergence time | June – high vigour | 7 | 3.75 | 0.020 |
| | | June – low vigour | 7 | 3.79 | 0.016 |
| | | December – high vigour | 7 | 6.94 | 0.005 |
| | | December – low vigour | 4 | 22.41 | 0.000 |
| | | 0 days | 3 | 3.10 | 0.038 |
| | | 5 day | 3 | 6.01 | 0.003 |
| | | 10 day | 3 | 14.27 | 0.000 |
### Table 4.3

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### Chapter 5

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Appendix II

Detailed Mann–Whitney results given by reference (Section, figure or table) with the W- and P-value for each test.

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Appendix III

Detailed binary regression results given by parameter tested

Relationship between buoyancy and time in seawater – single-noded fragments

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Log-Likelihood = -72.104
Test that all slopes are zero: G = 465.966, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

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<td>Pearson</td>
<td>10.0827</td>
<td>34</td>
<td>1.000</td>
</tr>
<tr>
<td>Deviance</td>
<td>12.3040</td>
<td>34</td>
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</tr>
<tr>
<td>Hosmer-Lemeshow</td>
<td>0.3587</td>
<td>7</td>
<td>1.000</td>
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</table>

Relationship between buoyancy and time in seawater – double-noded fragments

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.07142</td>
<td>0.105184</td>
<td>10.19</td>
<td>0.000</td>
<td>10.19</td>
<td>10.19</td>
<td>10.19</td>
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<tr>
<td>Days floating</td>
<td>-0.0274660</td>
<td>0.0017860</td>
<td>-15.38</td>
<td>0.000</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Log-Likelihood = -564.731
Test that all slopes are zero: G = 374.784, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Chi-Square</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>46.6888</td>
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<td>0.072</td>
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<td>0.086</td>
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<td>Hosmer-Lemeshow</td>
<td>36.2115</td>
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</table>

Relationship between buoyancy and time in seawater – triple-noded fragments

<table>
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<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.07314</td>
<td>0.158440</td>
<td>13.08</td>
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<td>13.08</td>
<td>13.08</td>
<td>13.08</td>
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<tr>
<td>Days floating</td>
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<td>0.0020509</td>
<td>-14.12</td>
<td>0.000</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Log-Likelihood = -342.878
Test that all slopes are zero: G = 304.339, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Chi-Square</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deviance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hosmer-Lemeshow</td>
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<td></td>
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</tbody>
</table>
### Relationship between viability and time in seawater –5°C

<table>
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<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.79474</td>
<td>0.54784</td>
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<td>0.001</td>
<td>0.99</td>
<td>0.95</td>
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<tr>
<td>days in sw</td>
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<td>0.0225536</td>
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<td>0.822</td>
<td>0.99</td>
<td>0.95</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Log-Likelihood = -38.875
Test that all slopes are zero: G = 0.051, DF = 1, P-Value = 0.822

### Goodness-of-Fit Tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Chi-Square</th>
<th>DF</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>4.80883</td>
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</tr>
<tr>
<td>Deviance</td>
<td>5.99544</td>
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<td>0.540</td>
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<td>Hosmer-Lemeshow</td>
<td>4.80883</td>
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<td>0.683</td>
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</table>

### Relationship between viability and time in seawater –10°C

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.07314</td>
<td>0.15844</td>
<td>13.08</td>
<td>0.000</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>days floating</td>
<td>-0.0289514</td>
<td>0.0020509</td>
<td>-14.12</td>
<td>0.000</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Log-Likelihood = -342.878
Test that all slopes are zero: G = 304.339, DF = 1, P-Value = 0.000

### Goodness-of-Fit Tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Chi-Square</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>16.4299</td>
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<td>0.995</td>
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<tr>
<td>Deviance</td>
<td>19.6048</td>
<td>34</td>
<td>0.977</td>
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<tr>
<td>Hosmer-Lemeshow</td>
<td>9.1479</td>
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<td>0.242</td>
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</tbody>
</table>
### Relationship between viability and time in seawater –15°C

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.85812</td>
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<td>3.59</td>
<td>0.000</td>
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<tr>
<td>days in sw</td>
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<td>-4.82</td>
<td>0.000</td>
<td>0.87</td>
<td>0.83</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Log-Likelihood = -40.642  
Test that all slopes are zero: G = 38.049, DF = 1, P-Value = 0.000

**Goodness-of-Fit Tests**

<table>
<thead>
<tr>
<th>Method</th>
<th>Chi-Square</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>3.77451</td>
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<td>0.805</td>
</tr>
<tr>
<td>Deviance</td>
<td>4.43248</td>
<td>7</td>
<td>0.729</td>
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<tr>
<td>Hosmer-Lemeshow</td>
<td>3.77451</td>
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<td>0.805</td>
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</table>

### Relationship between viability and time in seawater –25°C

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.41423</td>
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<td>0.72</td>
<td>0.61</td>
<td>0.85</td>
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</tbody>
</table>

Log-Likelihood = -18.979  
Test that all slopes are zero: G = 57.389, DF = 1, P-Value = 0.000

**Goodness-of-Fit Tests**

<table>
<thead>
<tr>
<th>Method</th>
<th>Chi-Square</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>0.328744</td>
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<td>1.000</td>
</tr>
<tr>
<td>Deviance</td>
<td>0.520060</td>
<td>7</td>
<td>0.999</td>
</tr>
<tr>
<td>Hosmer-Lemeshow</td>
<td>0.328744</td>
<td>7</td>
<td>1.000</td>
</tr>
</tbody>
</table>