Functional ecology of iceplant taxa and their role in eco-restoration of New Zealand sand dunes.

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ABSTRACT

Biological invasion by non-native plant species has often been cited as a cause of native biodiversity loss. While the outcome of species invasions depends on interactions between exotic and resident native species, most studies of biological invasions have focused solely on the direct negative impacts of non-indigenous species on native biota. Although investigations of the role of competition in shaping natural plant communities were dominant in the previous generations and are still popular, more recent experimental research has uncovered the striking influence of facilitation on community dynamics. This thesis aims to investigate competitive and facilitative influence of the invasive South African iceplant (*Carpobrotus edulis*) on *Spinifex sericeus*, a native foredune grass species, with particular reference to implications of these interactions for dune restoration in New Zealand. It further explores the growth rates, substrate preferences and mating systems of the exotic and native iceplant taxa found in New Zealand.

I begin by briefly outlining the influence of competition and facilitation on natural plant communities with reference to the role of facilitation in eco-restoration. I also give a few examples where exotic species have been found to facilitate native ones. Secondly, a neighbour removal experiment was conducted on coastal sand dunes with the main aim of studying the effects of *Carpobrotus edulis* on establishment of *Spinifex sericeus* at the foredune region. Finally, I compared the growth rates of the most widely distributed iceplant taxa in New Zealand in different substrates and the breeding systems of the exotic *Carpobrotus*.

Examples abound in literature of exotic plant species facilitating native ones especially in forestry. In the neighbour removal study, *Carpobrotus edulis* protected *Spinifex* seedlings against storm erosion, sandblasting and salt sprays while at the same time suppressing its leaf production. Suppression of *Spinifex* leaf production was more pronounced at top of the dune where stress elements is presumably more benign. There was no evidence of allelopathic suppression of *Spinifex* by *C. edulis*. Only *Carpobrotus chilensis* displayed some level of substrate preference by putting on relatively lower biomass in gravel. The exotic *Carpobrotus* spp. put on greater dry matter content than the native

Disphyma australe and the *Carpobrotus*-x-*Disphyma* hybrid. The hybrid displayed a faster vegetative growth rate whereas *D. australe* allocated relatively more biomass to the roots than the shoot. Both *Carpobrotus* spp. are self compatible and highly capable of intrageneric and intergeneric hybridisation.

Mass removal of the existing exotic iceplant stands from foredunes along high energy coasts is not advisable as they serve as useful stabilisers. The intergeneric hybrid is sexually sterile with sparsely spread stolons that could allow co-occurrence with other species and therefore is more suitable for foredune stabilisation. However, more research needs to be conducted on the ecology of the intergeneric hybrid.

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Finally, I wish to sincerely thank my dear wife for bearing the agony of separation during my first year of study, for convincing me to come over to New Zealand at a time when I felt I didn't have the energy to study, and for her constant support.

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Figure 1.1: *Carpobrotus edulis* plants dominating the foredune with some *Spinifex sericeus* (foreground) surviving among them. Photo taken by author at Queen Elizabeth Park in November 2010.

1.1. Abstract

Plant community structure is determined by both biotic and abiotic environmental conditions. Investigations of the role of competition in shaping natural plant communities were prevalent in previous generations and are still popular. However, more recent experimental research has uncovered the striking influence of facilitation on community dynamics. There is general consensus on the importance of facilitation and competition in natural communities, but the importance of competition in highly stressful environments is widely contested by plant ecologists. With the current climatic change, land-use change and widespread biotic exchange, natural ecosystems are threatened and ecological functions are being lost. In response to loss of biodiversity, ecological restoration enables us to regain at least some of the ecological services. Ecological restoration will normally aim at managing negative plant interactions while enhancing the positive ones. Here, I outline the influence of competition and facilitation on natural plant communities with reference to the role of facilitation in eco-restoration. I also highlight a few examples where exotic species have been found to facilitate native ones.

1.2. Introduction

Plant interactions play a key role in regulation of communities and ecosystems (Padilla and Pugnaire 2006), and may mediate the impacts of environmental change (Brooker 2006). Interactions among plants may have positive, negative or neutral effects. A negative interaction occurs when the presence of a neighbour has detrimental effects on the relative success of a plant. Ecologists are said to have been preoccupied with competitive processes for a long time (Bertness and Callaway 1994) with nearly all influential ecologists since Darwin having held the view that the natural world is shaped by conflict (Bruno, Stachowicz et al. 2003). Notable examples given by Bruno et al. (2003 pp 119) include "the mathematical models of interspecific competition developed by Lotka and Volterra, Gause's principle of competitive exclusion, and both Hutchinson's and MacArthur's work on niche and species packing". Examples of negative plant interactions include

exploitative competition and interference. Exploitative competition involves a struggle to pre-empt space and limiting resources such as light, water and nutrients (Callaway and Walker 1997) that determine growth, survival, and fecundity of individual plants (Lamb and Cahill 2008). On the other hand, interference includes strategies that improve competitive position of a species against competitors by killing, poisoning or intimidation (Levine 1976). Chemical-mediated growth inhibition (e.g. allelopathy) is therefore a form of interference.

Facilitation loosely refers to a relationship between two organisms in which at least one participant benefits and neither suffers (Bruno, Stachowicz et al. 2003). Though it was a central theme during the initial periods of the development of Ecology as a science, the topic of facilitation has suffered some neglect along the way (Brooker and Callaghan 1998). However, more recent research clearly indicates that the influence of facilitation on population- and community-level variables is at least as important as other factors (Bruno, Stachowicz et al. 2003; Padilla and Pugnaire 2006). Neighbouring plants may provide benefits for each other, such as nutrients, shade, more available moisture and protection from herbivores (Callaway 1998; Padilla and Pugnaire 2006), but the general importance of positive interactions to community diversity, structure and productivity is not always acknowledged (Bertness and Callaway 1994). Recently, there has been an increasing interest in facilitation, and its importance for plant community dynamics (Maestre, Bautista et al. 2003), in spite of which, little has been done to incorporate it into the conceptual framework of ecology (Bertness and Leonard 1997; Bruno, Stachowicz et al. 2003).

The study of plant interactions is as old as the discipline of Ecology itself. In this chapter, I attempt to explore the importance of competition and facilitation in determining plant community structure, drawing attention to the role of facilitation, particularly of exotic species in ecological restoration. This exercise is by no means exhaustive. Here, interference is treated as a mechanism of competition, and facilitation encompasses both commensalism and mutualism.   I begin by briefly examining the importance of competition and facilitation to community structure before highlighting the impacts of the interplay between the two processes. Finally, I sum up with examples where facilitation by exotic species has been used in ecological restoration. This chapter does not treat ecological restoration in its strict

sense (return to a pre-existing state) and so includes rehabilitation and reclamation as well (for terminologies see Aronson, Floret et al. 1993). While there is a lot of literature on facilitation and competition, there seems to be significantly less research documenting how exotic species influence ecological restoration (D'Antonio and Meyerson 2002).

1.3. Role of competition in shaping plant community structure

Competition has been a central component of many of the basic ecological theories that guided plant ecologists (Brooker 2006).   It is frequent among plants, and may be absent only in the early stages of the initial colonization (Bazzaz 1979) when density is low. Competition has also been found to influence both fitness components of individuals (Bengtsson, Fagerström et al. 1994) and the local plant species abundance (Bengtsson, Fagerström et al. 1994; Howard and Goldberg 2001).   It is generally believed that the structure and function of plant communities are strongly influenced by resource competition (Boyden, Binkley et al. 2005) since traits associated with competitive dominance also have effects on ecosystem nutrient cycling (Aerts 1999).   For instance, according to Aerts (1999), plants in nutrient-poor environments typically exhibit slow growth rates as well as low tissue turn-over and thus more nutrient retention ("stress tolerant" *sensu* Grime 1977).   The flipside of this is that those plants of nutrient-rich environments typically have greater resource capture and higher tissue turn-over, hence faster nutrient recycling. Therefore, by regulating nutrient recycling and in effect its availability to other species, competition influences the structure of plant communities. It has been suggested that for plant species to coexist they have to be differentiated in their abilities to compete for the various limiting resources (Tilman 1980). The principle of competitive exclusion posits that closely related taxa cannot coexist because they have similar requirements for which they compete more intensely than with their distantly related counterparts (Mayfield and Levine 2010).   This implies that competition results in local diversity because resource partitioning would allow unrelated taxa to coexist but not closely related ones with similar resource requirements.   On the other hand, Bengtsson et al. (1994) noted in a review that species richness generally increases when dominants are

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removed, supporting the assumption that dominant species often have negative effects on diversity. This seems to be in agreement with Grime (1977) that a superior competitor for one resource is also a superior competitor for all the other resources, in effect suppressing diversity. Although many plant scientists agree that interspecific competition is an important determinant of structure and dynamics of plant communities, there is much less concurrence on the mechanisms involved and its significance to community dynamics in different environments (Aerts 1999).

Environmental productivity is said to influence the intensity with which plants compete for resources as well as the specific resources for which they compete. According to Tilman (1988), the intensity of competition remains the same but the resources for which plants compete may change depending on habitat productivity. In fertile environments with dense canopies, competition is mainly for light and the successful competitor will have traits that lead to overtopping of its neighbours (Aerts 1999). In nutrient-poor environments competition is mainly for nutrients (Aerts 1999) but the importance, or even existence, of competition in such an environment has been strongly debated and persistently questioned throughout the history of ecological science (Fowler 1986; Grace 1993). This debate on the significance of competition to plant community structure has recently been dominated by the Grime and Tilman schools of thought, especially as relates to its importance in low productivity habitats (Grace 1991). Grime (1977) noted that the observed mortality of neighbours could not be exclusively a result of competition but could just as well be attributed to the capacity of a plant to exploit the features of the environment. Grime (1977) argues that in a nutrient-poor environment plant success is a result of nutrient capture and retention (also Aerts 1999) and not superior competitive ability, concluding that competition is rather unimportant in such habitats. Tilman (1989) on the other hand, demonstrated in an experiment involving bunchgrass (*Schizachyrium scoparium*) that competition may be strong regardless of habitat productivity, and that plant success is a result of its ability to deplete resources to levels too low for other species but to which it is tolerant (Tilman 1989). The Grime-Tilman debate (Thompson 1987; Tilman 1987; Thompson and Grime 1988) has been synthesised by Grace (1991) who concluded that their

disagreements are mostly about different aspects of competition; while Tilman discusses intensity of competition, Grime refers to its importance (see also Brooker, Kikvidze et al. 2005; Brooker and Kikvidze 2008; Kikvidze and Brooker 2010). Competition intensity refers to the reduction in the level of performance of a plant as a consequence of presence of a neighbour while competition importance refers to the impact of a neighbour as a proportion of the combined effects of all biotic and abiotic environmental factors (Welden and Slauson 1986). The persistent debate on competition is said to stem mainly from failure of researchers to distinguish between competition intensity and its importance (Brooker and Kikvidze 2008).

Another negative plant interaction is allelopathy, a non-resource interaction where one plant has negative effects on another through release of chemical compounds into the environment (Inderjit, Wardle et al. 2011). This phenomenon has been suggested as a mechanism for the remarkable success of invasive plants that frequently establish virtual monocultures where diverse communities once thrived (Hierro and Callaway 2003). For example, the diffuse knapweed (C*entaurea diffusa*) invades and dominates native bunchgrass communities of North America through allelopathic effects of its root exudates (Brooker 2006) to which native communities lack co-evolved tolerance (Callaway and Aschehoug 2000). Bazzaz (1979) noted that several members of pioneer plants produce toxins, mostly phenolic compounds that are inhibitory to several other species. Unlike resource competition that may produce diversity, allelopathy generally promotes the establishment of monocultures.

Competition plays an important role in biological invasions. Colonization, along with modification of many ecosystems throughout the world by exotic plants, and a resultant reduction in biodiversity, has become a commonly observed phenomenon (Vitousek 1990). A review of competitive interactions between native and exotic plants suggested that the spread and establishment of the exotic species is related to their ability to competitively suppress resident species (Levine, Vilà et al. 2003). The extent to which the success of an invader is explained by its competitive superiority, or the disturbances and dispersal that allow them to become established, is central to the debate on the importance of

competition and plant invasion and is valuable to the design of strategies to control invaders (Cuesta, Villar-Salvador et al. 2004). While there are many other explanations for plant invasions, such as enemy release (Keane and Crawley 2002), evolution of increased competitive ability (Blossey and Notzold 1995), and novel weapons hypothesis (Callaway and Ridenour 2004), competition still remains a central driver of biological invasions in addition to regulation of community composition.

1.4. Facilitative interactions among plants

Facilitative interactions have been demonstrated in a broad range of ecosystems, with most evidence coming from severely stressful environments such as semi-arid areas (Gomez-Aparicio, Zamora et al. 2004), salt marshes (Bertness and Shumway 1993; Didham, Tylianakis et al. 2007), and intertidal zones (Bertness and Leonard 1997). Direct positive interactions occur when neighbours modify environmental conditions, leading to positive effects (Bertness and Callaway 1994) for others. Presence of stress-tolerant plants can ameliorate potentially limiting physical stress in their immediate vicinity, thereby providing favorable sites where less tolerant species can then succeed (Bertness and Callaway 1994; King 2008).

The positive effect of an adult plant on seedlings of another species is called the "nurse-plant" effect (Bertness and Callaway 1994; Padilla and Pugnaire 2006) and has been found in harsh habitats, suggesting that habitat amelioration by neighbours is important for positive recruitment events (Bertness and Callaway 1994). Nurse plants concentrate nutrient cycling directly under their canopies (Callaway 1995; King 2008). For example, Kahi and colleagues (2009) observed that soil nitrogen content under canopy of mesquite (*Prosopis juliflora*) was 45% higher than in open areas. Other plants respond positively to such nutrient islands, leading to the formation and maintenance of vegetation patches around shrubs (King 2008). It has been experimentally determined that many shrubs acted as nurse-plants, resulting in increased survival of planted seedlings (Castro, Zamora et al. 2004; Gomez-Aparicio, Zamora et al. 2004). Generally, nurse-plant effects contribute to

recruitment and survivorship of regenerating beneficiary species and have been used widely in forestry (e.g. Castro, Zamora et al. 2004; Cuesta, Villar-Salvador et al. 2004; Gómez-Aparicio, Gómez et al. 2005; Sullivan, Williams et al. 2007; Smit, den Ouden et al. 2008). For instance, Aerts et al. (2007) found that seedlings of African wild olive (*Olea europaea*), survived better under shrub canopy of *Euclea racemosa* than in open fields.

There is improvement in soil moisture under the canopies of benefactor plants. This may be due to hydraulic lift (Richards and Caldwell 1987; Prieto, Martínez-Tillería et al. 2010) by the deeper roots of the larger plant. At night when transpiration is low, water absorbed by the roots of benefactor plants from the deeper soil layers tends to seep into the superficial layer (Caldwell and Richards 1989) where the beneficiary herbaceous species have their roots concentrated and consequently get access to this water. For example, Caldwell and Richards (1989) conducted a field experiment where they dipped roots of *Artemisia tridentata* (a deep rooted shrub) in water that has been highly enriched with hydrogen isotope, deuterium and observed that the stable isotopes appeared in the neighbouring tussock grass, *Agropyron desertorum* (shallow-rooted) confirming the phenomenon of hydraulic lift. Prieto and colleagues (2010) demonstrated that there was increased soil water potential in superficial layers after covering shrubs of five different species with opaque plastic bags for 48-72 hours. The shading effect of the nurse plant species also produces a microhabitat with lower temperature and wind speeds, as well as higher humidity, resulting in reduced transpiration demands and thermal stress (Holmgren, Scheffer et al. 1997; Aerts, Negussie et al. 2007) that further contributes to positive recruitment of the under-story beneficiary species.

Plants growing in oxygen limited soils are known to passively conduct oxygen from leaves to their roots that may then diffuse into the surrounding medium where it could oxidize harmful dissolved chemicals and ameliorate anoxia for other species (Callaway 1995). In a field and greenhouse experiment, Callaway and King (1996) found that narrow-leaved willow (*Salix exigua*) seedlings survived only in the presence of earenchymatous *Typha latifolia* at environmental temperatures of 11-12°C.   Black rush (*Juncus gerardi*), has also been observed to oxygenate the surrounding soil through its aerenchyma tissues that serve

as conduits for oxygen delivery to the rhizosphere (Hacker and Bertness 1999). This shows that under certain environmental conditions highly aerenchymatous species can aerate soil near its roots, improving growth of the less aerenchymatous neighbours that would otherwise not perform well in anoxic habitats like marshes (Callaway and King 1996).

Benefactor plants modify the substrate, making it more suitable for supporting other species. Sand-binding species stabilize the substrate for eventual colonization by successive sand dune plants (Lichter 2000). Leaf litter of such pioneer plants has been observed to improve soil structure and increase its cation exchange capacity, and water holding capacity (Hunter and Aarssen 1988). Later arriving species can then respond positively to the improved soil micro-conditions.

Protection from herbivores is an indirect form of facilitation where highly palatable species gain protection by growing among thorny (Callaway 1995) or less palatable neighbours (Callaway, Kikvidze et al. 2000; Brooker, Maestre et al. 2008). This phenomenon is widespread in rangelands where grasses find refuge among undesirable neighbours.

Co-flowering species have been shown to facilitate each other when the pollinator attraction of one species is greater than that of its neighbour (Callaway 1995; Padilla and Pugnaire 2006). In a field experiment, Hegland et al. (2009) found that positive interactions for pollinator attraction were more numerous than negative ones. In an experiment to test whether pollination of *Raphanus raphanistrum* was enhanced by other co-flowering species, Ghazoul (2006) found that pollination visits increased when it occurred with at least one of *Cirsium arvense*, *Hypericum perforatum* and *Solidago canadensis* compared to when it occurred alone.

There is widespread resource sharing among neighbouring plants through root grafts (Graham and Bormann 1966) and mycorrhizal networks (Hunter and Aarssen 1988; Selosse, Richard et al. 2006). An example is nitrogen transfer between *Casuarina cunninghamiana* and *Eucalyptus maculata* (He, Critchley et al. 2005). Woods and Brock (1964) found that radioisotopes of phosphorous and calcium introduced into the stumps of red maple (*Acer*

rubrum) appeared in 19 other neighbouring species. Although they did not come up with a definitive mechanism of the transfer, Woods' and Brock's (1964) experiment shows that transfer of materials among neighbouring plants is widespread. The breadth of literature on plant facilitation is extensive and the bottom line is that facilitation is a ubiquitous process in harsh environments where the presence of a neighbour enhances recruitment, survivorship, and performance of a beneficiary plant.

1.5. Competition-facilitation interplay and its influence on plant community structure

The composition and structure of a community are shaped by both abiotic factors and interactions among organisms, though the specific types of interactions partly responsible are usually less evident in plant communities (Holzapfel and Mahall 1999). In current models of plant community structure, it is widely accepted that positive and negative interactions operate simultaneously (Bertness and Callaway 1994; Callaway and Walker 1997; Holmgren, Scheffer et al. 1997; Brooker and Callaghan 1998). A growing number of studies indicate that the net outcome of most interactions between neighbouring plants is determined by the relative strength of facilitation and competition (Aguiar, Soriano et al. 1992; Callaway and Walker 1997; Holmgren, Scheffer et al. 1997; Holzapfel and Mahall 1999; Michalet, Brooker et al. 2006; Villarreal-Barajas and Martorell 2009). In highly stressful conditions, initial colonizers tend to ameliorate stress and facilitate further plant colonization (Bertness and Shumway 1993). However, the search for general patterns in the relationship between resource availability and the intensity or importance of competition has been controversial in population and community ecology (Boyden, Binkley et al. 2005). Reviews on these patterns (Callaway 1995; Bruno, Stachowicz et al. 2003; Brooker, Maestre et al. 2008) generally suggest that plant interactions shift from competition to facilitation along stress gradients (Bertness and Callaway 1994). Positive interactions are suggested to be more important in plant communities as abiotic stress or consumer pressure increases, while competition dominates when physical stress and consumer pressure are both relatively subtle (Bertness and Callaway 1994). The reasoning is that harsh environments may restrict plants from acquiring resources, and any amelioration

of these conditions will favour growth to the extent that it outweighs the competitive impacts of growing in close associations, whereas in benign environments amelioration of environmental conditions has a minimal impact and the balance is tipped towards negative effects (Olofsson, Jon et al. 1999). Grime (1977) reasons that while the prevalent plant coping strategy in productive, less disturbed environments is competition, stress tolerance is more important in persistently unproductive habitats. Brooker and Callaghan (1998) also claim that competition is rather unimportant in nutrient-poor environments and its intensity increases only with increasing productivity. Pugnaire and Luque (2001) compared biomass of understory species of the shrub *Retama sphaerocarpa* at a highly fertile valley bottom site and steeper rocky outcrops with their counterparts growing outside the shrub canopy and concluded that facilitation weakened with increase in soil fertility. Small-flowered forget-me-not (*Myosotis laxa*) was observed to have benefited significantly from soil oxygenation when grown with *Typha latifolia* at low soil temperatures, but the positive effects disappeared at higher soil temperatures and the interaction became competitive (Callaway and King 1996). Holmgren and Scheffer (2010) however, propose that facilitation is more important in mild, not severe environments, arguing that although the relative role of facilitation increases along stress gradient, its absolute effect is largest at moderately stressful conditions. They argue that under highly stressful conditions facilitation by a neighbour may not be sufficient enough to allow any growth as competition by the "benefactor" for the same resource may cancel out the effect of facilitation (Holmgren and Scheffer 2010).

The magnitude of competition experienced by species is said to vary among life-history stages and size asymmetry (Howard and Goldberg 2001), and may affect the outcome of interactions between plant species (Callaway and Walker 1997). In many cases, seedlings of beneficiary species are found spatially associated with nurse plants, whereas adults are not, suggesting that the importance of competition and facilitation shifts among the various life-history stages (Callaway and Walker 1997; Miriti 2006). Whereas naturally established seedlings of African wild olive (*Olea europaea* ssp. *Cuspidata*) were found restricted to sites under the canopy of pioneer shrub Bush guarri (*Euclea racemosa* ssp. *Schimperi*), the adults were not (Aerts, Negussie et al. 2007). Similarly, the sahuaro (saguaro)

cactus (*Carnegiea gigantean*) of the Sonoran Desert is commonly limited to areas beneath the canopy of its nurse-plant, the foothill paloverde (*Cercidium microphyllum*), but the close proximity was observed to lead to increased stem die-back and greater mortality of the nurse tree, *C. microphyllum* (McAuliffe 1984). Archer et al. (1988) found that once *Prosopis glandulosa* created habitat suitable for colonization by other woody perennials, the colonizers were able either to outcompete the original *Prosopis* nurse plant or to prevent it from reproducing in the clusters. Such patterns of nurse plant mortality indicate that some native species that begin their lives as the beneficiaries become significant competitors with their former exotic benefactors as they mature (Callaway and Walker 1997). These observations suggest that the positive effects of benefactors are strong when beneficiaries are young but competitive interactions dominate as the beneficiaries get older and larger (Callaway and Walker 1997; Schwinning and Weiner 1998; Miriti 2006). It is suggested that acquisition of resources usually depends more on plant size than on species identity (Bengtsson, Fagerström et al. 1994) and therefore asymmetry in competition is mainly a result of differences in plant size rather than species differences. Some benefactor species seem to play an altruistic role by contributing to survivorship of other species at their own expense. In summary, relationships between plant species can change from positive to negative with changes in size of the interacting species.

Other than degrees of environmental stress, ontogeny, and size asymmetry, shifts in dominance between facilitative and competitive outcomes also depend on density and successional stage. Thickets of early successional species often inhibit colonization by later successional species (Berkowitz, Canham et al. 1995) by using up resources and starving later arrivals (Bazzaz 1979).

1.6. Role of facilitation by exotic plant species in eco-restoration

Control of the introduction and spread of invasive exotic species is a highly costly and time-consuming undertaking. For instance, invasive species are reported to have cost New Zealand about $3.4 billion (just under 2% of GDP) in control expenditure and production losses in the year 2008 (Biosecurity 2009). In the United States, the annual costs of invasive species are estimated at between US$120-138 billion (Pimentel, Lach et al. 2000; Pimentel, Zuniga et al. 2005). In addition to economic costs, exotic plant invasions pose serious threats to native biodiversity and ecosystem functioning (Spence, Ross et al. 2010). It has been suggested that there is need for development of novel, low-cost and effective restoration techniques for maintaining these ecosystem functions and services (Hobbs, Arico et al. 2006). Facilitation, having been recognized as an important structuring force, is increasingly being considered as an ecological mechanism for developing vegetation restoration tools, particularly for severe and highly disturbed environments (Brooker and Callaghan 1998; Brooker, Maestre et al. 2008). Most restoration projects are typically preceded by mechanical or chemical removal of weed species (Berger 1993). For example, in reforestation, shrub cover is cleared before planting tree seedlings (Aerts, Negussie et al. 2007) as they are thought to compete with the seedlings (Gomez-Aparicio, Zamora et al. 2004). This may not be advisable given that shrubs may sometimes have positive impacts on the seedlings (Castro, Zamora et al. 2004) as demonstrated by studies referred to in the sections above. Removal of plants, even exotic ones, is said to have negative impacts on the ecosystem (Zavaleta, Hobbs et al. 2001; D'Antonio and Meyerson 2002). Moreover, clearing shrub cover is a disturbance that would create empty niches which may favour establishment of undesirable invasive species (Hierro and Callaway 2003). It is prudent to find ways of using the extant exotics positively as it may sometimes be necessary to use fast-growing exotic species in restoration of highly degraded sites (D'Antonio and Meyerson 2002).

Most studies of biological invasions have focused solely on the direct negative impacts of non-indigenous species on native biota (Rodriguez 2006). Incorporating facilitative interactions of invasive species into future ecological research will be important

to determine their relative strength in comparison to competition and changes in ecosystem processes and biodiversity (Rodriguez 2006). Some ecological traits of invasive exotic species, including nitrogen fixation, fast growth, and resistance to stress contribute to their ability for colonizing new areas (Noble 1989). On the other hand, these traits can be of relevance to fostering ecological restoration as an alteration of ecological conditions, and resource flow may promote the establishment of late successional species (Ehrenfeld 2003; Levine, Vilà et al. 2003; Ewel and Putz 2004). There are however, very few examples of research where facilitation by exotic species have been exploited for restoration. In a study at a degraded coastal pasture site in Puerto Rico it was observed that seedlings of several secondary forest species were abundant in the understory of exotic *Albizia lebbek* plantation but absent from grass control plots (Parrotta 1992), suggesting an important role played by such exotic plantations in forest regeneration. Similar observations were made by Chapman and Chapman (1996) in *Pinus* spp. and *Cupressus lusitanica* plantations, and under the canopies of the exotic *Acacia mangium* by Kuusipalo et al. (1995). While undisturbed forest habitats are important for conservation at a landscape scale, it has been suggested that exotic plantations may complement them by providing additional habitats for forest plant species (Hylander and Nemomissa 2009).

Invasive nitrogen-fixing plants can facilitate neighbouring plants. For example, in the lowland pampas of Argentina, the vegetative growth and aboveground biomass of native perennial grass *Paspalum dilatatum* increased in the presence of non-indigenous nitrogen-fixer, *Lotus tenuis* (Quinos, Insausti et al. 1998). Gorse (*Ulex europaeus*) is an invasive pioneer nitrogen-fixer introduced to New Zealand that has been observed to enrich the soil, making way for native forest regeneration (McQueen, Tozer et al. 2006; Sullivan, Williams et al. 2007). Gorse is also said to shade out invasive grasses in old fields, creating suitable microsites for regeneration of native woody species such as *Pittosporum eugenioides* that eventually replace it (Norton 2009). An invasive aquatic species (*Hydrilla verticillata*) that was originally thought to have an extremely high potential of eliminating submerged aquatic species in the United States has recently been reported to have beneficial effects on habitat quality, allowing native species to re-establish and maintain themselves (Hershner and Havens 2008).

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According to Van Aarde et al. (1996), Richards Bay Mining Company in South Africa removed all the vegetation from sand dunes before mining. After mining, the dunes were stabilized by sowing seeds of a mixture of exotic species such as babala grass (*Pennisetum americanum*), sun hemp (*Crotalaria juncea*), and a mixture of native grass seeds, forming a dense cover within a month. After 6-8 months the introduced grass species died off, leaving the local weeping love grass (*Eragrostis curvula*) after ameliorating surface microclimate for germination and subsequent establishment of the indigenous species (Van Aarde, Ferreira et al. 1996). Berens et al. (2008) quantified seed rain and seedling establishment of heterospecific woody species under fruiting exotic guava (*Psidium guajava*) trees in farms around Kakamega, Kenya and found that 93% of the seedlings were animal dispersed native species from the nearby Kakamega forest. Guava tree, though invasive in some regions, can serve as focal points for restoration in degraded areas by attracting seed dispersers (Berens, Farwig et al. 2008). Similarly, reclamation of derelict old quarries at Bamburi, Kenya using the exotic whistling pine (*Casuarina equisetifolia*) succeeded in ameliorating the salinity and nutrient deficiency, allowing regeneration of native bird-dispersed plants (Siachoono 2010). The abandoned gaping quarries at Bamburi are now a nature park where native plants are increasing in abundance and diversity (pers. observation). Fischer et al. (2009) tested whether the invasive *Cinchona* species had facilitative effects in simplified tropical Hawaiian systems and found that there were significantly more endemic herbs and shrubs under canopy of the invader than in uninvaded plots. Similar observations were made under invasive shrub *Pyracantha angustifolia* by Tecco and colleagues (2006).

Another area that may not have received sufficient attention is the mediation by exotic ecosystem engineers in creating micro-conditions suitable for native species colonization or substituting extinct native species by providing the same ecological services (Griffiths and Harris 2010). For example, Aldabra giant tortoises (*Aldabrachelys gigantea*) introduced to several small islands surrounding Mauritius, appear to have successfully substituted the herbivory and seed dispersal functions of native tortoises that recently became extinct (Griffiths, Jones et al. 2010). Taxon substitutions have been proposed as a new approach for ecological restoration (Donlan, Berger et al. 2006) and Hansen (2010 pp

124-125) has outlined proposals where extinct plant species could be substituted by related extant ones.

1.7. Research aims

Various studies (e.g. Castro, Zamora et al. 2004; Gomez-Aparicio, Zamora et al. 2004; Aerts, Negussie et al. 2007) have shown that restoration via nurse-plant facilitation is viable. However, there are only a few examples of restoration using facilitation by exotic species. There is need for more research into facilitative effects of exotic plants on native species and their possible role in ecological restoration.

This thesis aims to investigate competitive and facilitative influence of the invasive South African iceplant (*Carpobrotus edulis*) on native plants, specifically *Spinifex sericeus,* with particular reference to implications of these interactions for dune restoration in New Zealand. It further explores the growth rates, substrate preferences and mating systems of the exotic and native iceplant taxa found in New Zealand. The information gathered will add to the existing knowledge base on the ecology of iceplant taxa found in New Zealand and in advising coastal sand dune restoration practitioners.

1.8. Thesis structure

The remaining sections of the thesis are divided into three chapters. Chapter two investigates the competitive and facilitative effects of *C. edulis* on *Spinifex*. Chapter three examines the ecology of iceplant taxa found in New Zealand in terms of their in-situ growth, substrate preference and mating systems. Chapter four is a summary of the findings of the research and recommendations for future work. Chapters two and three have been prepared as manuscripts for journal submission and may differ in style, and contain some repetition.

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Chapter two

2. Mixed effects of invasive *Carpobrotus edulis* (Aizoaceae) on *Spinifex sericeus* and its implications for sand dune restoration

Figure 2:1: Seedlings of *Spinifex sericeus* (foreground) ready to be transplanted into plots where iceplant (*C. edulis*) was intact, removed or never existed. Photo taken by author on 15th November 2010 at Queen Elizabeth Park.

2.1. Abstract

New Zealand has experienced catastrophic losses to its native biodiversity as a consequence of the impacts of exotic species. While the outcome of species invasions depends on interactions between exotic and resident native species, it has been noted that most studies of biological invasions have focused solely on the direct negative impacts of non-indigenous species on native biota. Yet negative and positive interactions between neighbouring plants operate simultaneously. A research project involving neighbour removal was initiated on coastal sand dunes with the main aim of studying the effects of *Carpobrotus edulis* on establishment of *Spinifex sericeus* at the foredune region. *Carpobrotus edulis* protected *Spinifex* against storm erosion, sand-blasting and salt sprays while simultaneously suppressing its leaf production. Suppression of *Spinifex* leaf production was more pronounced at top of the dune where stress elements are presumably more benign. There was no evidence of allelopathic suppression of *Sinifex* by *C. edulis*.

2.2. Introduction

2.2.1. Invasive species as threats to native biodiversity

Invasion of natural communities by exotic species is often cited as the greatest threat to biodiversity, second only to habitat destruction (Vitousek, D'Antonio et al. 1997; Kolar and Lodge 2001; Shaw 2003). Increased international human travel and trade have led to widespread dispersal of plant and animal species (Mack, Simberloff et al. 2000) such that exotic plants have now colonized and changed many ecosystem properties throughout the world (Vitousek 1990). Invasive species result in global biotic homogenisation and loss of biodiversity through replacement of native, locally distributed species with widespread, non-native species (Olden, Poff et al. 2004). Globally, about 10% of introduced species are reported to have become naturalised, while those that have become invasive constitute around 1% (Williams and West 2000; Richardson and Pyšek 2006). Although the proportion of exotic naturalised plant species that successfully become invasive seems negligible, they

can cause substantial modifications to indigenous biodiversity and ecosystem functions (Williams and West 2000). Invasive species simplify communities, causing them to suffer a loss of functional diversity, potentially reducing community stability and resistance to environmental change (Olden, Poff et al. 2004). As many as 42% of endangered species are said to be on the brink of extinction, primarily due to invasive species (Pimentel, Zuniga et al. 2005) often stemming from strong competition (Wilcove, Rothstein et al. 1998). Such competition may further be underlain by a high propagule pressure (Lockwood, Cassey et al. 2005), contributing to the success of the invasive species to the detriment of native ones.

New Zealand has experienced catastrophic losses to its native biodiversity as a consequence of the impacts of exotic species (Jay, Morad et al. 2003) that have contributed to a decline of 59% of New Zealand's threatened native plant species (Dopson, de Lange et al. 1999). Over 25,000 species of plants have been introduced deliberately for agriculture, horticulture, forestry and as garden ornamentals, as well as accidentally (Taylor and Smith 1997). Many of these introduced species have gone on to establish populations in the wild so pervasively that naturalised exotic vascular plants now outnumber native New Zealand species (Taylor and Smith 1997; Williams and West 2000).

2.2.2. Threats to New Zealand coastal sand dunes

One distinctive feature of New Zealand's natural landscape that is now classified as a highly threatened ecosystem, partly by exotic species, is its coastal sand dunes (Sawyer 2004; Hilton 2006). The natural character of the coastal dunes has been lost from over 70% of their extent between the 1950s and 1990s (Hilton 2006) due to a combination of factors such as coastal development, farming, and forestry in addition to invasion by introduced plant and animal species (Hilton, Macauley et al. 2000). Comprehensive reviews of these threats have been carried out in recent years by researchers (e.g. Gadgil and Ede 1998; Hilton 2006). Frequent disturbance regimes by wave and wind erosion that create open niches make coastal sand dunes highly susceptible to invasions by exotic plants. The fact that a large population of New Zealanders live within 10 km of the coastline (Spence, Bergin

et al. 2007) may increase propagule pressure of exotic plants, some of which have been successful garden escapees. Sand dune erosion has been further exacerbated by inappropriate use of four-wheel drive vehicles and trampling by humans (Milne and Sawyer 2002). Yet, they are important ecosystems that are a popular place for recreation (Spence, Bergin et al. 2007). The fluid form of the sand dunes and their ability to regenerate provides excellent natural buffering of the coastline against storm actions (Carter 1991; Dahm, Jenks et al. 2005) – a function which will become increasingly important in the face of the expected rise in sea levels and greater frequency of storm events (NIWA 2008). Native sand dune plant species like pīngao (*Ficinia spiralis*) are of cultural significance to the Māori where they were used for weaving. Together with other foredune species such as *Spinifex sericeus* (Kowhangatara), they provide habitat for many rare and specialised plant and animal species like the endemic katipō (*Latrodectus katipō*) (Patrick 2002). Despite their conservation value, coastal dunes are constantly under threat from human constructions and leisure activities, and from biological invasions by exotic plants (Maltez-Mouro, Maestre et al. 2010).

2.2.3. Sand dune restoration efforts in New Zealand

In some early restoration projects in New Zealand, marram grass (*Ammophila arenaria*), the South African iceplant *Carpobrotus edulis*, and *Carpobrotus chilensis* were used to stabilise bare sand, because of their rapid growth, and tolerance to exposed conditions and sand burial. These exotic species have displaced native sand binding plants in the majority of sand dune areas (Partridge 1995), significantly altering the natural form and function of the dunes (Hilton, Macauley et al. 2000). *Carpobrotus edulis,* which is the focus of the present study, has been reported to be a problem species in areas outside its native range, including in California (Albert, D'Antonio et al. 1997; Vilà, Weber et al. 1998) and the Mediterranean Basin (Suehs, Affre et al. 2004; Vilà, Siamantziouras et al. 2008), displaying properties typical of an invader. *Carpobrotus edulis* often forms dense impenetrable mats which seem barely affected by herbivory or competition from native plants (Maltez-Mouro, Maestre et al. 2010). It causes smothering, thus suppressing growth

and regeneration of native flora (Bartomeus and Vilà 2009; Maltez-Mouro, Maestre et al. 2010). It is said to compete aggressively with native plant species for space and resources (D'Antonio and Mahall 1991; D'Antonio 1993). Because of its invasiveness and presumed threats to native flora, *Carpobrotus edulis* is considered an unwanted organism in New Zealand (Biosecurity New Zealand 2008).

Many New Zealand coastal community groups are taking action to restore their local patches of sand dunes; typically by removing the exotic species and planting foredune sand-binding grasses and sedges (e.g. the native *Spinifex* and pingaō, *Ficinia spiralis*). Yet these exotic plants may have some facilitative effect on the native species. Although *Carpobrotus edulis* is said to displace native foredune flora, few, if any studies, have documented how native pioneer plants that are well adapted to frequent disturbance events have been displaced by this adventive species. The displacement notion may have been inferred from the fact that *C. edulis* stands are simple, nearly homogenous communities with very few co-occurring native plants, which may not necessarily be a product of displacement but a colonization of an empty niche by the adventive species in the absence of propagules of native pioneer species. *Carpobrotus edulis* may as well have a role as a useful stabiliser, creating appropriate micro-conditions suitable for establishment of native species. However, there is little quantitative information on how it interacts with other fore-dune species.

2.3. Purpose of the study

The outcome of species invasions depends on interactions between exotic and resident native species (Diez, Sullivan et al. 2008). It has been noted that most studies of biological invasions have focused solely on the direct negative impacts of non-indigenous species on native biota (Traveset, Brundu et al. 2008). However, negative and positive plant interactions between neighbouring plants operate simultaneously (Bertness and Callaway 1994; Brooker and Callaghan 1998) and the net outcome is determined by the relative strength of each process (Michalet, Brooker et al. 2006; Villarreal-Barajas and Martorell

2009). Therefore, incorporating facilitative interactions of invasive species into ecological research is important to help determine the relative contribution of competition and facilitation to changes in ecosystem processes and biodiversity (Traveset, Brundu et al. 2008). On one hand, some ecological traits of invasive exotic species such as nitrogen fixation, fast growth, and resistance to stress, contribute to their ability to colonize new areas (Noble 1989). On the other, these traits can be exploited to foster ecological restoration, since alteration of ecological conditions and resource flow may promote the establishment of other species (Ehrenfeld 2003; Levine, Vilà et al. 2003; Ewel and Putz 2004).

I initiated a research project at Queen Elizabeth Park along Kapiti Coast, New Zealand where erosion of the dunes by the high seas during occasional storms has been of increasing concern to the management. Natural dune repair after storm events depends on presence of appropriate sand-trapping vegetation on the dune face (Bergin, Miller et al. 2007). While exotic species such as *C. edulis* have been used to stabilise dunes, it is deemed ineffective in repairing storm-damaged dunes between storm episodes (Bergin, Miller et al. 2007), probably because its prostrate growth form may not help trap as much sand as upright plants. To enhance self repair by the dunes, the management of Queen Elizabeth Park have initiated some restoration projects in gentler sections by removing *C. edulis* and planting *Spinifex sericeus*, a native stoloniferous sand-binding grass, in addition to pingaō (formerly *Desmoschoenus spiralis*) – now renamed *Ficinia spiralis* (Muasya and de Lange 2010). In line with the restoration goals of the management, the research project was initiated with the main aim of studying the effects of *Carpobrotus edulis* on establishment of *Spinifex sericeus* at the foredune region. The aims of this experiment were to determine (i) whether *C. edulis* has positive facilitative effects or negative competitive effects on *Spinifex*; (ii) whether the effects differ along the gradient of the dune face, (iii) if *C. edulis* has any allelopathic legacy that hampers establishment of *Spinifex.*

2.4. MATERIALS AND METHODS

2.4.1. Study site

Queen Elizabeth Park is located 40km North of Wellington, New Zealand between Paekakariki and Raumati South (40°56'S, 174°57'E and 40°58'S, 174°59'E). The terrain consists of undulating dunes characterised by steep seaward slopes exposed to prevailing westerly winds. There are two small streams that run into the sea at this section supplying some sand (Fig. 2.2) but the main sand supply seems to be from blowouts at the dune face that is blown inland at intervals by the westerly winds. The highest points of the dunes are elevated roughly between 4-10m above sea level with slope of the eroding foredune face ranging from 32-52°. The dunes of the park were recognised as "Recommended Area for Protection" in the 1992 Protected Natural Areas Programme Survey of Foxton Ecological District (Ravine 1992). The dune face is dominated by South African iceplant, *Carpobrotus edulis* interspersed with bare spaces. Other plants present include marram (*Ammophila arenaria*), spinifex (*Spinifex sericeus*), shore bindweed (*Calystegia soldanella*), and purple groundsel (*Senecio elegans*).
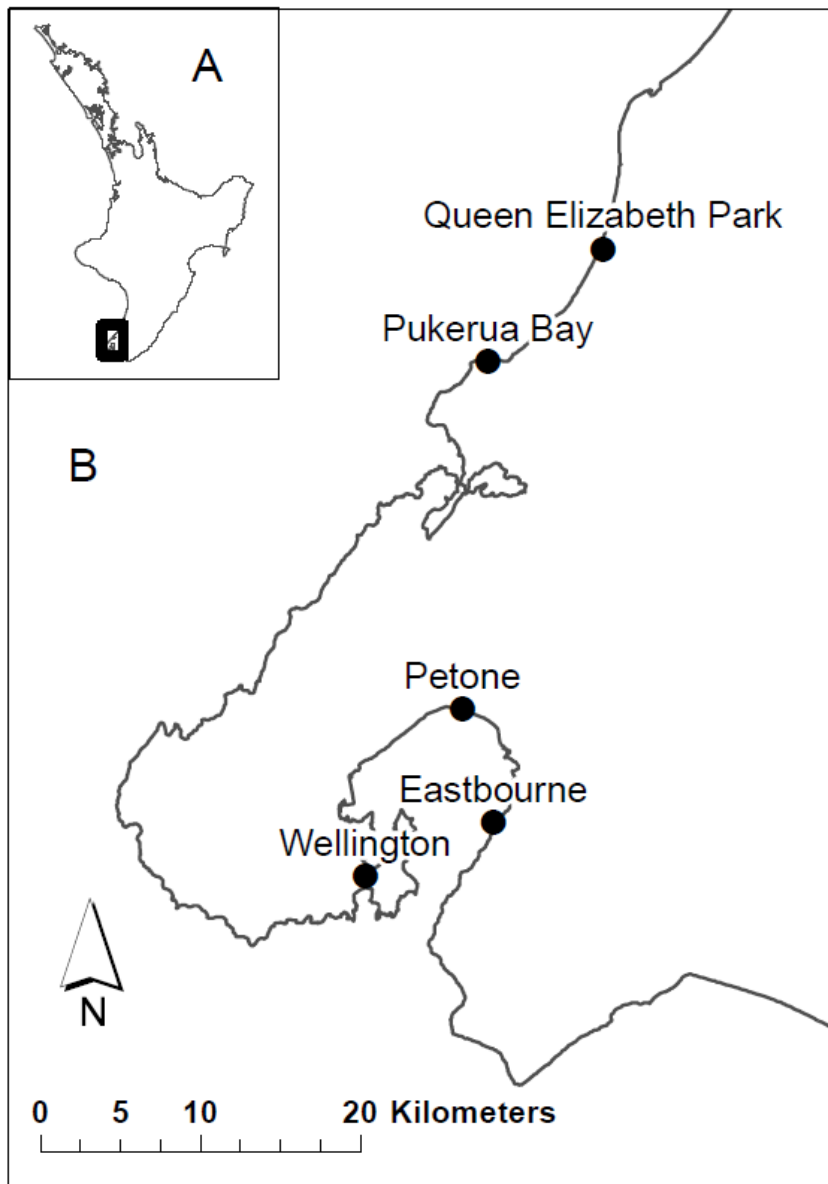
Fig. 2.2: Map of North Island, New Zealand (A) and Wellington region (B) showing the location of Queen Elizabeth Park.

2.4.2. Study species

2.4.2.1. Spinifex (*Spinifex sericeus*)

Spinifex is a short stoloniferous, dioecioes, perennial grass that is commonly found on the sand dunes along the coasts of Australia, New Zealand and New Caledonia. It is up to 40cm tall (Bergin, Miller et al. 2007) with strong creeping runners that produce roots and numerous upright leafy tillers at the nodes. The leaf blades are in-rolled, measuring 30-40 cm long and 7-8 mm wide when flattened (DERM 2011). The leaves are silvery on the upper surface, with a dense covering of short silky hairs on the underside. It is regarded as the most important pioneer sand-stabilising native plant in New Zealand (Connor 1984). It is tolerant of sand burial and salt spray (Maze and Whalley 1992). The upright leafy shoots reduce wind velocity, resulting in sand accretion. It grows well on all parts of the frontal dune and predominates particularly on the dynamic incipient foredunes, though it often appears to be less vigorous in the more stabilized semi-established and established dunes (Maze and Whalley 1992). The growth habit and ability of *S. sericeus* to grow vigorously in the most species-poor part of the foredune make it an ideal foredune stabilizer. For this reason, it is the main species used in planting programs for revegetation of foredunes around New Zealand.

2.4.2.2. South African ice plant (*Carpobrotus edulis*)

The South African iceplant is a succulent stoloniferous prostrate perennial that roots extensively at its nodes. It has its origin in the Karoo of South Africa but has now spread to the Mediterranean region, Australia, New Zealand and California (D'Antonio and Mahall 1991; Draper, Rossello-Graell et al. 2003; Suehs, Charpentier et al. 2006). It is a mat-forming species and seems to exclude other species by smothering them. Studies in the Mediterranean Basin and California have suggested that it alters soil chemistry to the detriment of native flora (Vilà, Tessier et al. 2006; Traveset, Brundu et al. 2008; Conser and Connor 2009). *Carpobrotus edulis* is a clonal species where growth is characterized by

vegetative production of numerous physically interconnected ramets that could quickly expand horizontally to efficiently colonize the surrounding area (Roiloa, Rodriguez-Echeverria et al. 2010). The ramets are physiologically integrated such that essential resources are translocated from established to developing ones (Noble and Marshall 1983; Price and Marshall 1999) and from those growing in favourable microhabitats to the ones growing under more adverse conditions (Hartnett and Bazzaz 1983; Shumway 1995; Stuefer, DeKroon et al. 1996; Roiloa and Retuerto 2006). Clones can therefore act as cooperative systems (Stuefer, DeKroon et al. 1996), buffering the negative effects of microhabitats, colonizing patches that otherwise would be unexploitable by independent plants (Salzman and Parker 1985; Yu, Dong et al. 2004; Roiloa and Retuerto 2006).

Carpobrotus edulis employs flexible sexual and asexual reproductive strategies (Suehs, Affre et al. 2004) to increase its invasion success (Baker 1974). It is tolerant to salt sprays, sand burial and water stress, making it an ideal foredune pioneer species. The species also seems to form a simplistic community that tends towards monoculture. Its status in New Zealand is that of an unwanted organism, probably because of its weed status in the aforementioned regions (Sheppard, Shaw et al. 2006; Andreu, Vilà et al. 2009) and the fact that it hybridises with the native iceplant, *Disphyma australe,* genetically modifying native flora (Chinnock 1971). *Carpobrotus edulis* was introduced as an ornamental plant which escaped from cultivation and was later used for sand dune stabilisation. The species is said to have naturalised in New Zealand around 1883 (Webb, Sykes et al. 1988; D'Antonio and Mahall 1991).

2.4.3. Experimental set-up

2.4.3.1. Experiment 1 (manual removal)

To investigate the effects of *Carpobrotus edulis* on restoration plantings of *Spinifex sericeus,* a manipulative field experiment was conducted. Study plots were assigned to three treatments, each replicated seven times (21 plots in total). The treatments involved planting *Spinifex*: (i) into stands of *C. edulis* (=Iceplant plots), (ii) into areas where *C. edulis* has been

removed manually (=Removal plots), and (iii) in open spaces where there was no pre-existing vegetation (=Bare plots). Twelve seedlings of *Spinifex sericeus* (obtained from Taupo Native Nursery in root trainer sleeves measuring 5cm x 5cm and 20cm in depth) were planted per plot. Six seedlings were planted at the bottom of the dune face and six near the top (= two subplots measuring 2m x 1.5m each). The distance between the subplots at the foot of the foredune and the ones towards the back varied between 2 – 4m according to the size of dune face. The seedlings were planted between 15th and 17th November 2010, spaced 40-50cm apart within a plot, since wider spacing is thought to increase vulnerability to wind erosion (Bergin, Miller et al. 2007). Seedlings were planted at a depth of at least 20 cm - equivalent to the depth at which the seedlings were in the root trainer sleeves. Two pellets of a slow release compound fertilizer (Agpro[TM]; 20:4:2; N: P: K) were applied in each planting hole before transplanting the seedlings. Since the dunes varied in shape and elevation above the sea level, the seedlings were not all planted at the same heights. Due to warm weather the plots were sprayed with 500 l of water from an industrial sprayer on one occasion at the end of November.

During the uprooting of the iceplant to create the plots for removal treatment, other plants such as clovers (*Trifolium* spp and *Medicago* spp), shore bindweed (*Calystegia soldanella*) and grasses (*Holcus lanatus* and *Lagurus ovatus*) that were within the iceplant crop were not targeted for removal but some still got removed due to soil disturbance. No specific effort was made to clear all the underground structures of *C. edulis* and as a result, some below ground tissues of the iceplant still remained. This would help in determining whether underground structures can regenerate to contribute to recolonization of the plots by the iceplant.

Survival of the *Spinifex* seedlings was assessed periodically at an interval of about four weeks. The location of each plant was mapped, and once survival rate seemed to have stabilised, a cumulative number of fully formed leaves per plant was recorded beginning 21st March 2011 at monthly intervals, as a proxy for plant performance.   Young leaves that were still rolled and not fully open, and dry ones, were excluded. Survival and leaf production data were not collected in the month of September 2011.

2.4.3.2. Experiment 2 (herbicide treatments)

Two additional types of removal plots were created on 10th May 2011 by spraying out iceplant using glyphosate (Agpro Glyphosate 360 at 10ml/Litre of water). During manual removal of live plants in November 2010 for experiment 1, transportation of the plant material to a dump site about 2km away proved labour intensive. This time the plants were chemically killed before manual removal to save on the effort required for disposal of the vegetative material and to minimize chances of exacerbating invasion. Dead debris of iceplant was left intact in three plots as mulching material, creating a treatment referred to as "dead debris intact". It was hoped that the plant debris left intact would moderate sand blasting and soil moisture loss, increasing survivorship and plant performance. On 25th of May 2011, dead debris of iceplant was removed manually from three of the plots to create the alternative treatment called "dead debris removed". 16 *Spinifex* seedlings were planted per plot, eight at the top and eight at the bottom section of each plot (two subplots) on 1st of June 2011, giving a total of 96 seedlings. However, the distance between the top and bottom band of the seedlings was 1-2m, due to size of the dune face. Planting techniques were the same as used in Experiment 1 except that planting depth was approximately 30cm (i.e. about 10 cm above the level of root trainer sleeves). Seedlings were chosen and assigned to treatments at random. The initial number of leaves per seedling was counted and it was subsequently determined that they were not significant different by treatment or location (all $P > 0.2$). Plant survival and cumulative number of leaves per plant were recorded periodically at 4-5 week intervals. Plant survival and leaf production data were not collected during the month of September, 2011.

2.4.4. Physical properties of dunes with and without iceplant

The slopes of the experimental plots were determined using a spirit level with a dial on a wooden plank 3m long to even out small depressions along the slope.

Soil samples were collected in October 2011 from under the canopy of iceplant and from bare sites both at the foot and the top of the dunes. Ten samples were collected per

site, five from the bottom of the dune and five from the top. Sample cores 4cm in diameter were obtained to a depth of 5cm and analysed gravimetrically for moisture, by combustion at 360 °C for organic matter content (Salehi, Beni et al. 2011), and by electrical conductivity for salinity .

2.4.5. Statistical analysis

Proportional survival of seedlings for each subplot in both experiments was calculated and transformed by applying square-root followed by arcsine to meet assumptions of ANOVA. The transformed survival data were analysed in SPSS (SPSS Inc. 2008). The arcsine transformed survival values were used as the dependent variable in a two-way ANOVA with "treatment" and "location" on the dune as the independent variables. A two-way ANOVA was also calculated for cumulative plant leaves, with log-transformed number of leaves as the dependent variable, and "treatment" and "location" as independent variables. Similarly, the differences in soil moisture, organic matter, electrical conductivity, and gradients of the plots were compared using General Linear Model in SPSS.

2.5. RESULTS

2.5.1. *Spinifex* seedling Survival

2.5.1.1. Experiment 1

Survival data for the first experiment was first collected on 15th December 2010 (four weeks after planting), at which point a total of 185 seedlings out of an initial 252 survived (73% survival). During the first month (December 2010), survival of seedlings was significantly higher for plants at the bottom of the dune face regardless of treatment ($F_{1, 36}$ = 5.423; *P*= 0.026; Table 2.1; Fig. 2.3), but this did not persist in the subsequent months. An iceplant plot (A4) had lost all the seedlings at its base by January. By February, one plot (C3) from the bare treatment had lost all the seedlings. A bare treatment plot (C4) also had no surviving seedlings at its base by March. Around the same time, B1, a removal plot lost all

its seedlings at the top of the dune. Survival rate declined gradually to 47% by March and remained stable through June. However, survival was consistently higher in the removal plots until July (Fig. 2.4).

Table 2.1: ANOVA table showing effect of treatment and location on *Spinifex* seedling survival for the month of December (one month after planting). Neither treatment nor location (or the interaction of the two factors) had any effect for the months of January through June.

Source	Df	Mean Square	F	Sig.
Treatment	2	.220	1.764	.186
Location	1	.676	5.423	**.026**
Treatment * Location	2	.010	.078	.925
Error	36	.125		
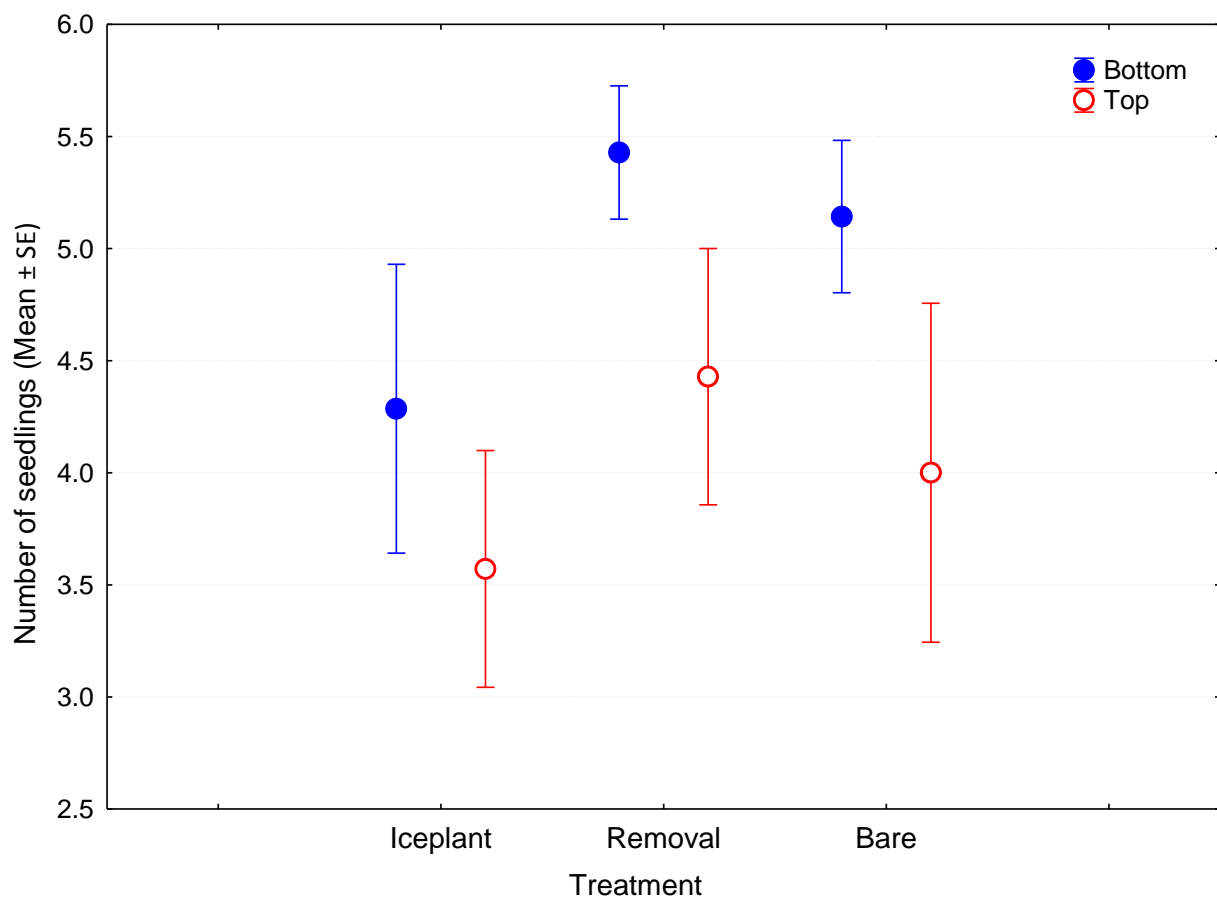
Fig. 2.3: Error plot showing that more *Spinifex* seedlings survived at the bottom of the dune than at the top in Experiment 1 during the month of December 2010.
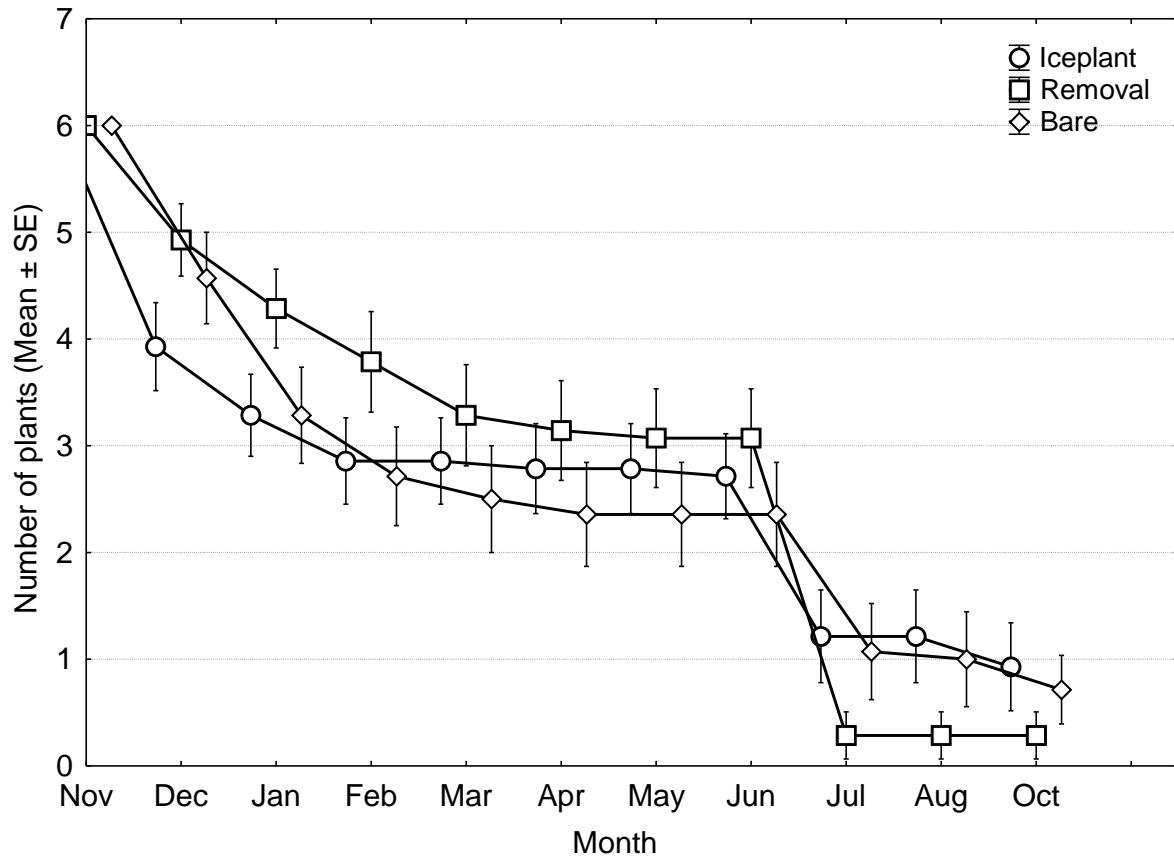
Fig. 2.4: A graph showing that treatment had no significant effect on *Spinifex* seedling survival except after July (the points are staggered).

There was a storm in July and in total only 36 *Spinifex* plants of the 114 from the previous month survived (17 in iceplant, 15 in bare treatment and 4 in the removal treatment) representing a mortality of 68.5% in just a single storm event. Plants worst affected by the storm were those in the removal treatment (Fig. 2.4) and those at the base of the dune (Fig. 2.5). *Spinifex* plants that survived were moved from the upper parts of the dune to just above the high tide mark.

Treatment did not have any effect on plant survival except in July where plants in the removal plots were worst affected by the storm, though statistically not significant ($F_{2, 36}$=3.07; *P*=0.06; Fig. 2.4). On the other hand, plant location had a highly significant effect on

survival during the same month ($F_{1, 36}$=22.323; P<0.001; Table 2.2), which is a consequence of storm disturbance rather than the continuous dynamics of environmental conditions at these parts of the dunes. The highly significant effect of plant location persisted in subsequent months (Table 2.2; Fig. 2.5).

Table 2.2: A summary ANOVA table showing effect of treatment and location on *Spinifex* survival for the months of July 2011 through October 2011. Location had a highly significant effect but treatment and interaction of the two factors did not.

	Treatment			Location		
Month	F	df	Sig.	F	df	Sig
July	3.070	2, 36	.059	22.323	1, 36	**< .001**
August	2.955	2, 36	.065	21.237	1, 36	**< .001**
October	1.286	2, 36	.289	13.569	1, 36	**.001**
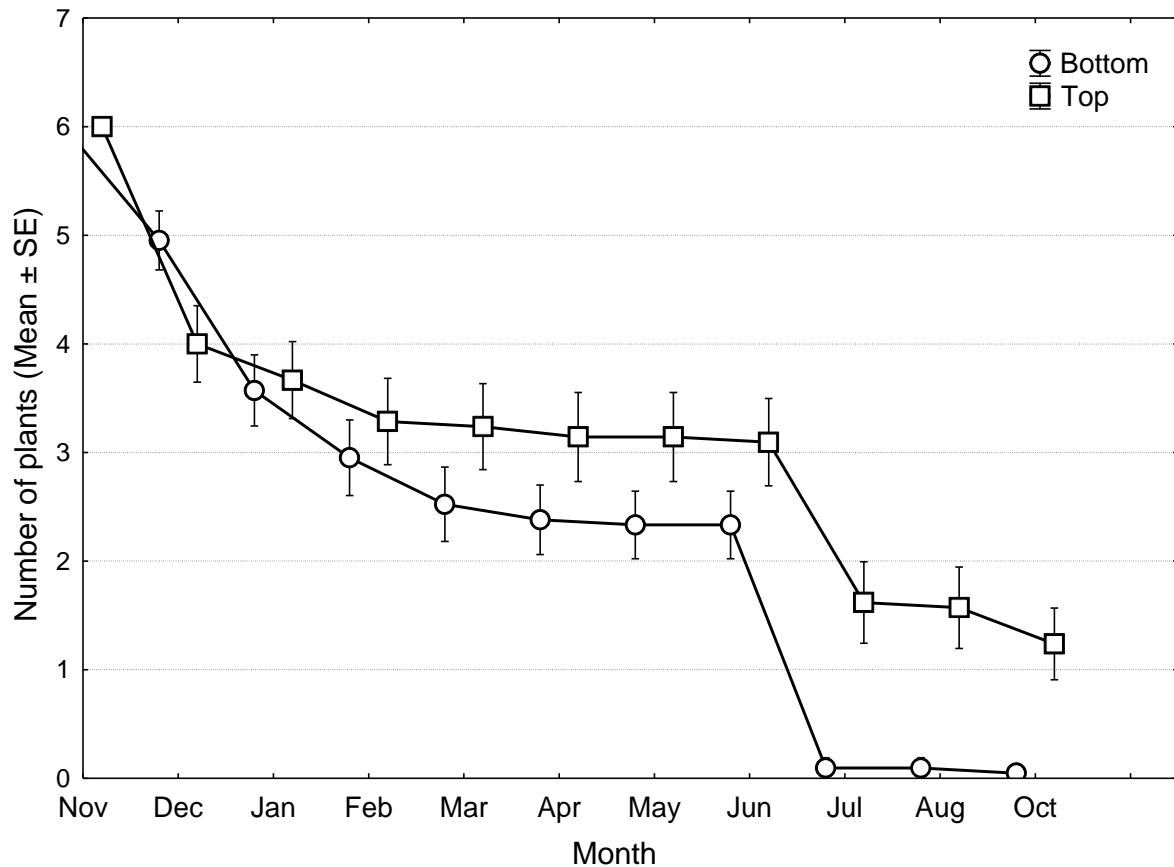
Fig. 2.5: *Spinifex* survival graph (points are staggered) showing that plant location had significant effect from July 2011 through to October 2011 (F $_{1, 36}$=22.323; *P*<0.001)

Spread of *Carpobrotus edulis* into neighbouring removal plots was minimal and no sprouts from buried structures were noted at the removal plots.

2.5.1.2. Herbicide treatments

Survival of seedlings in the modified removal treatment was poor since only 52% of the seedlings survived to July, just a fifth of which were at the bottom of the dunes. By October, total survivorship for this batch of plants had declined further to 34%. Using dead iceplant debris as mulching material did not have any effect on survival of *Spinifex* (Fig. 2.6; Table 2.3). However, location of the *Spinifex* seedlings mattered significantly, with those at the top of the dune surviving much better than those at the bottom (Fig. 2.6; Table 2.3).
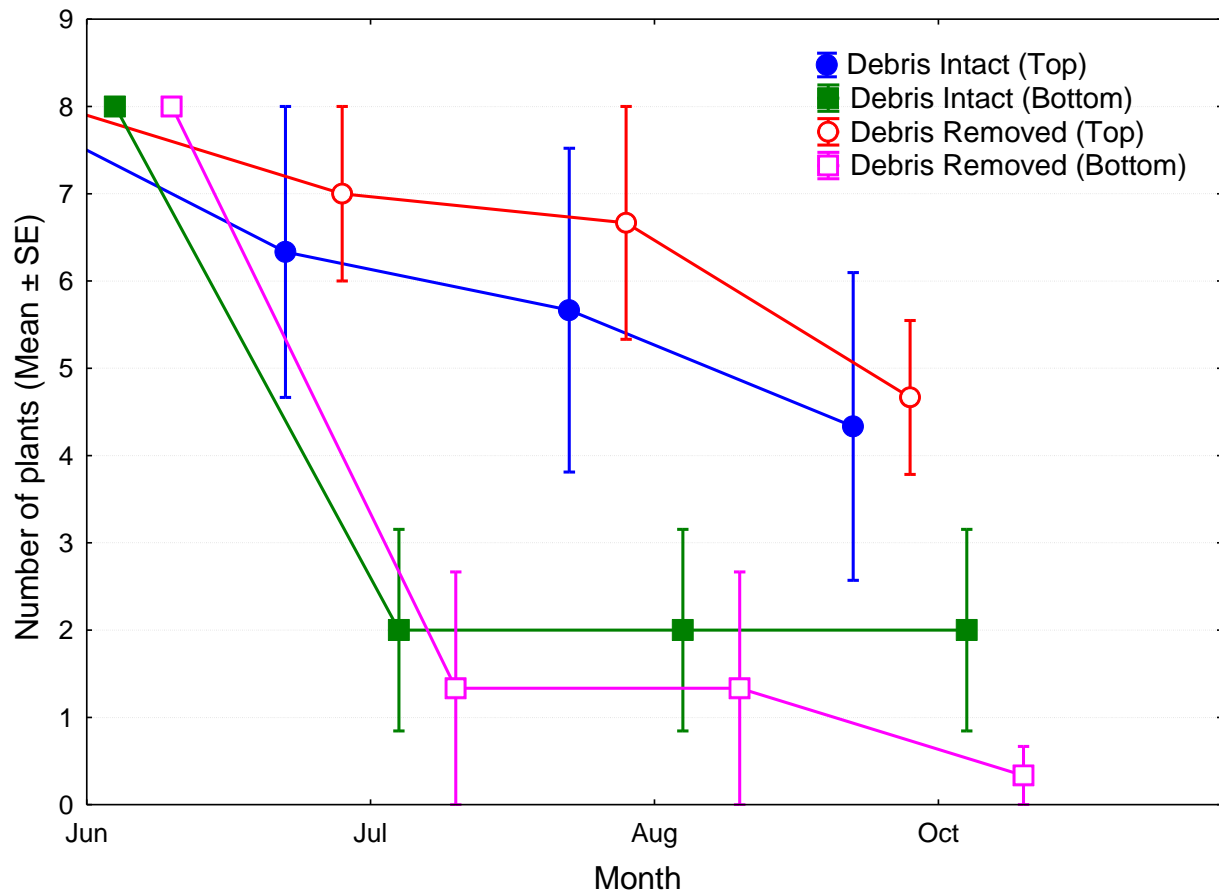
Fig. 2.6: Graph of seedlings in the herbicide treated plots showing that treatment had no significant effect on survival but plant location did (points are staggered).

Table 2.3: ANOVA table showing effect of treatment and location on *Spinifex* seedling survival in the herbicide removal experiment (Location had a significant influence due to storm but treatment or the interaction of the two did not).

	Treatment			Location		
Month	F	Df	Sig.	F	df	Sig
July	.031	1, 8	.864	14.0	1, 8	**.006**
August	.004	1, 8	.952	10.3	1, 8	**.012**
October	.514	1, 8	.494	9.2	1, 8	**.016**

2.5.2. Number of leaves produced by *Spinifex* seedlings

2.5.2.1. Experiment 1

On average, *Spinifex* plants in the removal plots had a higher number of leaves from month to month while the plants in iceplant stands had the lowest (Fig. 2.7). There was a highly significant effect of treatment on the number of leaves from March through to June (all $P < 0.002$; Fig. 2.6; Table 2.4). Plant location however, did not seem to have any effect on the number of leaves for the plants (Fig. 2.8) although plants at the bottom of iceplant plots had significantly higher numbers of leaves than their counterparts at the top of the dune until the condition was reversed by loss of plants in July ($F_{1, 201}=5.392$; $P=0.021$; Fig. 2.9). The interaction between treatment and location did not have any effect on leaf production.

Table 2.4: ANOVA table showing effect of treatment and location on the cumulative number of leaves produced by plants in experiment 1 from March 2011 through October 2011 (treatment mostly had a highly significant effect but location and interaction of the two factors did not).

		Treatment			Location		
Month	N	F	df	Sig.	F	df	Sig
March	115	6.537	2, 109	**.002**	.331	1, 109	.566
April	115	8.401	2, 109	**< .001**	.327	1, 109	.569
May	115	9.434	2, 109	**< .001**	.077	1, 109	.783
June	115	8.157	2, 109	**< .001**	1.011	1, 109	.317
July	36	4.835	2, 32	**.015**	.722	1, 32	.402
August	35	4.945	2, 31	**.014**	.005	1, 31	.945
October	27	3.997	2, 23	**.032**	.010	1, 23	.920
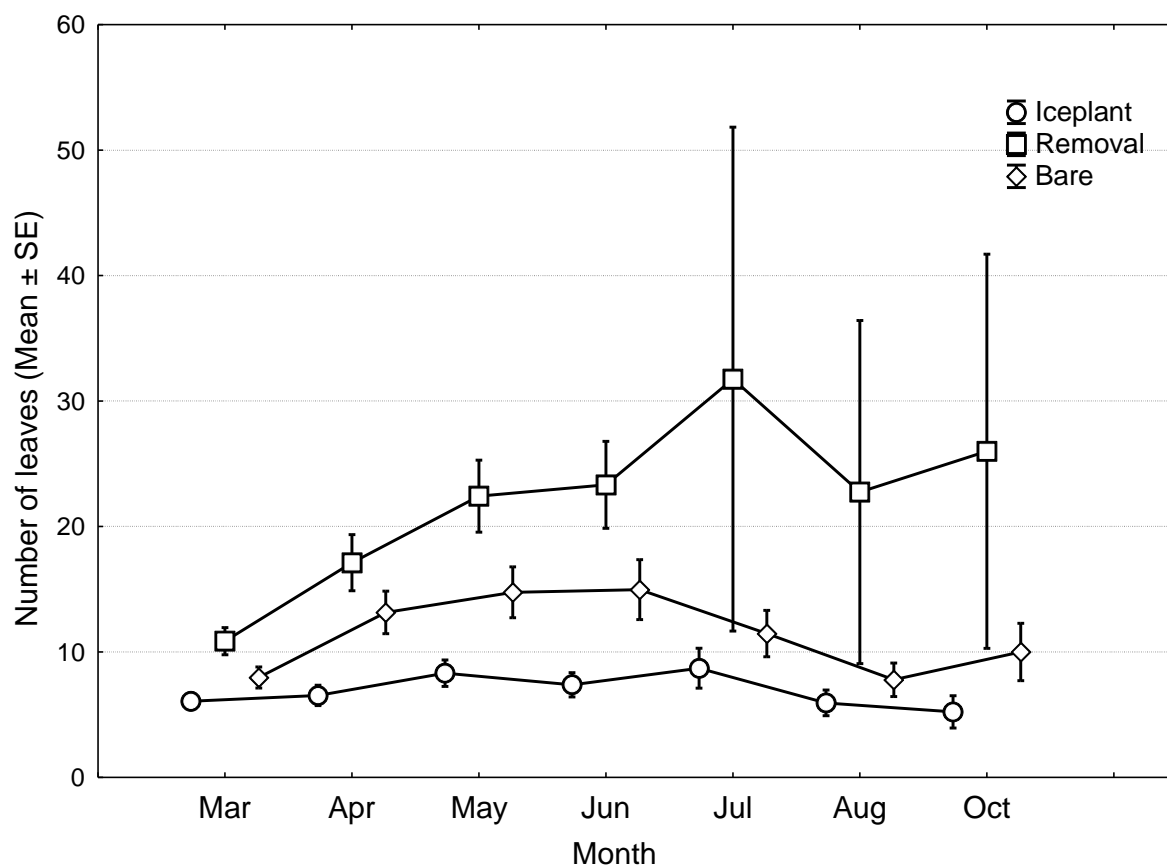
Fig. 2.7: Mean cumulative number of leaves produced by *Spinifex* plants in experiment 1 in relation to treatment over time (points are staggered). Treatment had a highly significant effect on number of leaves produced from March through to June (all *P* < 0.002).
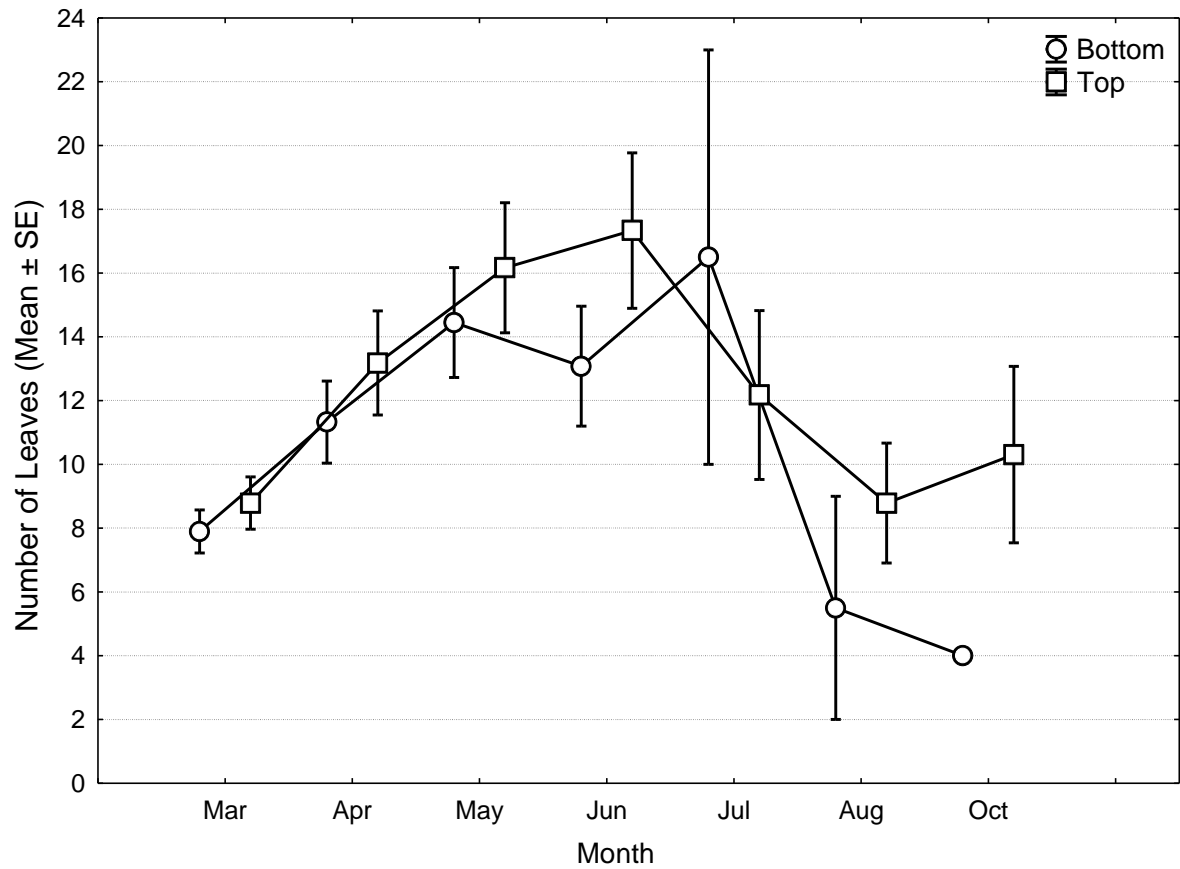
Fig. 2.8: Mean cumulative number of leaves per plant in Experiment 1 in relation to plant location over time (points are staggered). Plant location had no effect on number of leaves produced. The decline in leaf number from July to October was partially caused by dieback of surviving plants, but also from the loss of large plants to storm events.
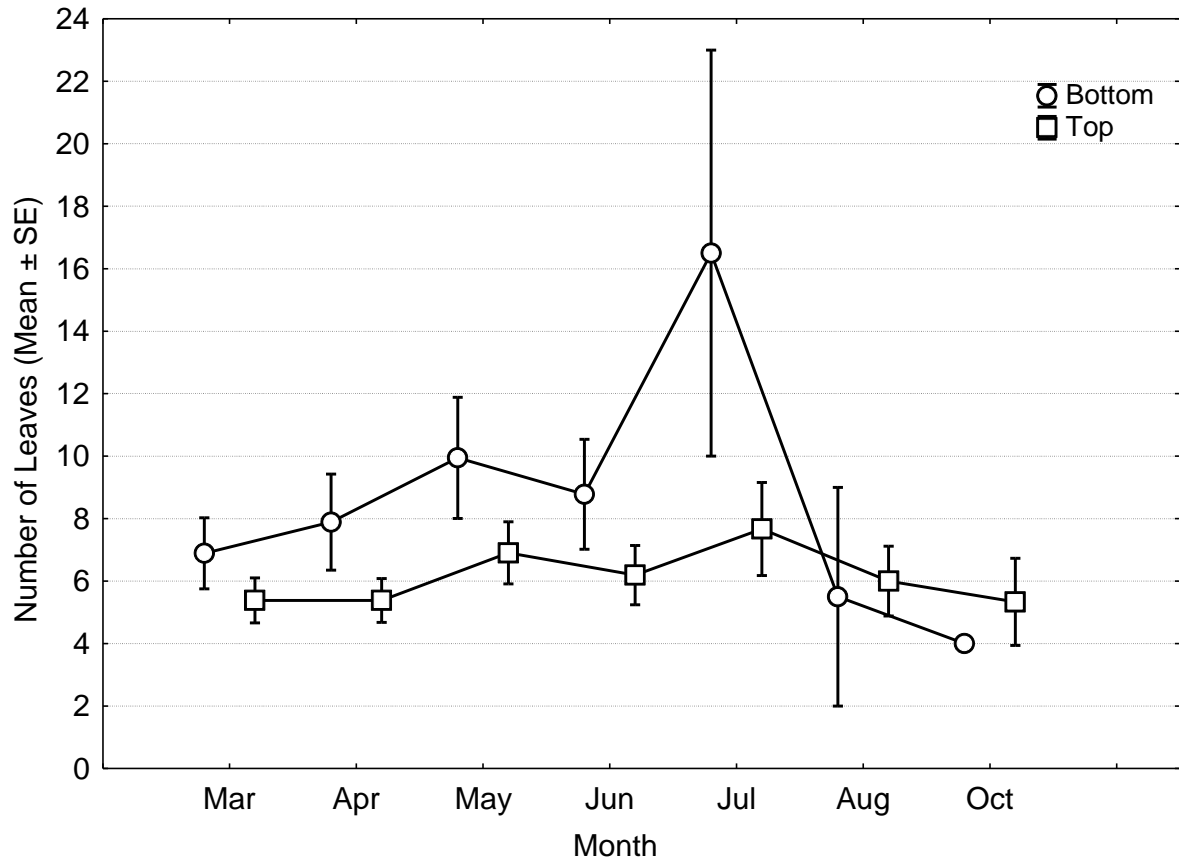
Fig. 2.9: Average number of leaves produced by each *Spinifex* plant in the Iceplant treatment of Experiment 1 in relation to plant location over time (points are staggered).

2.5.2.2. Experiment 2 (herbicide removal)

Leaf production of the seedlings in the herbicide-treated experiment was not affected by either treatment or location except in August where the interactive effect of treatment and location was highly significant ($F_{1, 43}$=9.864; *P*=0.003; Fig. 2.10; Table 2.5). This was because all plants except the ones at the top of "debris intact" plots lost a lot of leaves between July and August (Fig. 2.10).

Table 2.5: ANOVA table showing a highly significant interactive effect of treatment and location on survival of *Spinifex* plants in experiment 2 (herbicide removal) during August.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	4.299	1	4.299	1.390	.245
Location	5.519	1	5.519	1.785	.189
Treatment * Location	30.493	1	30.493	9.864	**.003**
Error	132.932	43	3.091		
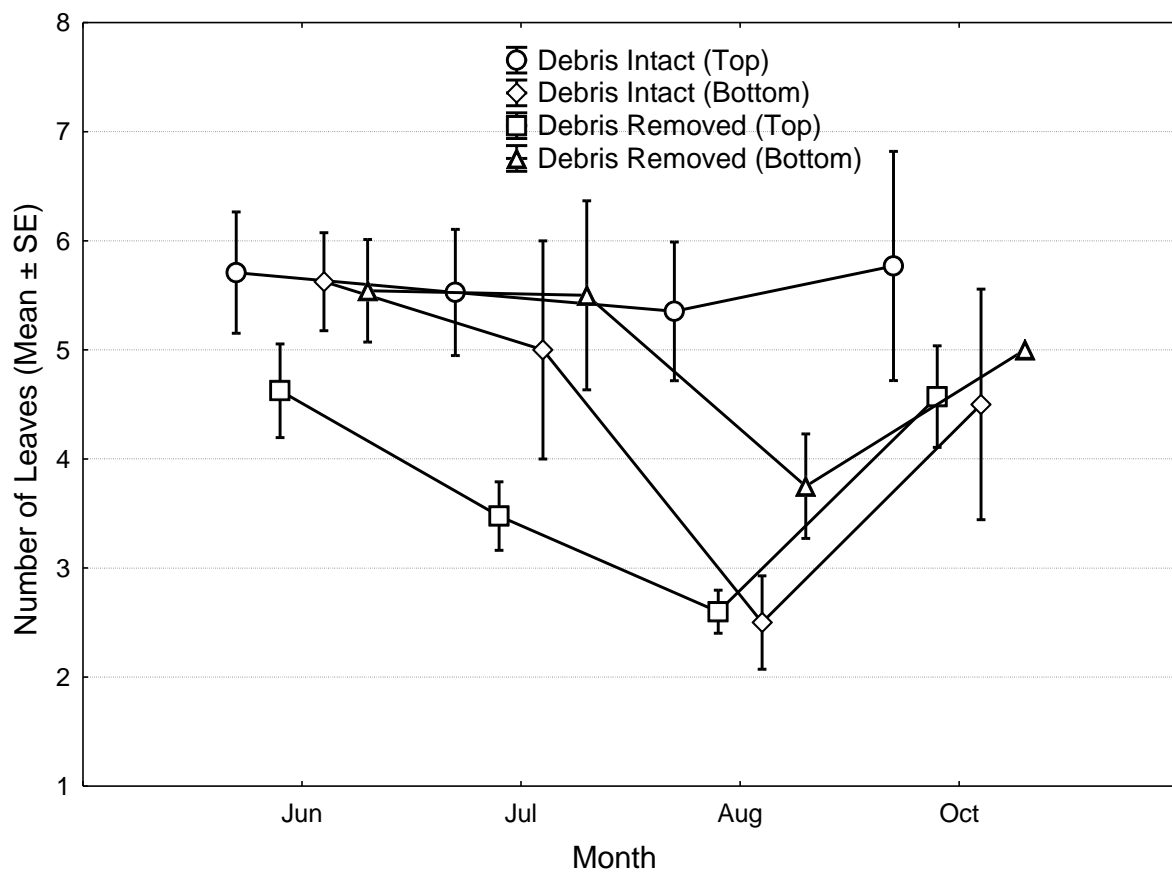
Fig. 2.10: Graph showing leaf production of *Spinifex* in the herbicide-treated plots (points are staggered). Plant location and treatment only had an effect in August.

2.5.3. Physical parameters

2.5.3.1. Slope gradient

Gradients in bare plots were significantly gentler (32° – 38°) than the removal (32° – 52°) and iceplant plots (38° - 50°) (Fig. 2.11).
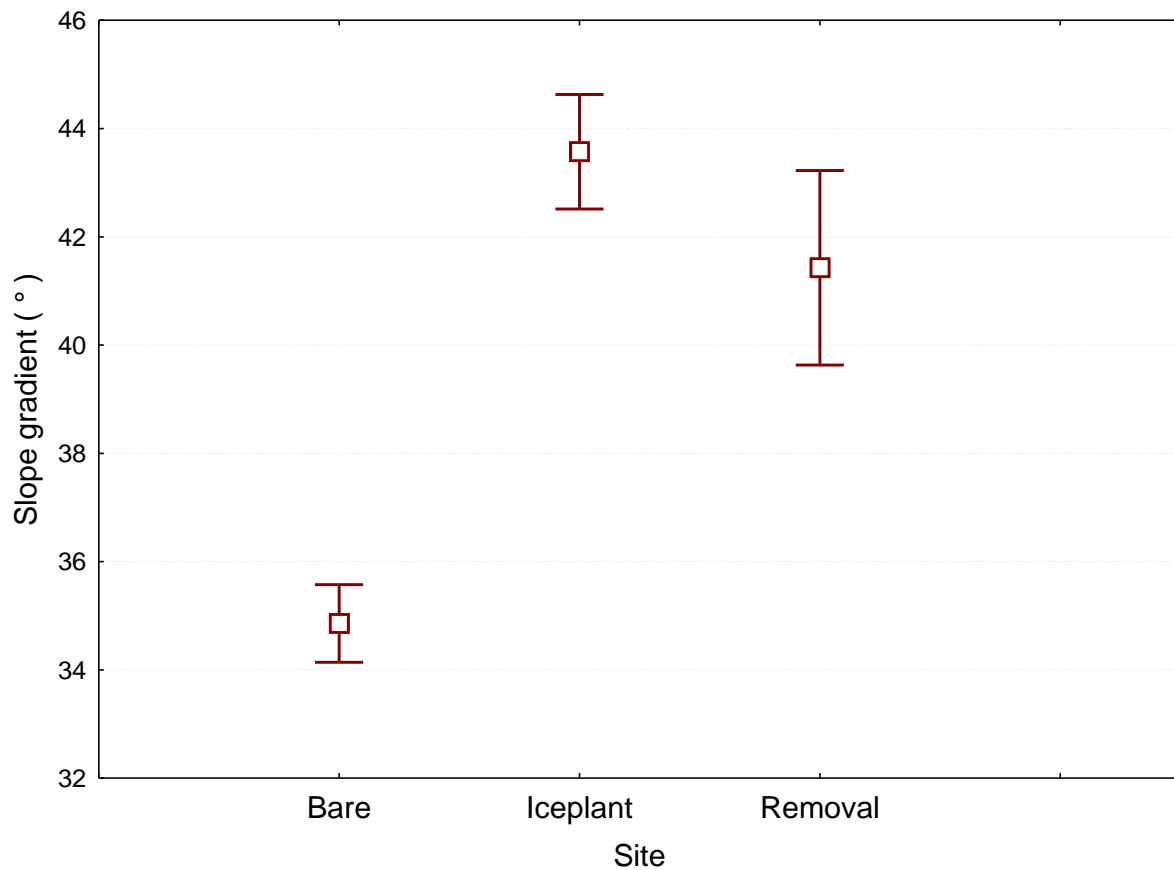
Fig. 2.11: Slope gradients (mean ± SE) of the plots of the different treatments in experiment 1 (Bare plots were significantly gentler).

2.5.3.2. Soil moisture and organic matter

There was a significantly higher amount of organic matter in the soil samples from the iceplant plots than in bare plots but not between locations within a site (Table 2.6; Fig. 2.12; $F_{1, 16}$=21.127; $P<0.001$). Soil moisture content did not differ either between sites or

locations (Fig. 2.13) although the difference approaches significance for bare plots ($F_{1,\ 15.62}$=4.53; P=0.066).

Table 2.6: Soil organic matter difference between bare and iceplant sites

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Site	.238	1	.238	21.127	**< .000**
Location	.002	1	.002	.182	.676
Site * Location	.015	1	.015	1.291	.273
Error	.180	16	.011		
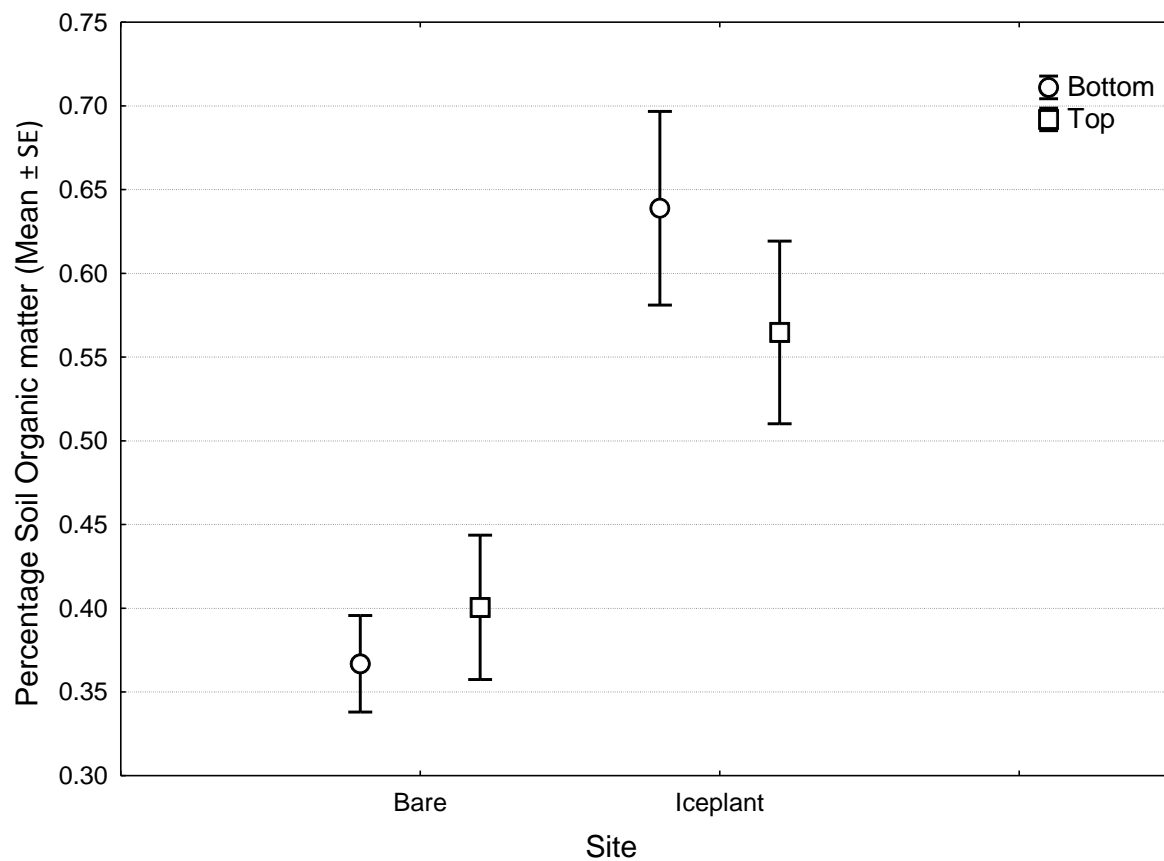
Fig. 2.12: Percentage soil organic matter at the bottom and top of the dunes of the iceplant and bare sites
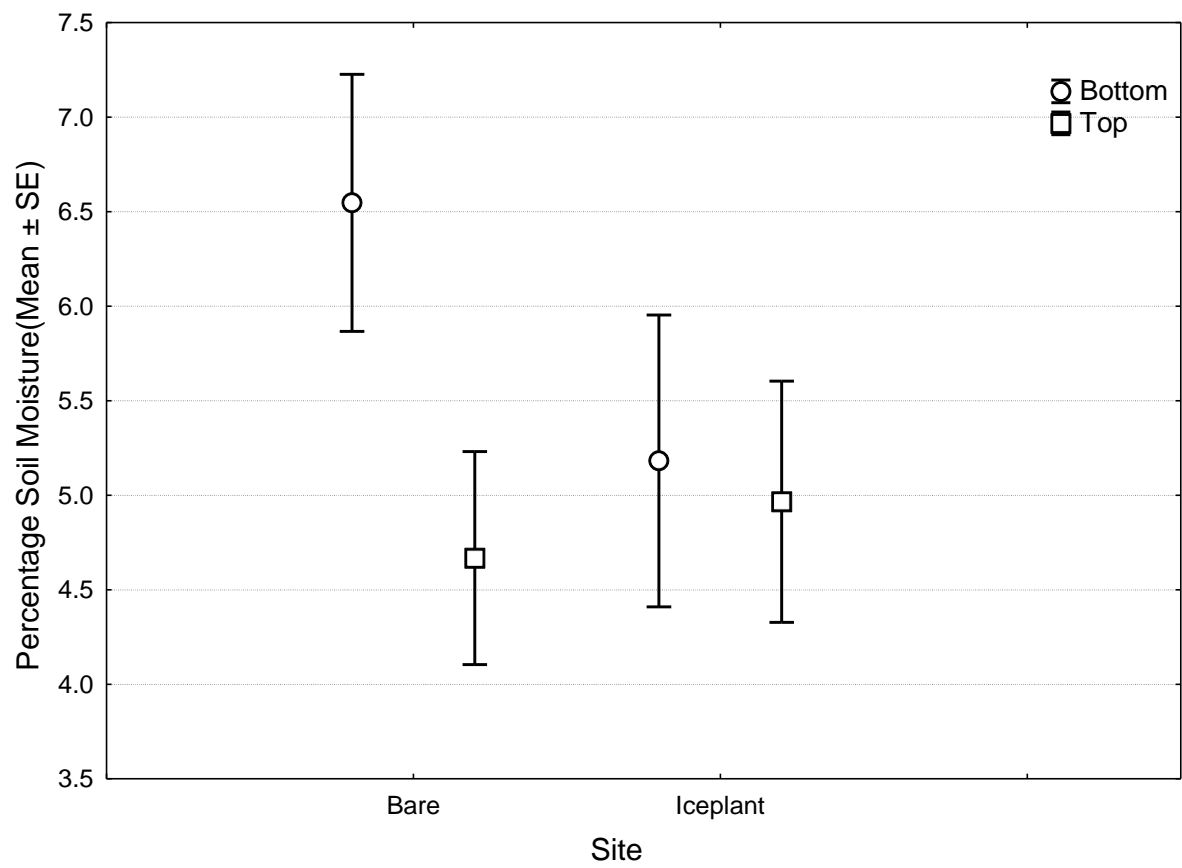
Fig. 2.13: Percentage soil moisture content of soil obtained from the two sites and locations of the dunes.

2.5.3.3. Electrical conductivity

Soil samples obtained from sites under the canopies of *C. edulis* were significantly more saline than the samples from sites in the bare plots ($F_{1, 16}$= 31.7; *P* < 0.001; Fig. 2.14). Although there was a slight trend in salinity in soil samples from the bottom of the dunes when compared with those from the top, this was not statistically significant.
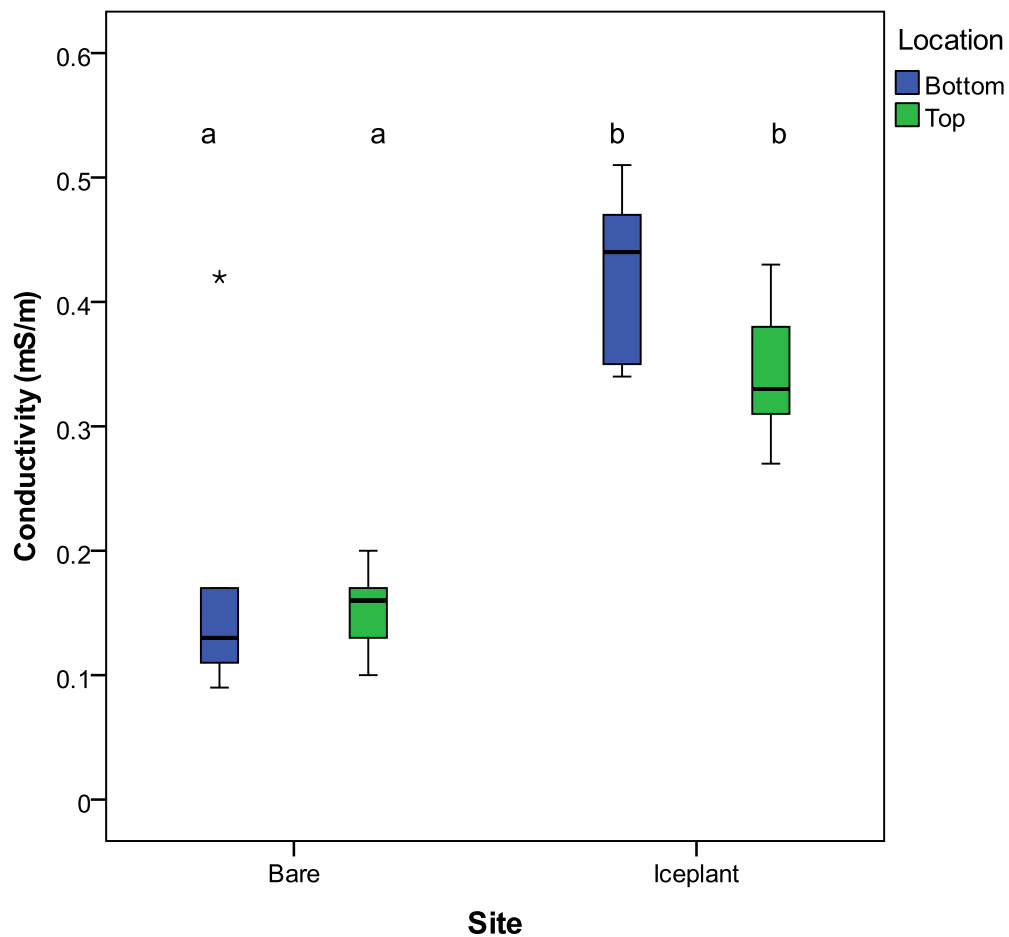
 Fig. 2.14: Electrical conductivity of soil samples obtained from the top and bottom of the dunes at the iceplant and bare sites.

2.6. DISCUSSION

2.6.1. Confounding factors to *Spinifex* seedling survival and growth

There was generally a low *Spinifex* seedling survival rate regardless of treatment. This was not surprising because it has been reported that species capable of vegetative propagation, such as *Spinifex sericeus,* often exhibit low levels of seedling survival (Cook 1979; Maze and Whalley 1992a). During the first month (December 2010), seedling survival was significantly higher at the bottom of the dune, regardless of the treatment, showing that *Carpobrotus edulis* did not have any influence on initial *Spinifex* survival.

The period during planting from November 2010 to early January 2011, was characterised by a dry spell, requiring us to spray the plots with about 500L of water two weeks after planting. Since soil moisture is one of the most limiting factors in sand dunes (Lichter 1998), drought may have had an influence on the overall initial survival. The higher survival of seedlings at the foot of the dune during the initial periods may be a result of more soil moisture due to inundation during high tides and wave splashes. Although this water is not expected to be readily available because of salinity, it has been observed from electrical conductivity measurements of macerated *Spinifex* tissue that it picks up some salt, either through leaves or by roots (Maze and Whalley 1992). Uptake of salt helps plants in water absorption or retention, enabling them to survive better. Therefore the ability of S*pinifex* to utilise salty water may have been responsible for better survivorship at the foot of the dune during the month of December 2010. Death of plants in plots A4, B1, C3 and C4 between January and March, can be attributed to drought, storm erosion and trampling by humans.

During planting it was noted that the soil under the iceplant and in the removal plots was moister than the soil in bare plots, though this was not empirically determined at the time. However, in subsequent months, soil samples collected from under the canopy of iceplant and in bare spaces did not show any significant difference in moisture content (Fig. 2.13). Variability in soil moisture changes through time (Wilson and Sykes 1999), and these results, do not rule out existence of a moisture gradient. Soil moisture content did not

differ between the bottom and the top of the dunes either, in agreement with Wilson and Sykes (1999), although other studies (e.g. Gooding 1947) suggest otherwise.

Planting technique is an important determinant of seedling survival, especially in sand dunes where the substrate is unstable. It is recommended that planting depth for *Spinifex* seedlings should be up to the lower third of crown (Auckland Regional Council) to guard against exposure of the root region by erosion. However, our seedlings were planted at the same level as they were in their root trainer sleeves. Therefore, insufficient planting depth coupled with storm erosion may also have had an impact on the initial overall survival since by January some seedlings had about 5cm of their root trainer soil exposed due to erosion. This root exposure was more pronounced for plants in the removal and bare treatments than in the iceplant treatment since surface erosion of sand is greater where there is a lack of above-ground vegetation.

Plant location on the dune and the presence/absence of dead debris interacted in their effect on *Spinifex* leaf production in the herbicide treated plots such that plants in the "debris intact" plots at the top of the dune put on the greatest number of leaves, although this effect was only significant in August ($P = 0.003$). The fact that location of the plants did not have any effect on their performance means that the 2-4m space between plants at the bottom and the top of the dune was not enough to produce a gradient to which plants could respond differentially. This is confirmed by the lack of difference in soil moisture and organic matter content, as well as in electrical conductivity between the two sampling locations separated by 5-8m (Fig. 2.12 – 2.14).

The factor that most significantly affected *Spinifex* seedling survival was storm disturbance that decimated plants nearer to high tide mark in the second week of July. The storm caused slumping of the dunes, creating a near-vertical scarp which destabilised even the plants that survived.

2.6.2. Positive effects of *C. edulis* on *Spinifex* seedlings

It is generally argued that in harsh environments like the sand dunes, facilitative processes are more important than competition to community structure (Bertness and Shumway 1993; Didham, Tylianakis et al. 2007). *Spinifex* plants in the removal and bare treatments of Experiment 1 had their leaves dying back from the tips, probably as a result of salt spray (Maze and Whalley 1992; Wilson and Sykes 1999). Abrasive leaf damage by sand blasting was also quite evident in the plants growing in absence of iceplant. Yura and Ogura (2006) observed that sand blasting followed by salt spray caused serious injury to *Imperata cylindrica* and *Miscanthus sinensis*. The leaves of *Spinifex* plants in the iceplant treatment, on the other hand, appeared intact, suggesting a buffering by iceplant against sand blasting and excessive salt spray. Normally, a stress-tolerant neighbour can ameliorate the harsh environmental conditions in its immediate vicinity, thereby providing favorable sites where other species can then succeed (King 2008). Subjective observations of the plants in the different treatments also showed that plants within the iceplant stands had leaves that appeared darker green than those in the removal and bare treatments. The positive effect of an adult plant on seedlings of another species is called the "nurse-plant" effect (Bertness and Callaway 1994; Padilla and Pugnaire 2006) and has been found in harsh habitats, suggesting that habitat amelioration by neighbours is important for positive recruitment events (Bertness and Callaway 1994). Therefore, the expectation that facilitation would be the dominant effect of extant adult plants on seedlings of other species at the fore dune seems plausible, at least in buffering against abrasive damage by sand blasting and necrosis, due to salt spray. Moreover, plants at the bottom of the iceplant plots (presumed to be the harsher section) had significantly higher numbers of leaves than their counterparts at the top of the dune (Fig. 2.9; $P = 0.021$). In this study, we have determined that soil salinity was higher under the canopy of the iceplant, which may cause the *Spinifex* seedlings internal toxicity or some difficulty in absorbing water (Wilson and Sykes 1999), rendering iceplant plots harsher. In contrast to Wilson and Sykes (1999) however, I did not find a significant difference in salinity of soil samples obtained from the bottom and the top of the dune.

Despite being statistically insignificant, *Spinifex* survival was higher in the manual removal plots until July. Soil sample analysis here showed that there was a higher percentage of organic matter under the canopy of iceplant, which may have improved soil micro-conditions to the benefit of the *Spinifex* seedlings. In addition, seedlings in removal plots consistently had significantly higher numbers of leaves (Fig. 2.7). Leaf litter of some plants has been observed to improve the structure of the soil, and increase its cation exchange capacity and water holding capacity (Hunter and Aarssen 1988).

Spinifex plants within iceplant plots best survived the July storm because of the soil-binding nature of the iceplant's extensive fibrous root system. Sand-binding species are said to stabilize the substrate, contributing to eventual colonization by successive sand dune plants (Lichter 2000). However, there is no evidence that *Carpobrotus* spp. lead to a succession resulting in dominance by native species.

2.6.3. Negative effects of *C. edulis* on *Spinifex* seedlings

The lessening abundance of many native species around the world has been considered to be the result of the better "competitive ability" of exotics (D'Antonio and Mahall 1991). A review of competitive interactions between native and exotic plants suggested that the spread and establishment of exotic species is related to their ability to competitively suppress resident species (Levine, Vilà et al. 2003). In the current study, there was a highly significant difference in *Spinifex* leaf production from March through June (Fig. 2.6; Table 2.4) with plants in the iceplant treatment having significantly lower numbers of leaves, suggesting that the presence of iceplant suppresses growth of *Spinifex sericeus*. In addition, *Spinifex* seedlings within the iceplant plots appeared more slender. *Carpobrotus edulis* has been reported to compete aggressively (D'Antonio 1990; Albert, D'Antonio et al. 1997; Vilà and D'Antonio 1998) and suppress the growth and establishment of other plants while achieving high rates of space colonization (D'Antonio and Mahall 1991; Suehs, Affre et al. 2004; Vilà, Tessier et al. 2006). Interspecific competition, being an important determinant of structure and dynamics of plant communities (Aerts 1999), has been

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suggested as a mechanism for the success of invasive plant species (Hierro and Callaway 2003) such as iceplant. It was reported that removal of *C. edulis* from around individuals of *Haplopappus ericoides* shrub species resulted in increased predawn xylem pressure, suggesting that the surrounding *C. edulis* was utilizing water that would otherwise have been available to the shrubs (D'Antonio and Mahall 1991). *Carpobrotus edulis* has a very dense fibrous root system concentrated in the upper 50 cm of the soil that makes it very effective at interfering with water uptake by other shallowly rooted species (D'Antonio and Mahall 1991).

Spinifex plants in the removal plots were worst affected by the July storm because they were located on a steeper unstable slope (Fig. 2.11) built by sand accretion activity of the previously extant iceplant. The disadvantage of iceplant for dune integrity against such a heavy storm is that cracks develop on the sand surface as a result of storm undercutting, leading to eventual slumping of the dune ridge (Carter, Hesp et al. 1990). This slumping led to the loss of almost all *Spinifex* plants at the foot of the dune (Fig. 2.5; Table 2.2).

Carpobrotus edulis is reported to indirectly interact with native species by altering soil chemistry (Conser and Connor 2009), causing changes in soil pH and nutrient regimes (Bartomeus and Vilà 2009). It has been suggested to have residual effects on the soil that inhibit reestablishment of native plant species after it has been removed (Conser and Connor 2009). However, *Spinifex* in our removal plots (where iceplant had been present) produced a significantly higher number of leaves than plants in the other treatments. In another removal study, *Haplopappus ericoides* shrubs were shown to have had a marked increase in canopy area after removal of *C. edulis* (D'Antonio and Mahall 1991). While this improved response may be attributed to elimination of competition, plants in the removal plots actually performed better that those in bare plots where there was no preexisting iceplant. This suggests that *C. edulis* did not have a net allelopathic legacy but may have actually improved soil micro-conditions. It has been put forward that for allelopathic chemicals to be important in structuring a plant community they must have had a significant resident time (Choesin and Boerner 1991). However, the residence time of iceplant at the field site is unknown and it may yet be too early for it to be able to significantly alter soil chemistry to the detriment of succeeding plants. In addition, biotic components of

ecosystem such as herbivores, competitors, pathogens and decomposers, (Inderjit, Wardle et al. 2011) as well as seasons (Barto and Cipollini 2009) can alter concentrations of allelochemicals. Response of plants to inhibitory exudates of neighbours is also said to be species-specific (Gómez-Aparicio and Canham 2008). Since we did not investigate these confounding factors we cannot claim that *C. edulis* is not allelopathic, but we can say that *Spinifex* plantings do not seem to suffer from allelopathic legacies of iceplant.

2.6.4. Soil seed bank legacy

Assessment of individual removal plots during each monthly visit over a period of one year did not reveal any iceplant seedling suggesting that in the short-term, buried seed bank is not a major route for its recolonization after eradication. However, soil cores were not analysed for presence or absence of seed bank.

2.7. Conclusion and management recommendations

As far as seedling survivorship is concerned, we conclude that low soil moisture, storm-induced erosion, steep slope and insufficient planting depth were the influential factors affecting *Spinifex* survival rather than the presumed negative effects of iceplant. In fact, the presence of iceplant ameliorated some of these negative effects. *Carpobrotus edulis* is a stabilizer and improved soil moisture and organic matter content for other plants that also provided buffering against salt spray, as evidenced by less leaf die-back of *Spinifex* within its stand. Additionally, *C. edulis* provided protection against abrasive leaf damage by sand-blasting, unlike in other treatments where plants had their leaves severely damaged.

On the other hand, *C. edulis* had a strong suppressing effect on biomass production, as shown by lower numbers of leaves and slenderer stems of *Spinifex* seedlings growing within its stands, and the long-term outcome may have been a failure of these *Spinifex* plants to thrive in the presence of iceplant. There was no evidence of allelopathy since plants in the removal treatment showed the best performance among the three treatments. If *C. edulis* is a highly competitive and allelopathic species, *Spinifex* plants in bare plots in the

absence of competition and the inhibitory legacy, would have shown the best performance. However, this was not the case.

Using iceplant as a mulching material did not, in general, result in better survival or growth performance of *Spinifex* seedlings.

Iceplant has been shown here to create steep slopes, despite its diminutive height which has been implicated in its unsuitability for sand accretion (Bergin, Miller et al. 2007). This portends poorly for the physical integrity of dunes in the face of storm disturbances, especially along high energy coasts.

Because of the constant storm disturbance along this coast, succession by other plants and establishment of pioneer seedlings is made difficult, and it is not advisable to remove the iceplant unless the dune morphology is mechanically reshaped to withstand wave actions. *Spinifex* seedlings may establish successfully at this field site if planted in winter on the upper parts of the dune face, away from wave impact. At other sites where moisture, not storm disturbance, is the main limiting factor, planting *Spinifex* closer to the foot of the dune, where inundation is more likely, may enhance seedling survivorship.

The combination of increased survival of *Spinifex* against storms, but lower growth in the presence of iceplant, suggests that an intermediate strategy for planting of *Spinifex* may be appropriate. As a result, I suggest that *Spinifex* could be interplanted into existing stands of iceplant if, at the time of planting, the iceplant is reduced to 25-50% of its original cover. This should still provide some nurse effect to aid the establishment of *Spinifex*, but also allow enough space to delay negative competitive effects. In the longer-term, we would like to see the *Spinifex* replace the iceplant. This may not occur through "natural" competition. Hence, to achieve the conversion, a second round of manual removal of iceplant may be necessary after 1-2 years, once the *Spinifex* has established.

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Chapter three

3. Substrate preference and breeding systems in introduced *Carpobrotus* spp., native *Disphyma australe* and their hybrid.

Fig. 3.1. Photos showing flower colours of the different iceplant taxa found in New Zealand

3.1. Abstract

Biological invasion by non-native plant species has often been cited as a cause of native biodiversity loss. Some exotic plant species tend to hybridise with related native taxa, altering genetic diversity while a majority grow aggressively to competitively displace the pre-existing native flora. In this study, I compared the growth rates of the most widely distributed iceplant taxa in New Zealand in different substrates and the breeding systems of the exotic taxa. The exotic *Carpobrotus* spp. put on greater dry matter content than the native *Disphyma australe* and the *Carpobrotus*-x-*Disphyma* hybrid. The hybrid displayed a fast vegetative growth rate whereas *D. australe* allocated relatively more biomass to the roots than the shoot. *Carpobrotus chilensis* put on relatively lower biomass in gravel but the other taxa did equally well across the different substrate types. Both *Carpobrotus* spp. are self compatible and highly capable of intrageneric and intergeneric hybridisation.

3.2. Introduction

Increased worldwide human travel and trade have moved species beyond their native ranges both intentionally and inadvertently (Mack, Simberloff et al. 2000), breaking down the biogeographic barriers that have shaped and maintained the major floral and faunal regions of the Earth (Vitousek, D'Antonio et al. 1997). Many of these species become established and proliferate in their new habitat (Vitousek, D'Antonio et al. 1997). When an introduced species establishes new self-perpetuating populations in the wild and becomes incorporated within the resident flora it is said to have naturalised (Richardson, Allsopp et al. 2000). Naturalised species that proliferate, spread and endure in ecosystems in which they were formerly absent to the detriment of the pre-existing native ecosystem are considered invasive (Mack, Simberloff et al. 2000). (Vilà, Siamantziouras et al. 2008) further suggested that a species should be considered invasive when it has spread more than 6 m away from the input focus in less than three years, and if its reproduction is vegetative through rhizomes or stolons. Naturalised species that have become invasive constitute only around 1% of all exotic introductions (Williamson 1996; Richardson and Pyšek 2006) but they can still

cause substantial modifications to indigenous biodiversity and ecosystem functions (Williams and West 2000). In due course, invasive species lead to a progressive and often irreparable homogenization of plant communities, with a single species dominating and influencing the processes of the whole ecosystem (Vitousek, D'Antonio et al. 1997; Mack, Simberloff et al. 2000). Nowadays, biological invasions by non-native plant species are considered to be among the most important triggers of biodiversity loss and one of the main drivers of environmental modifications (Sala, Chapin et al. 2000) at both the global and local scales (Zedda, Cogoni et al. 2010). Globally, biological invasions have been considered the second most important cause of change in the composition, structure, and functioning of natural ecosystems, after habitat destruction (Walther, Post et al. 2002; Simberloff, Parker et al. 2005; Thuiller, Richardson et al. 2005).

Many invasive plant species were deliberately introduced, often for their aesthetic value and sale as garden ornamentals (Carboni, Santoro et al. 2010). In New Zealand for example, almost all exotic plant species that have naturalised have been purposely introduced by people (Jay, Morad et al. 2003). The New Zealand Department of Conservation considers more than 240 naturalised exotic plant species as weeds that actually or potentially jeopardise the survival of nationally rare or endangered native plants (Green, 2000 as cited by Jay, Morad et al. 2003). Alien plant species are largely restricted to human dominated habitats (Affre, Suehs et al. 2010) as a result of both higher disturbance and propagule pressure (Chytrý, Pyšek et al. 2009). This is particularly pertinent for coastal dune communities, since human pressure on coastal zones with sandy interfaces around the world has progressively increased in the last 50 years (Curr, Koh et al. 2000). Although extreme abiotic factors such as low soil fertility, sand burial, and salt spray may represent a strong filtering mechanism on which species can survive in sand dunes (Wilson and Sykes 1999; Carboni, Santoro et al. 2010), a relatively large number of alien species have managed to successfully invade. This is because costal dune plant communities are very dynamic ecosystems, with many open spaces that allow the quick settlement of some exotic species with high colonizing ability (Campos, Herrera et al. 2004). Disturbances may take out competitively superior native species, eradicate natural enemies that curb invasion, and/or alter the resource base directly, in favour of non-native species (D'Antonio 1993).

In New Zealand, South African iceplant (*Carpobrotus edulis*) is among a highly invasive group of alien plants that were originally introduced as ornamentals (Weber 2003) but have gone on to naturally produce populations in the wild. They were later used for coastal sand dune stabilisation, because of their rapid growth rates and ability to cope with harsh dune environment (D'Antonio, Odion et al. 1993). Furthermore, they regularly form monoculture stands, suggesting a displacement of the pre-existing native flora. Other ecological impacts of iceplant include aggressive competition with native species (D'Antonio and Mahall 1991; Sheppard, Shaw et al. 2006), destabilization of native dune communities (Campos, Herrera et al. 2004; Acosta, Carranza et al. 2008), modification of soil pH (Vilà, Tessier et al. 2006) and likely the alteration of ecosystem function in terms of successional dynamics (Carranza, Carboni et al. 2010). Consequently, *C. edulis* is considered among the worst invasive alien species threatening biodiversity in Europe (Sheppard, Shaw et al. 2006) and is included in the list of the most invasive alien plants in the World (Weber 2003). Owing to the large extent of invaded areas in coastal communities, it is considered one of the most costly invasive species in Spain (Andreu, Manzano-Piedras et al. 2010). However, invasion success and impacts of *C. edulis* are reported to be habitat and context specific processes (D'Antonio 1993; Maltez-Mouro, Maestre et al. 2010). In New Zealand, as of October 2006, *C. edulis* and its hybrids are classified as unwanted organisms (Biosecurity New Zealand 2008), yet there is a paucity of information as to their impacts locally. Clonal growth form, flexible mating systems, and ability to hybridise with related taxa may be among the most important characteristics of the exotic iceplant species that contribute to their invasion success.

Hybridisation between native and non-native species as a consequence of species introduction (Abbott 1992) may result in invasive hybrid morphotypes that, in turn, have negative implications for the conservation of native species assemblages (Vilà, Weber et al. 1998). In the invasive ranges of the Aizoaceae, hybridizations have been recorded between *C. edulis* and different species: native *C. chilensis* (Molina) N.E. Br. in California (Albert, D'Antonio et al. 1997; Gallagher, Schierenbeck et al. 1997), native *C. virescens* (Haw.) Schwantes in Australia (Blake 1969 as cited by Verlaque, Affre et al. 2011), native *D. australe* in New Zealand (Chinnock 1972), and, introduced *C. acinaciformis* in France (Suehs, Affre et

al. 2004). While most of these hybridisations are intrageneric, it is only in New Zealand that it is intergeneric. It has been suggested that interbreeding increases the threat of extinction for a number of species due to hybridization introgression (Levin, Francisco-Ortega et al. 1996; Rhymer and Simberloff 1996). Hybrids can exhibit novel characters that neither parental species expresses (Rieseberg, Ellstrand et al. 1993; Rieseberg 1995) and may have advantages over parental individuals in certain habitats (Vilà and D'Antonio 1998). Novel gene combinations can extend the gene pool of a species and its ecological range, including the spread into new habitats (Lewontin and Birch 1966). A classic example of this is where seed-sterile *Spartina* X*townsendii*, an intrageneric hybrid of native *S. maritima* and exotic *S. alterniflora* in southern England, formed a fertile *S. anglica* by chromosome doubling (Ayres and Strong 2001). Hybridisation has the potential to dilute the gene pool of native species and create highly aggressive genotypes that may be undesirable in terms of management for native species (Ellstrand 1992; Rhymer and Simberloff 1996). It is believed to be a factor that allows the spread of plant species into areas they did not previously occupy (Abbott 1992) and hybrid vigour for vegetative growth may be one of the reasons for displacement of parental morphotypes or native species (Vilà and D'Antonio 1998). In the case of *S. anglica*, which has now become predominant in the estuarine salt marshes of the British Isles (Thompson 1991), neither of its parental species is present at its presumed site of origin in Hythe, England, and its native parent *S. maritima* is considered extremely rare or extinct in most of its former British range (Raybould, Gray et al. 1991).

One of the characteristics which increase the probability of successful invasion is the ability of clonal growth (Heger and Trepl 2003; Mack 2003) which is suggested as one of the most successful growth strategies in the plant world (Traveset, Moragues et al. 2008). *Carpobrotus* species are clonal plants capable of achieving high rates of space colonization (Sintes, Moragues et al. 2007), which suppresses the growth and establishment of other plants (D'Antonio and Mahall 1991; Albert, D'Antonio et al. 1997; Suehs, Affre et al. 2004; Vilà, Tessier et al. 2006). A characteristic of clonal growth that confers competitiveness is the efficient distribution of tasks among ramets which, coupled with phenotypic plasticity, can lead to enhanced resource exploitation (Stuefer, DeKroon et al. 1996). Clonal growth and its ability to survive in extreme environmental conditions make *C. edulis* a very

competitive plant with a subsequent negative impact on native flora (D'Antonio and Mahall 1991; D'Antonio, Odion et al. 1993; Moragues and Traveset 2005; Vilà, Tessier et al. 2006).

3.2.1. Aims of the study

To be able to understand the possible impacts of exotic iceplant species on native biodiversity, a useful starting point is the study of the mechanisms that are implicated for their success as invaders. The aim of this study is to compare growth rates and breeding systems of the iceplant taxa commonly found in New Zealand. The following research questions were explored in this study: (i) how do the field growth rates of the exotic and native iceplant, and their hybrids compare? (ii) What is the substrate preference of the various iceplant species? (iii) What is the potential implication of the growth rates on the native *Disphyma australe?* (iv) To what extent do flower phenology of the iceplant species overlap? (v) How do the exotic species differ in their mating systems and interbreeding with native *Disphyma*? This knowledge will then be evaluated in the context of environmental management of coastal dune habitats.

3.3. STUDY SPECIES

3.3.1. *Carpobrotus* spp. and their intrageneric hybrid

Carpobrotus species are members of Aizoaceae, many of which are native to the Karoo region of South Africa. They are succulent perennial plants with creeping, prostrate growth habits, capable of crawling over shrubs, fences and other obstacles (D'Antonio 1990). Leaves are opposite and sharply three-angled with variable keel serrations. *Carpobrotus* forms large mats on coastal rocks, cliffs, and sand dunes owing to its profuse clonal growth (D'Antonio 1990; 1993; Traveset, Brundu et al. 2008). Active growth occurs primarily along main axes, although lateral branches may also grow, particularly following death of the apical meristem of the main axis (D'Antonio 1990). Rooting occurs where nodes contact the soil and they spread radially with an individual branch elongating more than 1 m in a year

(D'Antonio 1990). Branches tend to grow over each other, resulting in the accumulation of up to 40 cm of live and dead plant material (D'Antonio 1990). A single individual of *C. edulis* has been reported to form dense, circular mats up to 20 m wide and over 50 cm deep (D'Antonio and Mahall 1991) that are nearly impenetrable and appear to totally eliminate all other vegetation in the immediate area (Campos, Herrera et al. 2004). *Carpobrotus chilensis* mats are said to be shallower in depth and have more bare space within them (Vilà and D'Antonio 1998).

Flowers are solitary and terminal, borne on vertical shoots and do not seem to require specific pollinators. They are unique within Aizoaceae in that they have fleshy indehiscent fruits (Albert, D'Antonio et al. 1997). In California, *C. edulis* hybridises with *C. chilensis* (N.E.Br.) and hybrids are also self-compatible (Vilà and D'Antonio 1998). Introgressive hybridization is very common (Vilà, Weber et al. 1998), occurring throughout coastal California between the non-native *Carpobrotus edulis* and the putative native *C. chilensis*, leading to a high abundance of invasive hybrid morphotypes that compete aggressively with native plant coastal species (D'Antonio 1990; Albert, D'Antonio et al. 1997; Vilà and D'Antonio 1998). The fleshy fruits bear a large number, often over a thousand, of small seeds (Bartomeus and Vilà 2009)that are eaten and widely dispersed by several mammals such as rabbits (D'Antonio 1990) and rats (Bourgeois, Suehs et al. 2005). *Carpobrotus* has a long-lived seed bank that can remain viable in the soil for at least two years (D'Antonio 1990).

There are two naturalized species of *Carpobrotus* (*C. edulis* (L.) N.E.Br., and *C. chilensis* (Molina) N.E.Br.) that occur widely in coastal parts of New Zealand (Heenan and Sykes 2010). These species have been introduced in different parts of the world as ornamental plants and for erosion prevention (Weber 2003). While *C. edulis* is native to South Africa, the origin of *C. chilensis* is not known (Albert, D'Antonio et al. 1997). *Carpobrotus chilensis* is no longer present in Chile (Schierenbeck, Symonds et al. 2005) if indeed this was its origin, as suggested by its scientific name. It is widely spread in California and is believed to be native in this region (Schierenbeck, Symonds et al. 2005). *Carpobrotus edulis* was introduced to New Zealand from South Africa as an ornamental plant and naturalised around 1883 (Webb, Sykes et al. 1988; D'Antonio and Mahall 1991). In New

Zealand they often occur in association with *D. australe* subsp. *australe* (Heenan and Sykes 2010). Of these two exotics, *C. edulis* and its hybrids are regarded as invasive in New Zealand.

3.3.2. *Disphyma* species

There are two species of the genus *Disphyma* native to New Zealand. Of these, *D. papillatum* is endemic to Chatham Island (Chinnock 1971; Webb, Sykes et al. 1988). The other, *Disphyma australe* (W.T. Aiton) N.E.Br is subdivided into two subspecies: *D. australe* subsp. *stricticaule* Chinnock is endemic to Kermadec Islands while *D. australe* subsp. *australe* occurs on North, South, Stewart and Chatham Islands (Chinnock 1976). *Disphyma maclavellatum* (Haw.) is of Australian origin but is considered naturalised (Webb, Sykes et al. 1988). There are two forms of *D. australe* subsp. *australe* that are distinguishable by the colour of their vegetative parts (Chinnock 1971). One produces red betanin pigments in response to changing environmental conditions while the other always remains green (Chinnock 1972). Unlike their exotic counterparts *Disphyma* spp. have dehiscent, hygrochastic capsules (Chinnock 1971). Very little is known about the ecology of *Disphyma*. An electronic search of the word "Disphyma" in the Web of Knowledge databases returns only 25 articles, most of which are floras that only mention presence/absence of the species.

3.3.3. *xCarpophyma mutabilis* Heenan and Sykes 2010

xCarpophyma mutabilis is the name given to the hybrid produced by intergeneric hybridisation between *C. edulis* and *D. australe* (Heenan and Sykes 2010). These plants have characteristics intermediate to their parents (Chinnock 1971; Heenan and Sykes 2010). They are highly sterile with chromosome count being 2n=27 (Chinnock 1972). Flowers rarely develop into fruits and if they do they are seedless (Pers. obs.; Chinnock 1972). Intergeneric hybridisation of *C. chilensis* and *D. australe* results in a hybrid named x*Carpophyma pallida* that is said to be found in the Canterbury region and on Chatham Island of New Zealand

(Heenan and Sykes 2010). x*Carpophyma mutabilis* is said to be the more common intergeneric hybrid in New Zealand (Heenan and Sykes 2010). Detailed morphological descriptions of these hybrids (as well as of their putative parents) have been given by (Chinnock 1972) and (Heenan and Sykes 2010)but they are quite similar and therefore difficult to distinguish. For this study, all the intergeneric sample plants were obtained from Wellington region and are therefore assumed to be x*Carpophyma mutabilis.*

3.4. METHODS

3.4.1. Experiment 1 (Common garden experiment – growth characteristics in different substrates)

Cuttings of the four different taxa of iceplant were obtained from 2-5 different populations around Wellington (Fig. 3.2; Appendix 1, Table 3) and planted in gravel (7.5mm diameter), coarse sand (commercial propagation sand No.2, 0.3-4.75mm) and fine sand (commercial propagation sand No.1, 0.15-2.35mm) in the Victoria University glasshouse between 13th and 19th June 2011. Samples of cuttings of each taxon were dried in an oven at 70°C for 48 hours to compare their initial biomass. Morphometric data (Chinnock 1972), especially flower colour, was used in identifying the different species earlier in the previous year when plants were flowering. It is possible that the two exotic species (*Carpobrotus* spp.) do not always (or often) exist as pure species in New Zealand and may in fact be thought of as different colour morphs or different ends of a hybrid swarm between *C. edulis* and *C. chilensis* (see Gallagher et al. 1997; Schierenbeck et al. 2005; Suehs et al. 2004). For the purposes of this thesis we continue to use the two names *C. edulis* for large yellow-flowered *Carpobrotus* and *C. chilensis* for large magenta-flowered *Carpobrotus*. Due to the limited distribution of *C. chilensis*, cuttings were only obtained from two populations. Each cutting was made up of two nodes with two true leaves for *Carpobrotus* and four true leaves for *Disphyma* and the hybrid. Leaves were considered true when they were fully open.
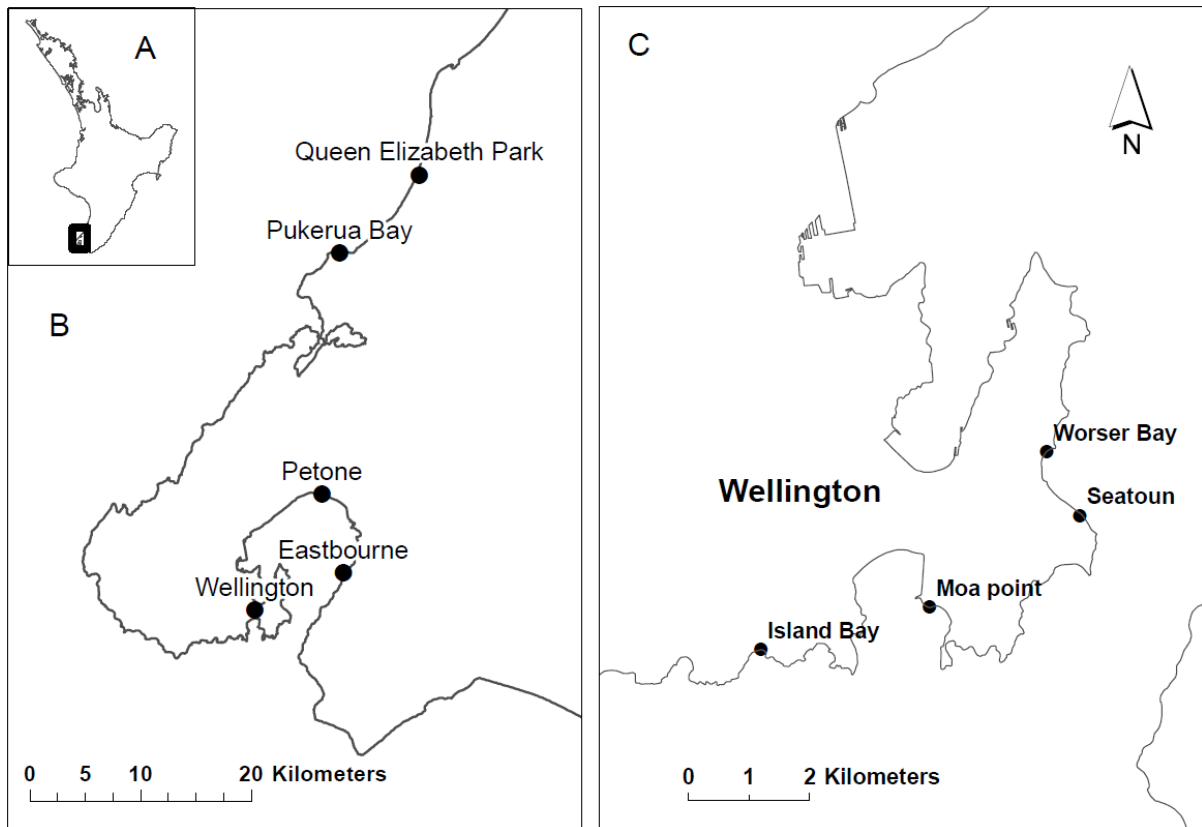
Fig. 3.2: Map of North Island New Zealand (A) showing Wellington region and sites from where the iceplant cuttings were obtained (B and C).

Twelve cuttings were obtained from each site and four of them planted in each medium except for *C. Chilensis* which was only found at two sites. Each cutting was planted in a container measuring 9cm X 9cm X 11cm in a medium formed by mixing two parts gravel or sand and one part potting mix. The cuttings were planted with one internode below the soil surface and the terminal node at soil-air interface. In total, 60 cuttings per species were distributed among the three substrate types. The cuttings were watered every second day for the period of the time that they were in the glasshouse (until the end of August) and moved around every two weeks to prevent positional effects.

On 4th of September all plants were relocated to the Victoria University Coastal Ecology Laboratory (VUCEL) at Island Bay. The pots were placed in trays holding 24 plants each arranged in an open area where they are exposed to natural weather elements. The trays were lined parallel to the coastline, 8m from the high tide mark so that they received

similar amounts of salt spray. The number of leaves, length and number of nodes of each plant were measured at the time of relocation and repeated at two-monthly intervals over the spring season. At the end of the experiment (28th December 2011) number of leaves, length of the main axis, number of main axis nodes, number of lateral branches and the number of nodes along lateral branches (Fig. 3.3) were recorded. Plants were then destructively harvested and separated into shoot and root portions. Roots were then thoroughly but gently washed under a running tap to remove all the soil. Each shoot and root was then dried in marked paper envelopes to a constant weight in an oven at 70°C.
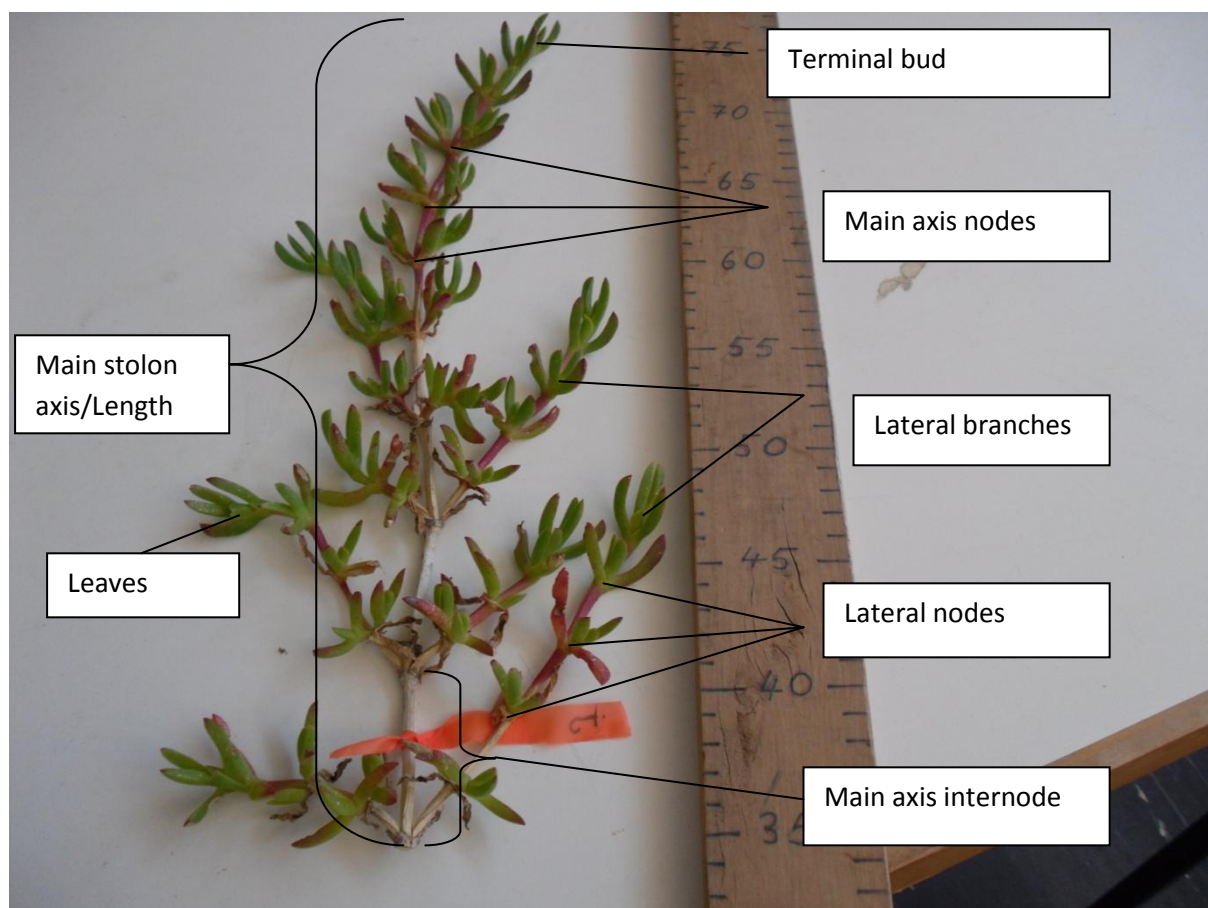
Fig. 3.3: Photo of a hybrid stolon showing morphological features referred to frequently in the sections that follow. The tag was originally placed just below the cut in September and the stolon harvested in December. Growth of the illustrated sample occurred over a three month period.

3.4.2. Experiment 2 (Field Growth monitoring)

On 10th of January 2011 one plant each of *C. edulis* and *C. chilensis* at the back dune were selected and fifty stolons per plant were tagged at a node just below the terminal bud. These were the only easily identifiable *Carpobrotus* plants in this section of the dune because they were in flower. Around the same time, ten stolons on each of four *C. chilensis* plants at the foredune were tagged at a node below the terminal bud. Ten stolons each were also tagged on five hybrid and *D. australe* plants at one and two nodes below each terminal bud respectively. The number of nodes along the main axis forward of the tag was counted at two to three month intervals. On 24th of September 2011, the total number of internodes, lateral branches and leaves forward of the tags were counted on all the stolons. Between 21st and 24th September 2011, ten additional stolons were tagged on the old *Carpobrotus* plants at Eastbourne. In addition, two new *C. chilensis* plants at the backdune and four new *C. edulis* plants (one at the foredune and the others at the rear) were selected and ten stolons tagged on each at the same site. Similarly, new tags were placed on an additional five stolons per plant of the old *D. australe,* and ten per plant on the hybrids at Day's Bay. Around the same time, ten stolons per plant of *C. edulis* at Queen Elizabeth Park were tagged on four plants each at the rear and foredunes. At the end of the experiment (after their vegetative growth characters were measured) 7-10 stolons tagged in September were harvested from a plant of each taxon, except *Disphyma australe* for conservation reasons. These stolons were transported quickly in air-tight zip-lock polythene bags and their fresh weight determined in the laboratory before they were dried to a constant weight at 70°C in an oven and their dry weights measured.

3.4.3. Experiment 3 (Mating systems)

A breeding experiment was conducted at Eastbourne between 17th and 18th of September 2011. Fifty flower buds each of *C. edulis* and *C. chilensis* were selected and assigned to five breeding treatments (10 flowers per treatment): (i) agamospermy (emasculated, stigmas clipped and flowers bagged), (ii) spontaneous self-fertilisation

(bagged flowers not manipulated), (iii) artificial self pollination (pollen donor was a different flower of the same individual plant), (iv) artificial pollination with the other congener, and (v) artificial pollination with *D. australe.* The flowers were selected before they opened up to ensure that no self pollination would have taken place naturally before manipulation. All flower buds were bagged using fine meshed nylon bags (0.25mm x 0.22mm) to exclude insect pollinators. However, no insecticides to exclude very small arthropods like mites and aphids that might crawl up the pedicels were applied, although no such potential pollinators were observed. Artificial self pollination involved dusting of pollen of other flowers of the same plant on to the stigmas of experimental flowers. During emasculation, the calyx and corolla were also removed since at that stage it was impossible to remove stamens only. However, I did not remove the corolla and calyx of the non-emasculated flowers to control for any independent effects these structures may have on seed production. Hand pollination was done about eight days later and on two consecutive days by plucking mature flowers of the pollen donor and dusting the fresh pollen onto the stigmas of the recipient using a fine painting brush. Care was taken to ensure that the brush used for one pollen donor was not used with the other. Hand pollination was done one flower at a time, after which the flower was bagged again. The flowers remained bagged until fruits were harvested on 3rd of January 2012. Each fruit was opened up and seeds removed from each carpel. The seeds were washed in a muslin bag under running water to remove the mucilaginous sap and the numbers of seeds contained were recorded.

In addition, five plants per taxon in fixed quadrats measuring 4m^2 were selected at Eastbourne and Day's Bay for monitoring of flower phenology starting 21st of September at about four week intervals until the end of December 2011. All twelve *D. australe* plants were monitored for flower phenology.

3.4.4. Statistical analysis

All data were analysed in SPSS statistical package (SPSS Inc. 2008).

3.4.4.1. Experiment 1: common garden experiment

For the cuttings, growth was measured as dry weights (root, shoot, total and root mass ratio), and net increase in stolon length, number of nodes and leaves over the field growth period (September to December 2011) at the Island Bay field site. Two-way ANOVA with the substrate and taxon as independent variables, including their interaction, and the measured growth parameters (biomass measurements, number of stolons, etc) as dependent variables were used. The data were then split by taxa and one-way ANOVA run with substrate as the fixed variable to test how plants of the same taxon performed in the different substrate types. Further one-way ANOVA was calculated after splitting the data by substrates to test how plants of the different taxa performed in the same substrate types. In addition, the source plant from which the cuttings were obtained was used as a random factor in a mixed model analysis. Bivariate correlation between biomass and other measured parameters was also assessed to determine which vegetative growth aspect best predicts biomass.

3.4.4.2. Experiment 2: field growth monitoring

Although there were some outliers, the data were mostly distributed normally and there was no need for transformation. For field growth experiments, the data were split into the different growth periods (January-December, January-September, and September-December) because additional plants were tagged in September and comprehensive measurements conducted periodically. Therefore, the January-September and September-December periods are subsets of the January-December period for most plants. Data for the September period are the difference of the other two periods for most plants and therefore some negative growth is expected. Nested ANOVAs were calculated in SPSS for the growth experiments with net incremental values of the measured parameters (increase in length, number of main axis nodes, etc) for each taxon as the dependent variable and the individual plants as a random variable nested within "taxon." Where variability in a parameter among taxa was found, the values were aggregated and post hoc tests run to

compare their performance. The data of the sample shoots destructively harvested were combined with those relating to the shoots of the cuttings and used in generating a linear regression model for estimating biomass (dry weights) of the field stolons.

3.4.4.3. Experiment 3: breeding and cross-pollination

Descriptive statistics and one-way ANOVA in SPSS was calculated for the number of seeds produced by the *Carpobrotus* spp. subjected to different breeding treatments with number of seeds as the dependent variable and the breeding treatment as the independent factor.

3.5. RESULTS

3.5.1. Growth of cuttings in the common garden experiment

Only 16 cuttings (6.7%) of the 240 planted died during the life of the experiment, mostly due to herbivory during exposure to field conditions. The initial biomass of the sample cuttings showed that *C. edulis* cuttings had a significantly higher biomass than those of the other taxa ($P < 0.001$). *Carpobrotus chilensis* also had a higher biomass than *D. australe* and the intergeneric hybrid ($P < 0.001$). However, there was no difference in the initial biomass of *D. australe* and the hybrid sample cuttings (Appendix 2, Table 1).

The final biomass (root plus shoot) was affected by the substrate ($F_{2, 200}=5.415$; $P = 0.005$; Table 3.1), taxon ($F_{3, 200}=77.930$; $P < 0.001$) and the interaction of these two ($F_{6, 200}=2.558$; $P = 0.021$). In post hoc tests there was no difference between the two *Carpobrotus* species, but both had a significantly higher total biomass than the hybrid and *D. australe* ($P < 0.001$). Comparisons of plants of different taxa growing in the same substrate showed that in all substrate types, *C. chilensis* and *C. edulis* plants accumulated greater

biomass than *D. australe* ($P < 0.001$) and the hybrid at various levels of significance except in gravel where biomass of *C. chilensis* did not differ from that of the hybrid (Fig. 3.4). The hybrid also had a higher total biomass than *Disphyma australe* ($P < 0.001$). Only *C. chilensis* showed a significant difference in performance across the various substrates ($F_{2, 54}=8.951$; $P < 0.001$; Table 3.2; Fig. 3.5) where those plants in coarse and fine sand did better than those in gravel ($P = 0.011$ and $P < 0.001$ respectively).

Table 3.1: Two-way ANOVA table showing that total dry weights of the cuttings varied with substrate type, taxa and interaction of the two factors (Type III Sum of Squares).

Source	df	Mean Square	F	Sig.
Substrate	2	4.8	5.415	.005
Taxon	3	68.9	77.930	<.001
Substrate * Taxon	6	2.3	2.558	.021
Error	200	.9		
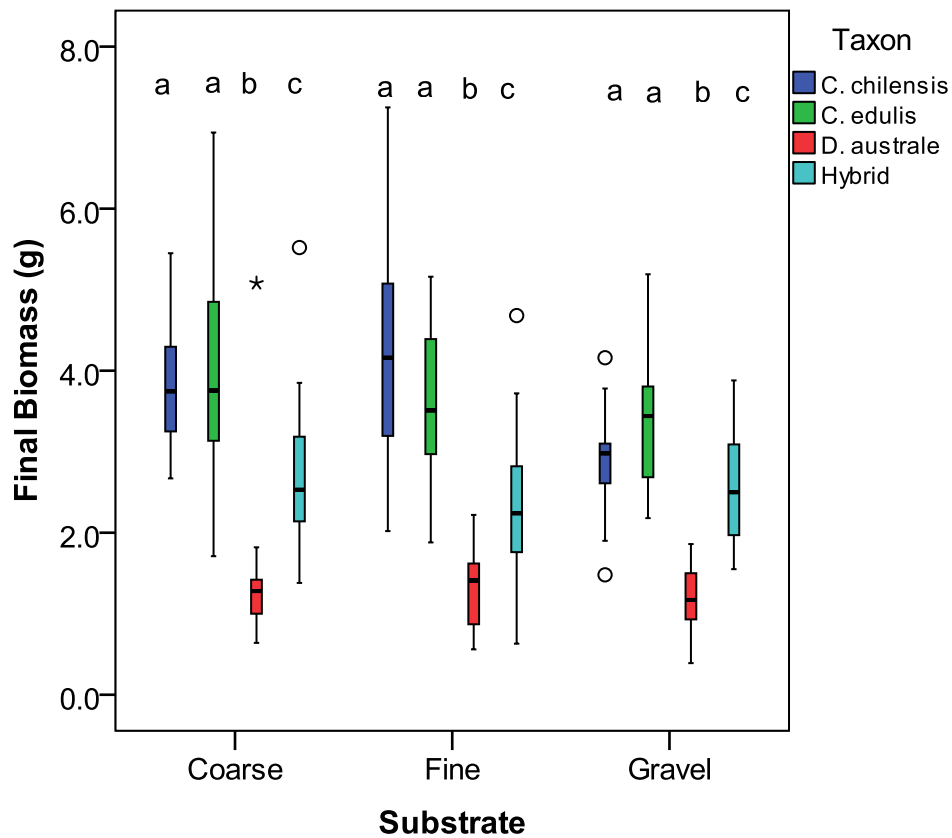
Fig.3.4: Boxplots showing final biomass of the cuttings of the various taxa growing in different substrate types. Different letters at the top of the bars indicate significant ($P < 0.05$) variability in post-hoc test between taxa in the same substrate type.

Table 3.2: Tukey HSD table showing that *C. chilensis* cuttings growing in gravel put on less final biomass than those growing in fine and coarse sand.

Taxon	(I) Subst	(J) Subst	Mean Difference (I-J)	Std. Error	Sig.
C. chilensis	Coarse	Gravel	.96	.31950	**.011**
	Fine	Gravel	1.33	.32346	**<.001**
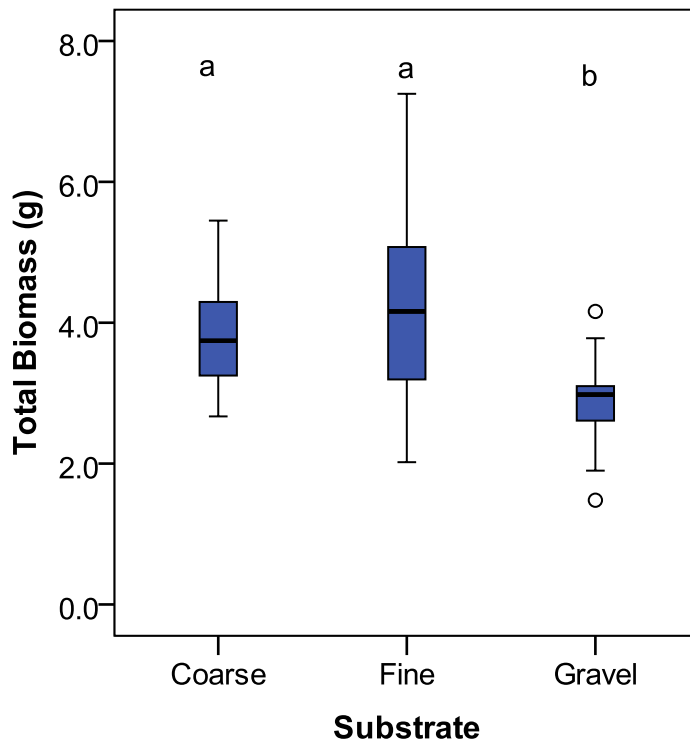
Fig. 3.5: Boxplots showing variability in final biomass of *C. chilensis* cuttings growing in different substrates (different letters above the bars represent significant differences at *P* < 0.05).

Biomass allocation to above- or below-ground structures, in terms of root mass ratio (root dry weight/total biomass), was not affected by the substrate but regardless, there was significant variability among the various taxa ($F_{3, 200}$=164.799; *P* < 0.001; Table 3.3). On the whole, there was no difference in RMR between *Carpobrotus* species. *Disphyma australe* had a significantly higher root mass ratio (RMR) than the other three (Fig. 3.6; *P* < 0.001), with the hybrid also having a higher RMR than the two *Carpobrotus* (*P* < 0.001). However, RMR did not differ between *D. australe* and the hybrid in fine sand. Plants of the same taxon growing in different substrate types did not differ in their RMR.

Table 3.3: Two-way ANOVA table showing root mass ratio of the cuttings was not affected by the substrate but varied among taxa.

Source	df	Mean Square	F	Sig.
Substrate	2	.003	2.618	.075
Taxon	3	.177	164.799	**<.001**
Substrate * Taxon	6	.001	1.136	.343
Error	200	.001		
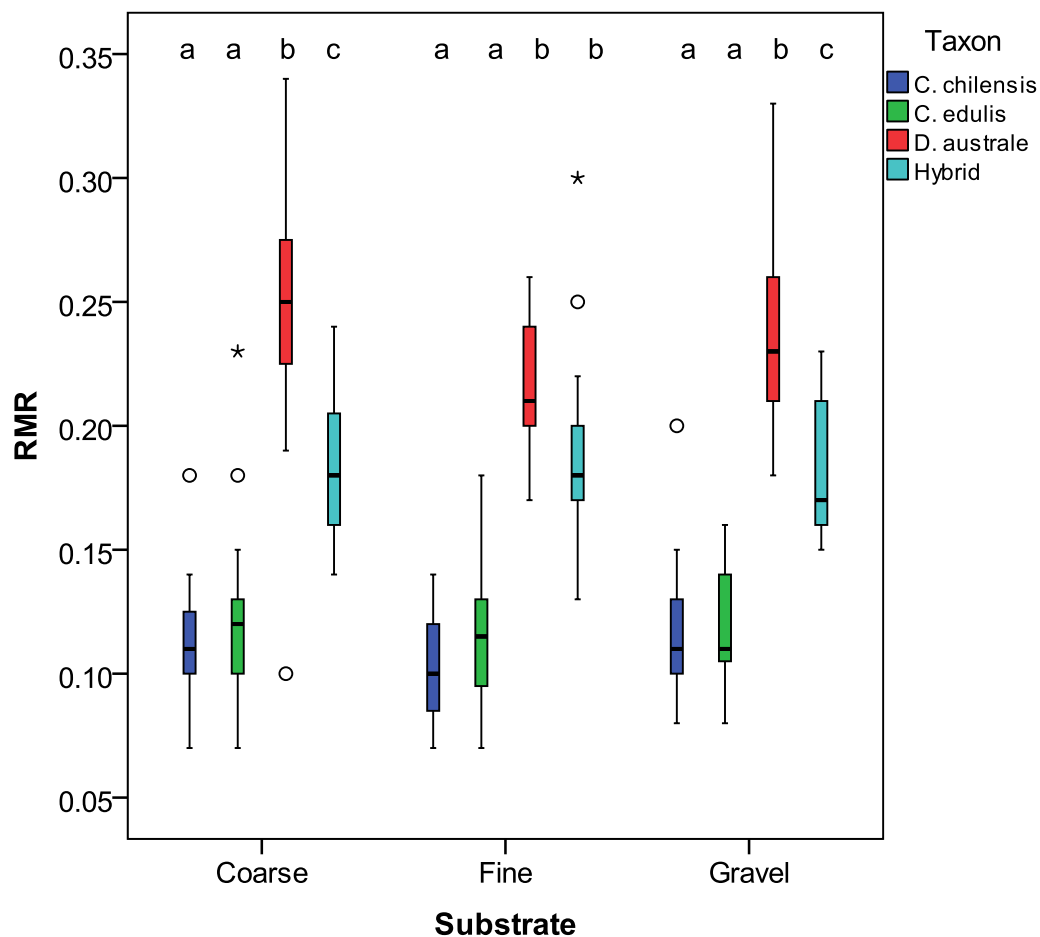
Fig. 3.6: The final root mass ratio (RMR) of cuttings of each taxon growing in the various substrates. Letters above the bars indicate variability among taxa growing in the same substrate at $P < 0.05$ significance levels.

Substrate type did not have any influence on the number of leaves produced in the field but there was significant variability among taxa ($F_{3, 212}$=61.093; $P < 0.001$; Fig. 3.7; Table 3.4), except between the two *Carpobrotus* species. Comparison of plants of the same taxon in different substrate types showed variability only among *C. chilensis* plants ($F_{2, 56}$=5.74; $P = 0.005$; Table 3.5) where plants growing in fine sand, on average, produced four more leaves than those in gravel ($P = 0.004$). There was significant variability among plants of different taxa growing in the same substrate type, except between the two *Carpobrotus* species. The hybrid produce more leaves than *Carpobrotus* in all substrates ($P < 0.001$), and more than *D. australe* in coarse sand ($P = 0.003$) as well as in gravel ($P = 0.004$). *Disphyma australe* performed better than *Carpobrotus* in coarse sand ($P = 0.002$). In addition, *D. australe* accumulated more leaves than *C. chilensis* ($P = 0.004$) and *C. edulis* ($P = 0.001$) in fine sand as well as gravel ($P = 0.001$ and $P = 0.004$ respectively). However, there was no difference between *D. australe* and hybrid in fine sand.
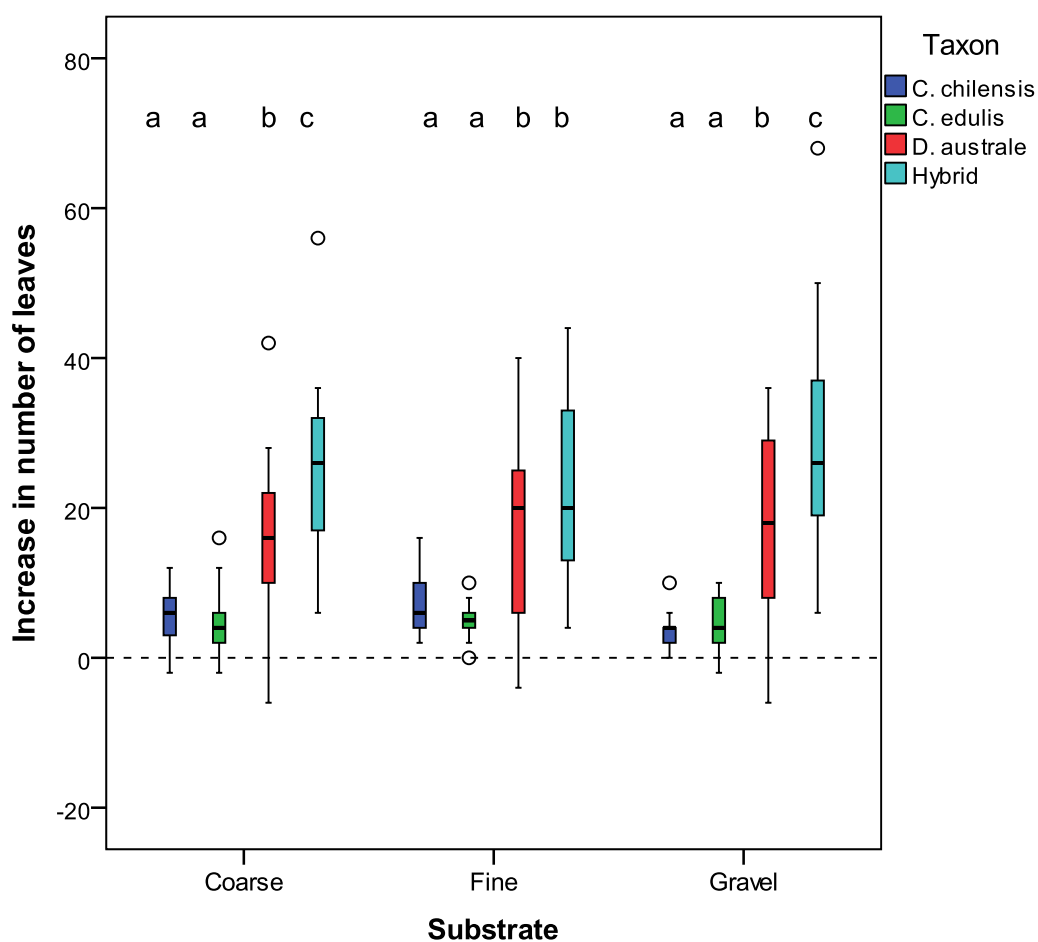
Fig. 3.7: Boxplots showing that the hybrid cuttings produced more leaves in the field than the other taxa and that *D. australe* produced more leaves than *Carpobrotus* spp. over the four month period. Different letters above the bars represent significant differences between taxa, within each substrate (*P* < 0.05).

Table 3.4: ANOVA table showing substrate and its interaction with taxon did not have any effect on increase in the number of leaves of cuttings over the four-month field exposure period.

Source	df	Mean Square	F	Sig.
Substrate	2	10.8	.117	.889
Taxon	3	5642.7	61.093	**<.001**
Substrate * Taxon	6	109.5	1.185	.315
Error	212	92.4		

Table 3.5: Summary of separate ANOVAs showing that over the four month field exposure period substrate had an effect on leaf increase of *C. chilensis* only.

Taxon	Source	df	Mean Square	F	Sig.
C. chilensis	Substrate	2, 56	68.1	5.740	**.005**
C. edulis	Substrate	2, 46	.6	.047	.954
D. australe	Substrate	2, 54	26.1	.161	.852
Hybrid	Substrate	2, 56	246.5	1.443	.245

The net increase in the number of nodes along the main axes did not differ with substrate type but variability among taxa was significant ($F_{3, 212}=89.969$; $P < 0.001$). Both the hybrid and *D. australe* had more nodes than *Carpobrotus* species ($P < 0.001$). The hybrid also produced more nodes than *D. australe* ($P = 0.041$). There was no variability within members of the same taxon growing in different substrates. Performance of plants of different taxa growing in the same substrate type showed that *D. australe* and the hybrid both had significantly more nodes than *Carpobrotus* species in all substrates ($P < 0.001$). There was no difference in the number of main axis nodes between the *Carpobrotus* species as well as between the hybrid and *D. australe*. In addition, there was a highly significant variability among taxa in terms of internode length (total length/ (main axis nodes-1)) ($F_{3, 212}=47.046$; $P < 0.001$; Table 3.6) with the hybrid cuttings having longer internodes than all the other taxa ($P < 0.001$; Table 3.4; Fig. 3.8). There was no difference in the internode lengths among the other three taxa.

Table 3.6: ANOVA table showing that the lengths of stolon internodes varied among the taxa but the substrate type in which plants were growing did not have any influence.

Source	df	Mean Square	F	Sig.
Taxon	3	13.432	47.046	**<.001**
Substrate	2	.005	.018	.982
Taxon * Substrate	6	.170	.596	.734
Error	212	.286		
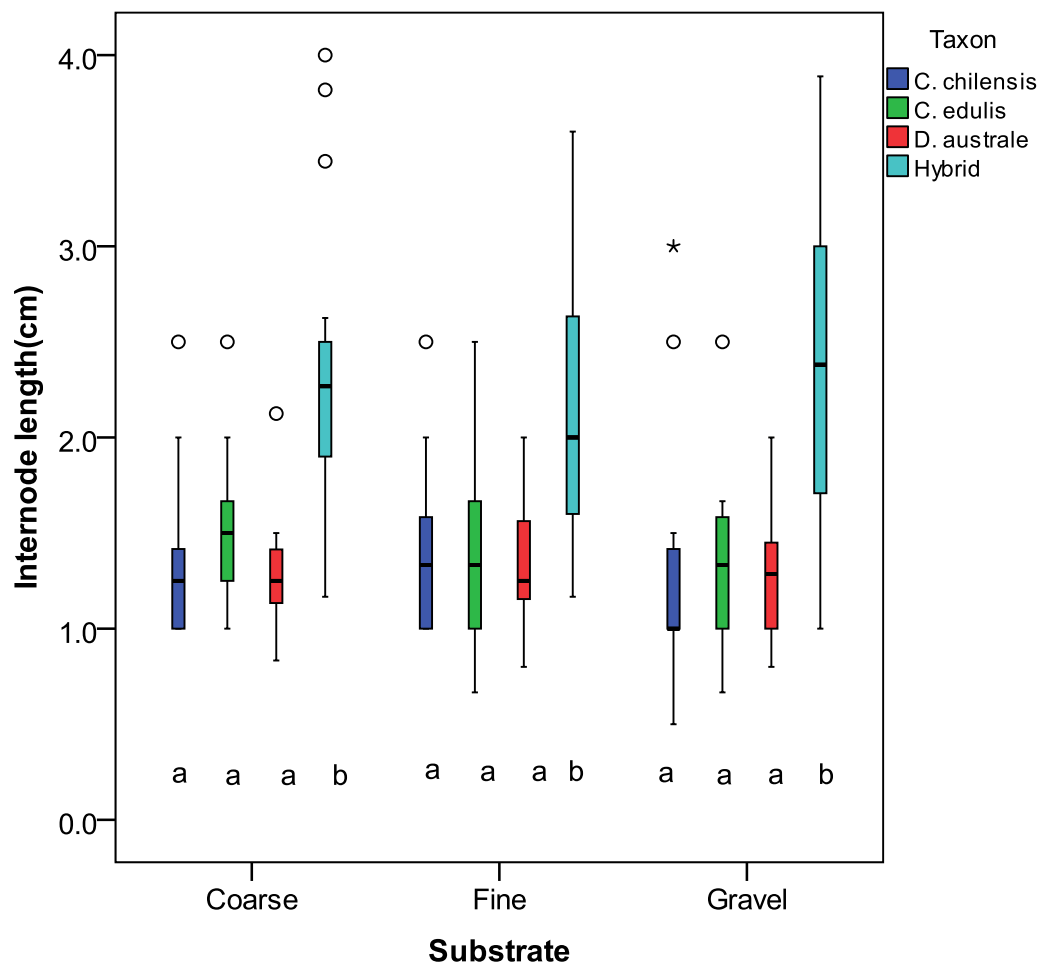
Fig. 3.8: Average internode length of cuttings of each taxon in the various substrates grown over a period of six months. The different letters below the bars indicate variability among plants of different taxa growing in the same substrate ($P < 0.001$).

Net increase in lengths of main axes did not differ with substrate type but variability among taxa was significant ($F_{3, 212}=68.834$; $P < 0.001$). The hybrid stolons were longer than those of the other taxa ($P < 0.001$) while *D. australe* stolons grew longer than *C. chilensis* ($P < 0.001$) and *C. edulis* ($P = 0.014$). There was no variability within members of the same taxon growing in different substrates. Between substrate comparisons (Fig. 3.9) showed that in gravel the hybrid outgrew all the other taxa ($P < 0.001$). In coarse and fine sand the hybrid grew longer than *Carpobrotus* ($P < 0.001$) as well as *D. australe* ($P < 0.001$; 0.003 respectively). *Disphyma australe* grew longer than *C. chilensis* in fine sand only ($P = 0.048$).
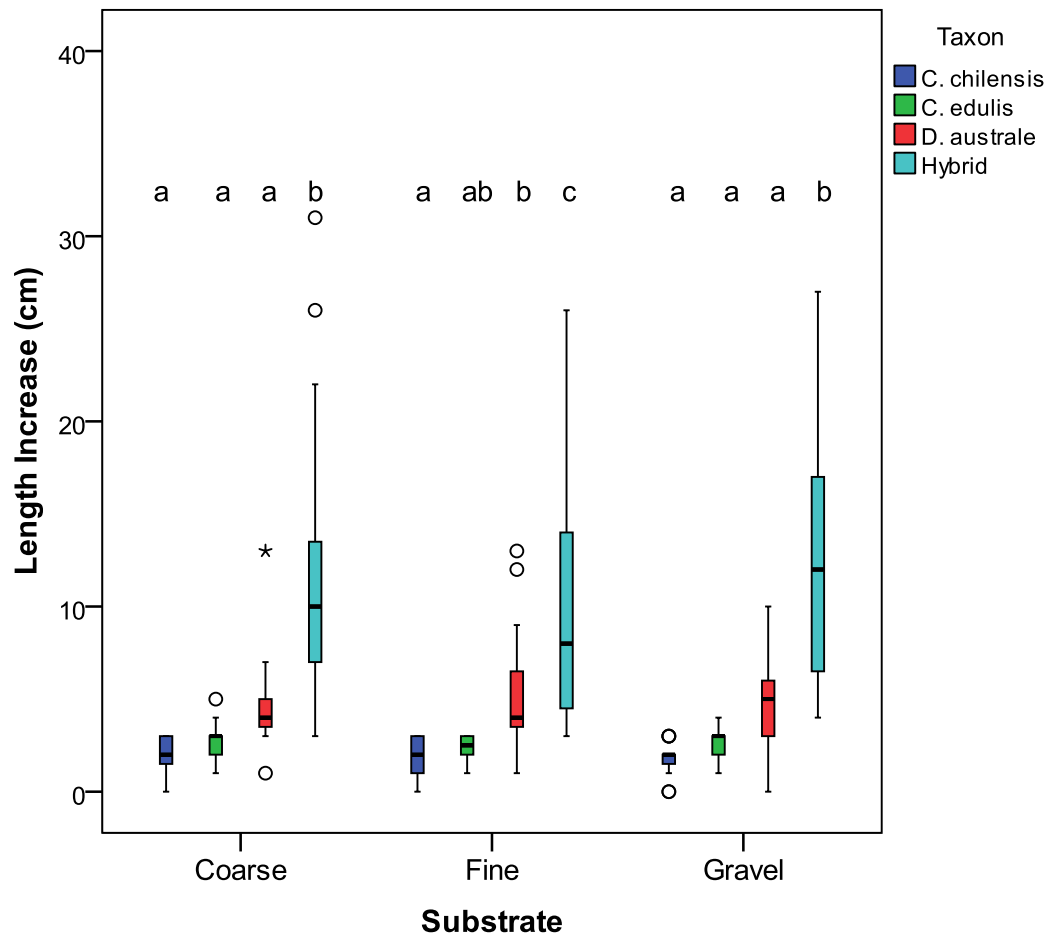
Fig. 3.9: The length by which stolons of the various taxa increased over the four-month period of exposure to field conditions. Letters above the bars refer to variability among taxa growing in the same substrate ($P < 0.05$).

An assessment of correlation between total biomass and net increase in leaves, nodes, and length of the cuttings showed positive relationships (Fig. 3.10; Table 3.7). However, increase in the number of leaves was stronger for all the taxa ($R^2 = 0.964$; $P < 0.001$).
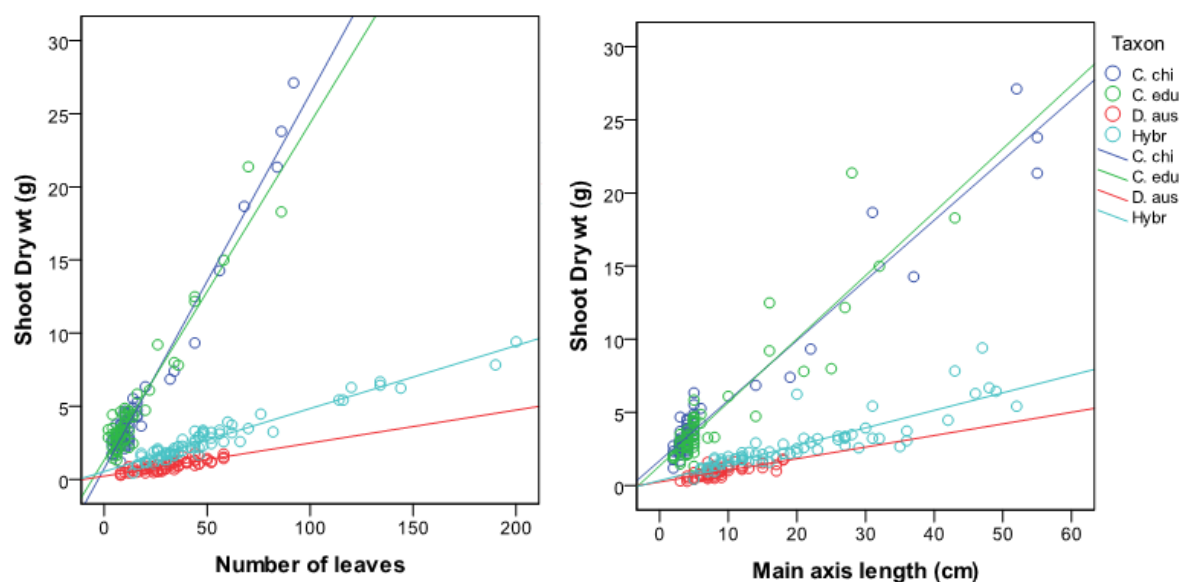
Fig. 3.10: A graph showing a positive correlation between shoot dry weight and number of leaves (Left), and stolon length (Right) of the cuttings and the stolons harvested from the field sample plants.

Table 3.7: Bivariate correlation (Pearson's R-squared) showing positive relationships between shoot dry weight and measured parameters of the cuttings and the stolons harvested from the field sample plants.

Taxon		N	Leaves	Nodes	Length
C. chilensis	Shoot Dry wt	67	.964	.952	.944
C. edulis	Shoot Dry wt	58	.920	.883	.831
D. australe	Shoot Dry wt	57	.702	.447	.588
Hybrid	Shoot Dry wt	68	.938	.813	.743

There were no variations in biomass accumulation among cuttings of the same taxon obtained from the different populations (Plants) when site was treated as a random variable and substrate as a fixed variable in a mixed effects ANOVA, except among the hybrids ($P < 0.001$) where plants obtained from Moa point put on more biomass than those from the other sites (Fig. 3.11).
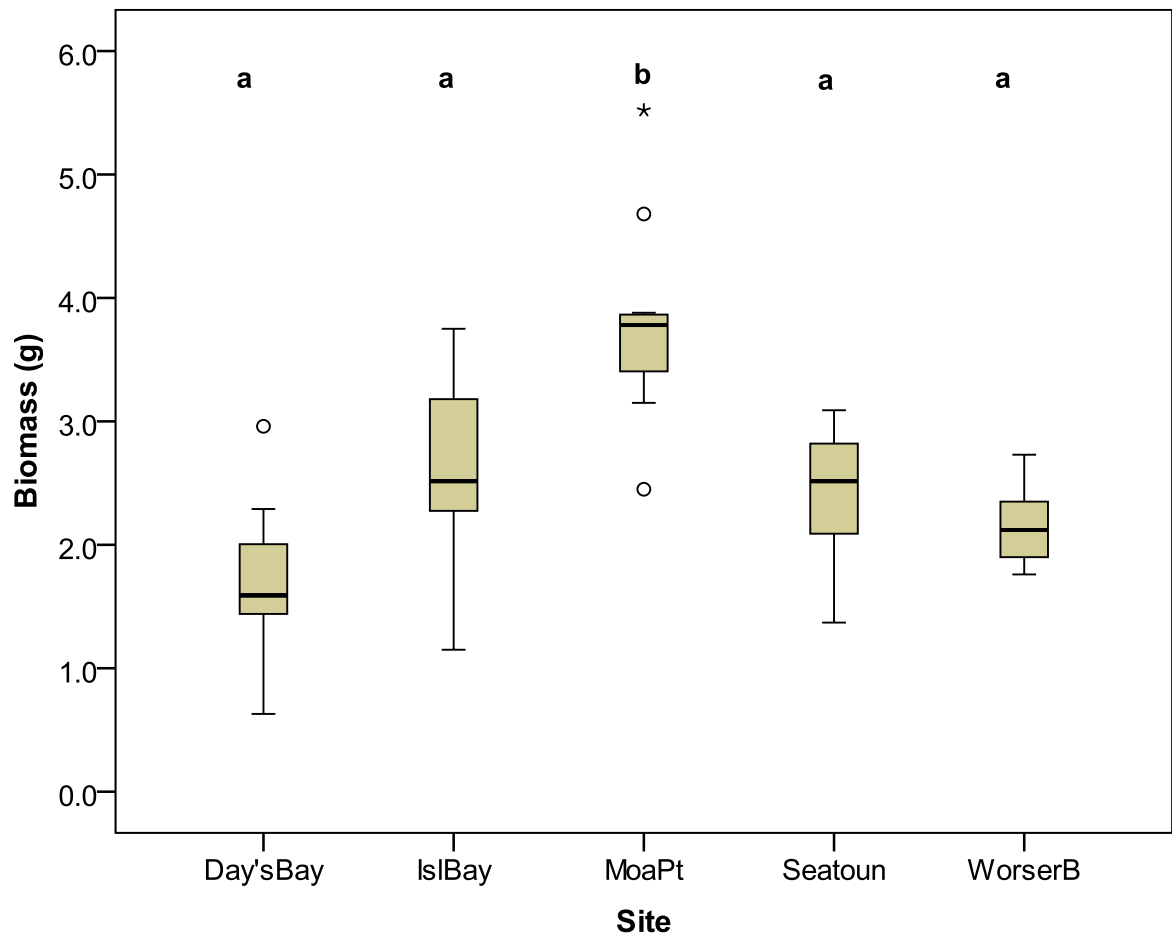
Fig. 3.11: Final biomass put on by the hybrid cuttings from the various populations over a period of six months indicating that cuttings from the plants at Moa Point put on higher biomass than the others.

3.5.2. Field growth monitoring

There was no significant variability in the number of leaves produced among the taxa over the total growth period of January-September ($P = 0.152$) and January-December ($F_{3, 9.01}$=3.753; $P = 0.053$; Table 3.8). There was however, a highly significant variability in the number of leaves among taxa for the period of September-December ($F_{3, 37.3}$=10.109; $P < 0.001$). In addition, there was significant variability among the different sample plants

within each taxon in terms of the number of leaves (all periods $P < 0.001$; Table 3.8). The hybrid plants were highly variable in each of the growth periods ($P \leq 0.002$), unlike plants of other taxa that mostly showed variability in number of leaves during the September-December period. There was no variability in the number of leaves among taxa during the January-September period. The hybrid produced more leaves than *D. australe* in the January-December and September-December periods ($P = 0.007$ and $P < 0.001$ respectively), and *C. edulis* in the September-December period ($P = 0.006$; Fig. 3.12; Table 3.9). During the September-December period, *C. edulis* also produced more leaves than *D. australe* ($P = 0.036$). *Disphyma australe* plants actually lost more leaves than they produced during the period (Fig. 3.12).

Table 3.8: Nested ANOVA summary table comparing the number of leaves produced by the field sample plants of each taxon over the various growth periods. There was significant variability among sample plants of the same taxon in all growth periods. Variability among taxa was only significant in the September-December period.

Period	Source	Error df	Df	MS	F	Sig.
Jan-Dec	Taxon	9.010	3	333913.5	3.753	.053
	Plant(Taxon)	111	11	54424.1	4.718	**<.001**
Jan-Sept	Taxon	9.276	3	60368.7	2.228	.152
	Plant(Taxon)	111	11	16332.9	5.481	**<.001**
Sept-Dec	Taxon	37.310	3	90276.8	10.109	**<.001**
	Plant(Taxon)	296	35	10511.5	6.619	**<.001**
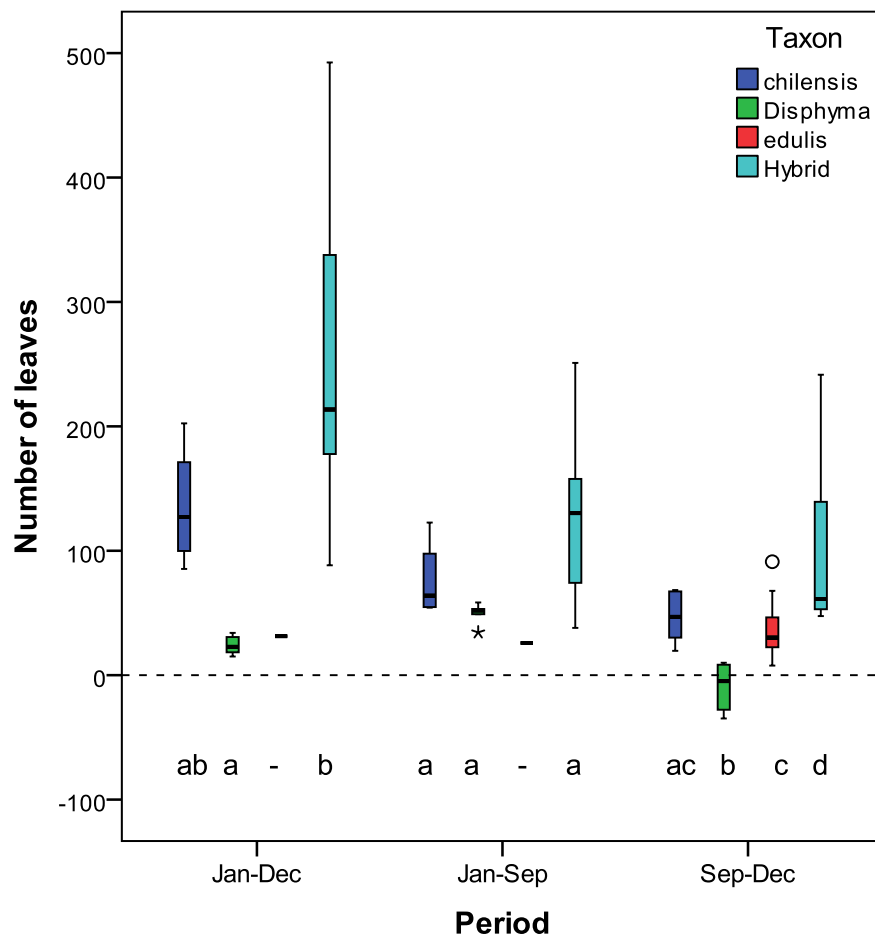
Fig. 3.12: Number of leaves produced by stolons of sample plants of the various taxa over each of the growth periods in the field growth experiment. The letters below the bars indicate variability among taxa at significance levels of *P* < 0.05. *Carpobrotus edulis* was not included in the comparison for the January-September and January-December periods because there was only one sample plant.

Table 3.9: Tukey HSD table comparing variability in the average number of leaves produced by plants of each taxon over the different growth periods in the field growth experiment. The table shows only combinations where variability was significant.

Period	(I) Taxon	(J) Taxon	Mean Difference (I-J)	Std. Error	Sig.
Jan-Dec	Hybrid	*Disphyma*	237.8	62.1	**.007**
Sep-Dec	*C. chilensis*	*Disphyma*	55.7	20.2	**.043**
	Hybrid	*Disphyma*	104.8	17.5	**<.001**
		C. edulis	58.1	16.4	**.006**
	C. edulis	*Disphyma*	46.6	16.5	**.036**

Lengths of the main axes stolons did not differ between the taxa, except during the September-December period ($F_{3, 36.5}$=7.588; *P* < 0.01; Table 3.10). However, there was a highly significant variability among plants of the same taxon over all of the growth periods (all periods *P* < 0.001), except *Disphyma australe* plants which differed in the lengths of their main axes only over the September-December period ($F_{9, 29}$=2.329; *P* = 0.041). In both the January-September and January-December periods, only the hybrid grew longer than *D. australe* (*P* = 0.003 and 0.008 respectively). During the September-December period, all the other taxa lengthened more than *D. australe* (least significant *P* = 0.029) and the hybrid lengthened more than *C. chilensis* (*P* = 0.026) while the two *Carpobrotus* species did not differ in length (Fig. 3.13; Table 3.11).

Table 3.10: Nested ANOVA table showing lengths of the main axes stolons did not vary among the taxa except in the September-December period but a highly significant variability among plants of the same taxon in each growth period.

Period	Source	Error df	df	MS	F	Sig.
Jan-Dec	Taxon	9.804	3	16815.9	2.888	.090
	Plant(Taxon)	111	11	3415.2	7.995	**<.001**
Jan-Sept	Taxon	9.902	3	7659.5	2.658	.106
	Plant(Taxon)	111	11	1681.7	8.731	**<.001**
Sep-Dec	Taxon	36.473	3	3586.7	7.588	**<.001**
	Plant(Taxon)	296	35	562.8	10.331	**<.001**

Fig. 3.13: Mean lengths of stolons of each taxon over the various growth periods in the field growth experiment. The letters below the bars indicate variability among taxa at significance levels of $P < 0.05$.

Table 3.11: A summary Tukey HSD test table comparing mean stolon lengths of plants of the different taxa over the various growth periods in the field growth experiment. The table shows only where significant variability was found.

Period	(I) Taxon	(J) Taxon	Mean Difference (I-J)	Std. Error	Sig.
Jan-Dec	Hybrid	*Disphyma*	54.2	12.2	**.003**
Jan-Sep	Hybrid	*Disphyma*	32.7	8.6	**.008**
Sep-Dec	*C. chilensis*	*Disphyma*	11.1	3.8	**.029**
	Hybrid	*C. chilensis*	11.3	3.8	**.026**
		Disphyma	22.4	3.3	**<.001**
	C. edulis	*Disphyma*	15.8	3.1	**<.001**

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The number of nodes along the main axes and lateral branches differed significantly among the various taxa during all the periods (least significant: $P = 0.037$; Table 3.12). Similarly, there was a high degree of variability among plants of the same taxon in all the periods ($P < 0.001$) except for *Disphyma australe* plants, which did not show variability in any of the growth periods. The hybrid plants generally produced more nodes than the other taxa in all the growth periods except for *C. chilensis* in the September-December period ($P = 0.057$; Fig. 3.14; Table 3.13). There was no difference among the other taxa in the number of their nodes in any of the periods.

Table 3.12: Nested ANOVA table showing significant variability among taxa and among plants of the same taxon in the total number of their nodes over each growth period.

Period	Source	df	MS	F	Sig.
Jan-Dec	Taxon	3, 9.283	235832.1	4.322	**.037**
	Plant(Taxon)	11, 111	32888.3	5.505	**<.001**
Jan-Sept	Taxon	3, 9.251	22370.3	5.147	**.023**
	Plant(Taxon)	11, 111	2623.9	5.398	**<.001**
Sept-Dec	Taxon	3, 36.848	84468.7	8.394	**<.001**
	Plant(Taxon)	35, 296	11919.3	8.252	**<.001**
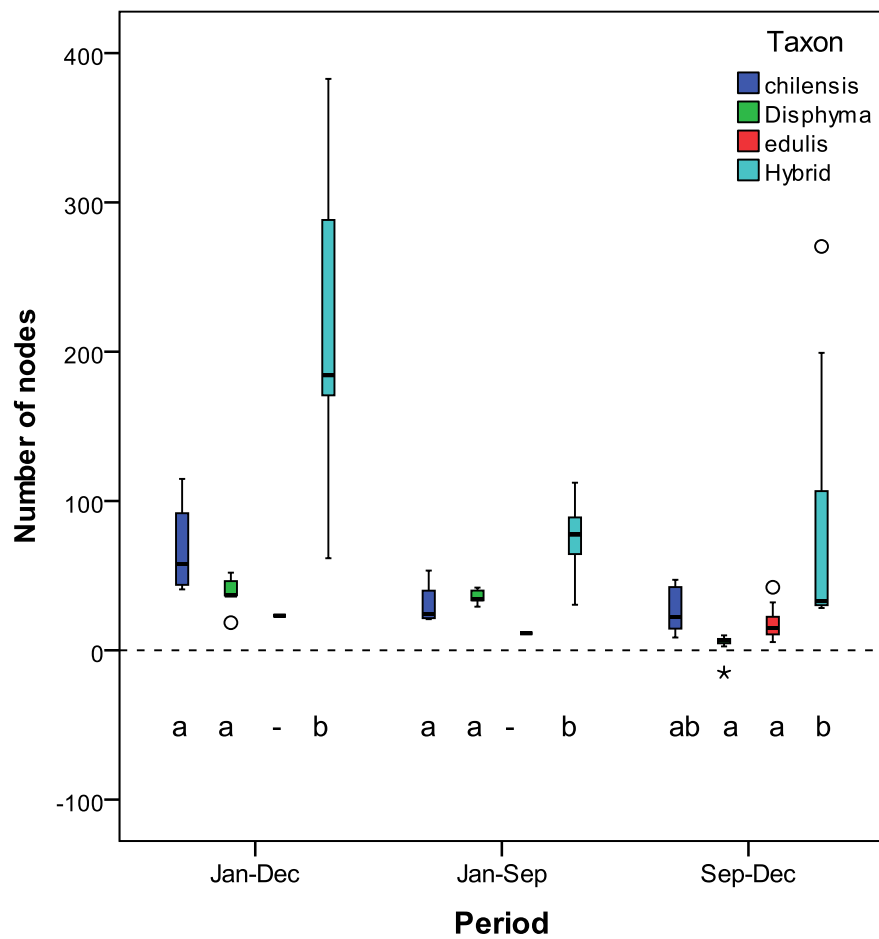
Fig. 3.14: Net number of nodes produced along the main axes of stolons of plants of each taxon during the various growth periods in the field growth experiment. The letters below the bars indicate variability among taxa at significance levels of *P* < 0.05.

Table 3.13: A summary Tukey HSD test table comparing the aggregate number of nodes produced along the main stolons and lateral branches by plants of the various taxa over the different growth periods in the field growth experiment. The table shows only where variability was significant ($P <$ 0.05) or approaching significance.

Period	(I) Taxon	(J) Taxon	Mean Difference (I-J)	Std. Error	Sig.
Jan-Dec	Hybrid	*C. chilensis*	149.7	51.1	**.034**
		Disphyma	179.5	48.2	**.009**
Jan-Sep	Hybrid	*C. chilensis*	44.0	13.6	**.020**
		Disphyma	39.0	12.8	**.027**
Sep-Dec	Hybrid	*C. chilensis*	60.5	22.9	.057
		Disphyma	82.4	19.9	**.001**
		C. edulis	68.2	18.7	**.004**

Number of lateral branches produced along the tagged main axes differed significantly among the taxa as well as between plants within a taxon during all or some of the growth periods (least significant P = 0.007; Table 3.14). The hybrid produced more lateral branches than *C. chilensis* in all the periods ($P <$ 0.023) and more than *D. australe* in January-December and September-December (both periods $P <$ 0.002; Fig. 3.15; Table 3.15). *Carpobrotus* spp. produced more lateral branches than *D. australe* during September-December ($P <$ 0.002). There were no differences in number of lateral branches between *C. edulis* and the hybrid and between the two *Carpobrotus* species in September-December.

Table 3.14: Nested ANOVA table showing that there was significant variability in the number of lateral branches produced by plants of the various taxa and among plants of the same taxon in all the growth periods in the field growth experiment.

Period	Source	df	MS	F	Sig.
Jan-Dec	Taxon	3, 9.283	2672.1	7.795	**.007**
	Plant(Taxon)	11, 111	207.8	5.160	**<.001**
Jan-Sept	Taxon	3, 9.251	1185.8	7.032	**.010**
	Plant(Taxon)	11, 111	102.7	4.934	**<.001**
Sept-Dec	Taxon	3, 36.848	775.1	11.804	**<.001**
	Plant(Taxon)	35, 296	78.0	9.180	**<.001**

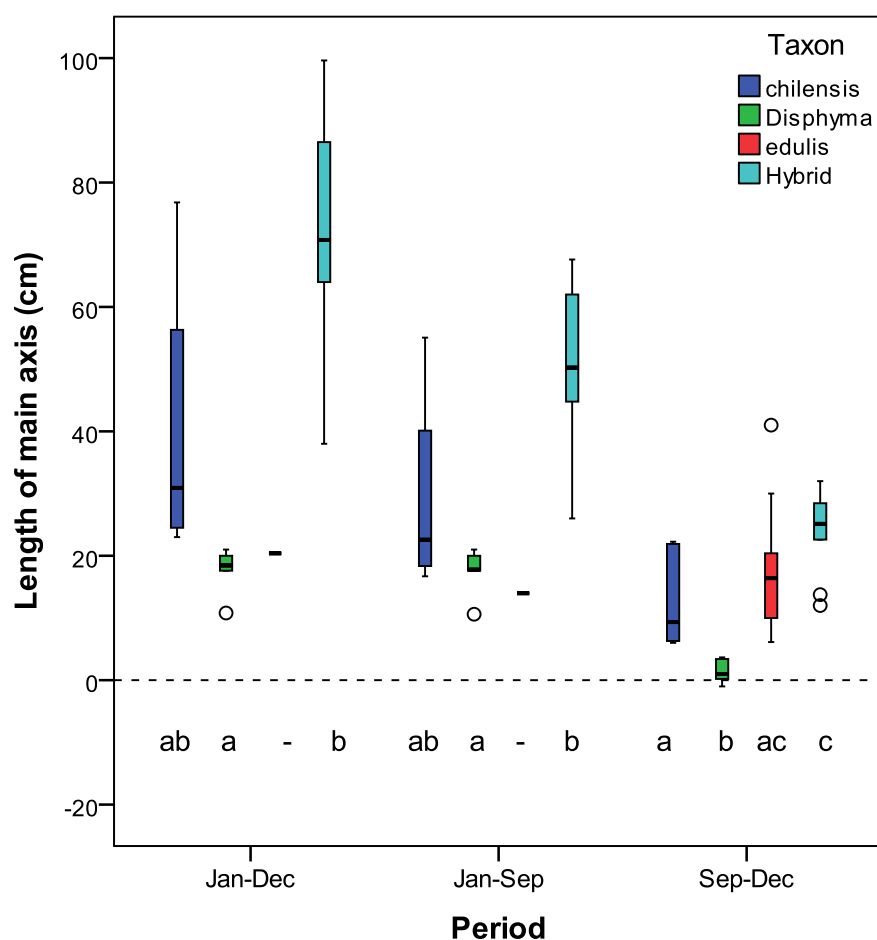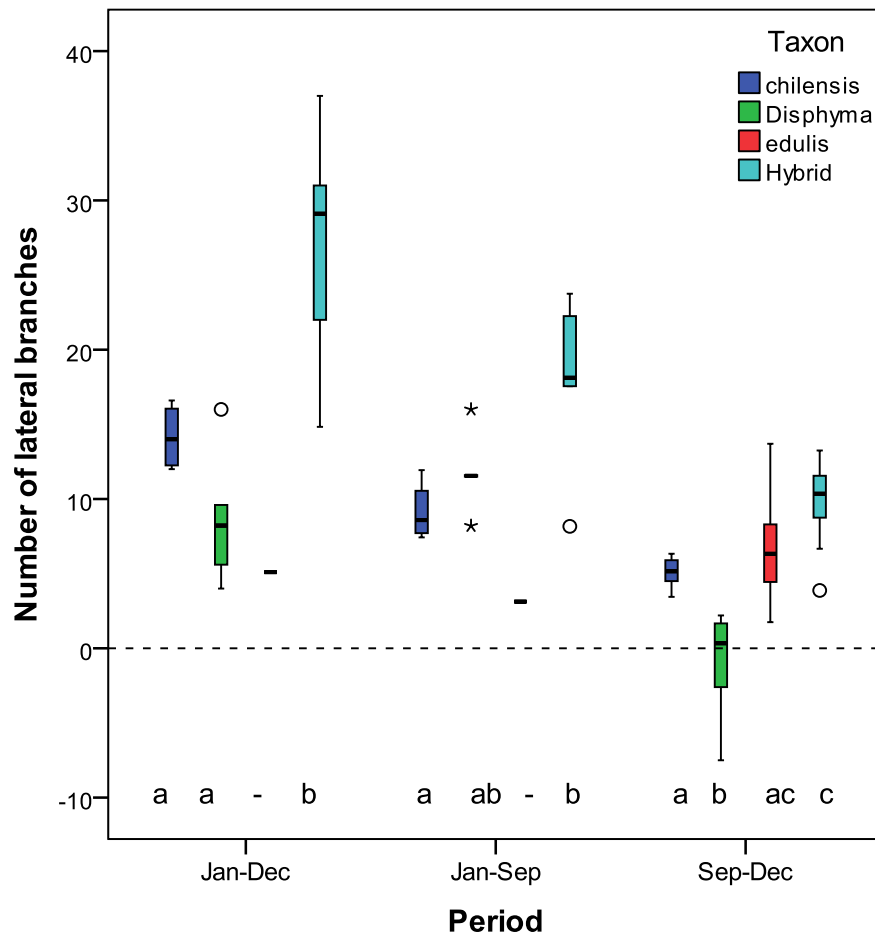Fig. 3.15: Number of lateral branches produced by a stolon of plant of each taxon over the various growth periods in the field growth experiment. The letters below the bars indicate variability among taxa at significance levels of *P* < 0.05. *Disphyma australe* showed loss of lateral branches in the September-December period.

Table 3.15: A Tukey HSD table comparing the mean number of lateral branches produced by plants of the various taxa in the different growth periods in the field growth experiment. The table only summarises where variability was significant.

Period	(I) Taxon	(J) Taxon	Mean Difference (I-J)	Std. Error	Sig.
Jan-Dec	Hybrid	*C. chilensis*	12.6	4.0	.023
		Disphyma	18.1	3.8	.002
Jan-Sep	Hybrid	*C. chilensis*	8.8	2.8	.023
Sep-Dec	*C. chilensis*	*Disphyma*	5.9	1.5	.002
	Hybrid	*C. chilensis*	4.7	1.5	.019
		Disphyma	10.6	1.3	<.001
	C. edulis	*Disphyma*	7.4	1.2	<.001

The sample stolons that were destructively harvested and their dry weights measured showed strong positive correlation between each of the measured parameters and the dry weight for each taxon. Similar observations were made for the experimental cuttings. Therefore, these parameters were all put in a multiple regression model to generate an equation for estimation of dry weight of the shoots of the field plants (Table 3.16). The estimated biomass was then analysed using a two-level nested ANOVA analysis with taxon as a fixed factor and individual plants as a random factor (Table 3.17). There was a highly significant variability among the different taxa ($F_{3, 39.5}$=9.192; $P < 0.001$) as well as among sample plants of the same taxon ($F_{35, 548}$=4.885; $P < 0.001$).

Table 3.16: Summary of multiple linear regressions used to estimate shoot dry weights of plants in the field growth experiment. The data were derived from number of leaves, number of nodes and main axis length of common-garden cuttings, and the sample stolons harvested from the field.

Taxon		Unstandardized Coefficients		T	Sig.
		B	Std. Error		
C. chilensis	(Constant)	.920	.185	4.975	**<.001**
	Leaves	.184	.038	4.807	**<.001**
	Nodes	.065	.107	.614	.541
	Length	.066	.070	.939	.351
C. edulis	(Constant)	1.346	.206	6.538	**<.001**
	Leaves	.247	.049	5.054	**<.001**
	Nodes	-.017	.128	-.130	.897
	Length	-.018	.075	-.248	.805
D. australe	(Constant)	.167	.141	1.184	.242
	Leaves	.016	.003	5.998	**<.001**
	Nodes	-.008	.028	-.284	.778
	Length	.038	.013	2.953	**.005**
Hybrid	(Constant)	.350	.103	3.388	**.001**
	Leaves	.032	.004	8.406	**<.001**
	Nodes	.004	.005	.788	.434
	Length	.035	.006	5.950	**<.001**

Table 3.17: Nested ANOVA table showing variability in estimated shoot dry weights among the various taxa and plants of each taxon in the field growth experiment.

Source	df	Mean Square	F	Sig.
Taxon	3	3477.3	9.192	**<.001**
Plant(Taxon)	35	464.8	4.885	**<.001**
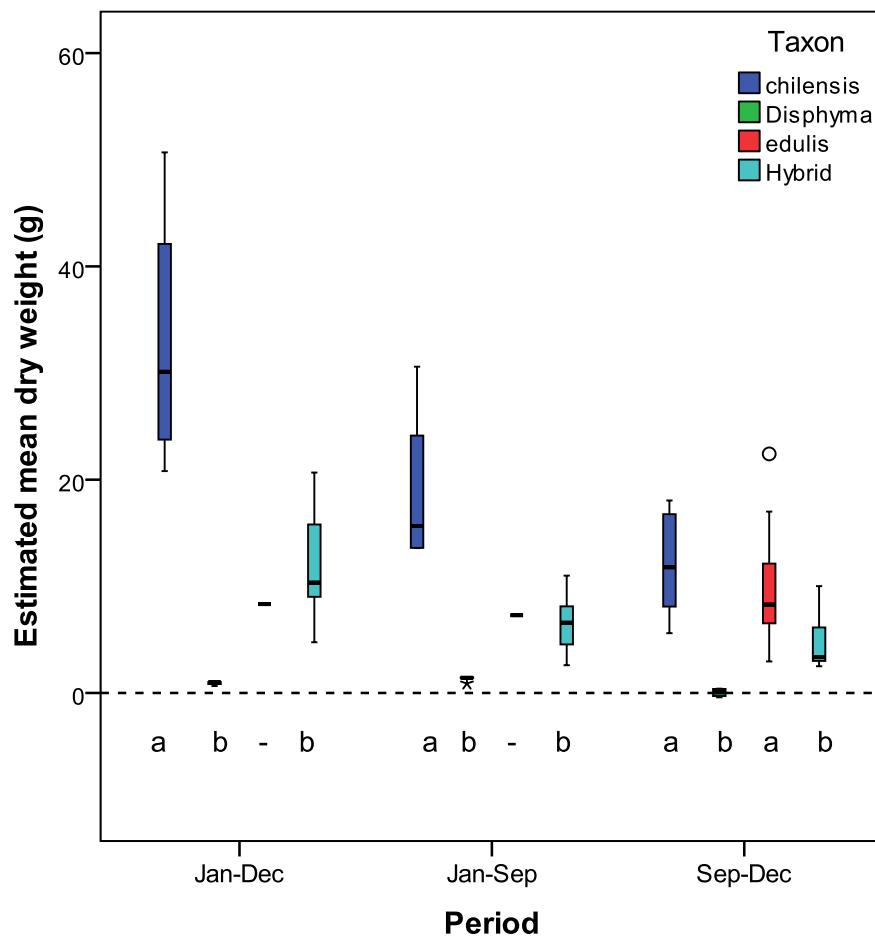
Fig. 3.16: Estimated shoot dry weight put on by the sample plants of each taxon over the various growth periods in the field growth experiment. The different letters below the bars indicate variability among taxa at $P < 0.01$. *Carpobrotus edulis* was excluded from the comparison in the Jan-Dec and Jan-Sep periods because there was only one sample plant.

On aggregating the dataset and running a post hoc test to discriminate the variability among taxa, it was found that all the other taxa had a significantly higher estimated dry weight than *D. australe* ($P < 0.001$; Fig. 3.16). *Carpobrotus edulis* did not vary from *C. chilensis* but both had a higher dry weight than the hybrid as well ($P < 0.05$; Table 3.18).

Table 3.18: A Tukey HSD table comparing the means of the aggregate estimated dry weights put on by the shoots of the various taxa over the different growth periods in the field growth experiment. The table summarises only where variability between taxa was significant.

Period	(I) Taxon	(J) Taxon	Mean Difference (I-J)	Std. Error	Sig.
Jan-Dec	*C. chilensis*	*Disphyma*	32.0	5.2	**<.001**
		Hybrid	20.8	5.2	**.005**
Jan-Sep	*C. chilensis*	*Disphyma*	17.5	3.1	**<.001**
		Hybrid	12.3	3.1	**.006**
Sep-Dec	*C. chilensis*	*Disphyma*	12.0	2.1	**<.001**
		Hybrid	7.4	2.1	**.005**
	C. edulis	*Disphyma*	9.9	1.7	**<.001**
		Hybrid	5.4	1.7	**.015**

While *C. edulis* is bigger in stature, its biomass was not higher than that of *C. Chilensis,* probably because it stores more water than organic compounds, as shown by the ratio of dry weights to fresh weights of the harvested sample stolons (Fig. 3.17).
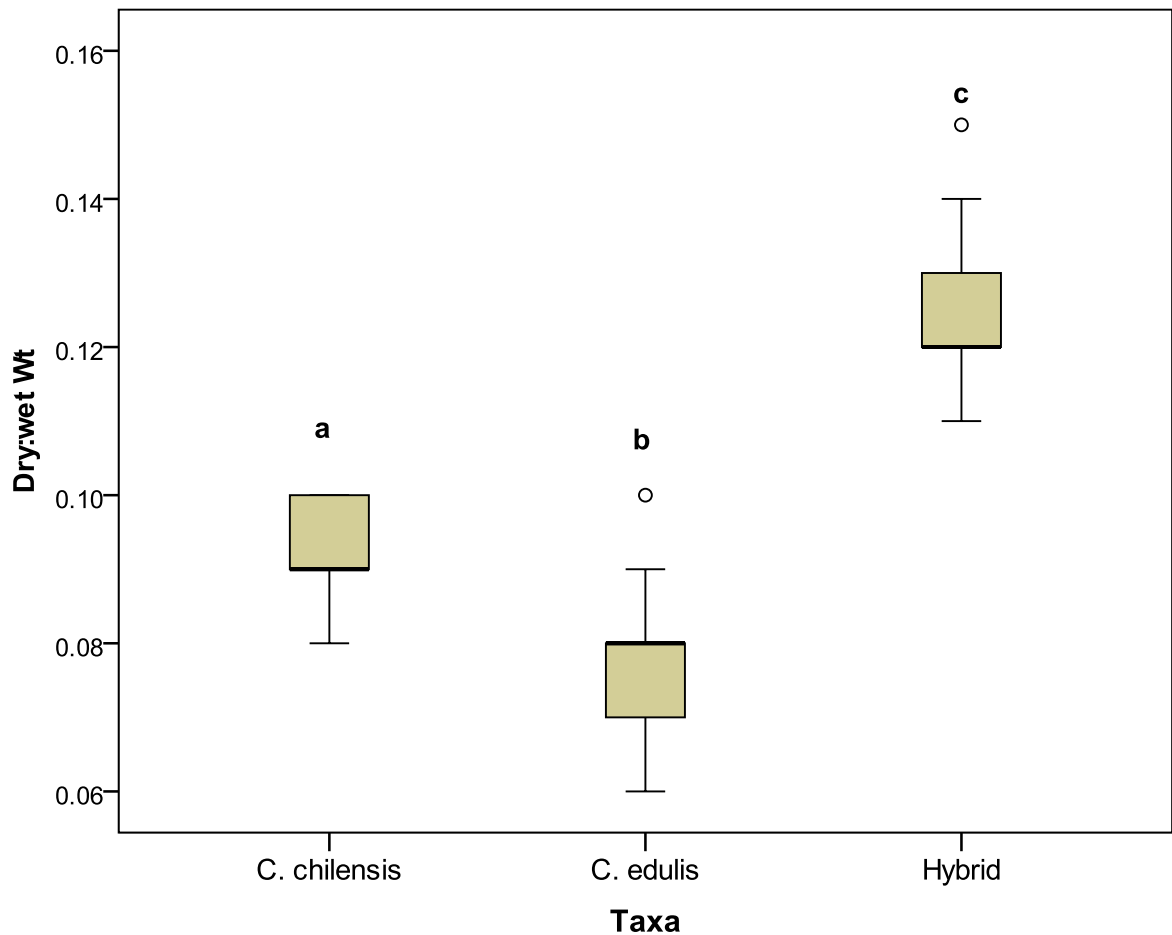
Fig. 3.17: The ratios of shoot dry weight of the stolons harvested from the field to their fresh weight showing that the hybrid was the least succulent, with the greatest dry weight: fresh weight ratio.

3.5.3. Breeding systems

The main flowering season was from September to November with peak flowering for all the taxa being October-November (Fig. 3.18). A few plants continued flowering into December. Some *C. edulis* plants also flowered from May to June, although at much lower density and at fewer sites.
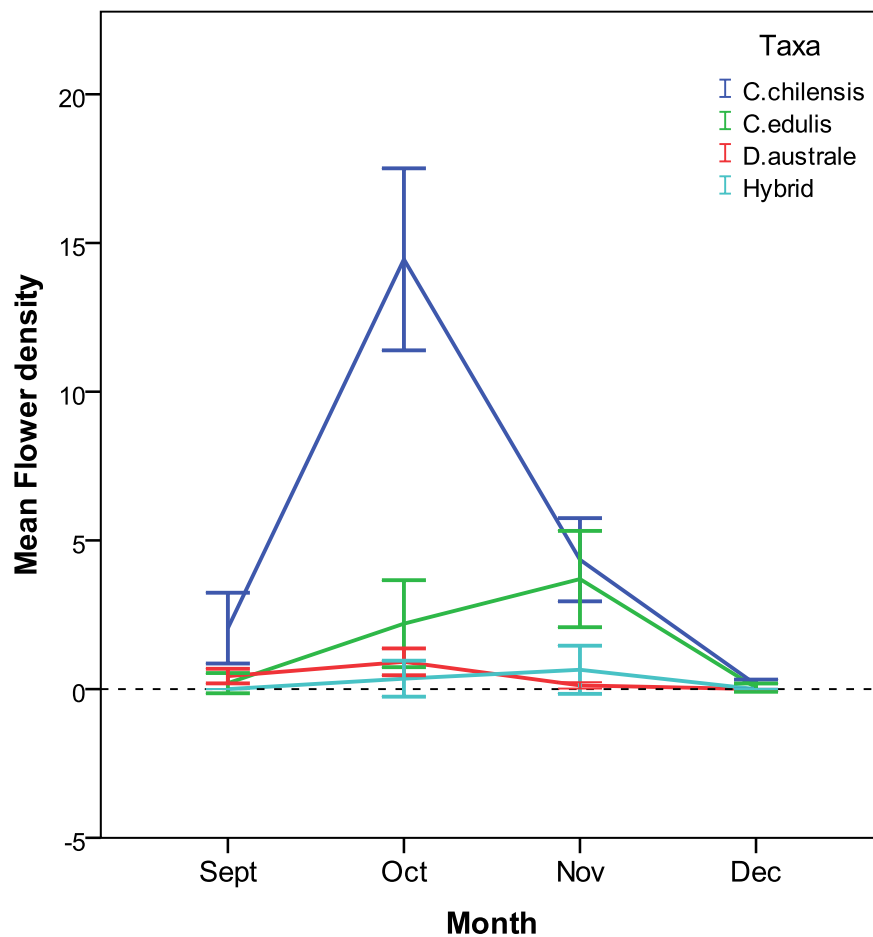
Fig. 3.18: Mean flower density per sample plant (number of flowers/m^2) of the various taxa during the flowering season of the year 2011.

It is important to note that although at least ten flower buds were assigned to each breeding treatment per taxon, not all were sustained to maturity due to human interference. The two exotic iceplant taxa responded differently to the mating experiments in terms of seed set ($F_{4, 60}$=15.833; $P < 0.001$). *Carpobrotus edulis* fruits did not produce any seeds in the agamospermy treatment while less than a third of fruits produced an average of only two seeds each in the spontaneous selfing treatment. In artificial selfing, three fruits from one plant produced seeds while the fruits of the other plant did not. *Carpobrotus edulis* produced a lot of seeds when supplied with pollen of *C. chilensis* and *D. australe.* *Carpobrotus chilensis* plants, on the other hand, responded positively to all treatments, although at low levels for agamospermy, where one of the two fruits produced only a single seed and the other produced 36 seeds (Table 3.19; Fig. 3.19).

Table 3.19: Descriptive statistics of number of seeds produced per fruit by the two *Carpobrotus* species subjected to different breeding treatments.

Taxon	Statistic	Agamospermy	Spontaneous selfing	Artificial selfing	X congener	X *D. australe*
C. chilensis	Mean ± S.D.	18.5 ± 24.7	442.0 ±87.2	317.2±97.5	377.6 ± 114.6	231.1 ± 36.9
	Range	1 - 36	32 5 - 568	212 - 422	234 - 545	160 - 285
	N	2	5	5	7	10
C. edulis	Mean ± S.D	.00	2.0 ± 3.5	77.7 ± 98.9	1310.4±694.5	493.8±323.0
	Range	0	0 - 9	0 - 202	805 - 2663	55 - 1095
	N	7	9	7	9	10
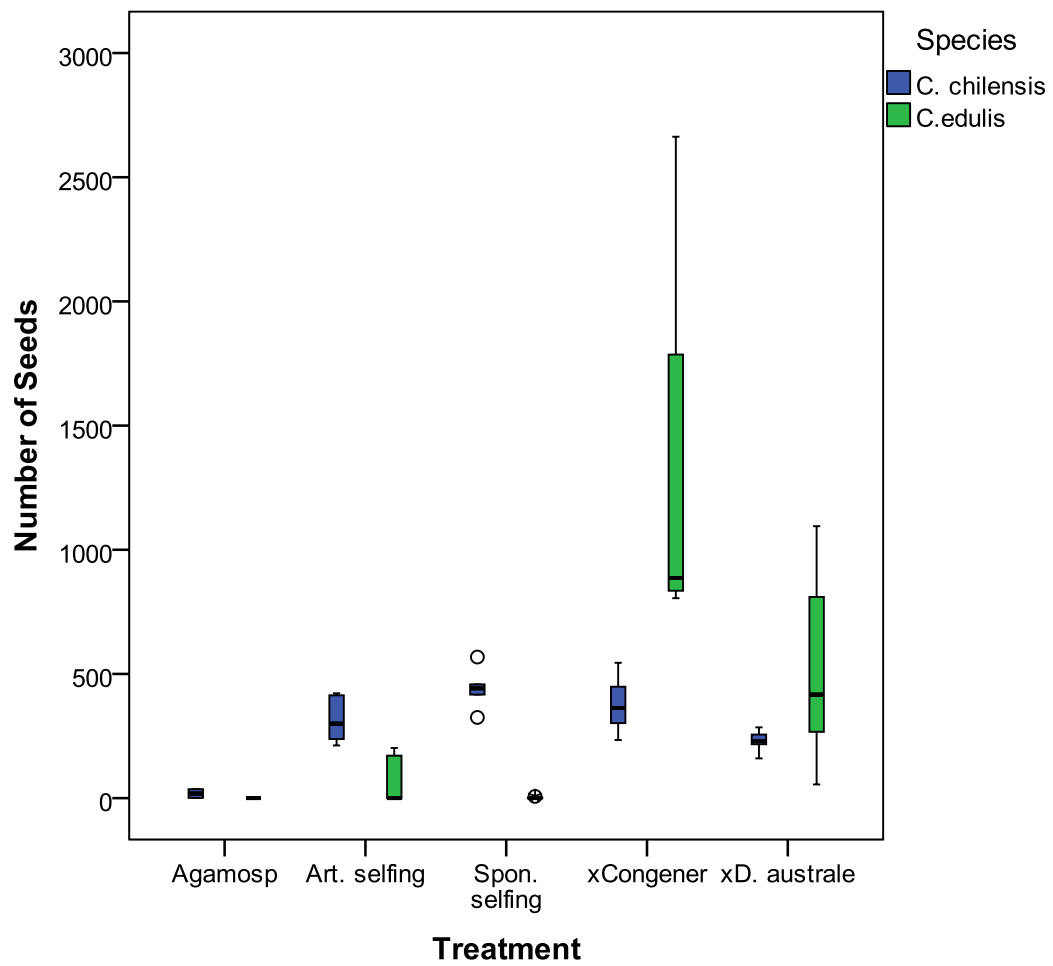
Fig. 3.19: Number of seeds per fruit produced by each of the two *Carpobrotus* species subjected to the different breeding treatments.

3.6. DISCUSSION

3.6.1. Growth comparison and substrate preference

The results of the experimental study showed that there was no difference in the final biomass of the two *Carpobrotus* spp., regardless of the substrate, although *C. edulis* cuttings had a higher indicative initial biomass. In a field study in several Mediterranean islands, (Traveset, Brundu et al. 2008) found that *Carpobrotus* species displayed similar growth rates in dune systems and rocky shores, concluding that their growth rate was more dependent on microsite type than on habitat type (Traveset, Brundu et al. 2008). The two exotics performed comparably in the field growth monitoring study. The actual and estimated final dry weights of the *Carpobrotus* cuttings were significantly higher than those of their hybrid and *D. australe* counterparts. This was not surprising because the same trend was evident from the initial biomass of the sample cuttings. The hybrids had significantly higher final dry weights (actual and estimated) than *D. australe* plants even though the initial dry weights of their sample cuttings did not differ. *Carpobrotus chilensis* cuttings growing in sand accumulated significantly higher dry weight and produced more leaves than those growing in gravel, showing some substrate preference. In terms of actual biomass allocation, *Disphyma australe* allocated relatively more to the roots than the shoot, compared to the other taxa, while the hybrid also invested relatively more in the roots than the two *Carpobrotus* spp.

In the present study, stolons of *Carpobrotus* spp. elongated by up to 130 cm over the eleven month field growth period, although there was high variability among stolons because of smothering of some by others. Similarly, the hybrids elongated by up to 127 cm over the same time period. The main stolons of iceplant species have the capacity to form functional individuals (ramets) by rooting at some nodes and spreading independently (Traveset, Moragues et al. 2008). The hybrids had the highest mean number of nodes along the main axes and lateral branches that could potentially root and form ramets. Such ramets act as cooperative entities with efficient distribution of tasks that can lead to enhanced resource exploitation in heterogeneous habitats (Stuefer, DeKroon et al. 1996).

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This kind of clonal growth has been proposed as one of the most successful strategies in the plant world (Traveset, Moragues et al. 2008), which together with other life history traits (Mack 2003) has been shown to increase the probability of successful invasion (Heger and Trepl 2003). The exotics and the hybrid (*xCarpophyma mutabilis* Heenan and Sykes 2010) seem to grow more aggressively than the native *D. australe*, whose longest stolon was 30cm. The high stolon elongation and branching rates allow the exotics and hybrids to rapidly occupy and dominate space (Sammul, Kull et al. 2004; Sintes, Moragues et al. 2007). Clonality further enhances access to unevenly distributed resources, thereby conferring competitiveness with subsequent negative consequences for the native flora (Stuefer, DeKroon et al. 1996).

The average number of lateral branches produced by an individual stolon over the period of just under a year was 16 for the hybrid, 9 for *C. chilensis*, 4 for *D. australe* and 5 for *C. edulis*. Lateral branches are vegetative propagules, which are an alternative means of dispersal that subsequently root and produce new individuals (Traveset, Moragues et al. 2008). It is suggested that vegetative propagules generally result in larger-sized plants with higher survival and growth rates than seeds (Nishitani, Takada et al. 1999). Success of iceplant propagules is further enhanced by clonal integration with the parent plant since resources can be translocated from the established parent to the developing ramets (Price and Marshall 1999). It is therefore not surprising that the hybrid, being sexually sterile, seems to have invested a lot in lateral branches as its only means of proliferation.

The parent plants (sites) from which the cuttings were obtained did not seem to have conferred differential advantages except for the hybrid plant from Moa point, whose cuttings performed better than the others in terms of total biomass. The two varieties of *D. australe* (green and red), did not differ in their performance in either of the substrate types, even though in nature the green one seems to predominate at rocky shores (K. Gould pers. comm.). *Disphyma australe* plants in the field growth study showed some negative growth for the period of September to December in all the measured variables, mainly as a result of smothering by the hybrid plants with which they grow at the study site.

The revelation by nested analysis that there was a high degree of variability among plants of the same taxon in all the measured characters suggests that, in future studies, more sample plants are needed to increase the sample size. However, the variability among plants in the current study may also be explained in terms of habitat heterogeneity (especially for *C. Chilensis*), where some plants were at the backdune where substrate is sandy, while others were at the foredune where soil structure is predominantly shingles. Furthermore, plants at the backdune, where the substrate is more stable, were intermingled with other plants that would normally curtail their growth compared to those at the foredune where storm and wave disturbance would ideally eliminate biotic resistance by removing the native residents.

The Eastbourne site where *Carpobrotus* spp were growing is about two kilometres from Day's Bay where *D. australe* and the hybrids were. The climatic conditions of these two sites are quite similar and not much ecotypic difference was expected.

3.6.2. Mating systems

A highly flexible mating system can be a key element in the establishment and success of invasive plants (Baker 1955). Aizoaceae species have been shown to have a high capability for intrageneric and intergeneric hybridization (Chinnock 1972; Hammer and Liede 1990). Mating experiments in the present study have shown that *Carpobrotus* species are highly fertile to intrageneric and intergeneric pollen.   However, *C. edulis* was not agamospermic but slightly self-fertile and needing intervention of a pollinator, while *C. chilensis* was slightly agamospermic and self-fertile, contrary to findings of (Vilà, Weber et al. 1998). It has been pointed out that ideal weeds (and by extension invasive plants) are self-compatible, but not completely autogamous or apomictic (Baker 1965 as cited by Vilà, Weber et al. 1998). Unfortunately, there was no time to germinate the seeds to test their viability, even using laboratory techniques. Self-fertilization and agamospermia are said to give the advantage for invasive species to start a seed-reproducing colony from a single individual and to build up a population quickly (Vilà, Weber et al. 1998). According to morphometric (Albert et al. 1997) and isozyme analyses (Gallagher et al. 1997), *C. chilensis*

appears to be hybridizing and backcrossing with *C. edulis* individuals (Vila and D'Antonio 1998), casting doubts on the species integrity of our experimental plants, whose exact source is unknown. In New Zealand, yellow-flowered *C. edulis* and magenta-flowered *C. chilensis* co-occur at most sites but there are no presence records of their intrageneric hybrids (Heenan and Sykes, 2010). Due to the general lack of morphometric differences, it seems possible that they may in fact be two colour morphs of a *C. edulis* and *C. chilensis* hybrid swarm.

Both *Carpobrotus* species in our breeding experiment were highly fertile to intergeneric *D. australe* pollen. Hybridization between native and non-native plants may result in invasive hybrid morphotypes that, in turn, have dramatic effects on the communities in which they occur (Thompson 1991). The hybrid cuttings in my trials performed better than those of the other taxa in the characters relating to vegetative growth such as number of leaves, number of nodes, main axes length and internode length. In the field growth study, performance of the hybrids, in terms of growth characters, was always better or comparable to those of the *Carpobrotus* spp. For example, they grew longer and produced more leaves than *C. edulis* but were not different from *C. chilensis*. Additionally, they produced more nodes and lateral branches than both *Carpobrotus* spp. The differential fitness of hybrid and parental genotypes can have important evolutionary consequences (Ellstrand and Schierenbeck 2000). Hybridizations between formerly allopatric species may result in new alien-derived genotypes (Verlaque, Affre et al. 2011) that are frequently competitive (Vilà, Weber et al. 2000) or stress tolerant (Milne and Abbott 2000). They may differently affect the structure and function of native ecosystems (Verlaque, Affre et al. 2011). For instance, *Spartina anglica* has been shown to cause sediment rise by accumulating large volumes of tidal sediment, making intertidal habitats more terrestrial (Ainouche, Fortune et al. 2009). Hybridisation has the potential to create highly aggressive genotypes that may be undesirable in terms of management for native species (Ellstrand 1992; Rhymer and Simberloff 1996). X*carpophyma mutabilis*, the intergeneric hybrid of *C. edulis* and *D. australe* is a perennial and clonal plant. Perennial hybrids will persist longer, giving more time for genetic stabilization opportunities to occur, especially if clonal reproduction is available (Ellstrand and Schierenbeck 2006). Clonality on

the other hand, would tend to stabilize lineages that would suffer sterility as F1 hybrids while fixing hybridity and novelty (Grant 1981). Alien species and their native-derived hybrids can also influence the evolution of natives by exposing them to novel interactions (Parker, Simberloff et al. 1999). For example, *Carpobrotus edulis* has been shown to interact indirectly with native vegetation by altering soil chemistry (Conser and Connor 2009), thereby exposing the natives to novel soil micro-conditions.

3.6.3. Implications of the Hybrid (x*Carpophyma mutabilis* Heenan and Sykes 2010) for *D. australe*

The field growth monitoring and experimental growth of the cuttings have shown that the hybrid is at least as aggressive as its exotic parent in space colonisation. *Disphyma australe* at the Day's Bay site seem to have been negatively affected by the hybrid where the hybrids grew over their stolons, smothering them in some seasons of the year. In addition, the fact that the hybrid cuttings in the common garden experiment did quite well in all the substrate types may mean that they are capable of growing in the same natural habitats as *D. australe*. Hybrid vigour for vegetative growth is thought to contribute to displacement of parental morphotypes or native species (Vilà and D'Antonio 1998). More research needs to be done on how the hybrids influence growth and survival of *D. australe*. If they were to grow intermingled, *Disphyma australe* is likely to stand no chance against the exotic mat-forming (Albert, D'Antonio et al. 1997)*Carpobrotus* spp. that have also been shown to crawl over other vegetation (D'Antonio 1990).

In other systems, hybrid genotypes originating from *C. edulis* and native congeners have been shown to present a major obstacle for managers (Vilà and D'Antonio 1998; Schierenbeck and Ellstrand 2009) with negative implications for the conservation of native species assemblages (Vilà, Weber et al. 1998). Human mediated gene flow between congeners or conspecific populations is regarded as a form of biotic homogenization both at the genetic and community level (Olden, Poff et al. 2004). However, little is known about how non-native taxa or the spread of their foreign alleles to native taxa may affect local

adaptation of a species, community composition, or ecosystem function (Levine, Vilà et al. 2003).

3.7. Conclusion and implications for management

The growth rates of the iceplant taxa in my common garden experiment suggest that all of them are capable of colonizing both sandy and shingle coastal habitats, although *C. chilensis* performed comparatively worse in gravel. The intergeneric hybrid displayed more aggressive growth characteristics than its putative parents but its stolons are slender and more sparsely spread, with sufficient spaces for other vegetation to grow. However, it negatively affected growth of *D. australe* in the field at a site where they were planted in an intermingled pattern. It is therefore possible that the exotic iceplant and the intergeneric hybrid may pose serious threats to native coastal plants. Thus, it is important to investigate the influence of other environmental filters such as salinity and sand burial on these taxa to be able to predict in which habitats they are more likely to be a management problem. Such a study would also help determine the ability of *D. australe* to tolerate sand burial and therefore its suitability for sand dune restoration. Due to its sexual sterility, the intergeneric hybrid is easier to manage since it does not leave behind any seed legacy and is therefore more suitable than *Carpobrotus* spp. for dune stabilisation.

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Chapter four

4.   Overall   conclusions   and recommendations

Fig. 4.1: *XCarpohyma mutabilis* (foreground) on the beach at Day's Bay, Wellington accumulating some sand. Photo taken by author in April 2012

4.1. Interactive effects of *C. edulis* on *Spinifex sericeus* and its role in sand dune restoration

This was a neighbour removal experiment aimed at investigating the effects of *C. edulis* on survival and growth of *Spinifex sericeus*. Presence or absence of *C. edulis* did not have any effect on initial *Spinifex* seedling survival. However, seedling survival was significantly higher at the sub-plots nearer the high tide water mark than those at the top. Given that the formative period after planting was characterised by drought, the only confounding factor that could have been responsible for varied survivorship at the two sub-plots is soil moisture, attributable to inundation during high tides, strong wave splashes, and water draining from inland.

Spinifex seedling survival was consistently higher in the removal plots (iceplant removed) until the July storm (eight months after planting), although the difference was not statistically significant. In addition, seedlings in these plots produced significantly higher numbers of leaves than those in plots of the other treatments. This suggests that the previously extant iceplant may have improved soil micro-conditions to which the incoming seedlings responded positively.

The most important determinant of *Spinifex* seedling survival in this experiment was storm disturbance. *Spinifex* plants within iceplant stands survived storm disturbance better than those in the removal and bare plots because of the soil-binding nature of the extensive fibrous root system of *C. edulis*. Seedlings in the removal plots were the worst affected by the storm because of the steeper slope built by sand accretion activity of the previously extant iceplant. Furthermore, these plots were rendered less stable by the removal of the iceplant. Steep slopes are generally more prone to slumping and eventual formation of vertical scarps after storm undercutting events. Location of the seedlings (toward the top or bottom of the dune face) also mattered since storm disturbance removed plants closest to the sea.

Other environmental conditions that limit growth and survival of plants on coastal dunes are sand-blasting and salt sprays. Sandblasting brings about abrasive damage to plant leaves, reducing photosynthetic surface and predisposing plant to infections. Salt

spray, on the other hand, has been observed to cause leaf necrosis beginning at the tips (Wilson and Sykes 1999). In this experiment, *Spinifex* seedlings in the removal and bare treatments had their leaves dying back from the tips, unlike those seedlings growing within the iceplant stands. This indicates that seedlings growing with iceplant neighbours were buffered from the harmful effects of sandblasting and salt sprays. Therefore, the expectation that facilitation would be the dominant effect of extant adult nurse plants on seedlings of other species at the harsher foredune zone (Padilla and Pugnaire 2006) seems plausible, at least in buffering against abrasive damage by sandblasting and necrosis due to salt spray.

Despite their better survival and less damage of leaf tips, *Spinifex* plants in the iceplant treatment (iceplant neighbour present) had significantly lower numbers of leaves and appeared more slender; confirming that presence of iceplant suppresses growth performance of native species (D'Antonio and Mahall 1991; D'Antonio, Odion et al. 1993). Salinity has been reported to cause internal plant toxicity (Wilson and Sykes 1999). In this study, soil salinity was higher under the canopy of *C. edulis* since salt particles trapped by leaves during salt sprays are then leached into the soil (Gormally and Donovan 2010). Accumulation of salts in leaves and eventual release into the soil after leaf senescence has been suggested as a mechanism by which *Mesembryanthemum crystallinum* (another member of Aizoaceae) contributes to desiccation and death of neighbouring plants (Conser and Connor 2009). *Carpobrotus edulis* has also been reported to have led to loss of floristic diversity at Berlangas, Portugal (Draper, Rossello-Graell et al. 2003). My inference from this is that *C. edulis* uses similar mechanisms to *M*. *crystallinum* to alter soil osmotic levels, to the detriment of neighbouring native plants. Comparison of the number of leaves produced by seedlings at the different sub-plots within a treatment showed that plants at the bottom of the iceplant plots had significantly higher numbers of leaves than their counterparts at the top of the dune, suggesting that competition from iceplant was less severe in the environmentally harsher section of the dune, and thus supporting the stress gradient hypothesis (Bertness and Callaway 1994; Brooker and Callaghan 1998).

Using dead debris of iceplant as a mulching material did not improve *Spinifex* survivorship. Presence of dead iceplant debris resulted in production of more leaves by

plants at the top of the dune only in August. The fact that location of the plants did not have much effect on their performance means that the 2-4m space between plants at the bottom and the top of the dune was not enough to produce a gradient to which plants respond differentially. This is confirmed by the lack of difference in soil moisture, soil organic matter and salinity between the two sampling locations separated by 5-8m.

It has been suggested that *C. edulis* has residual effects on the soil inhibiting reestablishment of native plant species even after its removal (Conser and Connor 2009). *Spinifex* seedlings in my removal plots (where iceplant had been present) produced significantly higher numbers of leaves than plants in the other treatments. While this improved response may be attributed to elimination of competition, plants in the removal plots actually performed better that those in bare plots where there was no preexisting iceplant. This suggests that *C. edulis* did not leave an allelopathic legacy, at least in the short-term but actually improved soil micro-conditions.

4.2. Growth, substrate preference and mating systems of the various iceplant taxa

The common garden experiment was set up to investigate growth performance of the three iceplant species in New Zealand and the intergeneric hybrid across three different substrates. The substrates in the experiment were made of one part potting mix and two parts sand or gravel, making them similar in initial fertility. However, because of differences in particle and interstitial pore size there would be variability among them in drainage and nutrient adsorption. It has been suggested that soil texture influences soil Carbon (C) and Nitrogen (N) accumulation as well as soil water dynamics (Hook and Burke 2000). Fine-textured soils tend to have higher water holding capacity and labile C and N than coarse-textured ones (Austin, Yahdjian et al. 2004). Gravel, having bigger particle size and interstitial space, is more freely draining and adsorbs less nutrients. On the other hand, fine sand with smaller particle size will be better at moisture retention and nutrient adsorption, whereas coarse sand will occupy the intermediate position. The differences in water retention and nutrient adsorption ability of the substrates make it possible to infer

tolerance of the taxa to moisture and nutrient deficits. The ratio of dry weight to fresh weight showed that *Carpobrotus* species were more succulent than the intergeneric hybrid, indicating that these exotics are more efficient at storing water (Rabas and Martin 2003) and may explain why substrate had little influence on growth rates of the two *Carpobrotus* species. It has further been found that water stress induces a weak Crassulacean Acid Metabolism (CAM) in *Carpobrotus edulis* (Earnshaw, Carver et al. 1987), but see (Rabas and Martin 2003).

According to the results obtained from the common garden experiment, only *C. chilensis* had some level of substrate preference, with plants growing in sand having significantly higher dry weight and producing more leaves than those in gravel. Despite this, they have been observed hanging down cliffs, e.g. at Taylor's Mistake near Christchurch, suggesting that the current distribution of the exotics is a product of human introduction rather than soil types. There was no difference in performance between the two *Carpobrotus* species, regardless of the substrate in the common garden experiment as well as in the field. The two exotics both had higher actual and estimated dry weights than the intergeneric hybrids and *D. australe,* with the hybrids in turn significantly outperforming *D. australe*.

The two varieties of *D. australe,* (green and red), did not differ in their performance in either of the substrate types, even though in nature the green one seems to predominate at rocky shores (K. Gould pers. comm.). Compared to the other taxa, *D. australe* allocated more biomass to its roots than shoot. The hybrid also invested more in the roots than the two *Carpobrotus* species. This implies that the hybrid and its native parent are better adapted morphologically to obtain water under desiccating conditions or in poor soils. Partitioning of more photosynthates to roots influences microbial biomass and hence, soil organic matter decomposition and nutrient recycling (Van Veen, Merckx et al. 1989). Therefore, *D. australe* contributes more to the "improvement" of soil micro-conditions which may not be advantageous to native sand-binders that normally prefer low nutrient environments.

In terms of characters relating to vegetative growth (number of leaves, number of nodes, main axes length, and internode length), the hybrid on the whole performed significantly better than the other taxa, both in the field and in the common garden experiment. In the common garden experiment, *D. australe* had better vegetative growth than the exotic *Carpobrotus* but experienced the least growth in the field study, due to smothering by the neighbouring hybrid. The hybrid that is sexually sterile seems to have invested a lot in lateral branches as its only means of proliferation and self perpetuation. Vegetative reproduction has been found to be positively correlated with invasiveness (Kolar and Lodge 2001) but the scope of my study was not extensive enough to conclude whether the intergeneric hybrid will displace *D. australe* or not. My observation is that its stolons are thin and spread sparsely enough to allow it to intermingle with other plants, more so than the *Carpobrotus* species or *D. australe*.

Mating experiments in the present study have shown that flowers of *Carpobrotus* species are highly fertile to intrageneric and intergeneric pollen. However, *C. edulis* was not agamospermic and only slightly self-fertile, needing intervention of a pollinator since spontaneous self-fertilisation did not occur in bagged flowers that were not manipulated. *Carpobrotus chilensis* was slightly agamospermic and self-fertile.

4.3. Recommendations for restoration of *Carpobrotus* dominated sand dunes

- Along high energy eroding coasts, such as Kapiti, removal of the exotic iceplant prior to establishment of native sand-binder species is not advisable. Seedlings of native sand-binders will most likely be lost before they establish especially if the slope of the dune face is steep, unless measures to mitigate erosion are put in place. For future plantings to succeed, gently sloping (probably less than 28°), low disturbance sites should be selected. Although options like mechanical dune reshaping have been used with satisfactory results on steep slopes (Bergin, Miller et al. 2007), I would personally favour reducing density of existing iceplant and planting *Spinifex*

in-between them. However, facilitation of *Spinifex* by *Carpobrotus edulis* in this study is not strong enough to warrant its retention as a nurse plant.

- If Spring is chosen as the planting season for native sand-binders, as is the case with my trials, planting should be done early in the season since the later months tend to be quite dry.

- *Spinifex* seedlings should be planted deep enough (at least 5cm above the level of root trainer sleeve) to enhance root contact with water and guard against root exposure by erosion. Care should be taken to make planting holes perpendicular to the horizontal plane, not to the slope incline.

- Conducting trials with the intergeneric hybrid iceplant on bare foredunes before in-planting native sand-binders may help mitigate erosion and enhance survivorship of the native seedlings. Seedlings of some native sand-binders are costly and a nurse plant may be desirable. This hybrid, being sexually sterile and therefore dispersal-limited, can be controlled by mechanical or chemical means if it proves undesirable. However more research needs to be conducted to establish its suitability.

- Even among the stands of invasive iceplant species, there were some invertebrates and reptiles. Dune reshaping, if deemed necessary, needs to include plans for some refugia for these animals.

- Human trampling was an issue at the Queen Elizabeth Park field site and therefore some form of fencing is desirable to enhance success. In urban settings where dunes lead onto the beach, adequate walkways should be provided.

4.4. Suggestions for future studies

- Building on my trials, large scale longer-term restoration trials can be undertaken to further investigate the facilitative and competitive effects of the various iceplant taxa on *Spinifex* at sites that are less disturbed, in order to eliminate the confounding effects of storm and human disturbance.

- Field trials on suitability or otherwise of the intergeneric hybrid and *Disphyma* for sand dune restoration may be conducted at sites that are less disturbed.

- Molecular studies to identify the iceplant taxa currently found in NZ and further investigation of their mating systems would go a long way in providing insights into species identity and hybridisation of the iceplant taxa. Isozyme analysis of the two *Carpobrotus* species in California has been done (Gallagher, Schierenbeck et al. 1997) and was attempted in my study (see appendix 3). Morphological characters alone, of which flower colour is the most important, are not enough to assume identity of the iceplant morphotypes because of introgressive hybridisation. Analysis of chloroplast DNA (cpDNA) data for *C. edulis*, *C. chilensis*, and their hybrid morphotypes, has been done by Schierenbeck et al. (2005) and can be used as a basis for further studies.

- One factor contributing to invasiveness of *C. edulis* that has been investigated in the Mediterranean region is the enemy release hypothesis (release from soil pathogens) (Van Grunsven, Bos et al. 2009). This can be further built on in the context of New Zealand. During my study, I observed scale insects presumed to be *Pulvinaria mesembryanthemi* (Appendix 4) which have infested all iceplant taxa in New Zealand. It would be interesting to investigate how these natural enemies affect the various taxa and as a possible bio-control measure.

- Comparing biodiversity of sites invaded by the exotic iceplant species and uninvaded/restored sites would provide useful additional information on them. Patterns of co-occurrence may shed some light on intimate associations. Over the course of my study I have observed the presence of leaf-roller caterpillars in terminal buds of the exotic iceplant taxa, most of which hatched into moths while a few hatched into flies upon incubation (Appendix 4). There may be a guild of native or exotic arthropods that depend wholly or partially on these plants to complete their

life cycles. Influence of *C. edulis* on soil biota has been studied by de la Pena et al. (2010) in Portugal and Spain but there is room to explore further in a New Zealand context.

- Studies of *Disphyma* have been few and far between, especially in recent years. (Chinnock 1971; 1972; 1976)studied species biology, but there is a need to develop on this and include autecological studies of the species.

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Appendices

Appendix 1: Geographical coordinates of the various sites

This appendix gives the Geographic co-ordinates (WGS84 geodatum) of some of the sites that were visited during the course of this study. Locations of some of experimental plots at Queen Elizabeth Park are given in Table 1 while Table 2 shows the locations of the sample *Carpobrotus* plants whose flower phonologies were studied during the main flowering season of 2011 (September-December). In addition, the locations of populations from which cuttings for experimental growth in different substrates are given in Table 3.

Table 1. Locations of some of the experimental plots at Queen Elizabeth Park

ID	Treatment	Geographical co-ordinates
QEP_A1	Iceplant intact	S40 57.466 E17458.069
QEP_A3	Iceplant intact	S40 57.475 E17458.065
QEP_A7	Iceplant intact	S40 57.519 E17458.044
QEP_B1	Iceplant removed	S40 57.472 E17458.066
QEP_B3	Iceplant removed	S40 57.478 E17458.063
QEP_B4	Iceplant removed	S40 57.485 E17458.06
QEP_B6	Iceplant removed	S40 57.519 E17458.045
QEP_B7	Iceplant removed	S40 57.521 E17458.045
QEP_C3	Bare	S40 57.492 E17458.058
QEP_C4	Bare	S40 57.494 E17458.058
QEP_C5	Bare	S40 57.504 E17458.052
QEP_C6	Bare	S40 57.509 E17458.049
QEP_C7	Bare	S40 57.517 E17458.047
SPRAY2	Iceplant sprayed	S40 57.59 E17458.006
SPRAY3	Iceplant sprayed	S40 57.601 E17458.001

Table 2. Locations of the *Carpobrotus* plants at Eastbourne whose flower phenologies were monitored from September to December 2011.

ID	Taxon	Geographical co-ordinates
EAS_CCPL1	*C. chilensis*	S41 17.515 E174 53.605
EAS_CCPL2	*C. chilensis*	S41 17.502 E174 53.609
EAS_CCPL3	*C. chilensis*	S41 17.492 E174 53.618
EAS_CCPL4	*C. chilensis*	S41 17.423 E174 53.702
EAS_CCPL5	*C. chilensis*	S41 17.467 E174 53.638
EAS_CEPL1	*C. edulis*	S41 17.48 E174 53.63
EAS_CEPL2	*C. edulis*	S41 17.408 E174 53.701
EAS_CEPL3	*C. edulis*	S41 17.397 E174 53.736
EAS_CEPL4	*C. edulis*	S41 17.377 E174 53.763
EAS_CEPL5	*C. edulis*	S41 17.392 E174 53.731

Table 3. Sites from where iceplant cuttings of the various taxa were obtained for growth comparisons in different substrates (WGS84 geodatum) (g=Green, r=Red)

ID	Site	Taxa	Geographic co-ordinates
EASTB01	Eastbourne	*C. edulis*	S41 17.397 E174 53.735
EASTB02	Eastbourne	*C. chilensis*	S41 17.514 E174 53.602
ISL01	Island Bay	*D. australe*(g)	S41 20.934 E174 45.152
ISL02	Island Bay	Hybrid	S41 20.882 E174 45.671
MOA1	Moa Point	*D. australe*(r), Hybrid, *C. edulis*	S41 20.167 E174 48.474
PET01	Petone	*D. australe*(g)	S41 13.547 E174 51.869
PUK01	Pukerua Bay	*C. chilensis*	S41 1.77 E174 53.699
QEP01	Queen Elizabeth Park	*C. edulis*	S40 57.5 E174 58.051
QEP02	Queen Elizabeth Park	*C. edulis*	S40 57.664 E174 57.971
SEAT01	Seatoun	*D. australe*(g)	S41 19.394 E174 50.184
SEAT02	Seatoun	Hybrid	S41 19.606 E174 50.338
WOR01	Worser Bay	*D. australe*(r), *C. edulis*	S41 19.075 E174 49.73
WOR02	Worser Bay	Hybrid	S41 19.061 E174 49.697
DAY01	Day's Bay	Hybrid, *D. australe*(r)	S41 16.764 E174 54.375

Appendix 2: Initial biomass of sample cuttings

In order to have an indicator of the initial biomass of the iceplant cuttings referred to in chapter two; I randomly selected ten cuttings of each taxon without regard to their source population and dried them in an oven for 48 hours at 70°C. One-way ANOVA with dry weight as the dependent variable and taxon as the independent variable showed that there was significant variability in the initial biomass of the various taxa (Table 1). After running a Post-Hoc test of the data, it was found that cuttings *C. edulis* had a significantly higher initial biomass than all the other taxa. *Carpobrotus chilensis* cuttings also had a higher initial biomass than *D. australe* and the intergeneric hybrid. However, there was no difference in the initial biomass of the cuttings of *D. australe* and the hybrid (Table 2; Fig. 1).

Table 1. ANOVA table showing variability in initial biomass among the taxa

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Taxon	7.570	3	2.523	91.525	**<.001**
Error	.992	36	.028		
Total	22.910	40			

Table 2. A Tukey HSD table comparing biomass of the cuttings of the various taxa

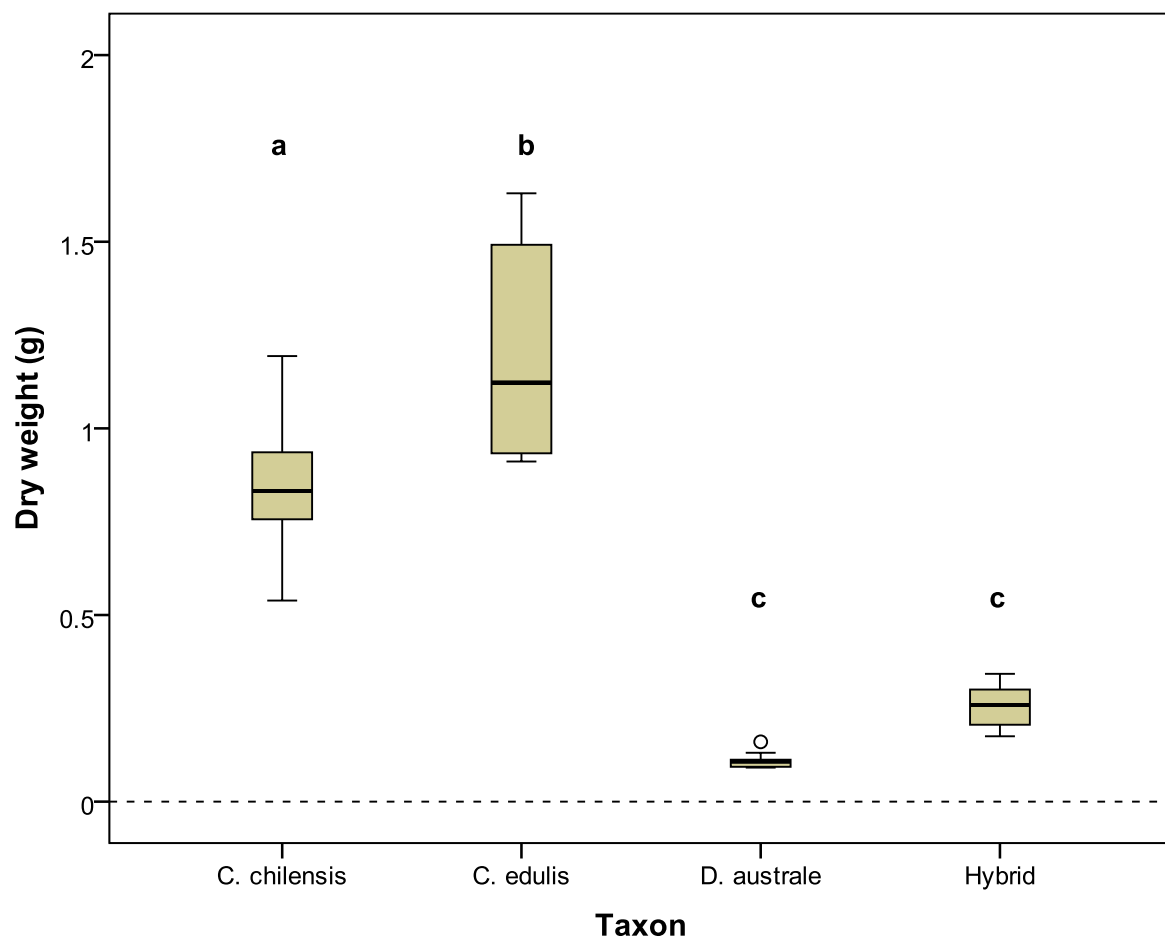| (I) Taxon	(J) Taxon	Mean Difference (I-J)	Std. Error	Sig.
C. chilensis	*D. australe*	.73*	.074	**<.001**
	Hybrid	.58*	.074	**<.001**
C. edulis	*C. chilensis*	.34*	.074	**<.001**
	D. australe	1.07*	.074	**<.001**
	Hybrid	.93*	.074	**<.001**
Hybrid	*D. australe*	.15	.074	.218

Fig. 1: Boxplots comparing the initial biomass of sample cuttings of the various taxa

Appendix 3: Allozyme Detection in *Disphyma* and *Carpobrotus*

Lesley Milicich and Guyo Gufu

Introduction

In New Zealand the yellow-flowered *Carpobrotus edulis* and magenta-flowered *C. chilensis* co-occur at most sites. *Carpobrotus chilensis* appears to be hybridizing and backcrossing with *C. edulis* individuals (Vila & D'Antonio 1998) according to morphometric (Albert et al. 1997) and isozyme analyses (Gallagher et al. 1997), although there are no presence records of the intrageneric hybrid in New Zealand (Heenan & Sykes 2010). The widespread hybridisation and backcrossing events of these congeners and their general lack of morphometric differences, make it possible that *Carpobrotus* plants present in New Zealand may in fact be two colour morphs of a *C. edulis* and *C. chilensis* hybrid swarm. The exotic *Carpobrotus* species are also known to hybridise with the native horokaka, *Disphyma australe* (Chinnock 1972). We obtained samples from different populations of the various taxa and conducted some allozyme analysis to determine whether the exotic *Carpobrotus* found in Wellington region were pure species or introgressant hybrid swarms before attempting artificial cross pollination experiments. We also wanted to find out the exotic parent of the presumed intergeneric hybrid (*Carpobrotus* x *Disphyma*).

Study taxa and sites

Cuttings of the following taxa were collected from the Wellington region: *Disphyma australe* (Worser Bay and Titahi Bay), the putative hybrid *Disphyma* x *Carpobrotus* (Worser Bay, Seatoun, Island Bay and Titahi Bay), *Carpobrotus* yellow flowered (Worser Bay, QE2 Park, Waikanae Beach, Peka Peka Beach, Te Horo Beach, Otaki Beach, Hokio Beach, Foxton Beach and Himatangi Beach) and *Carpobrotus* red flowered (Seatoun, Plimmerton, Paraparaumu Beach Golf Course, Hokio Beach and Foxton Beach). The cuttings were grown on in a glasshouse prior to allozyme analysis.

Materials and methods

Young leaf tips were ground in 75mM Tris/HCl extraction buffer pH 7.5, (Wendel and Weeden, 1989). The resulting crude protein extracts were absorbed on to 3mm x 15mm paper wicks and loaded on to 12% starch gels for enzyme electrophoresis. The extraction and electrophoresis steps were performed on ice.

Samples representing each of the four taxa above were loaded on to 11 different gel/electrode buffer systems: Amine citrate, AC, pH 6.0; Tris citrate1,2 & 3, TC 1,2 &3 pH 6.7, 7.0 and 8.0 respectively; Phosphate, PH, pH 6.7; Ridgeway, RW, pH 8.5; Histidine citrate, HC, pH 5.7; Citrate/histidine, C/H, pH 7.0; Lithium borate tris citrate, LB/TC, pH 8.3; Tris borate EDTA, TBE, pH 8.6 and Tris borate histidine citrate, TBHE, pH 8.6. References: AC, TC1 and RW, Allendorf et al., 1977; TC3, PH and TC2, Selander et al, 1971;TBE, LB/TC, HC and C/H, Wendel and Weeden, 1989; TBHE Suehs et al 2004.

Among 17 assayed enzyme systems, eight regularly showed scorable results: acid phosphatase (ACP; E.C. 3.1.3.2), adenylate kinase (AK; E.C/2.7.4.3), glucose phosphate isomerase (GPI; E.C. 5.3.1.9), isocitric dehydrogenase (ICD; E.C.1.1.1.42), malate dehydrogenase (MDH; E.C. 1.1.1.37), 6 phosphogluconate dehydrogenase (PGD; E.C. 1.1.1.44), phosphoglucomutase (PGM; E.C. 5.4.2.2 and shikimate dehydrogenase (SKD; E.C. 1.1.1.25).

The combinations which gave clearest activity were selected from these eleven gel/electrode buffer systems x eight stains (Table 1).

Table 1. The combinations of buffer systems giving optimal staining activity.

Gel/electrode buffer system	Optimal staining
C/H pH = 7.0	ACP, ICD, PGM & SKD.
TC2 pH = 8.0	MDH & GPI.
TBHE pH = 8.6	AK & PGD.

Samples of young leaf material from one plant from each of the following taxa/ locations (Table 2.) were applied to each of the three gels above.

Table 2. Order of the taxa for optimal comparison.

Sample lane	Plant and location
DYE	
1	*Disphyma australe* Worser Bay
2	*Disphyma australe* Titahi Bay
3	*Disphyma australe* Titahi Bay (long leaved)
4	Putative hybrid *Disphyma x Carpobrotus* Seatoun
5	Putative hybrid *Disphyma x Carpobrotus* WorserBay
6	Putative hybrid *Disphyma x Carpobrotus* Island Bay
DYE	
7	*Carpobrotus* yellow-flowered Worser Bay/Seatoun
8	*Carpobrotus* yellow-flowered QE2 Park
9	*Carpobrotus* yellow-flowered Waikanae Beach
10	*Carpobrotus* yellow-flowered Peka Peka Beach
11	*Carpobrotus* yellow-flowered Otaki Beach
12	*Carpobrotus* yellow-flowered Hokio Beach
13	*Carpobrotus* yellow-flowered Foxton Beach
14	*Carpobrotus* yellow-flowered Himitangi Beach
15	*Carpobrotus* sp. Te Horo Beach
16	*Carpobrotus* red-flowered Seatoun
17	*Carpobrotus* red-flowered Plimmerton
18	*Carpobrotus* red-flowered Paraparaumu Beach Golf Club
19	*Carpobrotus* red-flowered Hokio Beach
20	*Carpobrotus* red-flowered Foxton Beach
DYE	

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The dye, red food colouring, was applied to track the movement of the buffer front as well as to make identification of individual samples easier. One of each of the eight stains was performed on a slice of the gel indicated as giving optimum activity above.

Results

Differences were detected among the taxa *Disphyma australe*, the putative hybrid *Disphyma x Carpobrotus* and *Carpobrotus*. While variable alleles were found in the genus *Carpobrotus*, no consistent differences were found between the yellow-flowered and the red-flowered forms of the plant (Fig 1a- 1e).
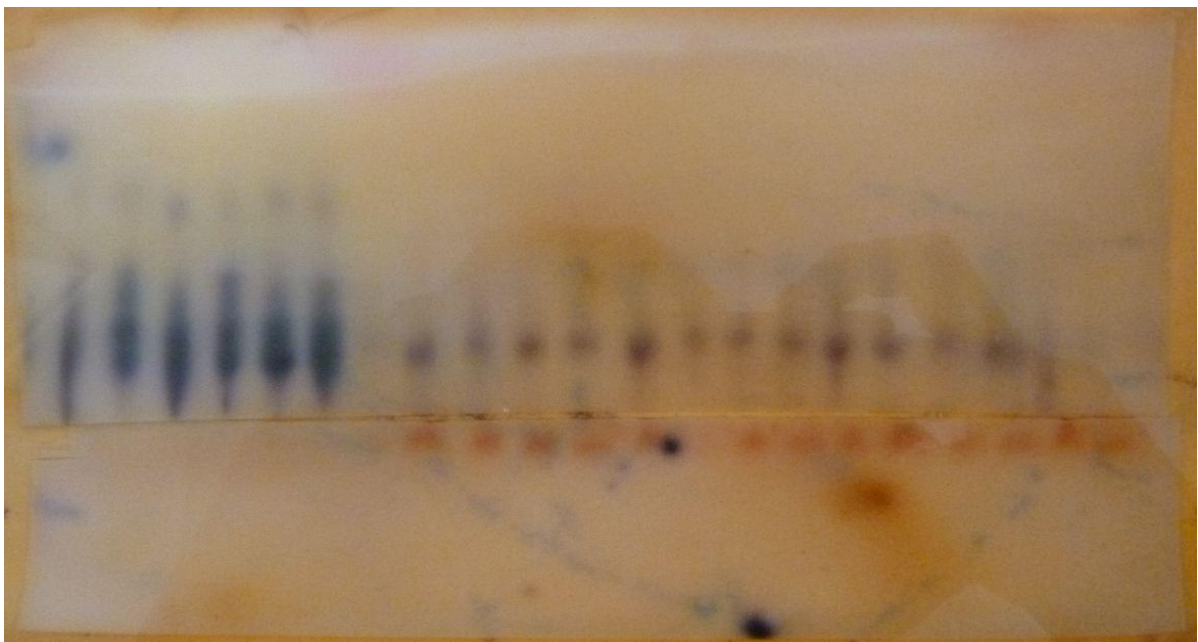

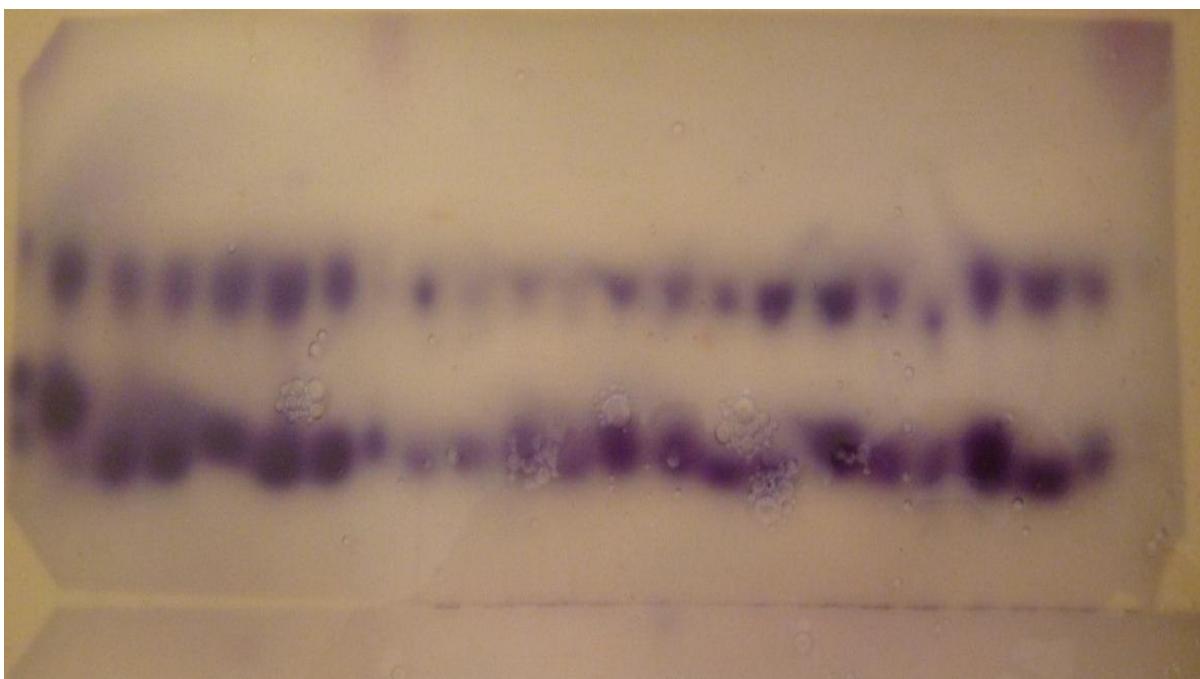Fig. 1a: ACP bands on CH gel/buffer electrode system

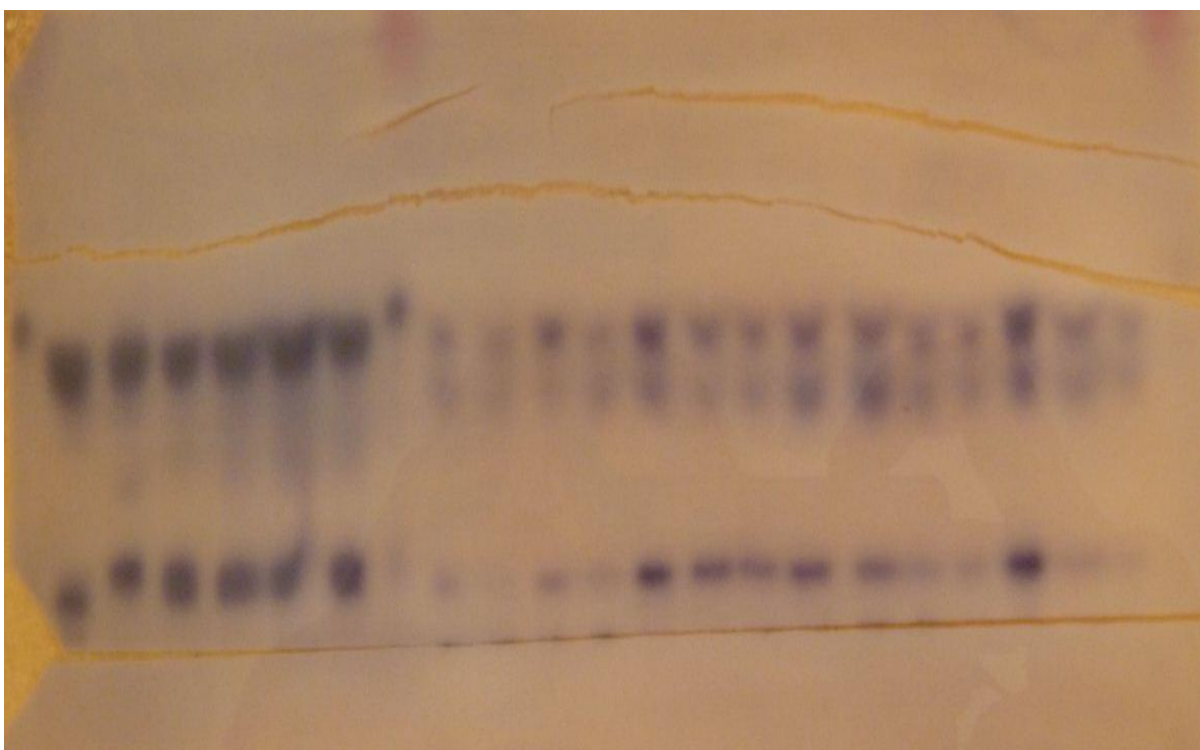Fig. 1b: GPI bands on TC2 gel/buffer electrode system

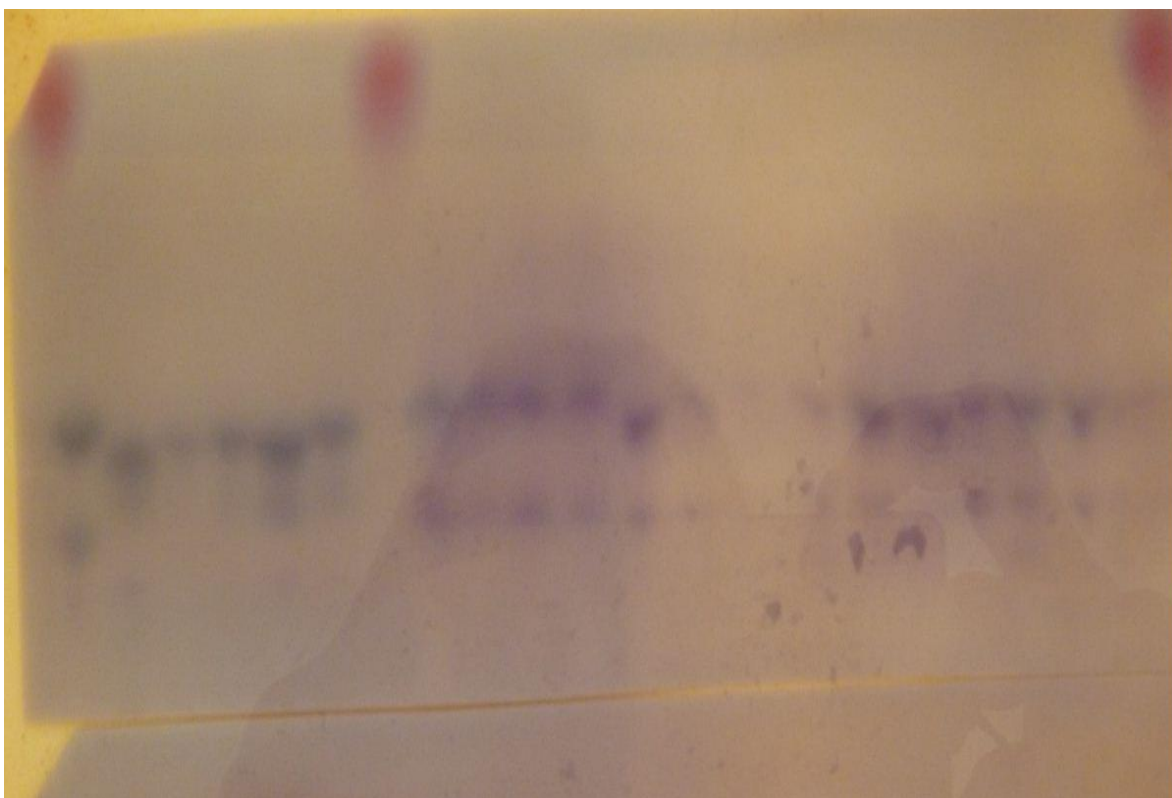Fig. 1c: MDH bands on TC2 gel/buffer electrode system

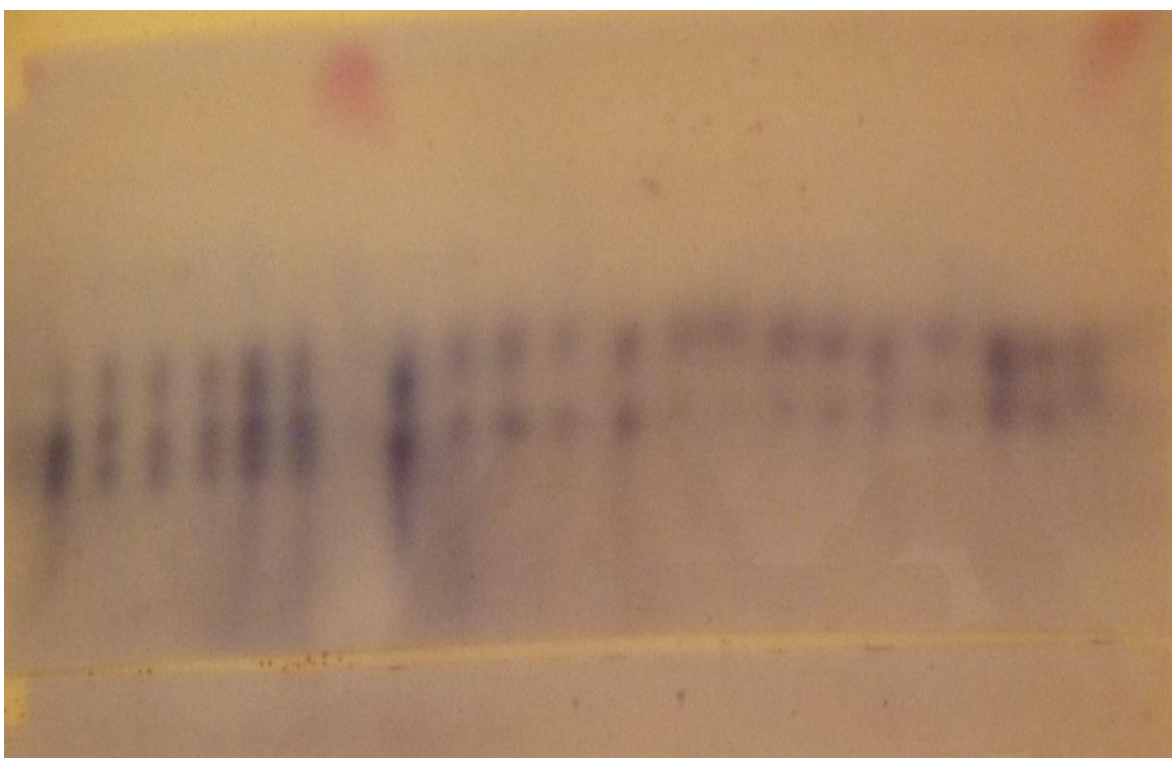Fig. 1d: PGD bands on TBHE gel/buffer electrode system

Fig. 1e: PGM bands on CH gel/buffer electrode system

Discussion and Conclusion

The two different colour morphs of *Carpobrotus* did not display any consistent differences in allozyme bands; hence the parent of the putative hybrid *Disphyma x Carpobrotus* could not be determined by allozyme analysis. The low variation within and between Carpobrotus taxa is contrary to the results of Gallagher et al., (1997). It is possible that the taxa introduced into NZ was a hybrid of *C. edulis* and *C. chilensis* which has retained variation for flower colour, but no detectable variation in allozyme bands. Further work with more sensitive genetic methods is required to investigate this hypothesis more fully.

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Appendix 4: Iceplant - Insect interactions

The terminal bud of the iceplant taxa initially appears as a single blade that bisects to form two leaves or sepals as it matures. Some caterpillars were observed between the terminal leaves of stolons of the exotic *Carpobrotus* at Eastbourne. It is seems that some lepidopteran lays its eggs before the bisection of the terminal bud such that the caterpillars are found in a tunnel between the two leaves that are held together with silk. When the terminal bud develops into a flower the caterpillar burrows a tunnel into the receptacle thereby destroying part or whole of the flower. The caterpillars were more abundant on *C. chilensis* plants than on *C. edulis*. They were also more abundant on plants at the backdune than those at the foredune. Samples of these caterpillars were collected with leaves of the plants where they occur and later incubated in test tubes with General Purpose Diet in the laboratory and the resultant insects preserved for identification. In general, most caterpillars hatched into moths while a few hatched into flies or wasps (Table 1; Fig. 1).

Table1: Summary results of the caterpillar incubation

Plant and part	No. of caterpillars	No. of pupae	No. of moths	No. of flies	No. of 'wasps'	Failed pupae	Failed to pupate
C. chilensis leaves	15	12	8	2	1	1	3
C. chilensis flowers	5	5	3	0	0	2	0
C. edulis leaves	10	6	6	0	0	0	4
C. edulis flowers	10	6	6	0	0	0	4

Fig. 1: A moth (left) and a wasp (right) that emerged from the incubated caterpillars

In addition, scale insects were observed to have infested some plants of all the iceplant taxa in the glasshouse and in the field (Fig. 2). At moa point where *C. edulis*, *D. australe* and their hybrid co-occur, the scale insects were found only on *D. australe* plants.

Fig. 2: *Disphyma australe* plant from the field and *C. edulis* plant in the glasshouse infested by scale insect (*Pulvinaria mesembryanthemi*).