Sustainable Exploitation of Saline Resources

ecology, ecophysiology and cultivation of potential halophyte crops

Arjen de Vos

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Arjen Coenraad de Vos

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Summary

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Nederlandse samenvatting

Summary

Saline agriculture can facilitate the adaptation to the increasing salinization and decreasing availability of fresh water. Also, many salt affected soils considered to be unsuitable for agriculture, can be turned into productive agricultural areas. In this way, salinization can be regarded as an opportunity for sustainable exploitation of saline resources, in contrast to the negative association that salinization often evokes. Although the concept of saline agriculture is not new, only limited research has been undertaken which has been insufficient to allow large scale saline agriculture. First, there is a need to evaluate the salt tolerance of plant species that can be cultivated as crops under saline conditions. Since most conventional crops are relatively sensitive to soil salinity and can only be cultivated under 'moderately' saline conditions (up to circa 20 % seawater salinity), the domestication of halophytes is an approach that should be considered. Knowledge of the ecology and ecophysiology of a species can help to evaluate/assess the salt tolerance and potential as a crop, but also to give insight into the optimal agronomic conditions for cultivation. In this regard, studying the ecology and ecophysiology of halophytes remains important. Next, the evaluation of the growth performance under field conditions is the imperative next step.

Chapter 1 focuses on different aspects of salinization, saline agriculture, and salt tolerance in general and an overview is given of the research questions which were addressed in the different chapters. The overall aim of this thesis is to contribute to the scientific knowledge of the ecology of (strandline) halophytes, their ecophysiological response to salinity, and their cultivation under field conditions.

Although it is well known that plant species occurring in the salt marsh habitat demonstrate high salt tolerance and potential for saline agriculture, relatively little is known about that of plant species occurring in the strandline habitat. In Chapter 2 it is shown that although salt spray is most important in determining the different vegetation characteristics of the strandline, the influence of soil salinity and incidental seawater flooding is considerable. Strandline plant species are exposed to enhanced levels of salt spray and occasional seawater flooding. The strandline species examined appeared to be moderately salt tolerant for increased root zone salinity and are able to survive short peaks of high salinity.

Crambe maritima, a typical strandline plant species, was exposed to various levels of airborne and soil borne salinity in a greenhouse experiment to assess tolerance to root zone salinity and salt spray (Chapter 3). Here, it is shown that salt spray caused no growth reduction in contrast to the root zone salinity treatments. Root zone salinities up to 20 % seawater salinity did not result in a decrease in relative growth, but a sharp decrease in growth was observed at 40 % seawater salinity which was mainly caused by the reduction in specific leaf area. Other morphological and physiological parameters changed with increasing salinity, but not any of these parameters could be linked with the observed growth reductions. Based on its growth response *Crambe maritima* can be classified as a salt spray tolerant plant that is sensitive to root zone salinities exceeding 20 % seawater salinity.

Two other species occurring in coastal habitats*,* namely *Diplotaxis tenuifolia* (L.) and *Cochlearia officinalis* (L.) may be assumed to be moderately salt tolerant as well. The growth performance under saline conditions was determined in a greenhouse experiment for *D. tenuifolia* and for *C. officinalis* (Chapter 4). The relative growth

rate at ≥ 40 % seawater salinity showed about 20 % reduction for both species, which could be contributed to a reduction in specific leaf area. In comparison with seven other Brassicaceae species, including salt sensitive and highly salt tolerant species, both *D. tenuifolia* and *C. officinalis* showed an intermediate salt tolerance. Both species can be classified as moderately salt tolerant with potential as vegetable crop for saline agriculture.

In Chapter 5 it is described how an experimental saline field site was created on Texel, The Netherlands. Seawater drip irrigation was applied to cultivate halophyte crop species under saline conditions. Soil salinity was monitored during the course of the experiment. The three populations of *Beta vulgaris* ssp. *maritima*, as well as *Plantago coronopus*, all showed continued growth under field conditions at 40 % seawater salinity. Differences in salt tolerance were found and analyzed between three populations of *Beta vulgaris* ssp. *maritima*.

By studying the ecology and ecophysiology of strandline halophytes various questions related to exposure to salinity, defining and calculation of salt tolerance and agronomic conditions for cultivation have been considered and analyzed. Based on hydroponic crop cultivation in the greenhouse, growth and ecophysiological parameters have been analyzed and salt tolerance has been assessed for *Crambe maritima*, *Diplotaxis tenuifolia* and *Cochlearia officinalis* which contributed to the understanding of the mechanisms of salt tolerance. In addition, field experiments with varying but controlled soil salinity are needed to test the plants' suitability as a new saline vegetable crop. The ecological, ecophysiological characteristics of the plant species studied, as well as the practice of cultivation of halophytes under saline field conditions, taste and edibility, as described and analyzed in this thesis contribute to the development of saline agriculture. Further development of sustainable exploitation of saline resources relate to market development, costs and benefits of saline crop products and imply upscaling of outdoor or indoor saline crop cultivation.

Nederlandse samenvatting

Van Zoute Kwel naar Zilte Bron

De titel van deze Nederlandse samenvatting, *Van Zoute Kwel naar Zilte Bron*, is een vrije vertaling van de Engelstalige titel van dit proefschrift. Deze titel omschrijft in het kort één van de doelstellingen van het voorbeeld transitieproject *Zilte Landbouw Texel*, waarvan de werkzaamheden van dit proefschrift een onderdeel van waren. In plaats van verzilting als een probleem te ervaren welke bestreden dient te worden (zoute kwel(ling)), kan verzilting ook als een kans voor duurzame zilte landbouw worden opgevat (zilte bron).

Zilte landbouw kan de adaptatie aan de toenemende verzilting en de afnemende beschikbaarheid van zoet water faciliteren. Daarnaast kunnen vele verzilte gebieden, waarvan werd gedacht dat deze onbruikbaar waren geworden, weer als productieve agrarische gebieden beschouwd worden. Op deze manier kan verzilting als een kans voor duurzame exploitatie van zilte bronnen worden gezien in plaats van de negatieve associatie waar verzilting meestal mee gepaard gaat. Hoewel het concept van zilte landbouw niet nieuw is, is de toe nu toe ondernomen actie ontoereikend geweest om grootschalige zilte landbouw te realiseren. Als eerste is er een noodzaak om de zouttolerantie van verschillende potentiële zilte gewassen te evalueren. Aangezien de meeste gangbare landbouwgewassen relatief gevoelig zijn voor verzilting en onder de beste omstandigheden alleen onder matig zoute condities (20 % zeewater saliniteit) nog gecultiveerd kunnen worden, zal ook de domesticatie van halofieten (planten welke onder natuurlijke zoute condities groeien en over een hoge mate van zouttolerantie beschikken) beschouwd moeten worden. Kennis van de ecologie en de ecofysiologie kan helpen om de zouttolerantie en potentie van een gewas in kaart te brengen. Ook kan met deze kennis een eerste aanzet van de agronomische aspecten van de cultivatie worden verkregen. Het onderzoeken van de ecologie en ecofysiologie blijft dus belangrijk. Hierna zal de groei onder zilte veldomstandigheden moeten worden onderzocht.

Hoofdstuk 1 richt zich op verschillende aspecten van verzilting, zilte landbouw en zouttolerantie. Daarnaast wordt er een overzicht gegeven van de onderzoeksvragen welke in de verschillende hoofdstukken worden behandeld. Het algemene doel van dit proefschrift is om bij te dragen aan de wetenschappelijke kennis van de ecologie van halofieten, hun ecofysiologische reactie op een toenemende zoutconcentratie en de cultivatie van halofieten onder zilte omstandigheden.

Het is bekend dat halofieten, welke in de kwelder voorkomen, over een hoge mate van zouttolerantie beschikken en ook potentie hebben als zilt landbouwgewas. Echter, dit is in mindere mate bekend voor planten welke in een 'vloedmerk-habitat' voorkomen. In Hoofdstuk 2 wordt aangetoond dat hoewel 'salt spray' of zoutsproei het meest bepalend is voor verschillende aspecten van de vegetatie van een vloedmerk, de invloed van bodemzout en zeewater inundatie aanzienlijk is. Vloedmerkplanten zijn blootgesteld aan verhoogde concentraties van zoutsproei en periodieke inundatie van zeewater. De onderzochte vloedmerkplanten schenen tolerant te zijn voor matige bodemzoutconcentraties en waren in staat om korte pieken van hoge zoutconcentraties te overleven.

In een kasexperiment is *Crambe maritima*, een typische vloedmerkplant, blootgesteld aan verschillende bodemzoutconcentraties en zoutsproei om de tolerantie voor beide type zoutbelasting te bepalen (Hoofdstuk 3). In dit hoofdstuk is aangetoond dat zoutsproei geen groeireductie veroorzaakte in tegenstelling tot bodemzout. Bodemzoutconcentraties tot 20 % zeewater saliniteit hadden geen effect op de groei, maar bij 40 % zeewater saliniteit trad een scherpe groeireductie op, wat vooral door de reductie van de 'specific leaf area' werd veroorzaakt. Andere morfologische en fysiologische parameters veranderden met toenemende zoutconcentratie, maar geen van deze individuele parameters kon duidelijk aan de groeireductie worden gekoppeld. Op basis van de groei-eigenschappen onder zoute condities, kan *Crambe maritima* geclassificeerd worden als zoutsproei-tolerant en gevoelig voor bodemzoutconcentraties boven 20 % zeewater saliniteit.

Van de twee plantensoorten *Diplotaxis tenuifolia* en *Cochlearia officinalis*, welke voorkomen in kusthabitats, werd aangenomen dat deze ook over enige mate van zouttolerantie beschikken. De groeiprestaties onder zoute condities van deze twee soorten is bepaald in een kasexperiment (Hoofdstuk 4). De blootstelling aan een saliniteit van ≥ 40 % zeewater resulteerde in een reductie van 20 % van de relatieve groeisnelheid bij beide soorten en deze reductie werd voornamelijk veroorzaakt door de 'specific leaf area'. In vergelijking met zeven andere Brassicaceae soorten, waaronder zoutgevoelige en zeer zouttolerante soorten, vertoonde zowel *D. tenuifolia* als *C. officinalis* een tussenliggende zouttolerantie. Beiden soorten kunnen geclassificeerd worden als matig zouttolerant met potentie voor zilte landbouw.

In Hoofdstuk 5 wordt een beschrijving gegeven van een zilte proeftuin, welke is gerealiseerd op Texel. Met behulp van zeewaterdruppel irrigatie zijn hier verschillende zilte gewassen gecultiveerd. De groei van *Beta vulgaris* ssp. *maritima* van drie verschillende populaties, de groei van *Plantago coronopus* en de bodemzoutconcentratie werd gedurende het experiment gemonitord. Zowel *Plantago coronopus* als de drie 'cultivars' van *Beta vulgaris* ssp. *maritima* vertoonden continue groei bij een bodemzoutconcentratie van 40 % zeewater saliniteit. Verschillen in zouttolerantie tussen de drie cultivars werd aangetoond en geanalyseerd.

Door het bestuderen van de ecologie en de ecofysiologie van halofieten, welke op vloedmerken voorkomen, zijn verschillende vragen over saliniteit, zouttolerantie en agronomische randvoorwaarden geanalyseerd en beantwoord. Door middel van verschillende kasexperimenten, zijn de groei, zouttolerantie en de onderliggende adaptaties van deze tolerantie onderzocht voor *Crambe maritima*, *Diplotaxis tenuifolia* en *Cochlearia officinalis*. Deze experimenten hebben bijgedragen aan het inzichtelijk maken van de mechanismen van zouttolerantie. Daarnaast zijn veldexperimenten nodig waarbij onder verschillende maar constante zoutconcentraties de potentie van zilte gewassen verder wordt onderzocht. De ecologische en ecofysiologische aspecten van de bestudeerde plantensoorten, de veldproeven onder zilte omstandigheden, maar ook hun smaak en eetbaarheid, welke allemaal zijn behandeld in dit proefschrift, hebben bijgedragen aan de verdere ontwikkeling van zilte landbouw. Verdere duurzame exploitatie van zilte bronnen is afhankelijk van marktontwikkeling, kosten-baten analyse van zilte producten en opschaling van de (binnen en buiten) teelt van zilte gewassen.

Contents

Chapter 1.

General introduction

Research framework

This thesis was carried out against the background of the project 'Saline Agriculture Texel' which was part of the 'BSIK-Transforum Agro&Groen-Living with Salt Water' research program. This innovative saline agriculture project served as an example to regard salinization as an opportunity instead of a threat. The adaptation to brackish and saline conditions by the development of saline agriculture was the main goal. It focused on the cultivation (methods) and marketing of new salt-tolerant crops, which was carried out in cooperation with the company Texelse Milieuvriendelijke NatuurProducten, led by Marc van Rijsselberghe. For this purpose, ecological field experiments and laboratory tests at the VU University Amsterdam were combined with halophyte crop cultivation experiments on the island of Texel. The main results of the project are described in the report *"Zilte Landbouw Texel-een voorbeeld transitieproject"* by De Vos *et al.* (2010a).

This first chapter focuses on different aspects of salinization, saline agriculture, and salt tolerance in general. First of all the salinization of arable land worldwide and in The Netherlands is discussed. This is followed by a brief analysis of the possibilities and potential of saline agriculture. The study of the salt tolerance of plant species and the ecophysiology of halophytes represent an important part of this thesis. The following chapters deal with these topics and some principles of salt tolerance will be discussed here. Finally, the aims and outline of this thesis are discussed.

Feeding the world of tomorrow

Soil salinization represents a serious threat to ecosystems and agricultural crop production of the world. Salinization can be described as an increase in the salt concentration to the extent that optimal soil use is no longer possible. This can be caused by either natural (primary) salinization or by man-induced (secondary) salinization. The latter is often the result of (improper) irrigation management. Although proper irrigation management can often prevent or decrease soil salinization, a lack of fresh water or financial means has globally led to the presence of a substantial area of saline soils. With current climate warming and increased evapotranspiration, global salinization will steadily continue (Rozema and Flowers 2008).

It is well known that ancient societies in Mesopotamia, the Indus Valley, China and South America were threatened or even destroyed by salinization (Jacobsen and Adams, 1958; Hillel, 2005; Szabolcs 2008) and today's society is also facing ever continuing and increasing soil salinization. Today, close to 1 billion hectares or 7 % of the world's total land area and about 20 % of the irrigated land is salt affected (Fig. 1.1A) and this area is still increasing (Szabolcs, 1994; Ghassemi *et al.*, 1995; Yensen, 2008).

In the arid and semi-arid regions of the world, the limited amount of precipitation in combination with the high evapotranspiration is the major cause of salinization, whereas in the temperate zones it is mainly caused by seepage of seawater into low-lying areas. A large part of The Netherlands is under the influence of salinization (Fig. 1.1B) and these areas coincide with former intertidal zones or reclaimed areas (Ter Voorde and Velstra, 2009). In these coastal areas, seawater is present as saline groundwater and located relatively close to the soil surface. These low lying areas are regularly flushed with fresh water from rivers and lakes, especially during dry and warm periods. Without this translocation of fresh water conventional
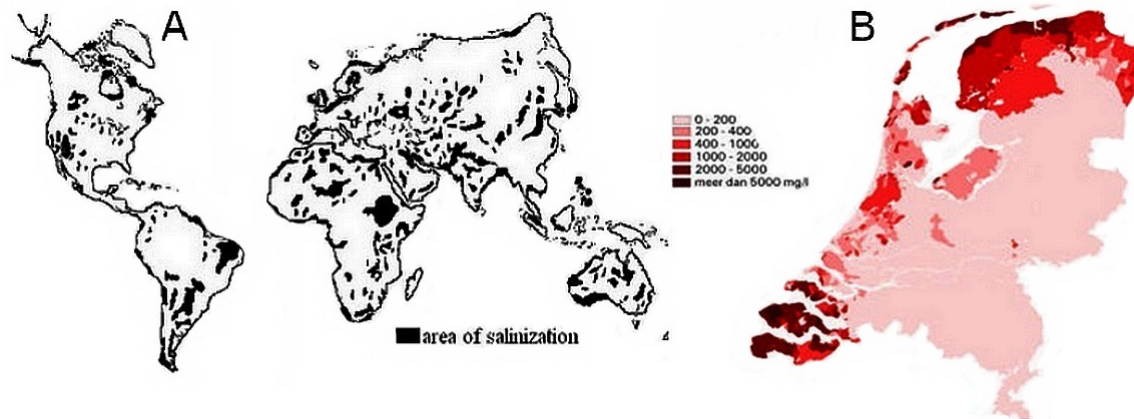
Figure 1.1. Global distribution of salt-affected soils worldwide (Fig. 1.1A) (based on Szabolcs, 1994 and Rozema and Verhoef, 1997) and average chloride concentration (in mg l^{-1}) of surface water during the month July in an extreme dry year in The Netherlands (Fig. 1.1B) (RIZA, 2004).

agriculture can be limited by brackish groundwater (Fiselier *et al*., 2003; Velstra *et al*., 2009). Through climate change, soil subsidence and increasing sea level there will be an increase of the influence of brackish water and a decrease in availability of fresh water in the coastal areas in The Netherlands (Fiselier *et al*., 2003; Velstra *et al*., 2009). It is expected that the area of salinization of arable land in The Netherlands will reach about 125.000 hectares (about 6 % of total agricultural area) in 10 years time (De Kempenaer *et al*., 2007).

Besides salinization, also the available amount of fresh water has to be considered since it is already a scarce and valuable resource in many countries. The amount of brackish water worldwide is about the same as the amount of available fresh water (both 1 % of the total amount of water), whereas 97.5 % is seawater (Rozema and Flowers, 2008; Corcoran *et al*., 2010). Fresh water shortage will increase in the nearby future, mainly due to the growing world population and rising prosperity (FAO, 2004; FAO and IFAD, 2006; Alcamo *et al*., 2007). Irrigation currently represents about 70 % of all water uses, but domestic use will claim an increasing part, resulting in lower fresh water availability for agriculture (FAO and IFAD, 2006; Alcamo *et al*., 2007). Together with the ongoing global salinization, this emphasizes the importance to explore the use of brackish water and seawater for agricultural purposes (Rozema and Flowers, 2008). With an area of about 1 billion hectares worldwide and 125.000 hectares in The Netherlands which is not suitable for conventional crop production, the potential of saline agriculture is clearly illustrated.

Saline agriculture
Saline agriculture is an alternative form of agriculture which uses arable land and water not suitable for most conventional crops. Here, saline and brackish water is considered to be a resource rather than a threat. Saline agriculture can also comprise aquaculture which focuses on the cultivation of (micro-)algae, seaweeds and animals like (shell)fish, although it mostly refers to the cultivation of terrestrial plants. In this thesis only saline agriculture that deals with the cultivation of terrestrial plants is considered.

The salt tolerance of many agricultural plant species has been evaluated and most herbaceous crops used at present day are relatively sensitive to increasing soil salinity. Out of 81 crops that were evaluated for salt tolerance the majority of the plant species already showed substantial growth reductions at a salt concentration of around

50 mM NaCl (10 % seawater salinity) and only two species did not show growth reductions at a salt concentration just above 100 mM NaCl (20 % seawater salinity). However, yields reduced dramatically with increasing salinity above this threshold (Tanji and Kielen, 2002). This sensitivity of conventional crops is illustrated by Table 1.1 where 6 different plant groupings are identified. Here, salinity levels above 140 mM NaCl are listed as extreme salinity and considered to be too saline for any crop. Another commonly used classification states that soil salinity below 40 mM NaCl can be classified as non saline, between 40 and 80 mM as moderately saline, between 80 and 160 mM as strongly saline and above 160 mM NaCl as very strongly saline (USSL, 1954). According to these commonly used classifications, extreme salinity starts from 140-160 mM NaCl, given that the salinity of seawater is in the range of 500 mM NaCl. These high salinity levels will indeed be too great for most common crops. However, most halophytes, which are adapted to grow in saline habitats, show little or no growth reductions at salinity levels of 200 mM NaCl (see Fig. 1.2).

Table 1.1. Soil salinity classification and plant salt tolerance grouping, based on Shaw (1999).

Soil salinity rating	Salt concentration (in dS m^{-1})	Salt concentration (in mM NaCl)	Salt concentration (in % seawater)	Plant salt tolerance grouping
non saline	< 1	< 10	2	sensitive crops
low salinity	1 – 2	10-20	2-4	moderately sensitive crops
medium salinity	2 – 5	20-50	4-10	moderately tolerant crops
high salinity	5 – 8.5	50-85	10-17	tolerant crops
very high salinity	8.5 – 14	85-140	17-28	very tolerant crops
extreme salinity	> 14	> 140	>28	generally too saline for crops

Salt concentration of the soil is commonly measured as electrical conductivity in the extract of the soil saturated paste (ECe) and expressed in dS m^{-1}, for more details see Rhoades *et al*. (1999). Salt concentration of seawater is in the range of 50 dS m^{-1} or 500 mM NaCl (1 dS m^{-1} ≈ 10 mM NaCl).

Since most conventional crops are relatively sensitive to soil salinity and the bioengineering approach has not delivered salt-tolerant cultivars of conventional crops, domestication of halophytes for saline agriculture to adapt to the global salinity problem is an approach that should be considered (Glenn *et al*., 1999; Flowers, 2004; Rozema and Flowers, 2008). This thesis will mainly focus on the ecophysiology and cultivation of coastal halophytes. Halophytes can be used as crops to produce fodder, food and biofuels as well as crops with an industrial application, ornamentals and for remediation, among others (Glenn *et al*., 1999; Rozema and Flowers, 2008; Yensen, 2008). Despite the great potential of saline agriculture, only little research has been carried out to develop the knowledge and know-how to successfully introduce saline agriculture as an adaptation to salinization (Shay, 1990; Choukr-Allah, 1996; Yensen, 2008). There is a need for innovations in agriculture and the cultivation of crops under saline conditions, which conditions have been considered to be unproductive until now, can increase the world's cultivated area to a great extent. This was illustrated by Niazi (2007) who compared the growth and ecophysiology of sea beet (*Beta maritima* ssp. *maritima*) with that of fodder beet *Beta maritima* ssp. *vulgaris,* and demonstrated relatively high salt tolerance of this crop. Fodder beet is now being successfully cultivated on salt affected soils in Pakistan and Mexico and used as cattle fodder crop.

Salt tolerance
The salt tolerance of a plant species is usually determined by assessing the growth performance under increased soil borne salinity (Maas and Hoffman, 1977). A

simplified classification is given in Figure 1.2 in which salt sensitive, salt tolerant and halophytic plant species are distinguished. In general, the growth of salt sensitive plant species is strongly reduced at even minor salt concentrations. Salt tolerant plant species can maintain optimal growth up to 50-100 mM NaCl but growth shows a decline at higher salinities and halophytic species may show enhanced growth at moderate salinity levels around 50-100 mM NaCl root zone salinity and are able to grow at salinity levels around half or even full strength seawater of around 500 mM NaCl (Glenn *et al.*, 1999; Breckle, 2002; Flowers and Colmer, 2008).
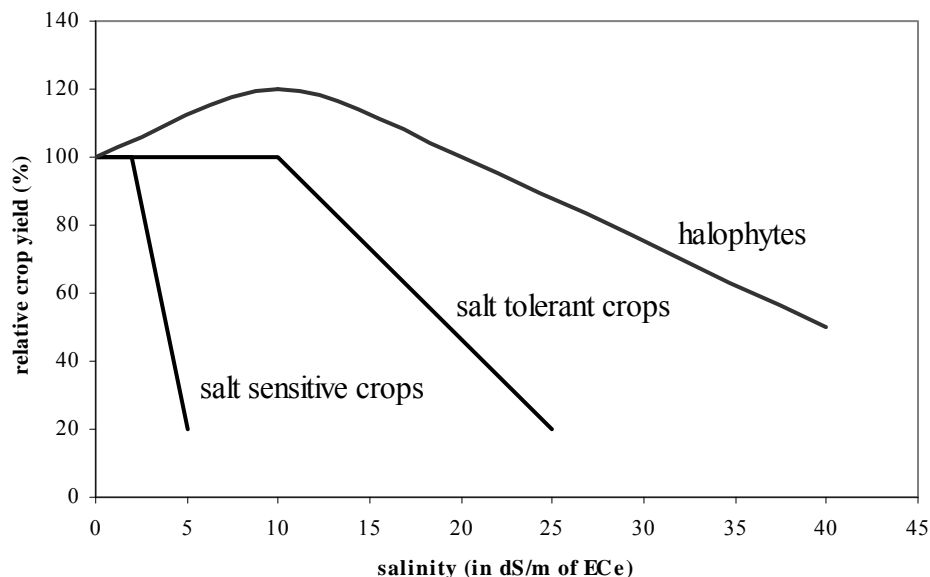

Figure 1.2. General effect of increasing salinity on the growth, expressed as relative crop yield (%), of salt sensitive crops, salt tolerant crops and halophytes (adapted from Maas and Hoffman (1977), Glenn *et al.* (1999) and Tanji and Kielen (2002)). The salinity of seawater is in the range of 50 dS m^{-1} ($\approx$500 mM NaCl).

Salt tolerance of halophytes

Halophytes are plants which can grow and reproduce in a saline environment of 200 mM NaCl and are often able to grow at seawater salinity levels of around 500 mM NaCl (Flowers *et al.*, 1986; Breckle, 2002; Flowers and Colmer, 2008). Halophytes represent only 1 % (Flowers and Colmer, 2008) to 2 % (Glenn *et al.*, 1999) of terrestrial plant species, but are present in 139 higher plant families (Yensen, 2008). It is estimated that around 3500 potential halophyte crop species exist worldwide (Yensen, 2008). Halophytes grow naturally on saline soils and have evolved various mechanisms to cope with salinity (Flowers *et al.*, 1977; Zhu, 2001; Munns, 2001; Munns *et al.*, 2002; Breckle, 2002; Ashraf and Harris, 2004; Parida and Das, 2005). In general, the tolerance of all halophytes to ionic as well as osmotic stress relies on controlled uptake, increased extrusion and compartmentalization of salts (Flowers *et al.*, 1977; Zhu, 2001; Flowers and Colmer, 2008). However, at high salinity levels growth reductions often occur. For more salt sensitive plant species the initial growth reduction is caused by the osmotic effect of salt outside the roots, and the subsequent growth reduction due to ionic stress is caused by the inability to prevent salt from reaching toxic levels in the transpiring leaves (Munns, 2005). As a consequence of these primary effects, secondary stresses such as oxidative damage and nutritional imbalance often occur (Zhu, 2001), also affecting plant growth. Part of the underlying mechanisms of the salt tolerance of halophytes remains unclear (Flowers and Colmer 2008). To understand these underlying principles of the salt tolerance of a given

species, it is necessary to unravel the plant response on the cellular, tissue and whole-plant level (Flowers *et al.*, 1986; Flowers and Colmer 2008; Munns and Tester, 2008). The salt tolerance mechanisms of plant species are further discussed in Chapters 3, 4 and 6.

Salt marsh halophytes
Tidal inundation exposes salt marsh plant species to high salinity on a daily basis and species occurring in this habitat are highly salt tolerant. Plant species occurring in the lower marsh elevation are exposed and adapted to high soil salinity as well as water logging conditions, whereas species occurring on higher marsh elevations are primarily exposed to high soil salinity (Cooper, 1982; Haines and Dunn, 1985; Rozema *et al.*, 1985; Pennings and Callaway, 1992; Lenssen *et al.*, 1995; Leendertse *et al.*, 1997; Colmer and Flowers, 2008). Different species that occur in salt marshes as well as in inland deserts can be cultivated using seawater irrigation, with *Salicornia bigelovii* (Glenn *et al.*, 1998), *Salicornia europaea* (O'Leary *et al.*, 1985) and different *Atriplex* species (O'Leary *et al.*, 1985; Pasternak *et al.*, 1985) as the most promising species.

Strandline halophytes
In the coastal zone, the strandline is the area where drift material is deposited by spring tides or storms. Unlike the salt marsh, where plant species are exposed to tidal inundation and thus to high salinity (Cooper, 1982; Colmer and Flowers, 2008), the strandline is out of reach of mean high tide and only rarely flooded with seawater. Reports on strandline soil salinity after flooding have been somewhat inconsistent. These salinity concentrations have ranged from the level of seawater (Ignaciuk and Lee, 1980; Greaver and Sternberg, 2007) or even exceeding that of seawater (Erickson and Young, 1995) to salt concentrations less than 10 % seawater following storm surges (Barbour and De Jong, 1977). Although strandline soil salinity occasionally shows periods of high salinity, these periods appear to be relative short in sandy soils and have been reported to occur outside the growing season (Boyce, 1954; Ignaciuk and Lee, 1980). The literature on strandline soil salinity during the entire growing season has been limited. The soil salinity has been reported to remain below 10 % seawater concentrations (Ignaciuk and Lee, 1980) and Barbour *et al.* (1985) concluded that the strandline is thus a non saline habitat. However, some strandline plant species have been reported to be salt tolerant such as *Beta vulgaris subsp. maritima* (Rozema *et al.*, 1993; Koyro, 2000) and *Cakile maritima* (Debez *et al.*, 2004; Megdiche *et al.*, 2007). Although the salt tolerance of plant species is usually based on the ability to tolerate soil salinity, the salt exposure at the strandline is mainly composed of salt spray. Salt spray is formed by seawater droplets breaking in the zone of heavy surf and the small droplets are transported landward by wind (Boyce, 1954). It has long been recognized that salt spray affects coastal plant communities (Oosting and Billings, 1942; Rozema *et al.*, 1982). The salts may enter the aerial organs of the plants, especially where small surface injuries are present (Boyce, 1954). In this way it can disrupt the water balance of plants (Munns, 1993), and cause necrosis or loss of leaves (Karschon, 1958), and lead to growth reduction (Tominaga *et al.*, 1991). Plant species growing in the vicinity of the tide line have adapted to salt spray in various ways (Barbour and De Jong, 1977; Rozema *et al.*, 1985; De Vos *et al.*, 2010b).

Research questions and thesis outline

The overall aim of this thesis is to contribute to the scientific knowledge of the ecology of (strandline) halophytes, their ecophysiological response to salinity, and their cultivation under field conditions. In Table 1.2 an overview is given of the research questions which were addressed in the different chapters.

Table 1.2. An overview of the research questions which were considered in the different chapters.

Area of research	Research questions
Ecology	*What is the effect of salt spray and soil salinity on the overall salt exposure of the strandline and the subsequent plant growth?* **(Chapter 2)**
	Can strandline plant species be recognized as salt tolerant based on their habitat? **(Chapter 2)**
Ecophysiology	*Does salt spray affect the growth of the strandline species* Crambe maritima*?* **(Chapter 3)**
	Does increased soil salinity affect the growth of the strandline species Crambe maritima*?* **(Chapter 3)**
	What are the morphological and physiological adaptations underlying these growth responses at higher salinities? **(Chapter 3)**
	What is the level of salt tolerance of Diplotaxis tenuifolia *and* Cochlearia officinalis *and do both species have potential as halophyte crops?* **(Chapter 4)**
	What are the morphological and physiological adaptations underlying these growth responses at higher salinities? **(Chapter 4)**
	Are there common adaptations within the Brassicaceae family concerning the salt response to increased salinity? **(Chapter 4)**
Cultivation	*What is the effect of the chosen irrigation regime, in combination with the evapotranspiration and precipitation, on the spatio-temporal variation of soil salinity?* **(Chapter 5)**
	Are there differences in salt tolerance between the tested varieties of Beta vulgaris *ssp*. maritima*?* **(Chapter 5)**

Ecology

Although it is well known that plant species occurring in the salt marsh habitat demonstrate high salt tolerance as well as potential for saline agriculture, relatively little is known about the salt exposure, the salt tolerance and crop potential of plant species occurring in the strandline habitat. The aim of this case study was to quantify the salt spray and soil salinity of the strandline during the entire growth season. By combining the long-term measurements of salt spray, soil salinity, mineral composition of plant and soil samples, leaf thickness, total vegetation coverage and species abundance, an attempt was made to elucidate the overall salt exposure of the

strandline. Secondly, an assessment was made about the salt tolerance of strandline plant species in general (Chapter 2).

Ecophysiology
A selection has been made from the different species occurring on the strandline to determine the ecophysiological response to airborne and soil borne salinity under greenhouse conditions. This was tested for *Crambe maritima* in Chapter 3 and for *Diplotaxis tenuifolia* and *Cochlearia officinalis* in Chapter 4. The aim of these experiments was to determine the effect of increasing salinity on different aspects of the growth and to obtain insight in the morphological and physiological adaptations underlying these growth responses.

Cultivation
There is a need to test the growth performance of potential halophyte crops under field conditions. An experimental saline field site was created on Texel, The Netherlands, where seawater drip irrigation was used to cultivate different crops under saline conditions. Here, the growth and cultivation potential of various halophytes in response to seawater irrigation was tested under field conditions and compared with previous performed greenhouse experiments (Chapter 5). The feasibility of conducting reliable field experiments with regard to soil salinity was an important aim. Secondly, the salt tolerance of three different varieties of *Beta vulgaris* ssp. *maritima* was compared and evaluated.

Finally, a general discussion about the research presented in this thesis and the implications is given in Chapter 6. The utilization of halophytes as agricultural crops and the perspective of saline agriculture will also be discussed.

Chapter 2.

Morphological and ecophysiological plant parameters in relation to spatial and temporal variation of airborne and soil borne salinity along the Afsluitdijk

Arjen C. de Vos[1]
Rob Broekman[1]
Jelte Rozema[1]

[1] *Institute of Ecological Science, Department of Systems Ecology, VU University Amsterdam De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

- *Background and Aims* Much literature on strandline salt exposure is based on relative short-term observations and has focussed on sandy soils. By combining different long-term measurements an attempt was made to elucidate the overall salt exposure of the strandline under field conditions.
- *Methods* The spatial and temporal variation in soil borne and airborne salinity was studied on a sea dike. Measurements were performed in two distinctive zones along a transect. These two zones, a lower and upper zone, were located about 7 and 14 m inland/landward from the mean tide line, at 2 and 4 m above mean seawater level, respectively. In this strandline habitat measurements of salt spray, soil salinity and soil mineral composition were performed during the growing season of three consecutive years. Plant coverage and abundance were also determined during this period. Leaf samples of *Crambe maritima* were collected during four years of which leaf mineral composition was determined. Leaf thickness of *Crambe maritima* was also measured.
- *Key Results* Results showed that along the small spatial scale, relative large differences between the two zones occurred in salt exposure, soil parameters and vegetation characteristics. In the lower zone, salt spray levels above 40 mg dm^{-2} day^{-1} were recorded during a total of 11 and 6 weeks for two consecutive years during the growing season of 26 weeks. Peak concentrations above 200 mg dm^{-2} day^{-1} were recorded once during the growing season and once outside the growing season during the entire period of measurements. In both years of continuous monitoring, inundation occurred in the lower zone in the middle of the growing season. In contrast with the lower zone, no inundation or salt spray levels above 40 mg dm^{-2} day^{-1} occurred in the upper zone during the growing season. The soil salinity of the lower zone can be classified as non-saline to moderately saline and only incidentally (after inundation with seawater) as saline. However, analyses of *Crambe maritima* leaves indicated that soil salinity did enhance leaf Na^+ concentrations. Despite differences in soil mineral composition, the plant mineral composition mainly differed in leaf Na^+ concentrations between the two zones. Differences in leaf thickness also reflected the difference in salt exposure. The percentage vegetation cover of the lower zone was almost 50 % lower compared to the upper zone and the absence of inland species in the lower zone also indicated that the actual salt exposure was greater than in the upper zone.
- *Conclusions* In the coastal zone on the Afsluitdijk, different soil parameters and vegetation characteristics are governed by the salt exposure. The influence of this salt exposure, mainly in the form of salt spray but seawater inundation as well, decreased rapidly with distance from and elevation above seawater level.

Key words: strandline, halophyte, salt spray, airborne and soil borne salinity, root zone salinity, *Crambe maritima*, leaf mineral composition, leaf thickness, vegetation cover/composition

INTRODUCTION

Much literature on strandline soil salinity has focussed on sandy soils. Rain rapidly leaches salts through coarse substrates lacking clay and organic matter. However, seawater inundation can deposit detritus and fine sediment on the sandy strandline and some strandline habitats consist of clay soil. Consequently, these soils enriched in clay and organic matter, are less easily leached. Also, the reported values of soil salinity and salt spray have often been based on relative short-term observations or measurements that were performed relatively far away from the mean tide line. This makes it difficult to assess the actual or average salt exposure of strandline plant species throughout the growing season. To elucidate the salinity of the strandline, the salt spray and soil salinity was monitored during three consecutive years on a sea dike in The Netherlands. This sea dike (the Afsluitdijk) resembles to some extent a pebble beach habitat, which is the natural habitat of *Crambe maritima*. The dike is covered with large basalt stones and the remaining space is filled with gravel. The dike is located in the Waddensea area which is enclosed by different islands, making it a somewhat sheltered location. Along this dike, measurements were performed in an upper and a lower zone, located 7 and 14 m from the mean tide line, respectively. The spatial and temporal variation in airborne salinity was correlated with wind speed and seawater level. Species composition, total cover and abundance and soil mineral composition was determined during the same period. Also, the effect of the salt exposure on leaf mineral composition of *Crambe maritima* was monitored during four consecutive years, from 2006 until 2009. By combining the long-term measurements of salt spray, soil salinity, mineral composition of plant and soil samples, leaf thickness, total vegetation coverage and species abundance, an attempt was made to analyse the overall salt exposure of the strandline in relation to the plant species occurrence and characteristics.

MATERIAL AND METHODS

Research site
The research site was located on the Afsluitdijk in The Netherlands (52°57'N and 5°05'E), where samples were collected of salt spray, soil and plants from July 2006 till September 2009. Along the dike, an upper and a lower zone were selected on the dike (Fig. 2.1). The dike itself is northwest oriented. The lower zone was located about 7 m inland from the mean tide line at 2 m above mean seawater level and the upper zone was located 14 m inland from the mean seawater level at 4 m above mean seawater level. The lower zone represents the strandline.

Salt spray measurement and inundation
The salt spray collectors were placed 20 meters apart with ten collectors per zone, covering a total distance of 200 meters, parallel to the coastline. The salt spray collectors were composed of polypropylene filter gauze placed over a plastic tube. This tube was placed (vertically) in a beaker to collect precipitation and prevent leaching of the collected salt spray (based on Doomen *et al.*, 2006). The collectors were fixed directly on the ground, with a height of 30 cm and the upper 20 cm being the exposed area (an image of a salt spray collector in the field is given in Picture 2.1).
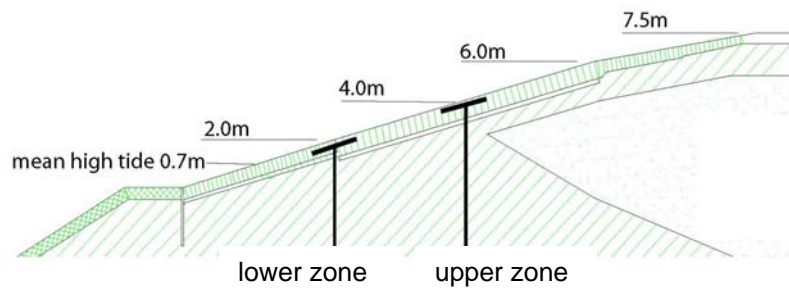
Figure 2.1. Cross-section of the Afsluitdijk (courtesy of Directorate General of Public Works and Water Management, the Netherlands) and the location of the lower and upper zone. Values are in meters above mean seawater level. The location of the salt spray meters of the lower and upper zone are 2.0 and 4.0 meters above mean seawater level and 7 m and 14 m inland from the mean seawater level (mean tide line), respectively.

The exposed surface area of the filter gauze was 1.14 dm^{-2}. The amount of salt spray was determined by vigorously shaking the beaker together with the filter gauze and a known amount of water for one minute. After this the electrical conductivity of the solution was determined and a standard curve was used to determine the concentration of NaCl. Captured salt spray was expressed as mg NaCl dm^{-2} day^{-1}. Inundation was recorded as the destruction of the salt spray collectors and the presence of drift material.

Picture 2.1. Image of a salt spray meter used to capture the salt spray on the Afsluitdijk.

Soil samples
Soil samples were collected within 1 m of a salt spray collector. Soil samples (n=2 for samples taken in 2006, n=3 for the rest) were composed of three consecutive subsamples and each subsample was collected at one salt spray collector (each subsample was composed of three soil samples of 20 cm deep). This part of the rooting zone mainly consisted of pebbles (± 3-5 cm), but these pebbles were not included in the soil samples.

Plant samples
Leaves of *Crambe maritima* were used to analyse plant mineral composition. Plant samples were collected similar to soil samples. Only the oldest, green, seaward facing leaf was collected, with one leaf per subsample and three subsamples per sample. In 2009 leaves of individual plants were harvested and analysed instead of combining subsamples. Leaf succulence was determined for individual leaves of 25 *C. maritima*

plants per zone. Measurements were performed non-destructively with a thickness gauge and expressed in mm.

Vegetation characteristics
Vegetation coverage and abundance was recorded near each salt spray collector, in 2 by 1 meter plots surrounding the collectors. Coverage and abundance data of vegetation was derived from visual cover estimations, ranging from 0 to 100 % with consecutive steps of 5 %. The growing season was defined as the period from the beginning of April until the end of September, 26 weeks in total.

Wind speed and seawater level
Data on wind speed was provided by the Royal Netherlands Meteorological Institute. Values of the highest mean hourly wind speed were used to calculate means for the two closest stations (De Kooy and Stavoren, both located about 20 km away from the research site). Inland wind was defined in relation to the northwest orientated dike, only wind direction northwest ±45° was used. Data on seawater level (recorded every ten minutes) was provided by the Directorate General of Public Works and Water Management, The Netherlands. Means were calculated for the two closest stations (Harlingen and Den Oever, located at 35 and 6 km away from the research site, respectively). Based on Doomen *et al*. (2006), it was decided to use only the maximum values of both wind speed and seawater level to test for correlations with salt spray. Seawater level was based on the NAP (Normaal Amsterdams Peil or Normalnull) reference height which is in use as a vertical reference and approximately equals mean seawater level at the present day. The mean difference in seawater level at high and low tide is about 1 m ± seawater level. Mean seawater level equals mean tide line.

Analysis of soil and plant samples
Soil samples were oven-dried at 70 °C for 48h. The extract of the saturated paste of the soil samples was used for analysis of conductivity (used as a measurement of salinity), pH and mineral composition of soil (Rhoades *et al*., 1999). Plant samples were also oven-dried at 70 °C for 48h. For extractions, leaf material was ground to a fine powder using a ball-mill (Retsch-MM200, Haan, Germany). Total P, Na^+, K^+ Ca^{2+} and Mg^{2+} concentrations were determined after digesting 100 mg plant material in 2 mL of a 1:4 mixture of 37 % (v/v) HCl and 65 % (v/v) HNO_3 in a Teflon cylinder for 6 h at 140 ºC. The volume was adjusted to 10 mL with demineralised water. Flame atomic absorption was used to determine the concentrations of Ca^{2+}, Mg^{2+}, Na^+ and K^+ in leaves as well as in the saturated extract of the soil samples (spectrophotometer Perkin-Elmer 1100B, Perkin Elmer Inc., Waltham, MA, USA). Phosphate concentrations were determined with a spectrophotometer (880nm) (Shimadzu UV-1601PC, Japan), using molybdenum blue as reagent (Murphy and Riley, 1962). Dry combustion with an elemental analyser (Carlo Erba NA1500, Rodana, Italy) was used to determine total C and N concentrations. The soil organic matter content was determined (soil samples of 14/07/2006) as the weight loss on ignition (4h, 650ºC), with samples measured in duplicate.

Statistical analysis
Statistical data analysis was performed using SPSS (version 15.0 for Windows, SPSS Inc. 2000, Chicago, IL, USA). Repeated measures analysis was used to test for possible differences in time and between zones, with different dates (temporal

variation) to test for within-subjects effects and different zones (spatial variation) to test for between-subjects effects. Periods with inundation, sampling occurring in just one zone, or sampling with n=2 were excluded from the analysis. Missing values of salt spray were estimated by calculating the means per period and per zone (for salt spray data the average missing values were n=1.24 for zone A and n=0.95 for zone B per date, with n=10 per date as maximum). Greenhouse-Geisser degrees of freedom corrections were used when assumptions about homogeneity of covariance were violated. One way analysis of variance was also used to test for differences in leaf thickness between individual leaves of *Crambe maritima*. The possible relationship between salt spray, inland wind speed and seawater level was analysed with linear regression and Pearson correlation. For this, the maximum value of either wind speed or seawater level during the period of measurement was used.

RESULTS

Salt spray

Measurements of seasonal variation of salt spray and records of seawater inundation for the period 2006-2008 are listed in Table 2.1. The level of salt spray in the lower zone exceeded 40 mg dm^{-2} day^{-1} on numerous occasions throughout the growing season. A salt spray level above 200 mg dm^{-2} day^{-1} was recorded during two periods

Table 2.1. Salt spray (mg dm^{-2} day^{-1}) and inundation from September 2006 until October 2008 on the Afsluitdijk of the lower and upper zone, located about 7 m and 14 m inland from the mean seawater level at 2 m and 4 m above mean seawater level, respectively.

Date [1]	lower zone [2]	upper zone	Date [1]	lower zone	upper zone
2006			**2007** continued		
07 - 12 Sep	71.8±16.3	3.5±0.3	11 Jul - 10 Aug	41.3±2.1	5.2±0.5
12 - 19 Sep	1.9±0.4	0.6±0.0	10 - 21 Aug	22.5±6.0	1.6±0.2
19 - 25 Sep	1.1±0.2	0.5±0.1	21 Aug - 12 Sep	55.7±4.1	5.9±0.8
25 - 29 Sep	10.3±1.4	0.8±0.1	12 - 27 Sep	41.3±7.0	4.5±0.3
29 Sep - 06 Oct	75.4±17.4	1.8±0.2	27 Sep - 15 Oct	4.9±0.7	1.6±0.2
06 - 13 Oct	inundation	24.1±2.9	15 Oct – 22 Nov	inundation	inundation
13 - 24 Oct	0.6±0.0	0.4±0.0	**2008**		
24 - 31 Oct	192.2±25.2	8.0±0.7	09 Apr - 09 May	0.6±0.0	0.5±0.0
31 Oct - 07 Nov	inundation	inundation	09 May - 19 Jun	0.4±0.1	0.6±0.1
2007			19 - 27 Jun	231.2±31.1	6.1±1.4
30 Mar - 04 May	7.5±0.6	2.5±0.1	27 Jun - 24 Jul *	94.7±0.5	8.4±1.7
04 May - 15 Jun	1.6±0.4	0.4±0.2	24 Jul - 15 Aug	20.3±1.9	2.1±0.1
15 - 27 Jun	inundation	40.8±6.6	15 Aug - 24 Sep	1.5±0.1	0.4±0.0
27 Jun – 11 Jul	57.4±6.7	5.1±1.1	24 Sep - 29 Oct	71.3±2.4	4.0±0.6

[1] difference in time (Date): $F_{(3, 53)}$ = 46.66; p< 0.001.

[2] difference between zones: $F_{(1, 18)}$ = 216.2; p< 0.001.

* Time*Zone interaction: $F_{(3, 53)}$ = 41.35; p< 0.001.

Values are means ±se, with n=6-10 except for the date 27 June – 24 July 2008 for which n=2 for the lower zone. This latter period (*) could also be listed as inundation since eight collectors were lost due to inundation. Data was analysed using repeated measures (Greenhouse-Geisser corrections) with the different dates (time) to test for within-subjects effects and the two zones to test for between-subjects effects. Dates with inundation and n=2 were excluded from the analysis.

in the lower zone, whereas the values for the upper zone did not exceed 10 mg dm^{-2} day^{-1} for the same periods. The maximum salt spray concentration recorded was 231 mg dm^{-2} day^{-1}, which occurred in the period 19-27 June 2008 in the lower zone. Also,

during the experiment the lower zone was inundated on four occasions, whereas the upper zone was inundated on only two occasions and only at the end of the growing season. The high levels of salt spray and inundation of the lower zone also occurred in the middle (June-July) of the growing season (April-September). The salt exposure of the upper zone was minimal compared to the lower zone. During the growing season, no inundation or salt spray levels above 40 mg dm^{-2} day^{-1} occurred in the upper zone. There was a significant difference in salt spray in time and between the two zones, and the interaction between time and zones was also significant (results listed in Table 2.1).

The correlation between salt spray, inland wind speed and seawater level for the lower and upper zone was analysed (Fig. 2.2). Both parameters showed a significant correlation with salt spray in both zones. The correlation in the lower zone appeared to be somewhat stronger with seawater level (r^2=0.31, F=8.79, P=0.008) than with inland wind speed (r^2=0.24, F=5.90, P=0.025). In the upper zone the difference between the correlation with seawater level (r^2=0.55, F=8.79, P<0.001) and inland wind speed (r^2=0.21, F=5.54, P=0.028) was more apparent. The correlation with maximum wind speed rather than with maximum inland wind speed was analysed as well (lower zone: r^2=0.18, F=4.50, P=0.047; upper zone: r^2=0.16, F=4.29, P=0.050),
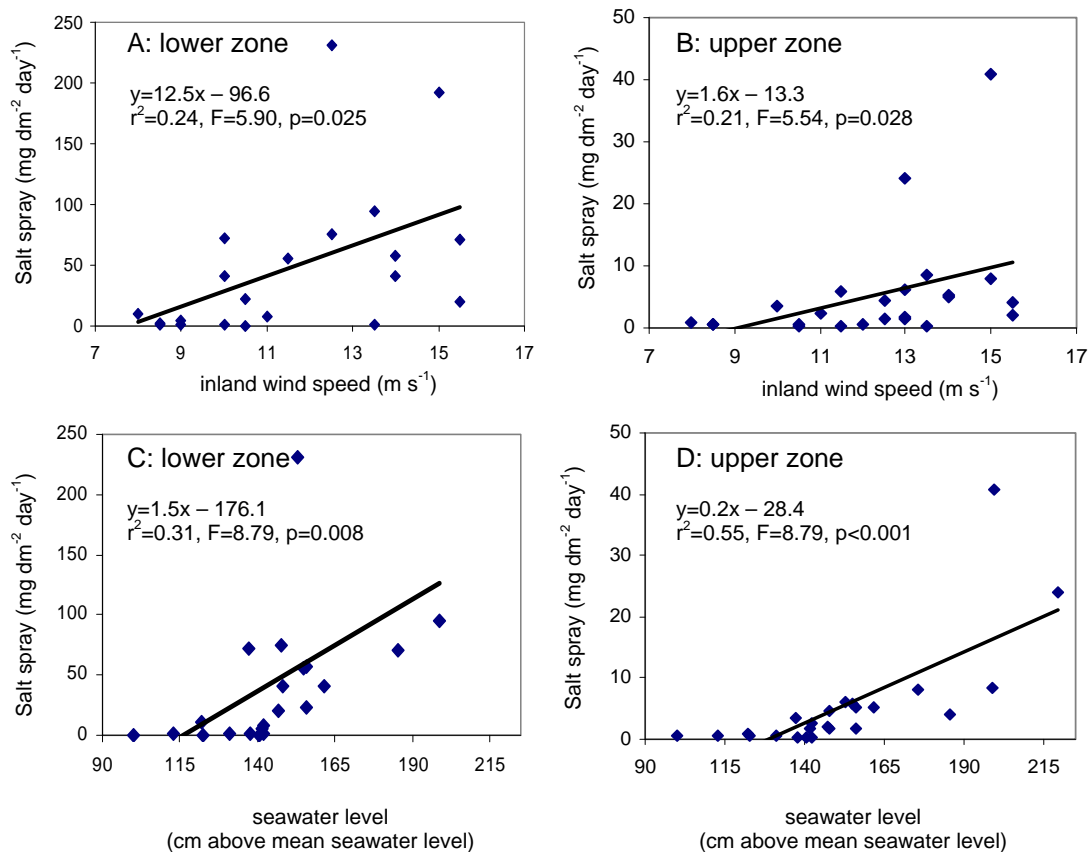
Figure 2.2. Correlation between the level of salt spray and maximum inland wind speed of the lower zone (A) and the upper zone (B), and the correlation between the level of salt spray and maximum seawater level of the lower zone (C) and the upper zone (D), The maximum is defined as the highest value of a specific day during the period of measurement. For A, B, C, D the number of observations are n=22, n=24, n=22 and n=24, respectively. Days with inundation were excluded from the analysis. Correlations were analysed with linear regression and Pearson correlation (r^2).

but these correlations were lower than with inland wind speed presented in Figure 2.2. By combining the data in figure 2 (inland wind speed * seawater level) the correlation with salt spray increased in both zones (lower zone: r^2=0.42, F=13.5, P=0.002; upper zone: r^2=0.58, F=29.4, P<0.001).

Soil parameters
Soil salinity levels of the lower zone ranged between 1.3 and 27.8 dS m^{-1} during the time of sampling (Table 2.2). Soil samples of the lower zone were collected on eleven occasions. Of these eleven soil samples, three were in the range of 1.3 to 1.6 dS m^{-1} and six in the range of 3.1 to 6.8 dS m^{-1}. On two occasions, namely 13/10/2006 and 24/07/2008, soil salinity increased to 19.3 and 27.8 dS m^{-1}, respectively. Soil salinity levels of the upper zone were lower compared to lower zone and did not exceed 3.5 dS m^{-1} during the time of sampling. The pH and saturation percentage (amount of water added per mass dry soil to obtain saturated paste) were also determined. The pH ranged between 7.5 and 8.1 for the lower zone and between 7.0 and 8.0 for the upper zone. The saturation percentage ranged between 60 and 90 % for the lower zone and between 100 and 129 % for the upper zone. This difference in saturation percentage between zones was significant (F(1,4)=39.9, P=0.003), whereas no difference in time was observed (F(4,16)=0.93, P=0.470). The soil organic matter content of the samples collected was 7.7±0.6 % and 29.5±4.8 % for the lower and upper zone, respectively.

The mineral composition of the soil samples between the two zones, with the exception of P, showed differences in the various mineral concentrations (Table 2.3). Concentrations of N were consistently lower in the lower zone compared to the upper zone, but no differences occurred in time. Concentrations of K$^+$, Ca^{2+}, Mg^{2+} and Na$^+$ showed differences between zones and also in time. The increased mineral concentrations in the lower zone appear to coincide with inundation, whereas the mineral concentrations in the upper zone remained more constant.

Table 2.2. Salinity of soil samples collected in the lower and upper zone on the Afsluitdijk, from July 2006 until October 2008.

	Salinity (dS m^{-1})			Salinity (continued)	
date [1]	lower zone [2]	upper zone	date	lower zone	upper zone
14/07/2006	3.1±0.7	1.9±0.1	30/03/2007	3.8±0.1	3.5±0.3
25/09/2006	6.8±1.4	-	21/08/2007	3.2±0.2	1.8±0.1
29/09/2006	-	1.8±0.0			
06/10/2006	1.5±0.0	-	09/05/2008	3.9±0.8	2.8±0.4
13/10/2006	19.3±0.5	-	24/07/2008	27.8±0.6	2.5±0.2
24/10/2006	1.6±0.1	-	31/07/2008	4.4±1.0	-
			29/10/2008	1.3±0.0	1.4±0.1

[1] Time (date) F(2,7)=536.2; p<0.001; [2] Zone F(1,4)=519.7; p<0.001; Time*Zone interaction F(2,7)=516.8; p<0.001
Salinity (dS m^{-1}) was measured in the soil saturated paste extract. Values are means ±se (n=2 for samples taken in 2006, n=3 for the rest). Data was analysed using repeated measures with the different dates (time) to test for within-subjects effects and the two zones to test for between-subjects effects. Dates with n=2 or sampling occurring in just one zone were excluded from the analysis. The salinity of the Waddensea is about 40 dS m^{-1} (Van Aken, 2001).

Mineral composition and leaf thickness of Crambe maritima
Plant mineral composition was determined for *Crambe maritima* leaves collected in September (Table 2.4). On one occasion the leaf mineral concentrations in the

beginning of the growing season, namely 09/05/2008, were determined as well. No difference occurred between the two zones in leaf N and K concentrations and also P

Table 2.3. Mineral composition (in μmol ml^{-1}) in 2006, 2007 and 2008 of the soil moisture of the soil samples collected in the lower and upper zone on the Afsluitdijk.

Date	zone	N [1]	P [2]	K$^+$ [3]	Ca^{2+} [4]	Mg^{2+} [5]	Na$^+$ [6]
14/07/2006	lower	164±16	0.01±0.00	1.26±0.09	1.50±0.01	1.76±0.24	21.6±5.9
	upper	-	-	-	-	-	-
25/09/2006	lower	160±15	0.01±0.00	1.59±0.21	3.77±0.44	5.83±1.36	36.6±5.9
	upper	-	-	-	-	-	-
29/09/2006	lower	-	-	-	-	-	-
	upper	583±18	0.17±0.07	1.84±0.49	3.50±0.14	2.53±0.09	4.8±0.3
06/10/2006	lower	145±0	0.04±0.00	0.84±0.17	1.57±0.02	2.20±0.20	7.6±0.5
	upper	-	-	-	-	-	-
13/10/2006	lower	186±5	0.06±0.02	5.89±0.55	9.04±1.22	22.25±3.70	168.1±21.7
	upper	-	-	-	-	-	-
24/10/2006	lower	193±8	0.12±0.01	0.71±0.12	0.41±0.04	1.33±0.16	14.8±0.9
	upper	-	-	-	-	-	-
30/03/2007	lower	219±18	0.05±0.01	1.10±0.09	0.86±0.07	2.37±0.20	28.9±0.2
	upper	456±6	0.08±0.02	1.35±0.23	0.87±0.14	2.14±0.39	27.8±1.5
21/08/2007	lower	304±4	0.13±0.05	0.37±0.27	0.49±0.04	1.75±0.11	22.1±2.1
	upper	490±3	0.13±0.02	1.28±0.34	1.97±0.33	2.38±0.10	6.6±1.9
09/05/2008	lower	228±29	0.04±0.01	1.24±0.18	1.79±0.26	3.52±0.65	28.8±5.6
	upper	479±9	0.05±0.01	1.21±0.09	3.27±0.80	4.75±1.08	12.9±1.8
24/07/2008	lower	223±17	0.10±0.04	10.80±0.96	10.33±0.43	26.70±1.25	309.3±9.6
	upper	528±146	0.07±0.01	1.28±0.14	2.35±0.09	3.26±0.13	12.8±0.3
29/10/2008	lower	252±24	0.06±0.01	0.71±0.02	0.85±0.03	1.74±0.07	6.9±0.3
	upper	299±45	0.09±0.02	1.29±0.23	2.77±0.23	2.10±0.01	2.6±0.1

[1] Zone F(1,4)=30.39; p=0.005, Time (date) F=(1,5)=1.84; p=0.239, Zone*Time F(1,5)=2.08; p=0.212

[2] Zone F(1,4)=0.60; p=0.481, Time (date) F=(2,6)=2.82; p=0.140, Zone*Time F(2,6)=0.46; p=0.605

[3] Zone F(1,4)=130.9; p<0.001, Time (date) F=(2,6)=66.7; p<0.001, Zone*Time F(2,6)=66.7; p<0.001

[4] Zone F(1,4)=15.2; p=0.018, Time (date) F=(2,7)=81.5 ; p<0.001, Zone*Time F(2,7)=73.0; p<0.001

[5] Zone F(1,4)=125.1; p<0.001, Time (date) F=(2,8)=189.1; p<0.001, Zone*Time F(2,8)=172.4; p<0.001

[6] Zone F(1,4)=547.4; p<0.001, Time (date) F=(1,6)=697.9; p<0.001, Zone*Time F(1,6)=683.9; p<0.001

Measurements were performed using the extract of saturated paste, except for N which was determined directly using dried soil samples (expressed as μmol g^{-1}). Samples were the same as those used for determining salinity. Values are means ±se (n=2 for samples taken from June 2006 until 6 October 2006, n=3 for the rest). Data was analysed using repeated measures (Greenhouse-Geisser corrections) with the different dates (time) to test for within-subjects effects and the two zones to test for between-subjects effects. Dates with n=2 were excluded from the analysis.

concentrations, although analysis of this latter potential difference resulted in a *P*-value of 0.052. Although no difference occurred between the two zones in leaf N and K concentrations, there was a difference in concentration in time. Concentrations of Ca^{2+}, Mg^{2+} and Na$^+$ differed between zones and in time, with leaf Ca^{2+} concentrations showing lower concentrations in the lower zone and Mg^{2+} and Na$^+$ showing higher concentrations in the lower zone compared to the upper zone. The Na$^+$ concentrations of leaves collected at the end of the growing season ranged between 2.35 and 3.89 mmol g^{-1} DW in the lower zone and between 0.50 and 1.95 mmol g^{-1} DW in the upper zone.

The thickness of *Crambe maritima* leaves was determined for the first seven leaves (Fig. 2.3). Both the thickness of the separate leaf numbers and the overall leaf thickness per zone showed increased values for the leaves of plants occurring in the lower zone. The overall leaf thickness of plants occurring in the lower zone showed an increase of 42 % compared to the leaves of plants occurring in the upper zone. The greatest difference between separate leaves of the two zones occurred in the youngest

leaf (leaf 7), which showed an increase in leaf thickness of 63 % in leaves of plants in the lower zone. The mean absolute leaf thickness of plants occurring in the lower zone was 1.05 mm with 1.55 mm as the greatest individual leaf thickness, whereas in the upper zone the mean absolute leaf thickness was 0.75 mm and the thickness of individual leaves rarely exceeded 1 mm.

Table 2.4. Mineral composition (mmol g^{-1} DW) of *Crambe maritima* leaves in the period 2006-2009, collected in the lower and upper zone on the Afsluitdijk.

Date	zone	N [1]	P [2]	K$^+$ [3]	Ca^{2+} [4]	Mg^{2+} [5]	Na$^+$ [6]
30/08/2006	lower	1.17±0.09	0.11±0.01	0.88±0.04	0.74±0.06	0.51±0.03	2.35±0.07
	upper	1.27±0.12	0.06±0.00	0.77±0.05	1.67±0.10	0.30±0.00	0.50±0.07
12/09/2007	lower	1.95±0.15	0.08±0.01	0.49±0.13	0.50±0.06	0.41±0.06	3.84±0.40
	upper	1.34±0.31	0.07±0.0	0.67±0.23	0.53±0.06	0.26±0.04	1.89±0.34
09/05/2008	lower	3.24±0.05	0.17±0.03	0.42±0.06	0.30±0.02	0.17±0.02	0.17±0.03
	upper	3.48±0.06	0.18±0.01	0.41±0.01	0.46±0.05	0.16±0.01	0.18±0.01
24/09/2008	lower	2.11±0.03	0.13±0.01	0.67±0.05	0.58±0.02	0.36±0.02	2.45±0.11
	upper	1.50±0.05	0.09±0.01	0.39±0.02	0.89±0.04	0.32±0.03	1.95±0.10
22/09/2009	lower	1.33±0.08	0.10±0.01	0.52±0.09	0.46±0.04	0.35±0.02	3.89±0.20
	upper	1.40±0.30	0.05±0.01	0.63±0.10	0.88±0.11	0.28±0.02	1.28±0.11

[1] Zone $F_{(1,4)}$=2.33; p=0.202, Time (date) $F_{(2,8)}$=42.76; p<0.001, Zone*Time $F_{(2,8)}$=2.35; p=0.159

[2] Zone $F_{(1,4)}$=7.49; p=0.052, Time (date) $F_{(4,16)}$=29.58; p<0.001, Zone*Time $F_{(4,16)}$=4.15; p=0.013

[3] Zone $F_{(1,4)}$=0.29; p=0.617, Time (date) $F_{(2,8)}$=3.32; p=0.070, Zone*Time $F_{(2,8)}$=1.34; p=0.316

[4] Zone $F_{(1,4)}$=179.5; p<0.001, Time (date) $F_{(2,7)}$=26.72; p<0.001, Zone*Time $F_{(2,7)}$=6.71; p=0.024

[5] Zone $F_{(1,4)}$=41.24; p=0.003, Time (date) $F_{(1,6)}$=18.92; p=0.004, Zone*Time $F_{(1,6)}$=4.12; p=0.083

[6] Zone $F_{(1,4)}$=188.1; p<0.001, Time (date) $F_{(2,6)}$=51.29; p<0.001, Zone*Time $F_{(2,6)}$=13.07; p=0.008

Values are means ±se (n=4 for samples taken in 2006, n=3 for 2007 and 2008, n=9 for the lower zone 2009 and n=7 for the upper zone 2009). Only green leaves facing seaward were collected and used for analysis. Data was analysed using repeated measures (Greenhouse-Geisser corrections) with the different dates (time) to test for within-subjects effects and the two zones to test for between-subjects effects. For statistical analysis, means were calculated per three salt spray collectors for 2009.

Vegetation coverage and species abundance

Results of vegetation coverage and species abundance are listed in Table 2.5. Vegetation coverage showed a difference between zones. The coverage percentage approximately doubled in the upper zone compared to the lower zone. No difference in vegetation coverage in time occurred. In both zones the dominant species were *Elytrigia atherica*, *Atriplex prostrata* and *Crambe maritima*, although on one occasion the dominant species in the lower zone was *Artemisia maritima*. The abundance of the species *Atriplex prostrata* and *Crambe maritima* not only showed a difference between zones but also in time. Another important difference between the two zones in species occurrence was formed by the less abundant species. In the lower zone, typical coastal species like *Plantago maritima*, *Honckenya peploides*, *Spergularia marina* and *Crithmum maritimum* were also observed, which did not occur in the upper zone. In the upper zone, non coastal (inland) species such as *Senecio viscosus*, *Pastinaca sativa*, *Solanum dulcamara* and *Convolvulus arvensis* were observed, which did not occur in the lower zone.
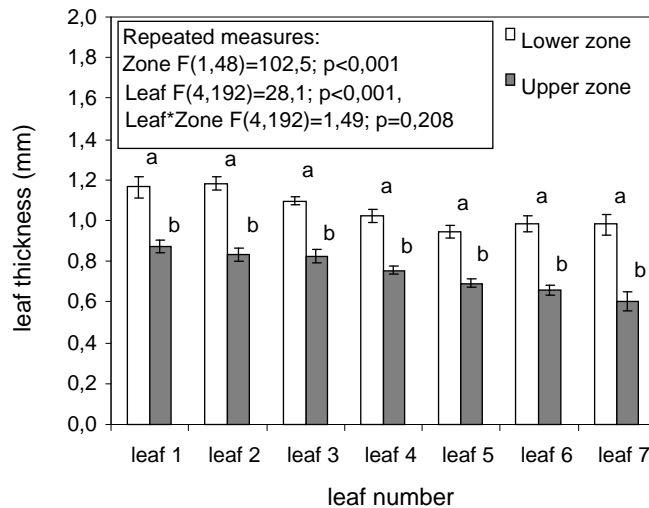
Figure 2.3. Leaf thickness of *Crambe maritima* plants, occurring in either the lower or the upper zone of the Afsluitdijk. Values are means±se with n=25 for the first 5 leaves, n=16 for leaf 6 and n=6 for leaf 7. Measurements were performed non-destructively in June 2006, with leaf 1 as the oldest leaf. Letters beside the columns show possible significant differences between the lower and upper zone, with each leaf number analysed separately. Different letters indicate a significant difference at $P<0.05$ (One-way ANOVA). Data was also analysed using repeated measures of the first 5 leaves with the different leaves to test for within-subjects effects and the two zones to test for between-subjects effects.

DISCUSSION

The level of salt spray differed between the lower and the upper zone and soil salinity, leaf Na^+ concentrations, leaf thickness, vegetation coverage and species abundance also showed differences. Each observation on its own indicated a difference in salt exposure between the two zones and by combining the individual observations the overall salt exposure of the strandline may be elucidated.

Salt spray
Although differences in salt spray along a gradient have been reported before, these gradients often started at the fore-dune habitat rather than at the strandline (Oosting and Billings, 1942; Boyce, 1954; Griffith, 2006). Also, in the elaborate study by Barbour (1978) measurements of salt spray started at 60 m from the shore. Since salt spray is formed by bubbles breaking in the zone of heavy surf and small droplets are transported land inwards by wind, the level of salt spray is, among others, correlated with distance from mean tide line (Boyce, 1954; Barbour, 1978; Rozema *et al.*, 1983b). The differences in salt spray reported here, occurred over a relative small distance between zones and, more importantly, were located close to mean tide line. The lower zone, which formed the actual strandline, was located only 7 m land-inwards from mean tide line and was elevated 2 m above mean seawater level. Barbour (1978) showed that salt spray can differ substantially over a relative small distance (measured in six rows over 20 m) and elevation accounted for the most

variation in salt spray. According to the correlation of Barbour (1978) between elevation and salt spray, the difference in salt spray should be about two-fold at 2 and

Table 2.5. Vegetation coverage and species abundance in 2006, 2007 and 2008 of the lower and upper zone on the Afsluitdijk.

	2006		2007		2008	
	Lower zone	Upper zone	Lower zone	Upper zone	Lower zone	Upper zone
Vegetation coverage [1]	36±4	63±7	36±4	72±3	36±6	65±4
Vegetation abundance						
Elytrigia atherica [2]	39±7	43±5	28±4	27±5	48±9	31±5
Atriplex prostrata [3]	28±5	5±2	38±4	19±2	16±5	34±5
Crambe maritima [4]	8±2	27±3	13±1	31±2	10±4	17±2
Artemisia maritima	5±4	-	-	-	16±8	-
Aster tripolium	11±6		2±2	-	2±1	-
Sonchus arvensis [5]	8±2	6±2	5±2	-	6±2	5±1
Tripleurospermum maritimum [6]	1±1	3±1	6±4	4±3	-	1±1
Cochlearia spp.	-	-	4±2	-	-	-
Atriplex portulacoides	-	-	2±2	-	1±1	-
Spergularia marina	-	-	-	-	2±2	-
Plantago major spp major	2±1	-	-	-	-	-
Crithmum maritimum	-	-	1±1	-	-	-
Leymus arenarius	-	3±2	1±1	-	-	-
Senecio viscosus	-	4±2	-	14±2	-	5±1
Diplotaxis tenuifolia	-	6±2	-	-	-	1±1
Pastinaca sativa	-	-	-	1±1	-	4±1
Rumex crispus	-	3±1	-	2±1	-	1±1
Solanum dulcamara	-	1±1	-	1±1	-	1±1
Convolvulus arvensis	-	1±1	-	-	-	1±1
Lamium album	-	1±1	-	-	-	-
Cymbalaria muralis	-	1±1	-	-	-	1±1
Taraxacum officinale	-	-	-	-	-	1±1
Cirsium vulgare	-	-	-	1±1	-	-

[1] Zone $F_{(1,18)}=40.20$; $p<0.001$, Time (date) $F=_{(2,36)}=0.51$; $p=0.603$, Zone*Time $F_{(2,36)}=0.51$; $p=0.603$

[2] Zone $F_{(1,18))}=0.73$; $p=0.403$, Time (date) $F=_{(2,36)}=3.14$; $p=0.056$, Zone*Time $F_{(2,36)}=1.80$; $p=0.180$

[3] Zone $F_{(1,18)}=5.13$; $p=0.036$, Time (date) $F=_{(2,36)}=6.25$; $p=0.005$, Zone*Time $F_{(2,36)}=19.19$; $p<0.001$

[4] Zone $F_{(1,18)}=74.62$; $p<0.001$, Time (date) $F=_{(2,36)}=4.39$; $p=0.020$, Zone*Time $F_{(2,36)}=2.83$; $p=0.072$

[5] Zone $F_{(1,18)}=1.22$; $p=0.284$, Time (date) $F=_{(1,18)}=0.71$; $p=0.410$, Zone*Time $F_{(1,18)}=0.13$; $p=0.722$

[6] Zone $F_{(1,18)}=0.01$; $p=0.922$, Time (date) $F=_{(1,18)}=1.74$; $p=0.203$, Zone*Time $F_{(1,18)}=0.71$; $p=0.412$

Plant coverage is expressed as a percentage of the total vegetation coverage per area. Species abundance is expressed as a percentage of species coverage per total vegetation coverage. Values are means ±se, with n=10 except for the period 2007 for which n=9. Data was analysed using repeated measures with the different dates (time) to test for within-subjects effects and the two zones to test for between-subjects effects.

4 m (elevation of lower and upper zone, respectively) above mean seawater level. However, the recorded mean difference in salt spray deposition between the lower and upper zone was about eleven-fold over the entire period of measurements. In this regard the recorded salt spray at the strandline (lower zone) was considerably higher than the correlations of salt spray, distance from mean tide line and elevation of

Barbour (1978) predicted. It was observed that waves breaking just in front of the strandline resulted in splash water which reached the lower zone. This splash water may be one possible explanation why salt spray levels between the lower and upper zone were so great. In contrast with the upper zone, the lower zone was exposed to repetitive salt spray levels of about 40-50 mg dm^{-2} day^{-1} and occasional salt spray levels above 200 mg dm^{-2} day^{-1} as well as inundation. Salt spray levels above 40 mg dm^{-2} day^{-1} were recorded in the lower zone during a total of 11 and 6 weeks for two consecutive years during the growing season of 26 weeks. Although the previous mentioned publications have measured salt spray further away from the mean tide line, some comparisons can be made. The salt spray intensity, measured during 15 consecutive weeks in spring, has been reported by Barbour (1978) to range between 1 and 200 mg NaCl dm^{-2} day^{-1}, with typical values between 10 and 50 mg NaCl dm^{-2} day^{-1}. Boyce (1954) reported maximum values of 8.4 mg dm^{-2} per hour at 20 m from mean tide, which also equals 200 mg dm^{-2} day^{-1}. So it appears that the maximum salt spray levels which are reported here are consistent with previous reported levels. However, it is possible that the levels of salt spray reported here are not the maximum levels that the strandline habitat, in general, potentially receives. First of all, the salinity of the Waddensea, where the research site was located, is about 80 % compared to the average of open ocean water (Van Aken, 2001). Secondly, the dike is located in the Waddensea which is enclosed by different islands, making the Waddensea a somewhat sheltered location. This results in a lack of (heavy) surf in front of the dike, whereas salt spray is the result of droplets breaking in heavy surf (Boyce, 1954). Also, salt spray was calculated as a mean per day whereas salt spray was measured over periods of at least one week. Conditions of high wind speed (which are responsible for high salt spray) lasted only one or two days, making it likely that the true peak in salt spray was considerably higher than the values listed in table 1. In contrast, the high values of salt spray reported by Barbour and De Jong (1977) were based on measurements during 12 hour periods.

Of the different correlations tested (Fig. 2.2), the level of salt spray was correlated more strongly with seawater level than with wind speed. Increased seawater level is the result of wind direction, wind speed and springtide, among others. Therefore, it seems likely that seawater level showed a stronger correlation with salt spray intensity than with inland wind speed. The correlation between the intensity of salt spray and seawater level was demonstrated by Barbour (1978) as well (r^2=0.43), but mean wind speed and elevation were reported to show greater correlations (r^2=0.59 and r^2=0.70, respectively). These latter correlations were based on mean values of wind speed and seawater level was based on 'mean higher high water tide level', whereas the correlations here were based on the maximum values during the time of measurements. These maximum values were chosen because Doomen *et al.* (2006) measured salt spray in The Netherlands as well and concluded that maximum wind speed during the time of measurement, rather than mean wind speed during measurements or mean daily maximum wind speed, showed the strongest correlation. The combination of inland wind speed and seawater level increased the correlation in both zones (as was shown in the section 'Results'). Although wind speed potentially influences seawater level, the direct effect of wind speed on salt spray is likely greater. Probably, this is the reason that the combination of seawater level and wind speed showed the greatest correlation with salt spray. All correlations were based on a minimal amount of observations of the more extreme values. When inundation was included as well the correlations of all parameters increased considerably. For this, inundation was set at a 'maximum salt spray' of 200

mg dm^{-2} day^{-1}. This level was chosen because this value was considered to be the maximum salt spray level according to literature (Boyce, 1954; Barbour, 1978). For the lower zone, the correlations changed from r^2=0.24 to r^2=0.40 for inland wind speed when inundation was included and from r^2=0.42 to r^2=0.59 for the combination of inland wind speed and seawater level.

Soil parameters

Soil salinity in the lower zone showed persistent salinity levels around 3-4 dS m^{-1} and occasional peaks after inundation. The increased concentration in soil salinity of 24 July 2008 is also the result of inundation, although in table 1 it is listed as salt spray. This level of salt spray was based on *n*=2, meaning that eight salt spray collectors were lost as a result of partial inundation. Salt concentrations in the range of 1.9-4.5 dS m^{-1} are classified as medium saline, at which level only moderately tolerant species are not affected (Shaw, 1999). According to the same classification, salt concentrations above 12.2 dS m^{-1} are considered as extremely saline, whereas in the lower zone concentrations of 19.3 and 27.8 dS m^{-1} were recorded as well. Another commonly used classification states that soil salinity below 4 dS m^{-1} can be classified as non saline, between 4 and 8 dS m^{-1} as moderately saline, between 8 and 16 dS m^{-1} as strongly saline and above 16 dS m^{-1} as very strongly saline (USSL, 1954). In contrast to the lower zone, soil salinity in the upper zone never exceeded 3.5 dS m^{-1}. Ignaciuk and Lee (1980) showed that the soil salinity of the strandline habitat is especially elevated between autumn and early spring in the temperate zone. The salt concentration of the strandline during the growing season in this investigation was about 1-10 dS m^{-1} (10-100 mM NaCl). Barbour *et al*. (1985) concluded that the leading edge of vegetation is a relative non-saline environment, except briefly following storm surges, although this leading edge of vegetation is often not comparable with the strandline. In relation to the salinities of the salt marsh, the soil of the strandline indeed shows a relative low salt concentration. The soil salinity of the lower zone was comparable with Waddensea salinity ($\pm$ 40 dS m^{-1}) on only one occasion, but in general the soil salinity of the lower zone was around 10 % seawater salinity. However, considering the classification of Shaw (1999) and USSL (1954), it can be concluded that strandline soil salinity can be classified as non to moderately saline and only occasionally highly saline.

The mineral composition varied for most minerals in both time and between zones. Since the increased mineral concentrations of K$^+$, Ca^{2+}, Mg^{2+} and Na$^+$ in the lower zone coincided with inundation, it seems likely that inundation influenced the mineral soil composition of the lower zone directly. Inundation did not change N or P concentrations in the soil. Possibly, the higher N concentration in the upper zone was the result of the greater amount of soil organic matter. Soil organic matter content was 7.7$\pm$0.6 % and 29.5$\pm$4.8 % for the lower and upper zone, respectively. As was mentioned before the dike resembles a pebble beach habitat and the deposited detritus and fine sediment is more easily retained than on a sandy shore. Based on the organic matter content and the saturation paste percentage (60-90 % for the lower zone and 100-129 % for the upper zone) a classification of the soil texture can be made. The soil of the lower zone can roughly be classified as clay (based on saturation percentage, see Rhoades *et al*. (1999), among others) and the soil of the upper zone as humus or peaty soil (commonly defined as > 25% organic matter). More importantly, the soil of both the lower and the upper zone was not composed of sand, but mostly of clay and organic matter. Soils which contain a high percentage of clay or organic matter adsorb more salt and are not as easily leached as sandy soils. This could have

resulted in longer periods of elevated soil salinity after inundation compared to sandy soils. However, as can be seen in Table 2.2, the elevated soil salinities after inundation were not recorded in samples collected about one week later. Possibly, this was caused by the large amount of pebbles (facilitating rapid leaching) and the above mentioned clay and peaty soil formed about 13±1 % (w/w, *n*=2 for the lower zone) of the sampled rooting zone.

Leaf mineral composition and leaf thickness of Crambe maritima

Analysis of the mineral composition of *Crambe maritima* leaves showed no difference in N, P or K^+ concentrations between the two zones (Table 2.4). Apparently, the difference in N and K^+ concentration of the soil samples did not result in a difference in the N and K^+ concentrations of the leaf samples. In a greenhouse experiment, De Vos *et al*. (2010b) determined the effect of salt spray and soil salinity up to 300 mM NaCl on the mineral composition of *C. maritima* leaves (see Chapter 3 of this thesis). Neither salt spray nor soil salinity was reported to affect the leaf P concentration. The leaf N concentration was only affected at 300 mM NaCl root zone salinity, but the concentration remained higher (2.65 mmol g^{-1} DW) than under the field conditions presented here (maximum of 2.11 mmol g^{-1} DW at the end of the growing season). The highest Na^+ concentrations of the leaves of *C. maritima* collected on the Afsluitdijk (only the oldest seaward facing leaves were collected) were comparable with the root zone salt treatment up to 200 mM NaCl. Continuous salt spray at a level of 80 mg dm^{-2} day^{-1} during 20 days under greenhouse conditions resulted in a Na^+ concentration of 2.79 mmol g^{-1} DW, whereas the highest Na^+ concentrations found in the leaves of *C. maritima* collected on the Afsluitdijk were 3.84 and 3.89 mmol g^{-1} DW. In this regard, it seems likely that root zone salinity also influenced the leaf Na^+ concentration under field conditions.

The effect of salt exposure is also apparent in leaf thickness. The overall leaf thickness of plants occurring in the lower zone on the Afsluitdijk showed an increase of 42 % compared to the leaf thickness of plants occurring in the upper zone. This difference is considerably greater than the maximum increase in leaf thickness (+ 18 %) of different salt spray treatments reported under greenhouse conditions (De Vos *et al*., 2010b). The mean absolute leaf thickness of plants occurring in the lower zone on the Afsluitdijk was 33 % greater than the leaf thickness of plants exposed to salt spray in this greenhouse experiment (1.05 mm on the Afsluitdijk and 0.79 mm in the greenhouse experiment). Possibly this is due to the fact that the greenhouse experiment was performed with seedlings, whereas mature plants (> 1 year) occurred on the Afsluitdijk. In the same greenhouse experiment, soil salinity of 200 mM NaCl caused the maximum recorded leaf thickness increase of 22 %, although this was not significant. An increase in leaf thickness is a typical response of salt-tolerant plants to salt spray (Boyce, 1954; Rozema *et al*., 1982; Barbour *et al*., 1985; De Vos *et al*., 2010b). Although it is not known what caused the difference between the greenhouse treatments and the field conditions, the increase in leaf thickness which was observed between plants occurring in the lower and upper zone is most likely caused by the difference in salt exposure.

Vegetation coverage and species abundance

The vegetation coverage and the abundance of the different species showed differences between the two zones. The coverage almost doubled in the upper zone compared to the lower zone, indicating that conditions were more favourable for plant growth in the upper zone, probably relating to a lower level of airborne salinity. The

presence of non-coastal (inland) species in the upper zone and typical coastal species in the lower zone also indicated that the growth conditions and the exposure to salt spray was probably less than in the lower zone. It is known that the highest concentration of salt spray is deposited on the windward side and less on the leeward side which forms somewhat protected/sheltered micro sites, and this difference in salt spray deposition can be as large as a factor 10 or more (Boyce, 1954; Barbour and De Jong, 1977; Barbour *et al.*, 1985). Possibly, the non-coastal species which were present in the upper zone occurred on the leeward side of large plants species like *Crambe maritima*. Vegetation coverage and especially the presence of specific species in the coastal habitat has long been recognized to be caused by different levels of salt spray and inundation tolerance, which results in the most salt tolerant species occurring closest to the tide line and less salt-resistant plants growing farther inland (Oosting and Billings, 1942; Barbour and De Jong, 1977; Rozema *et al.*, 1985). In the experiment described here, the two zones were located close together and the three most dominant species were alike in the two zones. Although the dominant species were similar in both zones, the abundance of the species *Atriplex prostrata* and *Crambe maritima* in the lower zone was apparent. Barbour *et al.* (1985) stated that the leading edge of strandline vegetation is subjected to seasonal changes and its position can move during the year and all vegetation is occasionally lost due to extreme events. This was also observed on the Afsluitdijk. Inundation often damaged the vegetation and inundation at the end of the growing season did result in a complete loss of vegetation. This is illustrated by the pictures on the title page of this chapter and the pictures in Appendix 2A.

Levels of salt spray and soil salinity recorded throughout the duration of the experiment are both believed to influence plant growth of salt sensitive species. Barbour and De Jong (1977) showed that salt spray of 50 mg dm^{-2} day^{-1} during three weeks resulted in 95-100% mortality of different salt spray sensitive species. Species adapted to a soil salinity of approximately 4 dS m^{-1} can be classified as moderately salt tolerant (Maas and Hoffman, 1977; Shaw, 1999). Besides the long-term moderate salt concentrations of the soil, the species occurring in the lower were also able to survive the peak concentrations after inundation. According to literature there is some evidence that beach and dune species show increased tolerance to soil salinity. However, in general, it is believed that beach and dune species are neither as tolerant to salinity as typical salt marsh halophytes, nor as salt sensitive as glycophytes (Barbour *et al.*, 1985) and strandline species can best be described as "aerohalophytes" (Rozema *et al.* 1982). *Crambe maritima* can be classified as a salt spray tolerant plant that is only sensitive to root zone salinities exceeding 100 mM NaCl or roughly 10 dS m^{-1} (De Vos *et al.*, 2010b). *Atriplex prostrata* showed leaf biomass reductions of 27 %, 69 % and 81 % at salinities of about 8, 16 and 30 dS m^{-1}, respectively (Wang *et al.*, 1997). Some other typical species of this strandline have been reported to show increased growth at salinities of 50 mM NaCl and show only moderate reductions at 200 mM NaCl (Lee and Ignaciuk, 1985). *Beta vulgaris subsp. maritima* at 400 mM NaCl showed a reduction of 21 % concerning relative growth rate (Rozema *et al.*, 1993) and about 50 % in whole plant fresh weight (Koyro, 2000), and *Cakile maritima* has been reported to be moderate tolerant (Barbour, 1970; Rozema *et al.*, 1982; 1983a) as well as highly salt tolerant (Debez *et al.*, 2004; Megdiche *et al.*, 2007). These growth performances seem to imply that strandline plant species are at least moderately salt tolerant and are able to survive short peaks of higher salinity. Based on their salt tolerance, many strandline plant species have the potential to become crops which can be cultivated under saline conditions.

Concluding remarks

In the coastal zone on the Afsluitdijk, different soil parameters and vegetation characteristics are governed by the salt exposure. The influence of this salt exposure, mainly in the form of salt spray but seawater inundation as well, decreased rapidly with distance from and elevation above seawater level. Results showed that between the two zones (differing only 2 m in elevation and 7 m in distance from mean seawater level) relative large differences occurred in salt exposure, soil parameters and vegetation characteristics. The long-term salt spray levels of the lower zone ranged between 0.4 and 231 mg dm^{-2} day^{-1}. Salt spray levels above 40 mg dm^{-2} day^{-1} were recorded during a total of 11 and 6 weeks for two consecutive years during the growing season of 26 weeks. Also, inundation was recorded in the middle of the growing season in both years. In the upper zone no inundation was recorded during the growing season and salt spray levels mostly remained under 10 mg dm^{-2} day^{-1}. On one occasion, during inundation of the lower zone, a maximum salt spray level of 41 mg dm^{-2} day^{-1} was recorded in the upper zone. The level of salt spray appeared to be stronger correlated with seawater level than with wind speed. Soil salinity levels of the lower zone were only temporarily elevated after inundation, reaching a maximum concentration of 28 dS m^{-1}. The overall leaf thickness of *Crambe maritima* plants occurring in the lower zone showed an increase of 42 % compared to the leaves of plants occurring in the upper zone, which is considerable greater than the increase in leaf thickness reported under greenhouse conditions. The highest Na$^+$ concentrations found in the leaves of *C. maritima* collected on the Afsluitdijk were 3.84 and 3.89 mmol g^{-1} DW. Compared to greenhouse salt treatments, it seems likely that root zone salinity also influenced the leaf Na$^+$ concentration under field conditions. The vegetation coverage in the upper zone was almost 200 % higher than that of the lower zone. In the lower zone only coastal plant species were observed whereas in the upper zone more non-coastal species occurred. Although it seems likely that salt spray is most important in determining the vegetation coverage and abundance, the influence of soil salinity and especially inundation can not be neglected. Strandline plant species are exposed to considerable levels of salt spray and occasional inundation and appear to be at least moderately salt tolerant and are able to survive short peaks of high salinity.

ACKNOWLEDGEMENTS

Appendix 2A. Pictures of the research site on the Afsluitdijk, which illustrate the changing vegetation coverage during the year (see dates on the pictures for time line). The relative large plant which can especially be seen on the picture of 04/05/2007, 09/05/2008 and 24/09/2008 is *Crambe maritima*.

Chapter 3.

Ecophysiological response of *Crambe maritima* to increased airborne and soil borne salinity

Arjen C. de Vos[1]
Rob Broekman[1]
Maartje P. Groot[1]
Jelte Rozema[1]

[1] *Institute of Ecological Science, Department of Systems Ecology, VU University Amsterdam De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

- *Background and Aims* There is a need to evaluate the salt tolerance of plant species that can be cultivated as crops under saline conditions. *Crambe maritima* is a coastal plant, usually occurring on the driftline, with potential use as a vegetable crop. The aim of this experiment was to determine the growth response of *Crambe maritima* to various levels of airborne and soil borne salinity and the ecophysiological mechanisms underlying these responses.

- *Methods* In the greenhouse, plants were exposed to salt spray (400 mM NaCl) as well as to various levels of root zone salinity (RZS) of 0, 50, 100, 200, and 300 mM NaCl during 40 days. The salt tolerance of *Crambe maritima* was assessed by the relative growth rate (RGR) and its components. To study possible salinity effects on the tissue and cellular level, the leaf succulence, tissue Na^+ concentrations, Na^+ / K^+ ratio, net K^+ / Na^+ selectivity, N, P, K^+, Ca^{2+}, Mg^{2+}, proline, soluble sugar concentrations, osmotic potential, total phenolics and antioxidant capacity were measured.

- *Key Results* Salt spray did not affect the RGR of *Crambe maritima*. However, leaf thickness and leaf succulence increased with salt spray. Root zone salinities up to 100 mM NaCl did not affect growth. However, at 200 mM NaCl RZS the RGR was reduced by 41 % compared to the control and by 56 % at 300 mM NaCl RZS. The reduced RGR with increasing RZS was largely due to the reduced specific leaf area (SLA), which was caused by increased leaf succulence as well as by increased leaf dry matter content (LDMC). No changes in unit leaf rate (ULR) were observed but increased RZS resulted in increased Na^+ and proline concentrations, reduced K^+, Ca^{2+} and Mg^{2+} concentrations, lower osmotic potential and increased antioxidant capacity. Proline concentrations of the leaves correlated strongly (r=0.95) with RZS concentrations and not with plant growth.

- *Conclusions* Based on its growth response *Crambe maritima* can be classified as a salt spray tolerant plant that is sensitive to root zone salinities exceeding 100 mM NaCl.

  Key words: *Crambe maritima*, halophyte, salt tolerance, ecophysiology, salt spray, root zone salinity, relative growth rate, specific leaf area, leaf succulence, Na^+, K^+, osmotic potential, proline, phenolics, antioxidant capacity.

INTRODUCTION

Crambe maritima L. or Seakale, belonging to the *Brassicaceae* family, is a potential salt-tolerant crop. It has been known as an edible plant for at least 300 years (Horwood, 1919). It is a long-lived perennial herb with an extensive, deep root system (up to 200 cm) and an underground stem resembling a tap root (Scott and Randall, 1976), referred to as tap root from here on (for more details see Péron, 1990). Leaves are covered with a thick waxy layer, which acts as an efficient water-repellent (Scott and Randall, 1976). At the end of the growing season the leaves and inflorescences die off, leaving only the underground root system and the leafless bud or tap root during the winter. When *C. maritima* is grown as a vegetable crop, etiolated sprouts are grown from this tap root, which are regarded as a highly prized and tasteful vegetable (Péron, 1990; Briard *et al.*, 2002).

Crambe maritima is mostly confined to coastal habitats with well-drained soils in North West Europe (Clapham *et al.*, 1962; Scott and Randall, 1976), a habitat occupied by only a few species. This is ascribed to considerable levels of airborne (Wells and Shunk, 1938; Rozema *et al.*, 1982) and soil borne salinity (Lee and Ignaciuk, 1985; Barbour *et al.*, 1985), high substrate mobility, sand blasting and burial, low water holding capacity and a low nutrient status of the substrate, except in the vicinity of detrital deposits (Boyce, 1954; Lee et al., 1983; Pakeman, 1990). This environmental variability of the habitat possibly explains the recorded variability in germination (Baron and Binet, 1964; Walmsley and Davy, 1997; Fusheng *et al.*, 1998) and the great phenotypic and genetic variability within and between populations of *C. maritima* (Briard *et al.*, 2002). Shingle beaches seem to be the preferred habitat of *C. maritima* (Scott & Randall, 1976). In The Netherlands establishment only occurred after construction of the basalt stone sea dikes in the 1950's (Mennema *et al.*, 1985). These dikes resemble an artificial pebble beach habitat. Since that time an explosive extension of *C. maritima* has been recorded, with the majority of the findings occurring on sea dikes (Mennema *et al.*, 1985; Van der Meijden, 2005). *Crambe maritima* often occurs on the driftline (Clapham *et al.*, 1962; Scott and Randall, 1976). The soil of these pebble beaches is believed to be well-drained, where the salts can readily pass through the root zone. However, it was demonstrated in the previous chapter that strandline soil salinity levels at (artificial) pebble beaches can be elevated periodically. Possibly, *C. maritima* is able to cope with saline conditions in the root zone during such periods. Since salt spray is an important natural selective abiotic factor on coastal plant communities (Wells and Shunk, 1938; Boyce, 1954; Barbour, 1978; Rozema *et al.*, 1982, 1983a) and *C. maritima* grows close to the ocean, it is expected to be adapted to salt spray. Since *C. maritima* is exposed to root zone salinity as well as salt spray in its natural environment, the ecophysiological effect of both types of salt exposure were examined in a greenhouse experiment.

The objective of this study was to determine the effect of airborne and soil borne salinity on the growth of *C. maritima* and to obtain insight in the morphological and physiological adaptations underlying these growth responses. The species studied is a coastal plant and a potential crop for saline agriculture. In a first experiment seedlings were exposed to salt spray to determine the effect of airborne salinity on plant growth and leaf thickness. In a second experiment plants were exposed to airborne as well as soil borne salinity and various responses at the whole plant and tissue levels were studied.

MATERIALS AND METHODS

Salt spray experiment
Seeds of *C. maritima* were collected in Normandy, France in 2005 and stored dry at room temperature. Seeds were germinated in March 2006 on peat soil (Seed Plot soil Jongkind, Aalsmeer, The Netherlands) and transplanted to individual pots also containing peat soil (potting soil No.5 Jongkind, Aalsmeer, The Netherlands). Seedlings were randomly assigned to either the salt spray treatment or the control treatment. The experiment was initiated 4 weeks after germination started, with one group sprayed with seawater with a concentration of 400 mM NaCl (measured as electrical conductivity (dS m^{-1}) and calculated as mM NaCl by means of a calibration curve) collected prior to the experiment at Zandvoort, The Netherlands (52º23'N 4º32'E) and one group sprayed with demineralised water as a control. Plants were

sprayed with a fine mist of seawater four times per day, which equalled on average 160 mg NaCl dm^{-2} leaf area day^{-1}, the controls were similarly sprayed four times per day with demineralised water. Plants were sprayed every 2.5 hours between 9 am and 4.30 pm. Plants were not sprayed in the weekend nor during holidays. Leaf length, number of leaves and leaf thickness was measured 8 weeks after germination, corresponding to 19 days of actual salt spray treatment. These measurements were repeated 13 weeks after germination, corresponding to 45 days of actual salt spray. In addition, 11 weeks after germination with 34 days of salt spray the leaf thickness of the second youngest leaf was measured and harvested to obtain fresh weight and leaf area to calculate leaf succulence expressed as L_{FW} / L_A. The thickness of each leaf was measured manually between the veins at the tip of the leaf, with an analogue thickness gauge (Mitutoyo No. 2046-08, Japan, accuracy 0.01 mm). Plants were watered with tap water during the experiment and grown under the same environmental conditions as the salt tolerance experiment listed below.

Salt tolerance experiment
Seeds were collected in September 2007 from a large population of *C. maritima* located on the Afsluitdijk (52º58'N 5º06'E) in The Netherlands, and stored dry at room temperature. Prior to germination seeds were placed on moist vermiculite in a closed plastic bag for 58 days at 5 ºC to break any potential dormancy and obtain uniform germination. After removal of the pericarp, seeds were placed on a Petri dish with filter paper (Whatman Grade 597) saturated with demineralised water for germination, which was defined as a visible, emerging radicle. Seeds that germinated within 9 days were transplanted into 4 litre polyethylene pots filled with 2-5 mm gravel, after which seedlings were allowed to grow for another 17 days. After this period only plants with a visible, second true leaf (< 2 cm in length) were used for the experiment so that the initial population was as uniform as possible. Plants were randomly divided into seven treatment groups consisting of five different Root Zone Salinity (RZS) treatments (0, 50, 100, 200, and 300 mM NaCl added to the nutrient solution), one salt spray treatment (root zone with 0 mM NaCl) which was sprayed twice a day with seawater (similar to the salt spray experiment) with an average of 80 mg NaCl dm^{-2} day^{-1}, and one control treatment sprayed with demineralised water as a control. The effect of salt spray and root zone salinity was analysed separately. The initial harvest consisted of 5 plants per treatment group just before salt addition started, 26 days after initial germination. This initial harvest was used to test for uniformity of the population at the start of the experiment and calculations of RGR. Plants were sprayed at 10 am and 4 pm. Plants were not sprayed in the weekend nor during holidays. Each pot contained one plant. Salt concentration was increased stepwise (50 mM NaCl per day) to avoid an osmotic shock. Plants were grown in a closed gravel/hydroponic system (Koyro, 2003), which was set up in a randomized block design (randomized weekly). The experiment was performed in April/May 2008 in a greenhouse with controlled temperature, humidity and light conditions: 22±2/16±2 ºC day/night, relative humidity 60/80% day/night, light intensity 300 PAR (µmol m^{-2} s^{-1}) at plant level, 14 h light day^{-1}. Nutrient solution, composed of 3 mM KNO$_3$, 2 mM Ca(NO$_3$)$_2$, 1 mM NH$_4$H$_2$PO$_4$, 0.5 mM MgSO$_4$, 20 µM Fe(Na)-EDTA, 1 µM KCl, 25 µM H$_3$BO$_3$, 2 µM MnSO$_4$, 2 µM ZnSO$_4$, 0.1 µM CuSO$_4$ and 0.1 µM (NH4)$_6$Mo$_7$O$_{24}$ in demineralised water, buffered with 2 mM MES, pH 6.0, adjusted with KOH, was supplied by continuous drip-irrigation and surplus water drained back to each of the individual 50-liter central reservoirs. Nutrient solutions were replaced

every two weeks. The final harvest occurred 40 days after salt addition was started and 5 plants per treatment were harvested.

Sample preparation
Plants were carefully uprooted, washed with running demineralised water for one minute and blotted dry and the fresh weight of the root, tap root, leaf stems and leaves were recorded. Following this, total leaf area was measured with a LI-COR 3100 (Li-Cor Inc, Lincoln, Nebraska, USA) leaf area meter and expressed as cm^2 $plant^{-1}$. Leaves were separated into old and new leaves, with the first two true leaves as the old leaves and from the third true leaf onwards as new leaves. Directly after measuring leaf area, the leaves were frozen in liquid nitrogen and freeze-dried for one week. The rest of the plant material was oven-dried at 70 ºC for 48 hours. Dried plant material was ground to a fine powder using a ball-mill (Retsch-MM200, Haan, Germany). The freeze-dried leaves were used to analyse nutrient composition, total phenolics, antioxidant capacity, proline and soluble sugars. Roots were analysed for nutrient composition only.

Measurement of morphological parameters and relative growth rate
The growth performance of *C. maritima* under airborne and soil borne salinity was used to evaluate the salt tolerance. The SLA, leaf weight fraction (LWF), leaf succulence, LDMC and leaf water content (LWC) were determined to study possible morphological adaptations. The RGR and the components ULR, LWF and SLA were estimated according to Hunt *et al*. (2002):

RGR=ULR*LWF*SLA (1)

with

ULR = (Δ W * Δ $\log_e L_A$) / (Δ L_A* Δ *t*) (2)

LWF = L_{DW}/W (3)

SLA = L_A/L_{DW} (4)

where Δ refers to the difference between values at the final and initial harvest, *t* is the salt treatment duration (days), W is the whole plant dry weight (g), L_{DW} is the total leaf dry weight (g), and ($\log_e$) L_A is the (natural logarithmic) value of leaf area (cm^2). The components leaf density (L_D) and leaf thickness (L_{Th}) increase linearly with the inverse of SLA:

1/SLA = L_D * L_{Th} (Poorter and Garnier, 2007). (5)

Leaf density was estimated by calculating LDMC because L_D ≈ LDMC (Garnier and Laurent, 1994; Wilson *et al*., 1999), and LDMC = L_{DW} / L_{FW} (Garnier *et al*., 2001), with L_{DW} as leaf dry weight (g) and L_{FW} as leaf fresh weight (g).
Leaf thickness (L_{Th}) can be estimated as:

L_{Th} = (SLA*LDMC)$^{-1}$ (6)

according to Vile *et al*. (2005). This leaf thickness can also be expressed as leaf succulence because $(SLA*LDMC)^{-1} = (L_A / L_{DW} * L_{DW} / L_{FW})^{-1} = L_{FW} / L_A$ and L_{FW} / L_A is often used as an estimate of leaf succulence (Jennings, 1976; Agarie *et al*., 2007). In this paper leaf succulence is calculated as

Leaf succulence = L_{FW} / L_A,   (7)

with L_{FW} as leaf fresh weight (g) and L_A as leaf area (cm^2).

Leaf water content was estimated according to the commonly used formula

LWC= $(L_{FW} - L_{DW}) / L_{FW}$,   (8)

with L_{FW} as leaf fresh weight (g) and L_{DW} as leaf dry weight (g).

Nutrient composition
The concentrations of total P, Ca^{2+}, Mg^{2+}, Na^+ and K^+, total C and N were determined as described in Chapter 2. Chloride was measured only in the supernatant of the extract of the osmotic potential, with a Sherwood Chloride Analyzer 926 (Sherwood Scientific Ltd, Cambridge, United Kingdom). Samples were measured in duplicate. The net selectivity of ion accumulation for K^+ over Na^+ was estimated from ion contents using the commonly used formula

$S = (K^+ / Na^+)_{leaves} / (K^+ / Na^+)_{medium}$ (9)

Osmolality and osmotic potential
Plant samples were frozen and stored at -18 ºC directly after harvest. Leaf discs were collected from the centre parts of fresh leaves of the whole plant, the frozen plant tissues were put into a syringe to thaw after which leaf sap was extracted. Of the supernatant 10 µl was utilized to determine osmolality. Osmolality was measured using a vapor pressure osmometer (Wescor 5500, Utah, USA). Samples were measured in duplicate. The osmotic potential was determined according to the Van't Hoff equation:

$\Psi_s(MPa) = -c(\text{mosmol kg}^{-1}) * 2.58 * 10^{-3}$ (10)

Ion contribution to plant osmotic potential was determined according to the Van't Hoff relationship:

solute potential = -C * R * T   (11)

with C = molarity of the solution (mol of solute kg^{-1} H_2O), R = gas constant (0.00831 kg mol^{-1} K^{-1}) and T = temperature (in K).

Soluble sugars
Soluble sugars were determined according to the Anthrone method (Yemm and Willis, 1954). For this, 40 mg plant material was mixed with 5 ml 80% ethanol and centrifuged at 4000g for 10 minutes. This was repeated twice and the supernatants were combined and demineralised water was added to a total volume of 25 ml. Of this, 250 µl was added to 2.5 ml Anthrone reagent (400 mg Anthrone in 6 ml 96%

ethanol, 60 ml demineralised water and 200 ml concentrated H_2SO_4). Samples were placed in a water bath for 7 minutes at 100 ºC. After the samples cooled down to room temperature the absorbance was measured at 625 nm using a spectrophotometer (Shimadzu UV-1601PC, Japan). Sucrose was used to make the standard curve and results are expressed in µmol equivalent sucrose g^{-1} DW. Samples were measured in triplicate.

Proline
The proline content was measured according to the method of Bates *et al*. (1973). Approximately 20 mg plant material was mixed with 2 ml 3 % sulfosalicylic acid and centrifuged for 10 minutes at 4000g. Of the supernatant 0.5 ml was mixed with 0.5 ml ninhydrin reagent and 0.5 ml glacial acetic acid, and placed in a water bath for 1 h at 100 ºC. After termination of the reaction in ice water, 1.0 ml toluene was added to extract the reaction mixture. The absorbance was measured at 520 nm using a spectrophotometer (Pharmacia LKB-Ultrospec3, Cambridge, UK). Proline was used to make the standard curve and results are expressed in µmol proline g^{-1} DW. Samples were measured in triplicate.

Total phenolics
The amount of total phenolics in the leaf tissue was determined by means of the Folin-Ciocalteu method (Waterman and Mole, 1994). The phenolic compounds were extracted by mixing 30 mg plant material with 5 ml 50% methanol, placed in a water bath for 1 h at 40 ºC and centrifuged at 4000g for 5 minutes. Of this, 100 µl was mixed with 3.90 ml demineralised water and 250 µl reagent was added. After 8 minutes 750 µl of sodium bicarbonate solution was added to stop the reaction. After 1 h the absorbance was measured at 760 nm using a spectrophotometer (Shimadzu UV-1601PC, Japan). Tannic acid was used as a standard and results are expressed in mg equivalent tanin g^{-1} DW. Samples were measured in duplicate.

Antioxidant capacity
Total antioxidant capacity was measured according to Re *et al*. (1999). For this method the preformed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$^{\cdot+}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of both lipophilic and hydrophilic antioxidants. This results in a decolorization which can be measured using a spectrophotometer. The extraction was made by mixing 30 mg plant material with 5 ml 50 % methanol, placed in a water bath for 1 h at 40 ºC and centrifuged at 4000g for 5 minutes. Of the supernatant 30 µl was mixed with 2970 µl of diluted ABTS$^{\cdot+}$ reagent. After 30 minutes the absorbance was measured at 734 nm using a spectrophotometer (Shimadzu UV-1601PC, Japan). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard and results are expressed in mg Trolox equivalent antioxidant capacity (TEAC) g^{-1} DW. Samples were measured in triplicate.

Statistical analysis
Data was analysed using the statistical program SPSS 15.0 for Windows (SPSS Inc. 2000, Chicago, IL, USA). Prior to statistical analysis, normality and homogeneity assumptions of the test were checked both by visual inspection of the residuals for undesired patterns and by Levene's test for homogeneity of variances. One-way ANOVAs were performed to test for differences between salt treatments for the different parameters. When necessary, values were log-transformed prior to analysis

or evaluated by nonparametric tests. When significant differences between means were found, Tukey's multiple range test was used to perform *post hoc* pair-wise comparisons between individual treatments. Linear regression was used to test for relationships between variables. Possible significant differences between treatments in leaf thickness in time and within treatments between old and new leaf Na^+ concentrations of the plant was evaluated by repeated measures ANOVA with Bonferroni adjusted pair-wise comparisons. In the salt tolerance experiment the effect of salt spray and root zone salinity was analyzed both separately as well as all treatments together which gave similar results. To obtain normality and homogeneity of the Na^+ concentration of roots, old and new leaves only differences between salt-exposed treatments were evaluated in the statistical analysis.

RESULTS

Salt spray experiment
Leaf length and number of leaves per plant of 8-week-old and 13-week-old plants were not affected during 19 days or 45 days of salt spray, respectively (Table 3.1). The differences in number of leaves and leaf length between week 8 and week 13 were minimal, indicating that growth in the first 8 weeks was greater than that from week 8 untill 13. Mean leaf thickness of the salt spray plants showed a significant increase in week 8, week 11 and week 13 as compared to the control treatment. A repeated measures ANOVA showed that there was no significant difference in leaf thickness for week 8, 11 and 13 (F=0.004, P=0.996) as a result of salt spray.

The second youngest leaf was a good indicator for whole plant leaf thickness because this leaf thickness in week 11 did not significantly differ from the whole plant leaf thickness in week 8 and 13. There was a strong positive relationship between leaf thickness and leaf succulence, with leaf thickness = 0.137+0.641*leaf succulence ±0.085 s.e., with r=0.77, F=85.4, P<0.001, n=61. Although mean leaf thickness showed a significant increase compared to the control in week 8, 11 and 13, this was only in the order of 11 %, 18 % and 12 %, respectively.

Table 3.1. Effect of salt spray on mean leaf length, number of leaves and leaf thickness of *Crambe maritima* plants, 8 and 13 weeks after germination, measured non-destructively and leaf thickness plus leaf succulence of the second youngest leaf 11 weeks after germination

Treatment	Whole plant			Second youngest leaf		Whole plant		
	8 weeks			11 weeks		13 weeks		
	leaf length (cm)	number of leaves	leaf thickness (mm)	leaf thickness (mm)	leaf succulence (mg FW mm^{-2})	leaf length (cm)	number of leaves	leaf thickness (mm)
Control	9.7±0.3[a]	4.0±0.1[a]	0.70±0.02[a]	0.67±0.02[a]	0.85±0.02[a]	11.7±0.3[a]	4.8±0.1[a]	0.69±0.02[a]
Salt spray	9.3±0.3[a]	3.9±0.2[a]	0.78±0.02[b]	0.79±0.02[b]	0.99±0.02[b]	12.4±0.4[a]	4.8±0.1[a]	0.77±0.02[b]

Treatments consisted of the control and the salt spray treatment. Plants were grown on potting soil. Values are means ± s.e (*n*=30). Means in the same column that have the same letter are not significantly different at *P* < 0.05 (one-way ANOVA). Measurements were performed on the same plants in week 8 and week 13 with 19 and 45 days of salt spray, respectively. Leaf thickness was measured with a thickness gauge and expressed in mm.

Salt tolerance experiment
Growth
Cold stratification at 5 ºC for 58 days resulted in the germination of 41±2 % (mean ± s.e., *n*= 26 with 20 seeds per Petri dish as *n*) within 9 days, of which 71±3 % germinated in the last 3 days. The initial harvest prior to salt addition showed no significant differences between treatment groups (one-way ANOVA *F*=0.64, *P*=0.70), demonstrating the uniformity of the population at the start of the experiment. The results of the final harvest of the 8-week-old plants, with either 20 days of actual salt spray treatment or 40 days of root zone salinity treatment, are listed in figure 3.1. The whole-plant dry weight production (Fig. 3.1A) of the salt spray treatment plants showed no significant difference with the control and the 0, 50 and 100 mM NaCl RZS treatments. After 40 days of RZS exposure, whole-plant dry weight production showed no significant difference between the 0, 50 and 100 mM treatments. Compared to the 0 mM RZS treatment the dry weight production at 200 mM NaCl was reduced by 78 %. No significant difference occurred between the 200 and 300 mM NaCl treatments. Results of mean leaf dry weight production and mean leaf area showed similar responses as whole plant dry weight production (see Appendix 3A).
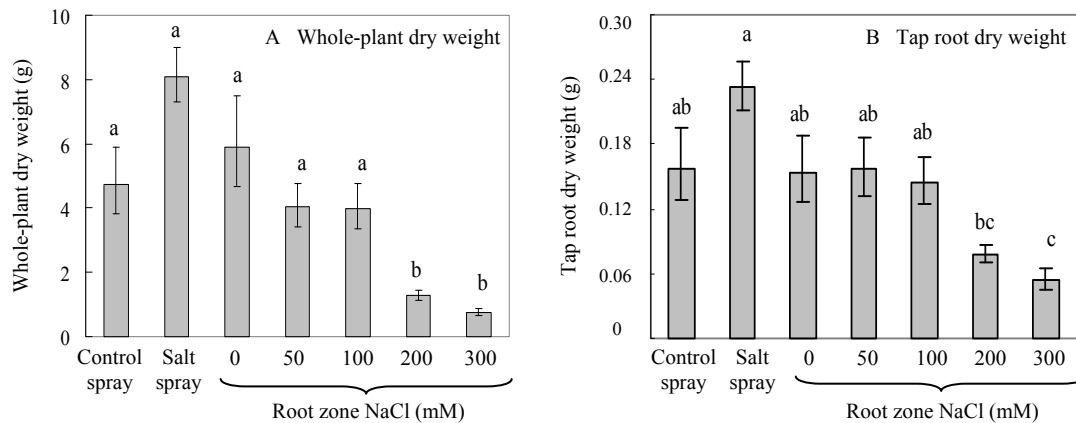

Figure 3.1. Dry weight production of the whole-plant (A) and the tap root (B) of all treatments of the 8-week-old *Crambe maritima* plants with 20 days of salt spray or 40 days of root zone salinity ranging from 0 to 300 mM NaCl. Plants were grown on gravel culture with hydroponics. Values are the means of five replicates ± s.e. Different letters indicate a significant difference at *P*<0.05 (Tukey's test).

Tap root dry weight production (Fig. 3.1B) of the salt spray treatment showed no difference with the control treatment and the 0, 50 and 100 mM NaCl RZS treatments. The 0, 50 and 100 mM treatments did not differ from the 200 mM NaCl RZS treatment. No difference occurred between the 200 and 300 mM treatments.
As expected, the Relative Growth Rate (RGR, Table 3.2) showed results similar to the whole-plant dry weight production with no significant difference between the salt spray and the control spray treatment, as well as the 0, 50 and 100 mM RZS treatments. The RGR decreased 41 % between the 0 and 200 mM RZS NaCl treatment. Although the ULR showed a mean increase at moderate salinities and a decrease at the highest salinities, none of these differences were significant. The only difference occurred between the highest value of ULR (salt spray treatment) and the lowest value (0 mM NaCl RZS treatment), although the difference in ULR between the 100 and 300 mM NaCl RZS was almost significant (*P*=0.08). The LWF showed

no differences between treatments. Of the components of RGR, it was SLA that showed the largest decline for both the salt spray and RZS treatments compared to the controls. The SLA of the 100, 200 and 300 mM NaCl RZS treatments significantly differed from the 0 mM treatment and showed a maximum decline of 44% with increasing salinity. For leaf succulence the control spray treatment only differed from the 200 mM RZS treatment, although the P-values between the control spray and the salt spray treatment (P=0.051) and the 100 mM RZS (P=0.054) almost showed significant differences. Within the LDMC only the 300 mM treatment showed an increase, similar to the LWC which was only affected at the 300 mM NaCl RZS level.

Table 3.2. Relative growth rate, its components and leaf water content (LWC) of *Crambe maritima* in response to salt spray or increased (0-300 mM NaCl) root zone salinity

Treatment	RGR (mg g⁻¹ day⁻¹)	ULR (mg cm⁻² day⁻¹)	LWF (g g⁻¹)	SLA (cm² g⁻¹)	Succulence (g cm⁻²)	LDMC (mg g⁻¹)	LWC (%)
Control spray	101.2 ± 5.0^a	0.90 ± 0.11^{ab}	0.76 ± 0.02^a	153.0 ± 10.1^{ab}	0.087 ± 0.004^a	77.1 ± 5.3^a	92.3 ± 0.5^a
Salt spray	113.3 ± 3.5^a	1.18 ± 0.03^b	0.76 ± 0.00^a	127.5 ± 5.0^{bc}	0.108 ± 0.004^{ab}	73.3 ± 2.3^a	92.7 ± 0.2^a
0	110.2 ± 6.5^a	0.83 ± 0.08^a	0.78 ± 0.02^a	173.7 ± 11.3^a	0.092 ± 0.005^{ab}	63.6 ± 1.1^a	93.6 ± 0.1^a
50	97.9 ± 4.2^a	0.93 ± 0.09^{ab}	0.75 ± 0.01^a	145.4 ± 12.1^{ab}	0.100 ± 0.002^{ab}	70.9 ± 6.1^a	92.9 ± 0.6^a
100	94.7 ± 4.5^a	1.00 ± 0.07^{ab}	0.77 ± 0.01^a	125.2 ± 8.9^{bc}	0.108 ± 0.003^{ab}	75.5 ± 4.9^a	92.5 ± 0.2^a
200	65.2 ± 3.2^b	0.78 ± 0.04^a	0.72 ± 0.02^a	$117.5\pm5,8^{bc}$	0.112 ± 0.006^b	76.8 ± 2.5^a	92.3 ± 0.2^a
300	48.9 ± 3.8^b	0.70 ± 0.04^a	0.72 ± 0.03^a	96.6 ± 2.2^c	0.103 ± 0.008^{ab}	102.7 ± 5.5^b	89.7 ± 0.6^b

Plants were grown on gravel culture with hydroponics. Values are means ± s.e., (*n*=5). Means in the same column that have the same letter are not significantly different at $P < 0.05$ (Tukey's test). RGR= ULR*LWF*SLA, 1/SLA= leaf succulence (L_{FW}/L_A)*LDMC (L_{DW}/L_{FW}), LWC= (L_{FW} - L_{DW}) / L_{FW}.

Na$^+$ concentration
The tap root Na$^+$ concentration of the salt spray treatment showed no difference as compared with the 50 and 100 mM RZS treatments (Fig. 3.2). Also, the Na$^+$ concentration of the old leaves of the salt spray treatment did not differ from that of the 50, 100 and 200 mM RZS treatments. The largest differences in the Na$^+$ concentration occurred in the new leaves. The Na$^+$ concentration of the new leaves of the salt spray treatment was lower than that of the RZS treatments. There were no differences in Na$^+$ concentrations of the tap root and the old leaves between the RZS treatments. When comparing the Na$^+$ concentrations of the new leaves there were no between the 50, 100 and 200 mM treatments, but all values differed from the 300 mM treatment.

Repeated measures ANOVA (results not shown in Figure 3.2) was used to test for differences between the Na$^+$ concentration of the old and new leaves. Only the salt spray, 0, 50 and 100 mM NaCl RZS treatments showed significant lower Na$^+$ concentration in the new leaves compared the Na$^+$ concentration in the old leaves. The highest Na$^+$ concentration occurred in the 300 mM NaCl treatment, reaching a concentration of 4.74 mmol g^{-1} DW in the new leaves, representing 11 % of the total dry weight. Chloride concentrations were only determined in the leaf sap (Fig. 3.3A).
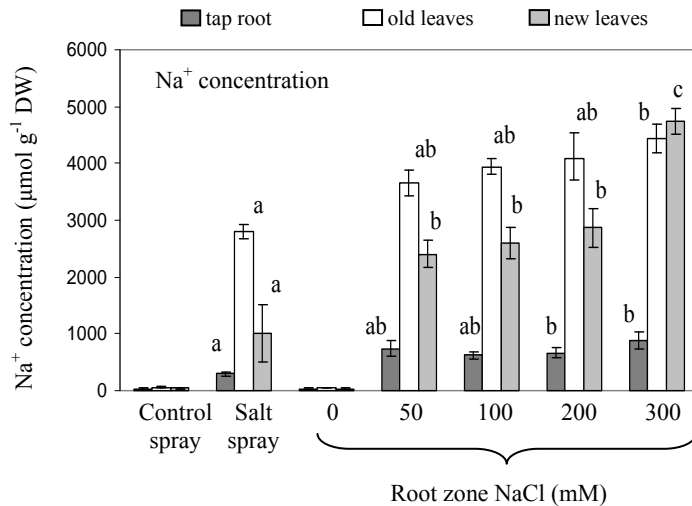
Figure 3.2. Sodium concentrations of the tap root, old and new leaves of 8-week-old *Crambe maritima* plants with 20 days of salt spray or 40 days of root zone salinity ranging from 0 to 300 mM NaCl. Values are means of 4 replicates ±s.e. Letters beside the columns show possible significant differences in the Na^+ concentrations of either root, old or new leaves between treatment groups. Different letters indicate a significant difference at *P*<0.05

K^+, Ca^{2+}, Mg^{2+}, N, P concentrations, Na^+-K^+ ratio and net K^+ / Na^+ selective uptake of the new leaves

The Na^+, K^+, Ca^{2+}, Mg^{2+}, N and P concentrations were determined for the tap root, old and new leaves. No large differences occurred between these three compartments. Because the mineral or nutrient composition of the actively growing parts is most important, only results of the new leaves are shown (Table 3.3). Salt spray showed a negative effect on the K^+ concentration of the new leaves, but only to a minor extent when compared to the RZS treatments. The effect of salt spray on the Na^+, Ca^{2+}, Mg^{2+}, N and P concentrations, Na^+-K^+ ratio and net K^+ / Na^+ selective uptake of the new leaves also appears to be minimal when compared to the RZS treatments.

Concentrations of K^+, Ca^{2+} and Mg^{2+} were greatly reduced under all RZS treatments. The reductions of the K^+ and Mg^{2+} concentrations were in the order of 50 to 70 % for the 50, 100 and 200 mM NaCl treatments and 75 to 85 % for the 300 mM treatment. The reduction of the Ca^{2+} concentration was in the order of 60 % for the 50 and the 100 mM NaCl treatments and 80 % for the 200 mM. The increase in the Na^+- K^+ ratio was similar between the 50, 100 and 200 mM NaCl treatments and showed a 400 % increase at 300 mM RZS compared to the 100 mM treatment. The net K^+ / Na^+ selectivity increased compared with the 0 mM NaCl treatment, but no differences occurred between the 50, 100, 200 and 300 mM NaCl RZS treatments. The difference in net K^+ / Na^+ selectivity between the 50 mM and 200 mM NaCl RZS treatments was almost significant with *P*=0.051. No significant difference occurred in the P concentration between RZS treatments with values falling in the range of 0.20 to 0.30 mmol g^{-1} DW (data not shown). For the N concentration only the 300 mM NaCl treatment showed a significant reduction with a mean N concentration of 2.65 mmol g^{-1} DW, whereas the other treatments were in the range of 3.50-4.00 mmol N g^{-1} DW (data not shown).

Table 3.3. The Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations, Na^+-K^+ ratio and net K^+ / Na^+ selectivity of the new leaves of *Crambe maritima* in response to salt spray or increased (0-300 mM NaCl) root zone salinity

Treatment	mmol Na^+ g^{-1}	mmol K^+ g^{-1}	mmol Ca^{2+} g^{-1}	mmol Mg^{2+} g^{-1}	Na^+-K^+ ratio	K^+ selectivity
Control spray	0.05±0.02[a]	1.49±0.06[a]	1.06±0.6[a]	0.16±0.00[a]	0.03±0.01[a]	0.15±0.04[a]
Salt spray	1.01±0.11[b]	0.90±0.19[b]	0.99±0.09[a]	0.24±0.00[b]	1.44±0.39[b]	0.00±0.00[b]
0	0.03±0.06[a]	1.47±0.09[a]	1.16±0.07[a]	0.17±0.01[a]	0.02±0.00[a]	0.16±0.03[a]
50	2.40±0.23[c]	0.46±0.04[bc]	0.46±0.03[b]	0.08±0.01[c]	5.48±0.91[c]	1.55±0.28[c]
100	2.59±0.13[c]	0.49±0.07[bc]	0.43±0.04[b]	0.10±0.01[c]	5.68±0.91[c]	2.98±0.51[c]
200	2.87±0.42[c]	0.51±0.10[bc]	0.24±0.02[bc]	0.08±0.00[c]	7.18±2.09[c]	6.20±1.70[c]
300	4.74±0.26[d]	0.20±0.06[c]	0.12±0.01[c]	0.04±0.00[d]	29.41±9.26[d]	2.00±0.75[c]

New leaves were defined as the third true leaf and onwards. Values are means ± s.e ($n = 4$) and are expressed per gram dry weight of the leaves. Means in the same column that have the same letter are not significantly different at $P < 0.05$ (Tukey's test). Concentrations of P and N were also determined with no significant differences in P concentrations and for the N concentration only a significant difference for the 300 mM NaCl treatment.

Osmotic potential, leaf sap Na^+ and Cl^-, proline and soluble sugars
The osmotic potential of the leaf sap lowered with increasing RZS. All values of the osmotic potential of the leaf sap were lower than that of the irrigation water (indicated by the line inside the columns in Figure 3.3B). Between the 100 and 200 mM NaCl RZS treatments the osmotic potential of the irrigation water increased by a factor 2.0 and that of the leaf sap by a factor 2.3. The total NaCl concentration of the leaf sap increased 3.1-fold between these two treatments. When comparing the total NaCl concentrations of the leaf sap (Fig. 3.3A) only the 200 mM differed from the 50 and 100 mM RZS treatments (indicated by the letters above each column). The Cl^- concentration of the leaf sap was different for the RZS treatments, whereas the Na^+ concentration between the 50 and 100 mM RZS did not differ (indicated by the letters beside the columns). Ion contribution to plant osmotic potential was 2, 10, 19 and 31% for chloride and 1, 38, 32 and 39 % for sodium for the 0, 50, 100 and 200 mM NaCl RZS treatments, respectively (calculated according to equation 11). Salt spray increased the proline concentration (Fig. 3.4) compared to the control spray and the 0 mM NaCl RZS treatment and was in the range of the proline concentrations of the 50 and 100 mM NaCl treatment. Proline concentrations of the RZS treatments showed a gradual and significant increase. The greatest increase in proline concentration compared to the control was found in the 300 mM NaCl treatment where proline concentrations increased 25-fold compared to the 0 mM NaCl treatment. A linear regression ANOVA showed that proline concentration (in µmol g^{-1} DW) was strongly correlated to RZS concentrations (NaCl in mM) (r=0.95, F=154.4, P<0.001). The soluble sugar concentrations showed a gradual but not significant decrease with increasing salinities with values ranging from 116 to 175 µmol g^{-1} DW (data not shown).
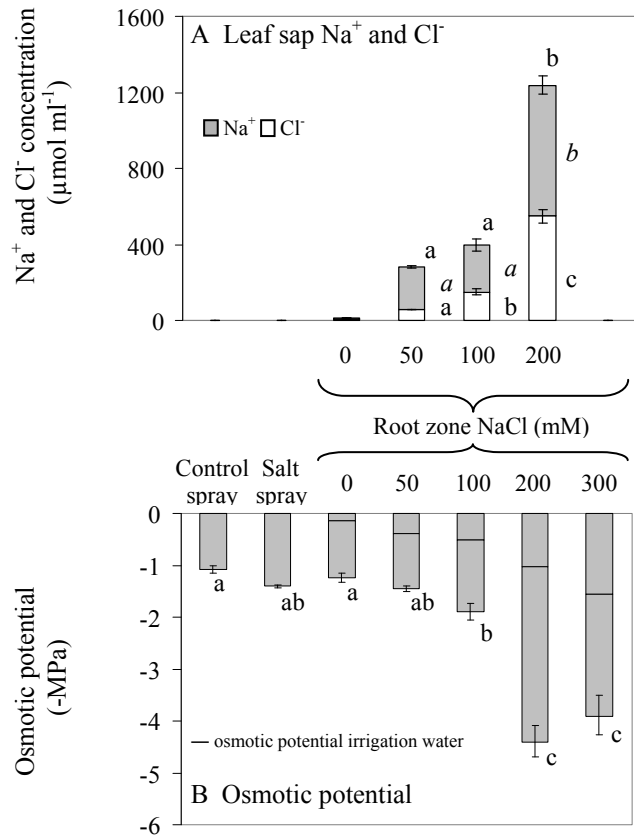
Figure 3.3. Chloride and Na$^+$ concentrations (A) and osmotic potential of the leaf sap of fresh leaves of 8-week-old *Crambe maritima* plants with 20 days of salt spray or 40 days of root zone salinity ranging from 0 to 300 mM NaCl. Differences between treatments in overal NaCl concentrations were evaluated by repeated measures ANOVA, the rest with one-way ANOVAs. Values are the means of 3 replicates ± s.e. Different letters indicate a significant difference at $P<0.05$ (Tukey's test). Note that Na$^+$ and Cl$^-$ concentrations combined do not give the total NaCl concentration per plant.

Antioxidant capacity and total phenolics
The mean antioxidant capacity of the leaves of *C. maritima* increased with increasing RZS compared to the 0 mM NaCl treatment with the exception of the 300 mM NaCl treatment, but no differences occurred amongst the 50, 100, 200 and 300 RZS treatments (Fig. 3.5A). The mean antioxidant capacity increased by 68, 77, 61 and 48 % in the salt treatments 50, 100, 200 and 300 mM NaCl compared to the 0 mM NaCl treatment, respectively. The antioxidant capacity of the salt spray treatment showed no difference with the control spray treatment or the 0 mM NaCl RZS. No differences were found in the total phenolics concentration between the treatments (Fig 3.5B).
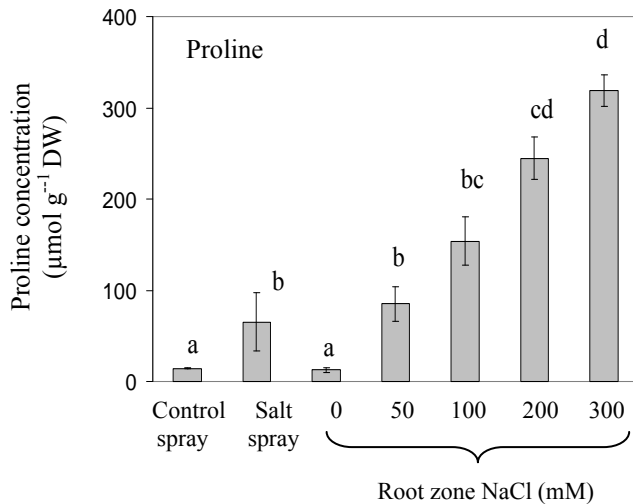
Figure 3.4. Proline concentrations of the freeze-dried leaves of 8-week-old *Crambe maritima* plants with 20 days of salt spray or 40 days of root zone salinity ranging from 0 to 300 mM NaCl. Plants were grown on gravel culture with hydroponics. Values are means ± s.e, with $n= 4$. Different letters indicate a significant difference at $P<0.05$ (Tukey's test).

DISCUSSION

The aim of the experiments was to determine the growth response of *C. maritima* to various levels of airborne and soil borne salinity and the ecophysiological mechanisms underlying these responses. For this, an overview of the overall effects on the measured parameters is given in Figure 3.6. The salt spray treated plants did not show growth reductions and showed only small physiological changes as compared to the root zone salinity treatment. Because no growth reductions occurred up to 100 mM NaCl RZS only the effects of $\geq$ 200 mM RZS are listed in Figure 3.6. The effects of airborne and soil borne salt treatments are discussed separately.

Effect of salt spray on growth
The salt spray intensities in our experiments represented a moderate intensity (80 mg NaCl dm^{-2} day^{-1} during 20 days; salt tolerance experiment) and a high intensity (160 mg NaCl dm^{-2} day^{-1} during 19 and 45 days; salt spray experiment) compared to the levels found in the natural habitat of *C. maritima* (1-200 mg NaCl dm^{-2} day^{-1} according to literature (Barbour, 1978; Griffiths, 2006) and based on results of Chapter 2, Table 2.1, page 14 of this thesis). The salt spray experiment demonstrated that high salt spray intensity applied during 19 or even 45 days did not affect leaf length and total leaf number per plant (Table 3.1). The salt tolerance experiment showed that dry weight production and RGR were not affected after 20 days exposure to moderate intensity salt spray (Table 3.2). The results even indicated a trend that salt spray might have affected the growth of *C. maritima* in a positive way, which has been reported for various strandline species by Rozema *et al*. (1982).
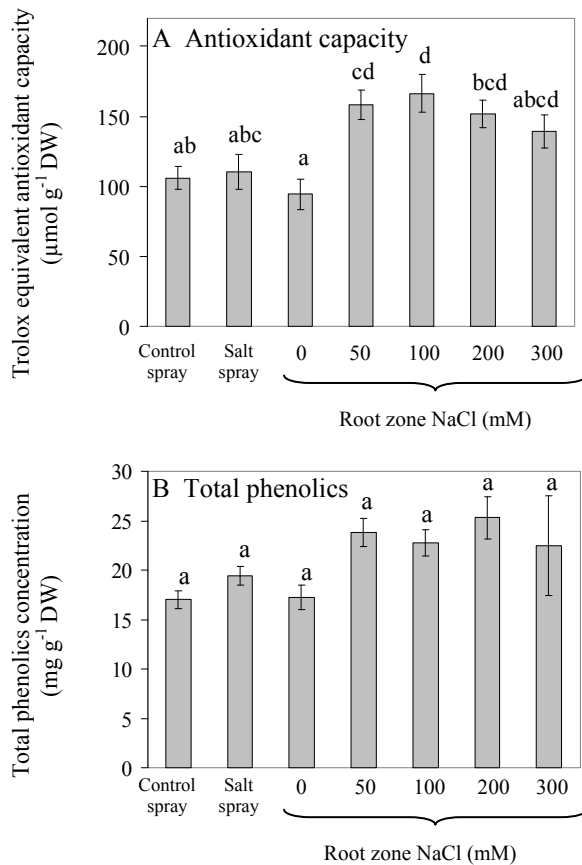
Figure 3.5. Trolox equivalent antioxidant capacity (A) and total phenolics concentration (B) of the leaves of 8-week-old *Crambe maritima* plants with 20 days of salt spray or 40 days of root zone salinity ranging from 0 to 300 mM NaCl. Plants were grown on gravel culture with hydroponics. Values are means ± s.e, with *n*= 4. Different letters indicate a significant difference at *P*<0.05 (Tukey's test).

Ecophysiological adaptations to salt spray

Salt spray caused only minor physiological responses in the plants as compared to the RZS treatments and the salt spray tolerance of *C. maritima* seemed to be based on preventing salt from entering the leaves. Leaves of *C. maritima* are covered with a thick waxy layer acting as an efficient water-repellent (Scott and Randall, 1976). Water droplets can roll off the leaves easily, but the salt spray in our experiment was applied as a fine mist, which remained on the leaves. The salt spray treatment resulted in lower Na^+ concentrations in the plants as compared to the root zone salinity treatments. Salt spray resulted in an increase in leaf thickness and leaf succulence (Table 3.1), which was comparable with the leaf thickness increase of another Brassicaceae, the highly salt-tolerant *Cakile maritima* (Debez *et al*., 2008). The overall increase in leaf thickness is a typical response of salt tolerant plants to salt spray (Martin and Clements, 1939; Boyce, 1951, 1954; Rozema et al., 1982) and increased leaf thickness or succulence has been interpreted as adaptation of halophytes in terms of conservation of internal water, efficient water storage and dilution of accumulated salts (Storey and Wyn-Jones, 1979; Flowers *et al*., 1986;

Breckle, 2002; Dimmit *et al.*, 2005; Munns, 2005; Koyro and Lieth, 2008; Munns and Tester, 2008).
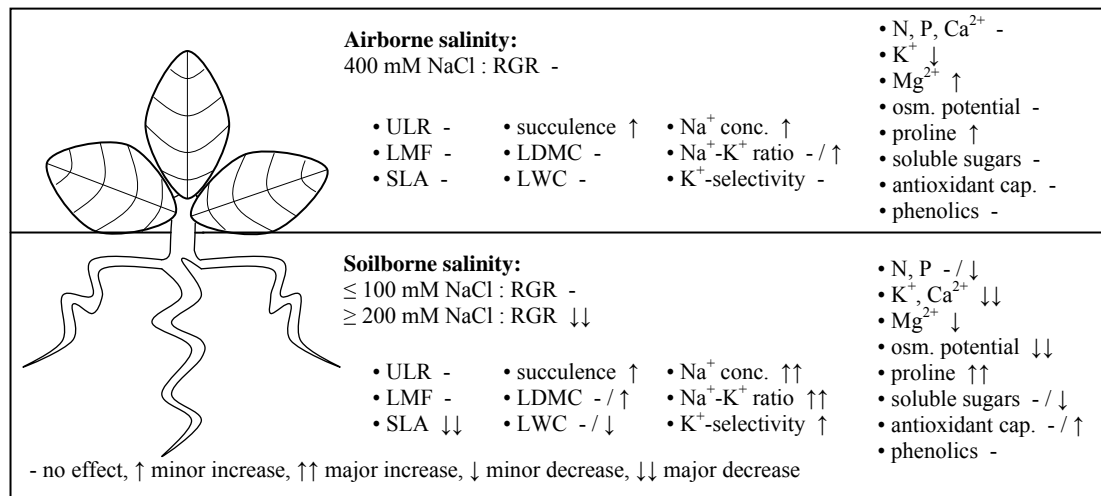

Figure 3.6. Overall effects of airborne and soilborne salinity on the measured parameters of *Crambe maritima*. For soilborne salinity the effects of ≥ 200 mM NaCl are listed to evaluate the observed growth reduction of the 200 and 300 mM NaCl treatments.

Effect of root zone salinity on growth

Crambe maritima was relatively sensitive to soil borne salinity above 100 mM NaCl and was only just capable of maintaining growth at salt concentrations of around 200 mM NaCl. Some halophytes show enhanced growth at moderate salinity levels around 50-100 mM NaCl RZS and are able to grow at salinity levels around half or even full strength seawater of around 500 mM NaCl (Flowers *et al.*, 1986; Breckle, 2002; Bell and O'Leary, 2003; Koyro and Lieth, 2008), but *C. maritima* showed no enhanced growth at moderate salinities and growth was greatly reduced at the 200 mM NaCl level. Although the growth after 40 days of salt exposure did not differ at 300 mM NaCl compared to the 200 mM treatment, there are indications that *C. maritima* is not able to adapt to this level of salinity. Compared to the 200 mM treatment the mean RGR of the 300 mM treatment was 25 % lower, the leaf succulence started to decrease, the Na^+ concentration and the Na-K ratio of the new leaves was considerably higher, whereas the net K^+ / Na^+ selectivity and the nutrient concentrations of N, K^+, Ca^{2+} and Mg^{2+} showed a sharp decrease.

Ecophysiological adaptations to root zone salinity

Many of the physiological traits measured, proline concentration, Na^+ concentration and Na^+ / K^+ ratio all responded to the root zone salinity treatments but none of these appeared to result in a change in ULR. The reduction in RGR with increasing salinity was mainly caused by a decreasing SLA. All other determined parameters responded to increasing salinity, but it appeared that no single parameter could explain the observed growth reduction between 100 and 200 mM NaCl RZS.

The reduction in RGR with increasing salinity was mainly caused by a decreasing SLA, because decreases in ULR and LMF were only minor and not significant (Table 3.2). In general, variation in RGR is strongly correlated with SLA and can be considered as the prime factor determining interspecific variation in RGR (Lambers and Poorter, 2004) and shoot and leaf morphology are more plastic and more important determinants of leaf assimilation capacities than leaf chemistry and assimilation rates (Niinemets, 1999). Also, a gradual decrease of SLA with increasing

salinity has been reported for *Lycopersicon esculentum* (Knight *et al.*, 1992) and for *Aster tripolium* (Shennan *et al.*, 1987). The inverse of SLA, 1/SLA, is the product of leaf succulence and LDMC (Poorter and Garnier, 2007) and leaf succulence in our RZS experiment showed a maximum increase of 22% with increasing salinity. An increase in leaf succulence can compensate for the negative effects of salinity on leaf cell metabolism to some extent (Munns and Tester, 2008). This also seems to be true for the 50 and 100 mM NaCl RZS where there was a trend that the reduction in SLA was compensated by an increase in ULR. However, the increased internal surface for CO_2 absorption does not necessarily lead to higher CO_2 uptake rates because the CO_2 resistance as well as stomatal resistance can increase even more with salinity, resulting in a decrease in photosynthesis (Longstretch and Nobel, 1979; Flowers, 1985; Geissler *et al.*, 2009). Although the SLA showed the greatest reductions with increasing salinity, the SLA of the 200 mM NaCl RZS treatment only showed a minor decrease compared to the 100 mM NaCl RZS treatment, whereas the reduction in RGR showed a major decrease. The ULR changed from 120 % to 94 % (compared to the 0 mM NaCl RZS treatment) for the 100 and 200 mM treatments, respectively, so it appears that the observed growth reduction between the 100 and the 200 mM NaCl RZS was caused by the combined reduction in SLA and ULR. The SLA and LDMC are considered to reflect a fundamental trade-off in plant functioning between a rapid production of dry weight (high SLA, low LDMC species) and an efficient conservation of nutrients (low SLA, high LDMC species) (Garnier *et al.*, 2001). In this regard, *C. maritima* adapted to increased salinity by changing from fast-growth to slow-growth with conservation of water and nutrients.

The ability of controlled uptake, increased extrusion and compartmentalization of Na^+ is likely primarily determining the salt tolerance of halophytes (Flowers *et al.*, 1977; Zhu, 2001; Munns, 2005; Flowers and Colmer, 2008; Munns and Tester, 2008). No visible salt glands or bladders were detected on the leaves of *C. maritima* and no mention of such structures was found in the literature (Scott and Randall, 1976). Increased root zone salinities resulted in increased Na^+ concentrations, decreased K^+ concentrations and increased Na^+ / K^+ ratios in *C. maritima*. The results showed that Na^+ readily entered the plants at 50 mM NaCl RZS but concentrations up to 200 mM NaCl were comparable with concentrations found in the highly salt-tolerant Brassicaceae species *Cakile maritima* (Debez *et al.*, 2004; Megdiche *et al.*, 2007) and *Thellungiella halophila* (Ghars *et al.*, 2008). So the measured concentration of Na^+ on leaf tissue level does not inevitably result in major growth reduction as observed in *C. maritima*. Possibly, the amount of Na^+ transported into the vacuoles differed amongst the species. Halophytes must be able to select K^+ from a medium in the root zone dominated by Na^+ to maintain adequate K^+ nutrition or, in other words, to maintain a low Na^+ / K^+ ratio in the cytosol (Breckle, 2002; Xiong and Zhu, 2002; Wyn Jones and Gorham, 2002; Flowers and Colmer, 2008). Dependent on the plant species this ratio can range from 1 to 46 when concentrations of the whole leaf are considered (Flowers *et al.*, 1986; Flowers and Colmer, 2008), although in the Brassicaceae species *Cakile maritima* (Debez *et al.*, 2004; Megdiche *et al.*, 2007) and *Thellungiella halophila* (Ghars *et al.*, 2008) this ratio is around 4 at 200 mM NaCl and around 8 at 500 mM NaCl. The Na^+ / K^+ ratio of *C. maritima* was about 7 at 200 mM NaCl and a similar ratio resulted in considerable growth reductions in *Cakile maritima* and *Thellungiella halophila*. Although this ratio was reached at different salt concentrations for the different species, it does emphasizes the importance of a low Na^+ / K^+ ratio to maintain high growth rates. Based on these results the growth reduction of *C. maritima* at 200 mM NaCl seems to be, at least partially, due to an

increased Na^+ / K^+ ratio. However, it was expected that growth reduction by an increased Na^+ / K^+ ratio would result in ionic stress of which the physiological effects would reduce the ULR. The results (Table 3.2) showed no effect on the ULR, making it unlikely that ionic effects caused the observed growth reductions.

It is likely that osmotic effects are not responsible for the observed growth reductions with increasing root zone salinities. *Crambe maritima* was able to sufficiently lower its osmotic potential with NaCl present in the leaf tissue. All values of the osmotic potential of the leaf sap were lower (more negative) than that of the irrigation water and at the 200 mM NaCl treatment level the leaf sap NaCl concentration accounted for 70 % of the leaf sap osmotic potential. Compatible solutes like proline must accumulate in the cytosol to balance the osmotic pressure and allow turgor maintenance of cells (Yeo, 1983; Zhu, 2001; Breckle, 2002; Wyn Jones and Gorham, 2002; Munns and Tester, 2008). It is likely that proline is an important solute for *C. maritima*, but the proline concentration in our experiment was strongly correlated to the soil borne NaCl concentration and not to the RGR of *C. maritima*. Proline accumulation in coastal plant species was demonstrated under field conditions, but this accumulation, in the absence of salinity stress, was linked with drought stress (Smirnoff and Stewart, 1985).

Salinity could also have directly affected nutrient uptake (Grattan and Grieve, 1999; Ullrich, 2002; Xiong and Zhu, 2002; Abdelgadir *et al*., 2005). The differences in K^+ and Mg^{2+} concentrations between the 100 and 200 mM NaCl RZS were relatively small, so it is unlikely that nutrient deficiencies were responsible for the observed growth reductions. The decrease in Ca^{2+} concentration between the 100 and 200 mM NaCl RZS indicated that the role of Ca^{2+} may be important in the salt tolerance in *C. maritima.*

Salinity can induce oxidative stress by the production of toxic reactive oxygen species (ROS) in plants. Compounds that detoxify ROS, like enzymes, small molecule antioxidants and polyphenols, may have an essential role in adapting plants to salinity stress (Zhu, 2001; Xiong and Zhu, 2002; Ashraf and Harris, 2004; Parida and Das, 2005; Halliwell, 2006; Munns and Tester, 2008). Our results showed that with increasing salinity there was an increase in TEAC but not in total phenolics (also used as a measurement of oxidative activity). The level of increase of TEAC was similar amongst salt treatments, showing that this reaction was not specific to the actual salt concentration. It is not known if these levels are the maximum that *C. maritima* can produce or that these levels were sufficient to cope with the potential oxidative stress. Salt sensitive plant species, like *Lactuca sativa*, show a decrease in total phenolics and antioxidant capacity with increasing salinity (Chisari *et al*., 2010). This will decrease the ability of a plant species to detoxify ROS whereas this detoxification can improve plant salt tolerance (Zhu, 2001). However, genetic differences in salinity tolerance are not necessarily due to differences in the ability to detoxify ROS (Munns and Tester, 2008) and further work is required to establish the general validity of these protective mechanisms in salinity tolerance (Ashraf and Harris, 2004).

Suitability as a crop for saline agriculture
When *C. maritima* is grown as an agricultural crop, root cuttings are used as the propagation method and the etiolated sprouts that grow from the root stem form the actual crop (Péron, 1990; Briard et al., 2002). It is not known if these root cuttings and etiolated sprouts respond in the same way to increased salinity as the seedlings in our experiment. However, no growth or tap root dry weight reduction occurred up to the 100 mM NaCl root zone salinity level in our experiment, indicating that *C. maritima*

can be cultivated under this salt concentration without loss in yield. Also, no growth reductions are expected when *C. maritima* is grown in a salt spray rich environment.

Concluding remarks
Based on the growth performance it can be concluded that *C. maritima* is salt spray tolerant and can endure the salt spray levels which it encounters in its natural habitat. Salt spray caused only minor physiological responses in the plants as compared to the root zone salinity treatments, although leaf thickness and leaf succulence significantly increased with salt spray.

Root zone salinities up to 100 mM NaCl did not result in a decrease in growth, but a sharp decrease in growth was observed at 200 mM NaCl. *Crambe maritima* responded to increasing soil salinity by lowering the RGR. The observed growth reduction at 200 mM NaCl RZS was mainly caused by the reduction in SLA. The lower SLA was caused by increased leaf succulence as well as increased LDMC. All other determined parameters responded to increasing salinity, but it appeared that no single parameter was clearly linked with the observed growth reduction between 100 and 200 mM NaCl RZS.

ACKNOWLEDGEMENTS

Appendix 3A. Leaf dry weight production and leaf area of the whole-plant of all treatments of the 8-week-old *Crambe maritima* plants with 20 days of salt spray or 40 days of root zone salinity ranging from 0 to 300 mM NaCl. Plants were grown on gravel culture with hydroponics. Values are the means of five replicates ± s.e. Different letters indicate a significant difference at $P<0.05$ (Tukey's test).

Treatment	leaf dry weight (g)	leaf area (cm^2)
control spray	4.80±1.55[ab]	692±183[ab]
salt spray	8.39±1.07[a]	1085±167[a]
0	4.98±1.35[ab]	827±201[ab]
50	3.37±0.57[b]	477±75[b]
100	3.35±0.50[b]	414±65[b]
200	1.00±0.14[c]	116±14[c]
300	0.58±0.11[c]	55±9[c]

Chapter 4.

Relative importance of leaf traits for the salt tolerance of the Brassicaceae species *Diplotaxis tenuifolia* and *Cochlearia officinalis*, grown in hydroponic culture

Arjen C. de Vos[1]
Rob Broekman[1]
Cátia C. de Almeida Guerra[2]
Jelte Rozema[1]

[1] *Institute of Ecological Science, Department of Systems Ecology, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*
[2] *University of Aveiro, Department of Biology, 3810 Aveiro, Portugal*

- *Background and Aims* Since *Diplotaxis tenuifolia* (L.) and *Cochlearia officinalis* (L.) can be found in coastal and maritime habitats, they will be exposed to saline conditions. Although these saline conditions are not as extreme as in the salt marsh environment, it was assumed that both species may be salt tolerant to some extent. Both species have potential to be developed to vegetable saline crops. A greenhouse experiment was conducted to determine the level of salt tolerance and the mechanisms underlying this level of tolerance.

- *Methods* Plants were grown hydroponically in a greenhouse and harvested at the end of the vegetative growth period. The absolute growth, the relative growth rate (RGR) and its components as well as the Na^+ concentration, the net K^+ / Na^+ selectivity and Na^+ / K^+ ratio and possible nutritional imbalances were determined for *D. tenuifolia* and for *C. officinalis* in response to increasing salinity.

- *Key Results* No growth reductions occurred up to 100 mM NaCl for *D. tenuifolia*, whereas *C. officinalis* showed a 37 % decrease in total dry weight at the 100 mM NaCl level, corresponding with a 9 % decrease in RGR. The RGR at higher salinity levels ($\geq$ 200 mM NaCl) showed reductions around 20 % for both species. The reductions in RGR were mainly due to reductions in specific leaf area (SLA), which were similar for the two species. The reductions in SLA were caused by an increase in leaf succulence which showed a maximum increase of 63 % at the 300 mM NaCl level for *D. tenuifolia* and 72 % at the 400 mM NaCl level for *C. officinalis*. The shoot Na^+ concentration at 200 mM NaCl most likely did not result in toxic effects which affected growth. In comparison with seven other Brassicaceae species, including salt sensitive and highly salt tolerant species, both *D. tenuifolia* and *C. officinalis* showed an intermediate response to increasing salinity. Both species were able to survive the highest salinity treatment (300 mM NaCl for *D. tenuifolia* and 400 mM NaCl for *C. officinalis*) and can be classified as salt tolerant with potential as vegetable crops for saline agriculture.

- *Conclusions* The growth reductions of both species at high salinity ($\geq$ 200 mM NaCl) were largely due to changes of leaf morphology (decrease of SLA, increase of leaf succulence) rather than toxic leaf Na^+ concentrations. In this regard, the relative importance of leaf traits for the salt tolerance of *Diplotaxis tenuifolia* and *Cochlearia officinalis* was demonstrated.

Keywords halophyte - salt tolerance - relative growth rate - leaf traits - specific leaf area - leaf succulence - Na^+ / K^+ ratio -

INTRODUCTION

As was pointed out in chapter one, halophytes represent only 1% (Flowers and Colmer, 2008) to 2% (Glenn *et al.*, 1999) of the number of terrestrial plant species, but are present in 139 higher plant families (Yensen, 2008). This paper focuses on one plant family in an attempt to compare possible mutual traits between two closely related species and also possible common traits among other related Brassicaceae species. Among these Brassicaceae species, at least *Thellungiella halophila (salsuginea)* (M'rah *et al.*, 2006; Ghars *et al.*, 2008; Alemán *et al.*, 2009), *Cochlearia anglica* (Le Saos, 1978), and *Cakile maritima* (Debez *et al.*, 2004; Megdiche *et al.*,

2007) have been classified as halophytes and the effect of salinity on growth has been evaluated for *Arabidopsis thaliana* (Ghars et al., 2008; Alemán *et al.*, 2009), *Brassica spp*. (He and Cramer, 1992), and *Crambe maritima* (De Vos *et al.*, 2010b), among others. In this paper, two other Brassicaceae species with the potential to be used as saline vegetable crops and with presumed salt tolerance will be evaluated, namely *D. tenuifolia* (L.) and *C. officinalis* (L.).

Diplotaxis tenuifolia (L.) (Perennial wall rocket or Wild rocket) is an intensively studied and commonly cultivated vegetable crop (Bianco, 1995; D'Antuono *et al.*, 2007), but as far as the authors are aware the salt tolerance has not been investigated. It is mainly used as a leaf vegetable, but also contains antifungal oil and has therapeutic properties (Padulosi, 1995; Rodriguez *et al.*, 2006). *Diplotaxis tenuifolia* is a perennial plant originating from the Mediterranean and Asian region but has a cosmopolitan distribution at present day, occurring on sandy and chalky soils in coastal areas and plains (Weeda *et al.*, 1987; Warwick, 1995; D'Antuono *et al.*, 2007). Since *D. tenuifolia* occurs in coastal areas and occasionally can be found on the strandline or floodline (personal observations) it is assumed that *D. tenuifolia* will be salt tolerant to some extent.

Cochlearia officinalis ssp. *officinalis* (Common scurvy grass), often named *C. officinalis* (L.) and in this paper referred to as *C. officinalis*, is classified as a typical halophyte confined to maritime and saline habitats (Weeda *et al.*, 1987; Pegtel, 1999). This biennial to short-lived perennial plant species occurs especially in the coastal areas of Northwest Europe, but subspecies also occur in the arctic region. *Cochlearia officinalis* is mostly confined to brackish water conditions, whereas the closely related *C. officinalis* ssp. *anglica* or *C. anglica* can be found in the more saline salt marshes (Weeda *et al.*, 1987; Van der Meijden, 1996). Previous experiments have evaluated the effect of NaCl on plant growth (Le Saos, 1978) and different parameters of roots of *C. anglica* (Binet, 1985; Prud'homme *et al.*, 1990) and the effect of increasing salinity on the germination of *C. officinalis* and *C. anglica*, among others (Pegtel, 1999), but not the effect of increasing salinity on the whole-plant growth of *C. officinalis*. *Cochlearia officinalis* contains considerable levels of ascorbic acid or vitamin C (Gustafson, 1954; Hughes, 1990; Buckland *et al.*, 1991) and glucosinolates (Wynne Griffiths *et al.*, 2001) and can be used as salad plant (Small, 2006). It was used in the past by mariners to combat scurvy, a disease resulting from vitamin C deficiency (Maat, 2004).

The objective of this study is to evaluate the response of *D. tenuifolia* and *C. officinalis* to increasing salinity by determining the effect on the growth, leaf succulence and mineral composition. The salt tolerance of a plant species is usually based on its ability to continue growth under increasing salinity (Maas and Hoffman, 1977). In general, the osmotic effect of increased salinity in the root zone will cause the initial growth reduction and the subsequent growth reduction due to ionic stress (after the uptake of salt) is caused by ion accumulation up to toxic levels in the transpiring leaves (Munns, 1993; Munns and Tester, 2008). A common approach to analyse growth is to use the RGR and its components unit leaf rate (ULR), leaf weight fraction (LWF), and SLA, with RGR=ULR*LWF*SLA (Hunt *et al.*, 2002). This comparative approach is used to analyse the physiological and morphological attributes that lead to variation in growth (Lambers and Poorter, 2004; Poorter and Garnier, 2007).

To evaluate possible (toxic) ionic effects on plant growth with increasing salinity the Na^+ concentration, the net K^+ / Na^+ selectivity and Na^+ / K^+ ratio and possible nutritional imbalances were determined. Characteristically, halophytes are

able to accumulate sufficient Na^+ for the purpose of osmotic adjustment and support relatively high rates of net Na^+ uptake without injury to the plant (Flowers and Colmer, 2008). However, all plants suffer from high (shoot) concentrations of Na^+, largely as a result of the ability of Na^+ to compete with the uptake of the macronutrient K^+ (Maathuis and Amtmann, 1999; Tester and Davenport, 2003). One of the key determinants of plant salt tolerance is likely the capacity to maintain a high cytosolic K^+ / Na^+ ratio with increasing salinity (Maathuis and Amtmann, 1999). Halophytes are able to maintain this relative high K^+ / Na^+ ratio (low Na^+ / K^+ ratio) by means of selective uptake of K^+ from a medium dominated by Na^+ (Wyn Jones and Gorham, 2002; Flowers and Colmer, 2008). In salt tolerant plants the K^+ selectivity increases with increasing salinity (Wyn Jones and Gorham, 2002) and this ability is commonly expressed as net K^+ / Na^+ selectivity. Salinity can also directly affect nutrient uptake resulting in nutritional disorders which affect plant growth (Grattan and Grieve, 1999).

Thus, the effect of increasing salinity on the Na^+ concentration, the net K^+ / Na^+ selectivity, the Na^+ / K^+ ratio and possible nutrient imbalance, can reveal important mechanisms that are characteristic for salt tolerant plants. By determining the effect of increasing salinity on the different components of growth and the mineral composition, possible common characteristics between the species *D. tenuifolia* and *C. officinalis*, as well as other Brassicaceae species, were evaluated.

MATERIAL AND METHODS

Experimental growth conditions
Seeds of both *D. tenuifolia* and *C. officinalis* were obtained from Vreeken's Zaden (Dordrecht, The Netherlands) and germinated on peat soil (Seed Plot soil Jongkind, Aalsmeer, The Netherlands). Seedlings were transplanted after the emergence of the first true leaf. Seedlings of *D. tenuifolia* were transferred to a hydroponic culture system 15 days after sowing and were allowed to grow for an additional 6 days before salt addition started. Seedlings of *C. officinalis* were transferred to the hydroponic culture system 19 days after sowing and were allowed to grow for another 7 days before salt addition started. Plants were grown in 1-liter polyethylene pots filled with a continuously aerated nutrient solution as described in Chapter 3. The experiment was performed in Januari/March 2008 in a greenhouse with controlled temperature, humidity and light conditions: $22\pm2/15\pm2$ ºC day/night, relative humidity 60/80 % day/night, light intensity 300 PAR (μmol m^{-2} s^{-1} at plant level, 16h light day^{-1}). Salt concentration was increased by 50 mM NaCl per day to a maximum of 300 mM NaCl for *D. tenuifolia* and 400 mM NaCl for *C. officinalis*. The salinity treatments consisted of 0, 50, 100, 200 and 300 mM NaCl for *D. tenuifolia*, and 0, 50, 100, 200 and 400 mM NaCl for *C. officinalis*.

Plant analysis
Just before addition of salt, 25 plants of *D. tenuifolia* and 20 plants of *C. officinalis* were harvested to obtain values of initial dry weight and leaf area. During the experiment the fresh weight of the plants was measured non-destructively and recorded weekly. For this, plants were carefully removed from the hydroponic culture, blotted dry, weighted, and placed back in the nutrient solution. Plants were harvested at the end of the vegetative growth period, which was defined as the emergence of the flowering stem for *D. tenuifolia* and as discontinued formation of new leaves for *C.*

officinalis (for *C. officinalis*, no flowering occurs in the first year (Weeda *et al*. 1987). The final harvest occurred 20 days after salt addition was started for *D. tenuifolia* (*n*=8) and 37 days for *C. officinalis* (*n*=5). Plants were carefully washed with running demineralised water for 1 min, blotted dry and separated into leaves, leaf stems (only *C. officinalis*) and roots and the fresh weights were recorded. The leaf area was recorded with a LI-COR 3100 area meter (Li-Cor Inc, Lincoln, Nebraska, USA). Following this, leaves were frozen in liquid nitrogen and lyophilized before analysis of dry weights and mineral composition. The rest of the plant material was oven-dried at 70 ºC for 48 hours.

The relative growth rate (RGR) and its components (ULR, LWF, SLA, LDMC and leaf succulence) were estimated with the formulas given in Chapter 3. The thickness of *D. tenuifolia* leaves was measured with a thickness gauge (Mitutoyo No. 2046-08, Japan, accuracy 0.01 mm).

Mineral composition
The concentrations of total P, Ca^{2+}, Mg^{2+}, Na^+ and K^+, total C and N were determined as described in Chapter 2 and net K^+ / Na^+ selectivity was determined as described in Chapter 3.

Statistical analysis
Statistical data analysis was performed using SPSS (version 15.0 for Windows, SPSS Inc. 2000, Chicago, IL, USA). One-way ANOVA was used to test for differences between salinity treatments. For this, the two species were analysed separately. Normality and homogeneity assumptions of the test were checked both by visual inspection of the residuals for undesired patterns and by Levene's test for homogeneity of variances. When assumptions were violated log transformed values or non parametric tests (Mann-Whitney U) were used. Two-way ANOVA was used to test for differences between species (for values $\leq$ 200 mM NaCl), using salinity and species as the factors.

RESULTS

Plant growth
During the experiment the fresh weight of the whole plant was recorded non-destructively every week after salt addition started (Fig. 4.1). The results showed that growth continued at all salinity levels, although at the highest salt concentration both plant species showed minimal growth. The total fresh weight of the final harvest of *D. tenuifolia* showed no significant differences between the 0, 50 and 100 mM NaCl treatments, but declined by 64 % at the 200 mM NaCl level compared to the 0 mM NaCl treatment. For *C. officinalis* the fresh weight already declined by 46 % at the 100 mM NaCl level and by 74 % at the 200 mM NaCl level. The mean maximum fresh weight was about 23 grams after 20 days for *D. tenuifolia* and 65 grams after 37 days for *C. officinalis*. After these 20 days, flower stems started to appear within all salinity treatments for *D. tenuifolia*, indicating the ability to reproduce at all salinity treatments. For *C. officinalis* this ending of the vegetative growth period (after 37 days) was defined as the discontinued formation of new leaves since no flowering occurs in the first year.

The total dry weight of the final harvest (see Appendix 4A) showed similar results as the fresh weights with no reductions up to 100 mM NaCl for *D. tenuifolia*.

However, the dry weight of *C. officinalis* was reduced by 44 % at the 100 mM NaCl level compared to the 0 mM NaCl level. The total dry weight of the final harvest of
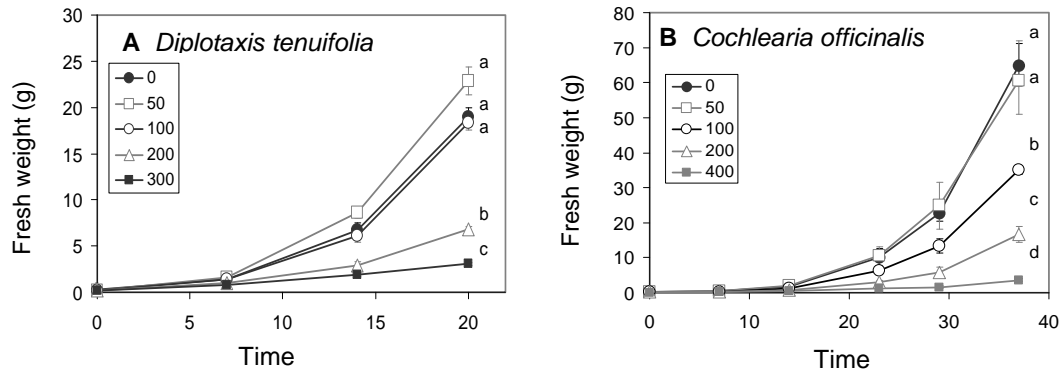

Figure 4.1. Time course (days) of total fresh weight (in grams) of hydroponically grown plants of *Diplotaxis tenuifolia* (A) and *Cochlearia officinalis* (B) in response to increasing salinity (mM NaCl). Plants were finally harvested 20 days (*D. tenuifolia*) or 37 days (*C. officinalis*) after salt addition started. Values are means of five replicates ± s.e.. Values of the final harvest were used to test for differences between treatments (One-way ANOVA). Different letters indicate a significant difference at P<0.05 (Tukeys's test).

the 0 mM NaCl treatment was 1.52 g and 5.47 g for *D. tenuifolia* and *C. officinalis*, respectively. At the 200 mM NaCl level the dry weights were reduced by 60 % for *D. tenuifolia* and 70 % for *C. officinalis*. The maximum RGR of *D. tenuifolia* was around 220 mg g^{-1} day^{-1} and 170 mg g^{-1} day^{-1} for *C. officinalis* (Fig. 4.2) and both species showed comparable significant differences with the data in Figure 4.1. Compared with the RGR at 0 mM NaCl, the RGR at the 100 mM NaCl level showed no change for *D. tenuifolia* and a decrease of 9 % for *C. officinalis*. The RGR at the 200 mM NaCl level showed reductions of 21 % and 19 % for *D. tenuifolia* and *C. officinalis*, respectively, and a maximum reduction of 34 % at the highest salinity concentration of 300 mM NaCl level for *D. tenuifolia* and 4 2% at the 400 mM NaCl level for *C. officinalis* compared with the RGR at 0 mM NaCl. The ULR of *D. tenuifolia* showed an increase of 25 % at the 100 mM NaCl level and did not decrease at higher salinities compared to the 0 mM NaCl level. The ULR of *C. officinalis* showed a non-significant increase of 23 % and 8 % at the 50 and 100 mM NaCl level compared to the 0 mM NaCl level, respectively, and only showed a significant decrease of 26 % between the 50 and 400 mM NaCl treatment. The LWF remained constant for *D. tenuifolia* and showed an increase of 37% at the 400 mM NaCl level for *C. officinalis*. The SLA showed a gradual reduction with increasing salinity in both *D. tenuifolia* and *C. officinalis*, with a maximum decrease of 54 % at the 300 mM NaCl level for *D. tenuifolia* and 54 % at the 400 mM NaCl level for *C. officinalis*. Results of the two-way ANOVA (referred to in Fig. 4.2 as 'between species') showed that the difference in RGR between the two species was highly significant and this difference was caused by the difference in LWF and not in ULR or in SLA.

The leaf succulence of both species showed an increase with increasing salinity with a maximum increase of 63 % for *D. tenuifolia* and 72 % for *C. officinalis* at the highest salinity levels (Fig. 4.3). The LDMC of both species did not change at the 200 mM NaCl level. The LDMC of *D. tenuifolia* did not change significantly, and only the increase of 25 % between the 100 and 400 mM NaCl level of *C. officinalis* was significant. Results of the two-way ANOVA (referred to in Fig. 4.3 as 'between species') showed no difference in LDMC between the two species, whereas the

difference in leaf succulence was highly significant. The LWC (data not shown) showed similar results as the LDMC, with no significant differences for *D. tenuifolia* (values ranging from 90 % to 93 %) and for *C. officinalis* the only significant change was between the 100 and 400 mM NaCl level with LWC changing from 91 % to 89 %, respectively. The leaf thickness of *D. tenuifolia* (data not shown) increased by 27 %, 54 %, 84 % and 107 % for the 50, 100, 200 and 300 mM NaCl treatments, respectively.
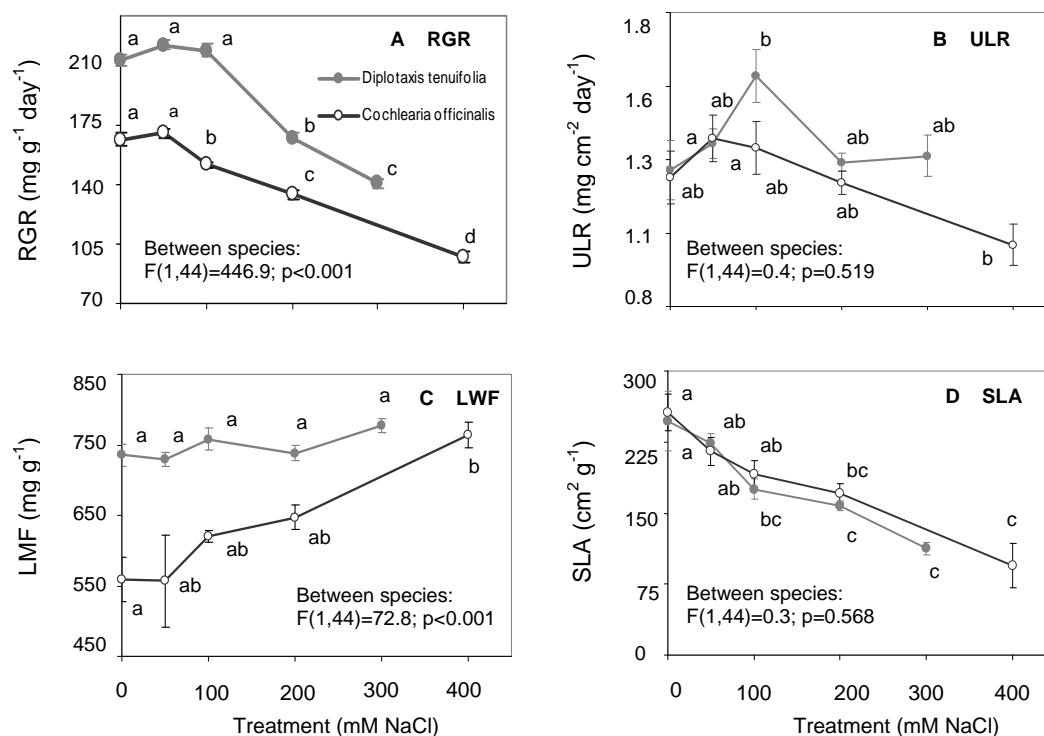

Figure 4.2. The RGR (A) and the components ULR (B), LWF (C), and SLA (D) of *D. tenuifolia* and *C. officinalis* in response to increasing salinity (mM NaCl). Plants were finally harvested 20 days (*D. tenuifolia*) or 37 days (*C. officinalis*) after salt addition started. Values are means±s.e. with *n*=8 for *D. tenuifolia* and *n*=5 for *C. officinalis*. Different letters indicate a significant difference between salinity treatments at *P*<0.05 (One-way ANOVA with Tukeys's test as *Post hoc*) with species analysed seperately. Possible differences between the two species (≤ 200 mM NaCl) were analysed with two-way ANOVA and referred to in the figure as 'between species'.

Mineral composition

The results of the mineral composition of *D. tenuifolia* are listed in Table 4.1. The 100 mM NaCl treatment resulted in a decrease of 18 %, 52 % and 37 % (as compared to the 0 mM NaCl treatment) in total N, K^+ and Ca^{2+} concentration, respectively. At the 200 mM NaCl level K^+, Ca^{2+} and Mg^{2+} concentrations showed major reductions as compared with the 0 and 100 mM NaCl treatment. The total content of N and P of the 200 mM NaCl treatment showed similar concentrations as those of the 100 mM treatment. At the 300 mM NaCl level all mineral concentrations showed major reductions. The total leaf content of C was also determined but showed no reductions with increasing salinities and ranged from 26 to 28 mmol g^{-1} DW (data not shown). The results of the mineral composition of *C. officinalis* are listed in table 4.2. For *C. officinalis*, the 100 mM NaCl treatment resulted in a decrease of 48 %, 68 % and 49 % of K^+, Ca^{2+} and Mg^{2+}, respectively. These concentrations decreased even further at the

200 mM NaCl level, whereas N and P were not affected. Nitrogen concentrations were reduced at the 400 mM NaCl level. Total C ranged from 26 to 30 mmol g^{-1} DW (data not shown) with treatments 200 and 400 mM NaCl showing a significant reduction as compared to the 0 mM treatment, but no difference occurred as compared with the other salt treatments.
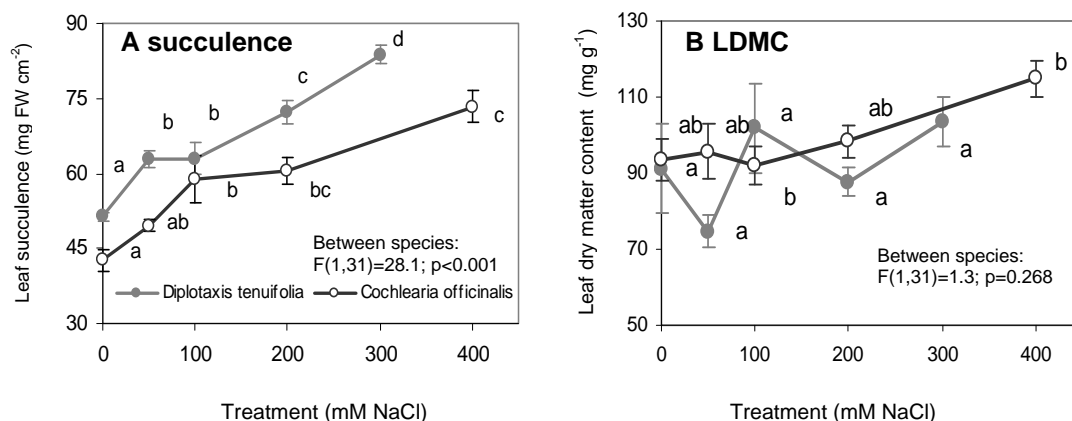

Figure 4.3. Leaf succulence (A) expressed as L_{FW}/L_A (in mg FW cm^{-2}) and leaf dry matter content (B) expressed as L_{DW}/L_{FW} (mg g^{-1}) of *Diplotaxis tenuifolia* and *Cochlearia officinalis* in response to increasing salinity (mM NaCl). Plants were harvested 20 days (*D. tenuifolia*) or 37 days (*C. officinalis*) after salt addition started. Values are means of five replicates ± s.e.. Different letters indicate a significant difference at P<0.05 (Tukeys's test) with species analysed seperately. Possible differences between the two species (≤ 200 mM NaCl) were analysed with two-way ANOVA and referred to in the figure as 'between species'.

Na$^+$, Na$^+$ / K$^+$ ratio and net Na$^+$ / K$^+$ selectivity

The Na$^+$ concentration in the leaves of *D. tenuifolia* and *C. officinalis* readily increased with increasing Na$^+$ concentration of the nutrient solution (Table 4.3). The maximum Na$^+$ concentration in *D. tenuifolia* of 5 mmol g^{-1} DW was reached at the 300 mM NaCl level, which represented 11 % of the total biomass (DW). *Cochlearia officinalis* showed similar values at the highest salt treatment with the Na$^+$ concentration representing 13 % of the total biomass. Both species showed the greatest increase in the net K$^+$ / Na$^+$ selectivity at the lower salinity treatment levels. At the highest salinity levels this selectivity started to decrease, whereas the Na$^+$ uptake continued. This resulted in a sharp increase of the Na$^+$ / K$^+$ ratio in both species at the higher salt concentrations (≥ 200 mM NaCl). Results of the two-way ANOVA (referred to at the bottom of each column in Table 4.3) showed that although the Na$^+$ concentration and net K$^+$ / Na$^+$ selectivity differed significantly, this did not result in a difference in the Na$^+$ / K$^+$ ratio between the two species.

DISCUSSION

Plant growth

Halophytes can be defined as plants that survive to reproduce in environments where the salt concentration is around 200 mM NaCl or more (Flowers and Colmer, 2008). Flower stems were produced within all salinity treatments of *D. tenuifolia* and for *C. officinalis* growth continued even at the 400 mM NaCl level making it likely that

plants will survive to reproduce at the 200 mM NaCl level (for *C. officinalis*, no flowering occurs in the first year (Weeda *et al*., 1987)). So, based on their growth

Table 4.1. Mineral composition of *D. tenuifolia* leaves in response to increased (0-300 mM NaCl) root zone salinity treatments, expressed as μmol g^{-1} DW

treatment	N	P	K$^+$	Ca^{2+}	Mg^{2+}
0	5067±56^a	489±34^a	1938±57^a	636±39^a	122±6^a
50	4502±97^{ab}	438±40^a	1174±71^b	507±36^{ab}	98±7^a
100	4133±318^b	416±37^a	937±113^b	399±16^b	99±7^a
200	3853±196^b	400±29^a	309±43^c	132±14^c	66±4^b
300	2608±129^c	174±13^b	142±13^d	40±5^d	32±3^c

Treatment levels are expressed in mM NaCl. Plants were harvested 20 days after salt addition started. Plants were grown hydroponically with NaCl added to the nutrient solution. Values are means ± s.e. (*n*=5). Means in the same column that have the same letter are not significantly different at *P* < 0.05 (Tukey's test)

Table 4.2. Mineral composition of *C. officinalis* leaves leaves in response to increased (0-400 mM NaCl) root zone salinity treatments, expressed as μmol g^{-1} DW

treatment	N	P	K$^+$	Ca^{2+}	Mg^{2+}
0	3838±129^a	176±14^a	1784±57^a	512±61^a	197±14^a
50	4003±216^a	161±24^a	1023±109^b	260±10^b	136±8^b
100	4255±217^a	177±10^a	929±111^b	163±9^c	100±5^c
200	3822±92^a	143±17^a	462±53^c	97±5^d	58±4^d
400	2521±115^b	109±17^a	199±24^c	40±7^e	26±3^e

Treatment levels are expressed in mM NaCl. Plants were harvested 37 days after salt addition started. Plants were grown hydroponically with NaCl added to the nutrient solution. Values are means ± s.e. (*n*=5). Means in the same column that have the same letter are not significantly different at *P* < 0.05 (Tukey's test)

performance under increasing salinity both *D. tenuifolia* and *C. officinalis* can be classified as halophytes. No growth reductions were recorded for the 100 mM NaCl treatment of *D. tenuifolia*, whereas the RGR of *C. officinalis* was reduced by 9 % by this treatment. The reductions in RGR at the 200 mM NaCl level were comparable between the two species, with reductions of 21 % and 19 % for *D. tenuifolia* and *C. officinalis*, respectively. Since RGR=ULR*LWF*SLA and only SLA showed a decrease at the 200 mM NaCl salinity level, it can be concluded that this decrease in SLA is responsible for the decrease in RGR at this salinity level. The SLA decreased by 36 % and 33 % at the 200 mM NaCl level for *D. tenuifolia* and *C. officinalis*, respectively. In general, a 10 % increase in RGR is associated with an increase of 7.8 % in SLA or an increase of 2.4 % in ULR. Also, a 10 % increase in SLA is associated with a 4 % decrease in ULR (Lambers and Poorter, 2004). In the case of both *D. tenuifolia* and *C. officinalis* reductions in RGR were associated with 2-fold greater reductions in SLA, assuming that the general correlations according to Lambers and Poorter (2004) also apply to reductions rather than increases in RGR and its components. These greater reductions (for *D. tenuifolia* and *C. officinalis*) are possibly due to the fact that ULR showed a 2-fold greater increase with decreasing SLA compared to the general values of Lambers and Poorter (2004). One explanation may be that the low values of SLA of Lambers and Poorter (2004) were derived from naturally slow-growing species which invest more in protective compounds and structure, resulting in lower values of ULR. The decrease in SLA in dicotyledonous halophytes is often the result of an increase in succulence and in the amount of photosynthetic active parenchyma tissue (Koyro, 2000; Geissler *et al*., 2009), thereby

increasing the assimilation capacity per unit area (Vile *et al.*, 2005; Munns and Tester, 2008) and thus maintaining a relative high RGR with a relative low SLA. But although it appeared that the values of SLA and especially the increased values of leaf succulence were able to increase the values of ULR at low salinities, the decreasing

Table 4.3. The Na^+, Na^+ / K^+ ratio and net K^+ / Na^+ selectivity of *D. tenuifolia* and *C. officinalis* in response to increased (0-300 or 400 mM NaCl) root zone salinity treatments, with Na^+ expressed as $\mu mol\ g^{-1}$ DW

| treatment | Na^+ | | Na^+ / K^+ ratio | | net K^+ / Na^+ selectivity | |
	D. tenuifolia	*C. officinalis*	*D. tenuifolia*	*C. officinalis*	*D. tenuifolia*	*C. officinalis*
0	32 ± 8^a	132 ± 12^a	0.02 ± 0.01^a	0.07 ± 0.01^a	0.6 ± 0.3^a	0.1 ± 0.0^a
50	1603 ± 108^b	2755 ± 33^b	1.38 ± 0.10^b	2.83 ± 0.32^b	11.5 ± 0.7^b	5.8 ± 0.6^{bc}
100	2431 ± 121^c	3388 ± 79^b	2.75 ± 0.36^c	3.95 ± 0.63^b	12.2 ± 1.6^b	8.5 ± 1.0^c
200	4503 ± 451^d	4397 ± 106^c	15.98 ± 3.39^d	9.93 ± 0.95^c	4.6 ± 0.8^c	6.6 ± 0.7^{bc}
300	4955 ± 536^d	nd	37.32 ± 5.78^e	nd	3.7 ± 0.5^c	nd
400	nd	5707 ± 549^d	nd	31.36 ± 7.72^d	nd	4.6 ± 0.9^b
	$F(1,32)=17.8; p<0.001$		$F(1,32)=0.9; p=0.362$		$F(1,32)=11.0; p=0.02$	

Treatment levels are expressed in mM NaCl. Plants were harvested 20 days (*D. tenuifolia*) or 37 days (*C. officinalis*) after salt addition started. Values are means±s.e. with $n=5$. Means in the same column that have the same letter are not significantly different at $P < 0.05$ (Tukey's test), nd = not determined. At the bottom of each column the results of the two-way ANOVA to test for difference between species (≤ 200 mM NaCl) are given

values of SLA at higher salinities are held responsible for the observed reductions of RGR in both *D. tenuifolia* and *C. officinalis*. Similar trends were also reported for *Crambe maritima* (De Vos *et al.*, 2010b) and *Beta vulgaris spp maritima* (Chenopodiaceae / Amaranthaceae) (Rozema *et al.*, 1993) and decreasing values of LAR (LAR = leaf area ratio = SLA * LWF) and not ULR were also responsible for the observed growth reduction of *Brassica napus* (He and Cramer, 1993). However, a reduction in RGR due to a reduction in ULR and not SLA with increasing salinity has been reported for *Asteriscus maritimus* (Asteraceae) (Rodriguez *et al.*, 2005) and *Atriplex portulacoides* (Chenopodiaceae / Amaranthaceae) (Redondo-Gómez *et al.*, 2007), among others. Thus, a reduction in RGR with increasing salinity can be caused by a reduction in SLA as well as by a reduction in ULR and the particular component that is reduced appears to be a plant species specific adaptation. Since changes in leaf traits are linked with the osmotic effect of NaCl (which resembles a water-stress effect) rather than the ionic effect (Munns, 1993; Munns and Tester, 2008), it appeared that the osmotic effect of increased salinity affected the growth of both *D. tenuifolia* and *C. officinalis*. The growth of most salt sensitive plants will show an immediate decrease at a salt concentration of 40 mM NaCl as a reaction to the osmotic effect of NaCl in the root zone (Munns and Tester, 2008). It is likely that the osmotic effect of salinity has caused the growth reductions in *D. tenuifolia* and *C. officinalis*. Nevertheless their ability to maintain considerable growth rates at the 200 mM NaCl level justifies classifying both species as salt tolerant.

The analysis of the components of SLA, namely leaf succulence and LDMC with 1/SLA≈leaf succulence*LDMC, showed that the increased leaf succulence (Fig. 4.3A) was responsible for the decreased SLA in both species. No significant changes in LDMC were recorded at the 200 mM NaCl level, whereas the leaf succulence showed an increase of 41 % and 42 % for *D. tenuifolia* and *C. officinalis*, respectively. The leaf succulence showed a maximum increase of 63 % for *D. tenuifolia* and 72 % for *C. officinalis* at the highest salinity levels. Characteristically, halophytes are able

to accumulate sufficient amounts of Na^+ for the purpose of osmotic adjustment, which is associated with succulence in dicotyledonous halophytes (Flowers *et al.*, 1986). Increased leaf thickness or succulence with increased salinity results in conservation of internal water, efficient water storage and dilution of accumulated salts (Flowers *et al.*, 1986; Munns and Tester, 2008, among others). This increased leaf succulence with increasing salinity has also been reported for the Brassicaceae species *Cakile maritima* (Debez *et al.*, 2004), *Crambe maritima* (De Vos *et al.*, 2010b) and *Thellungiella halophila* (M'rah *et al.*, 2006) and indeed appears to be a common adaptation among Brassicaceae species.

Values of the relative growth (expressed as a percentage compared to the 0 mM NaCl treatment, based on dry weight) of *D. tenuifolia* and *C. officinalis* were plotted against previous reported values of other Brassicaceae species exposed to increasing salinity (Fig. 4.4A). This relative growth showed no difference between *D. tenuifolia* and *C. officinalis* (two-way ANOVA: $F_{(1,44)}=0.2$; $p=0.658$). It is difficult to interpret the different values of Figure 4.4 due to differences in the cultivation methods and the time of salt exposure between the species (20 days for *D. tenuifolia*, 37 days for *C. officinalis*, 180 days for *Cochlearia anglica* (Le Saos, 1978), for *Cakile maritima* 6 weeks (Debez *et al.*, 2004) and 20 days (Megdiche *et al.*, 2007), for *Thellungiella halophila* 14 days (M'rah *et al.*, 2006), 18 days (Aleman *et al.*, 2009), 28 days (Inan *et al.*, 2004, only used for net K^+ / Na^+ selectivity) and 3 weeks (Ghars *et al.*, (2008), 25 days for *Brassica napus* and *B. carinata* (He and Cramer, 1992), for *Arabidopsis thaliana* 3 weeks (Ghars *et al.*, 2008) and 18 days (Aleman *et al.*, 2009) and 40 days for *Crambe maritima* (De Vos *et al.*, 2010b)). However, it appears that some comparisons can be made. The relative growth of *D. tenuifolia* and *C. officinalis* was higher at low salinities (50 mM NaCl) compared to that of *Brassica napus* and *Brassica carinata* although the growth of *C. officinalis* was comparable with that of the two *Brassica spp* at the 100 mM NaCl level. *Diplotaxis tenuifolia* and *C. officinalis* were able to maintain growth at the higher salt concentrations ($\geq$ 200 mM NaCl) but this relative growth was considerably less than the reported growth of *Thellungiella halophila*, *C. anglica* and *Cakile maritima*. The growth performance of *Cakile maritima* used in Figure 4.4 was based on the maximum growth performance under salinity reported in two different publications. However, the effect of increasing salinity on the growth of *Cakile maritima* reported by Rozema *et al.* (1982, 1983a) was considerably lower compared to the data used for Figure 4.4. Rozema *et al.* (1983a) reported a strong reduction in growth at 60 mM NaCl and a 68% reduction at 300 mM NaCl (Rozema *et al.* 1982). This latter reduction is comparable with the other strandline species *Crambe maritima* (De Vos *et al.*, 2010b) and the reductions of *D. tenuifolia* and also *C. officinalis* in the experiment reported here. Also the Na^+ concentration (4.3 mmol g^{-1} DW), the K^+ concentration (0.33 mmol g^{-1} DW) and the Na^+ / K^+ ratio (13) reported by Rozema *et al.* (1982) were comparable with *Crambe maritima*, *D. tenuifolia* and also *C. officinalis* at 300 mM NaCl. In this regard only *T. halophila* and *C. anglica* (although exposure to NaCl did not exceed 200 mM NaCl) can be regarded as euhalophytes (for discussion of classification of halophytes see Breckle (2002)) and the strandline species *Cakile maritima*, *Crambe maritima*, *D. tenuifolia* and the brackish water halophyte *C. officinalis* represent a group of intermediate salt tolerance. Based on their growth performance under increasing salinity these latter species do not perform equally well as many halophytes (Glenn *et al.*, 1999; Flowers and Colmer, 2008), but considerably better than salt sensitive species (Maas and Hoffman, 1977; Tanji and Kielen, 2002). This seems likely considering that the strandline habitat is only occasionally exposed to seawater

floodings and as a result, soil salinity is usually low to moderate (Lee and Ignaciuk, 1985; Barbour *et al.*, 1985; Greaver and Sternberg, 2007). As was discussed before, strandline species are exposed more frequently or even continuously to airborne salinity in the form of salt spray (Boyce, 1954; Rozema *et al.*, 1985; Griffiths, 2006) and strandline species can be described as aerohalophytes (Rozema *et al.*, 1982).

Mineral composition
Concentrations in total N, P and C showed no or minimal reductions with increasing salinity and it appeared that these changes were not responsible for the observed growth reductions of *D. tenuifolia* as well as *C. officinalis*. For *D. tenuifolia* the 100 mM NaCl treatment resulted in a considerable decrease in K^+ and Ca^{2+} concentrations, although biomass (Fig. 4.1) and RGR (Fig. 4.2A) were not affected by these reductions. At the 200 mM NaCl level, where dry weight biomass was reduced by 60 %, K^+, Ca^{2+} and Mg^{2+} concentrations showed major reductions as compared with the 0 and 100 mM NaCl treatment. For *C. officinalis* the concentrations of K^+, Ca^{2+} and Mg^{2+} were reduced at the 100 mM and especially at the 200 mM NaCl level. Possibly, the reductions in shoot concentrations of K^+, Ca^{2+} and Mg^{2+} have affected the growth of *D. tenuifolia* and *C. officinalis*. However, a negative effect of this nutritional disturbance would probably have resulted in a decrease in photosynthetic capacity and thereby in a decrease in ULR. This decrease was not observed for *D. tenuifolia* or for *C. officinalis* at the 200 mM NaCl level. Similarly, the difference in salt tolerance between two other Brassicaceae species, namely *Brassica napus* and *B. carinata*, could not be related to ion effects or nutritional disturbance (He and Cramer, 1993) although changes in LAR (*B. napus*) and NAR (=ULR) (*B. carinata*) were observed. Le Saos (1978) demonstrated that the growth of *Cochlearia anglica* without additional Ca^{2+} was gradually reduced under increasing salinity with a reduction of 75-80 % in biomass at 170 mM NaCl, whereas the addition of 13.5 mM $CaCl_2$ resulted in increased biomass production up to 85 mM NaCl and a decrease of 20% at 170 mM NaCl. So with increasing salinity *C. anglica* only showed a typical halophytic growth response when grown with sufficient Ca^{2+} concentrations. Possibly *C. officinalis* will also show increased salt tolerance when grown with Ca^{2+} concentrations of around 10 mM in the nutrient solution. However, supplemental Ca^{2+} (up to 10 mM) did not improve the salt tolerance of the other Brassicaceae species *B. carinata* and *B. napus* (Schmidt *et al.*, 1993). Grattan and Grieve (1999) reviewed the relations between salinity and mineral nutrition in plants and concluded that these relations are extremely complex. It is clear that elevated Na^+ inhibits the uptake of other nutrients, but the effects of nutrient deficiencies are probably not as severe as the direct effects of Na^+ toxicity (Tester and Davenport, 2003). Conclusive remarks about the effect of reductions in shoot concentrations of K^+, Ca^{2+} and Mg^{2+} on the growth of *D. tenuifolia* and *C. officinalis* are difficult without additional experiments which focus on the effect of nutritional disturbance.

Na^+, Na^+ / K^+ ratio and net K^+ / Na^+ selectivity
Both species showed similar results concerning the Na^+ concentration, the Na^+ / K^+ ratio and the net K^+ / Na^+ selectivity (Table 4.3). The Na^+ concentration in the leaves increased with increasing Na^+ concentration of the nutrient solution. As was mentioned before, halophytes are able to accumulate sufficient amounts of Na^+ for the purpose of osmotic adjustment (Flowers *et al.*, 1986). No visible salt glands or bladders were observed on the leaves of both species and the high shoot Na^+ concentrations indicated that Na^+ remained within the leaves. The shoot Na^+

concentration (and net K^+ / Na^+ selectivity and the shoot Na^+ / K^+ ratio) of *D. tenuifolia* and *C. officinalis* were plotted against previous reported values of other Brassicaceae species exposed to increasing salinity (Fig. 4.4B, 4.4C, 4.4D, respectively). The shoot concentrations of Na^+ of the different species were more or less comparable, although at the highest NaCl concentrations it appeared that only *T. halophila* and *Cakile maritima* were able to maintain constant levels. The shoot Na^+
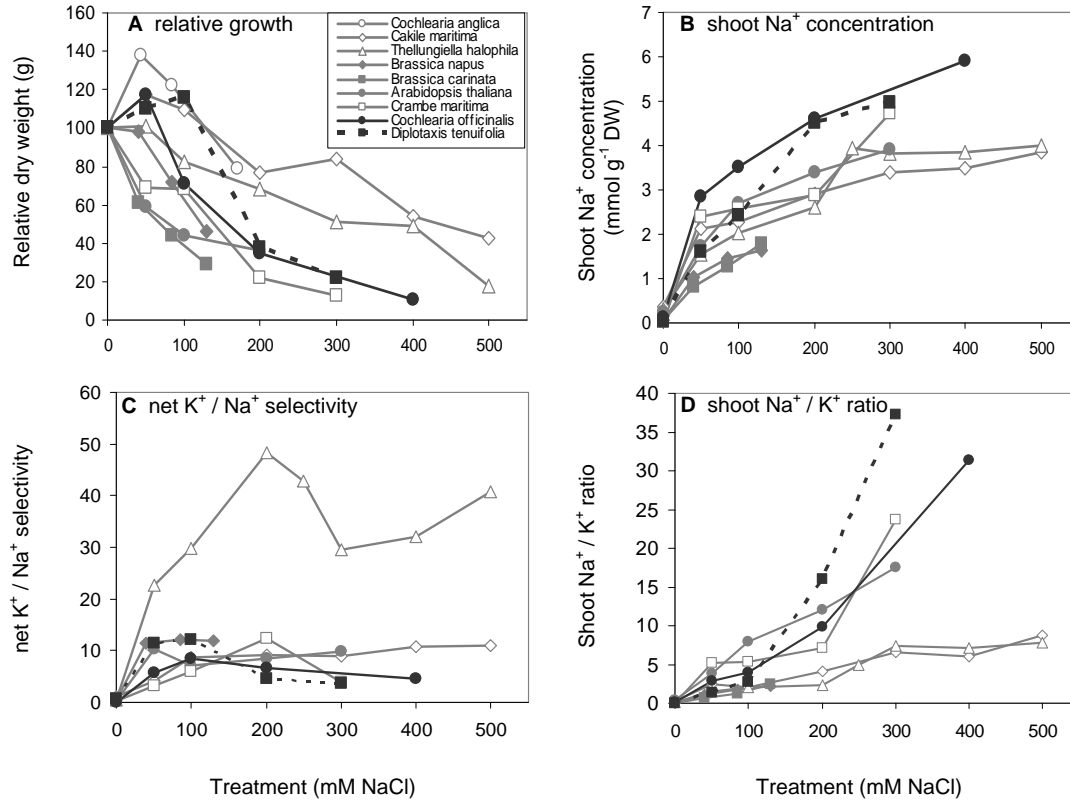

Figure 4.4. The relative growth of *D. tenuifolia* and *C. officinalis* and other Brassicaceae species (A:expressed as a percentage compared to the 0 mM NaCl treatment, based on dry weight), shoot Na^+ concentration (B), net K^+ / Na^+ selectivity (C) and the Na^+ / K^+ ratio (D) in response to increasing salinity (mM NaCl). Data of *Cochlearia anglica* was calculated from Le Saos (1978), of *Cakile maritima* from Debez *et al*. (2004) and Megdiche *et al*. (2007), of *Thellungiella halophila* from Inan *et al*. (2004; only used for net K^+ / Na^+ selectivity), M'rah *et al*. (2006), Aleman *et al*. (2009) and Ghars *et al*. (2008), of *Brassica napus* and *B. carinata* from He and Cramer (1992), of *Arabidopsis thaliana* from Ghars *et al*. (2008) and Aleman *et al*. (2009) and of *Crambe maritima* from De Vos *et al*. (2010). Means were calculated when more than one value per concentration was obtained within a species.

concentration reached 4.5 and 4.4 mmol g^{-1} DW at the 200 mM NaCl level for *D. tenuifolia* and *C. officinalis*, respectively. Sodium concentrations in the Brassicaceae species *Cakile maritima* and *Thellungiella halophila* never exceeded 4.0 mmol g^{-1} DW which were exposed up to 500 mM NaCl (Fig. 4.4B), whereas values for other halophytes were in the range of 5 mmol g^{-1} DW (Glenn *et al*., 1999). So the shoot Na^+ concentration at 200 mM NaCl does not inevitably result in major growth reduction as observed in *D. tenuifolia* and *C. officinalis*, although it is not known if Na^+ is indeed transported into the vacuoles. However, it is generally assumed that salt tolerant plants are able to compartmentalized Na^+ and Cl^- in vacuoles (Flowers *et al*., 1977; Wyn Jones and Gorham, 2002; Munns and Tester, 2008). Many halophytes are able to maintain relative constant Na^+ concentrations at high salinity levels ($\geq$200 mM NaCl)

(Glenn *et al*., 1999; Ghars *et al*., 2008; Aleman *et al*., 2009, among others), whereas it appeared that the Na^+ concentration in *D. tenuifolia* and *C. officinalis* readily increased with increasing salinity (Table 4.3). However, in general, the ionic stress starts when salt (Na^+) accumulates to toxic concentrations resulting in the death of old leaves (Tester and Davenport, 2003; Munns and Tester, 2008), but no such senescence or necrosis of leaves was observed for *D. tenuifolia* or *C. officinalis*.

In salt tolerant plants the K^+ selectivity increases with increasing salinity (Wyn Jones and Gorham, 2002). Both *D. tenuifolia* and *C. officinalis* showed an increase in the net K^+ / Na^+ selectivity at the lower salinity treatment levels (Table 4.3). However, at salinity levels ≥ 200 mM NaCl this selectivity started to decrease, whereas the selectivity of halophytes shows no decrease at higher salinities (Flowers and Colmer, 2008). This decrease in K^+ selectivity resulted in a sharp increase in the Na^+ / K^+ ratio in both species at the higher salt concentrations (≥ 200 mM NaCl), whereas one of the key determinants of plant salt tolerance is likely the capacity to maintain a high cytosolic K^+ / Na^+ ratio with increasing salinity (Maathuis and Amtmann, 1999). The net K^+ / Na^+ selectivity was calculated for various Brassicaceae species (Fig. 4.4C), which showed that *T. halophila* was able to increase this selective uptake to a much greater extent than the other Brassicaceae species. The value of the net K^+ / Na^+ selectivity of *Cakile maritima* was relatively low due to high concentrations of K^+ in the nutrient solution. However, the selective uptake by *Cakile maritima* showed no reductions at higher salinities, whereas most other species (besides *T. halophila)* showed reductions at higher salinities. The ability of *T. halophila* (and to a lesser extent *Cakile maritima*) to maintain relative low concentrations of Na^+ and high concentrations of K^+ resulted in a relative low Na^+ / K^+ ratio in the shoot compared to the other species (Fig. 4.4D). Although the Na^+ / K^+ ratio can range from 1.8 to 46 in dicotyledonous halophytes (Flowers *et al*., 1986) it appears that a Na^+ / K^+ ratio below 10 is favourable for growth in highly salt tolerant Brassicaceae species (Fig. 4.4D). In this regard the increase in the Na^+ / K^+ ratio between the two highest salinity treatments (which increased from 16 to 37 and from 10 to 31 for *D. tenuifolia* and *C. officinalis*, respectively) indicated the inability of the two species to maintain a relative low Na^+ / K^+ ratio with increasing salinity. The controlled uptake and compartmentalization of Na^+, increased net K^+ / Na^+ selectivity and the ability to maintain a low Na^+ / K^+ ratio in the cytosol are important mechanisms in the salt tolerance of halophytes (Tester and Davenport, 2003; Flowers and Colmer, 2008; Munns and Tester, 2008). Thus, although *D. tenuifolia* and *C. officinalis* can be classified as halophytes according to the definition of Flowers and Colmer (2008) their minimal growth, the relative high shoot Na^+ concentration, the reduced net K^+ / Na^+ selectivity and Na^+ / K^+ ratio at NaCl concentrations of ≥ 200 mM indicate that these species are less adapted to withstand high soil salinity levels than most halophytes.

Saline crops

Both *D. tenuifolia* and *C. officinalis* seem suitable for cultivation under saline conditions up to 100 mM NaCl based on their growth response and their ability to maintain considerable growth rates under saline conditions up to 200 mM NaCl. Since *D. tenuifolia* is already in use as an agricultural crop (Bianco, 1995; D'Antuono *et al*., 2007), no constraints are foreseen for the introduction as a saline crop. Also, the genus *Diplotaxis* has been recognised as a potential source of useful characters for breeding, cultivated *Brassica* species (Sharma *et al*., 2002), so the salt-tolerant *D. tenuifolia* may contribute in the development of more salt-tolerant *Brassica* species. Currently

the authors of this chapter are involved in larger scale field experiments to further develop practise of saline agricultural which is based on the results presented here. On a small regional scale the cultivation and selling of saline (100-150 mM NaCl) cultivated *Diplotaxis tenuifolia* has been started and preliminary results indeed show the crop potential for saline agriculture.

Table 4.4. Overall effect of increasing root zone salinity on measured growth and physiological parameters of *D. tenuifolia* and *C. officinalis*

treatment	RGR	ULR	LWF	SLA	succ.	LDMC	Na^+	Na^+/K^+	K-select	K^+	Ca^{2+}	Mg^{2+}
Diplotaxis tenuifolia												
0	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	↑	-	↑	↑	↑↑	↓	-	-
100	-	↑	-	↓	↑	-	↑↑	↑↑	↑↑	↓	↓	-
200	↓↓	-	-	↓↓	↑↑	-	↑↑↑	↑↑↑	↑	↓↓	↓↓	↓
300	↓↓↓	-	-	↓↓	↑↑	-	↑↑↑	↑↑↑↑	↑	↓↓↓	↓↓↓	↓↓
Cochlearia officinalis												
0	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	↑↑	↑↑	↑	↓	↓	↓
100	↓	-	-	-	↑	-	↑↑	↑↑	↑↑	↓	↓↓	↓↓
200	↓↓	-	-	↓↓	↑	-	↑↑↑	↑↑↑	↑	↓↓	↓↓↓	↓↓↓
400	↓↓↓	↓	↑	↓↓	↑↑	↑	↑↑↑↑	↑↑↑	↑	↓↓	↓↓↓↓	↓↓↓↓

RGR= ULR*LWF*SLA, 1/SLA= leaf succulence (L_{FW}/L_A)*LDMC (L_{DW}/L_{FW}), LWC= (L_{FW} - L_{DW}) / L_{FW}. Treatment is in mM NaCl. The abbreviations succ and K-select stand for leaf succulence and net K^+ selectivity, respectively. Results of N and P are not shown due to the insignificant difference between the 100 and 200 mM NaCl treatments. Symbols stand for: – no effect, ↑ small increase, ↑↑ great increase, ↑↑↑ major increase and ↑↑↑↑ for greatest increase, ↓ small decrease, ↓↓ great decrease, ↓↓↓ major decrease and ↓↓↓↓ for greatest decrease.

It is likely that different opinions about the reported and observed bitter taste of *C. officinalis* apparently relate to chemical characteristics of varieties of *C. officinalis* rather than a difference in personal tastes (Small, 2006). This bitter taste of *Cochlearia* leaves may restrict its use as a saline crop. *Cochlearia officinalis* grown in this experiment with hydroponic culture was predominantly bitter-tasting and was considered non-usable as a vegetable crop, whereas *C. anglica* collected in a salt marsh close to Holwerd, The Netherlands, was predominantly sweet-tasting (personal observations). *Cochlearia officinalis* sampled in 2009 along the coast of Svalbard, Norway, was lacking the bitter taste (Rozema, personal communication). The soluble sugar concentration in *C. anglica* was on average 16 % of the total dry weight, which is 5-10 times higher than in other halophytes such as *Salicornia stricta* and *Aster tripolium* (Binet, 1971). Since the 170 mM NaCl treatment was the highest salt concentration in the experiment of Le Saos (1978) it is unknown how the growth of *C. anglica* will respond to salinities above 200 mM NaCl. *Cochlearia officinalis* is confined to brackish water conditions and already showed a high level of salt tolerance, so possibly the salt marsh species *C. anglica* is even more salt-tolerant. Also, the germination under saline conditions of *C. anglica* was least affected whereas *C. officinalis* showed the greatest reduction (Pegtel, 1999). Natural hybridization is common among the different *Cochlearia* species (Weeda *et al*., 1987; Pegtel, 1999) and additional research may reveal more salt tolerant species and provide potential varieties for saline agriculture. Compared to four other *Cochlearia* species the coastal *C. hollandica* (*C. officinalis*C. anglica*) showed the greatest shoot dry weight production under non-saline conditions (Pegtel, 1999). *Cochlearia hollandica* seems to be a likely candidate for saline agriculture and further research may provide a *C. hollandica* variety with high yield and sweet-tasting properties under saline

conditions, which can be introduced as a saline crop. Although *D. tenuifolia* and *C. officinalis* have crop potential for saline agriculture, their shoots contained relative high Na^+ concentrations which will influence the taste and possibly the application as vegetable crops.

Concluding remarks
In Table 4.4 the overall effects of increasing salinity on the different parameters determined for *D. tenuifolia* and *C. officinalis* are summarized. In short, the following can be concluded. The growth of *D. tenuifolia* was not affected up to 100 mM NaCl, whereas a variety of changes occurred in mineral concentrations. The higher salinity treatments ($\geq$ 200 mM NaCl) affected most of the determined growth parameters. Although the concentrations of many essential minerals showed reductions, it is unlikely that these were responsible for the observed growth reductions for both *D. tenuifolia* and *C. officinalis*. The shoot Na^+ concentration at 200 mM NaCl most likely did not result in toxic effects which affected growth. The reduced net K^+ / Na^+ selectivity and Na^+ / K^+ ratio at concentrations $\geq$200 mM NaCl indicated that *D. tenuifolia* and *C. officinalis* are less adapted to high soil salinity levels than other more salt tolerant halophytes. The growth reductions of both species at high salinity ($\geq$ 200 mM NaCl) were largely due to changes of leaf morphology (decrease of SLA, increase of leaf succulence) rather than toxic leaf Na^+ concentrations. In this regard, the relative importance of leaf traits for the salt tolerance of *Diplotaxis tenuifolia* and *Cochlearia officinalis*, was demonstrated. In comparison with seven other Brassicaceae species, ranging from salt sensitive to highly salt tolerant, both *D. tenuifolia* and *C. officinalis* showed an intermediate growth response to increasing salinity. However, both species were able to survive the highest salinity treatment and can be classified as salt tolerant with potential as vegetable crops for saline agriculture.

ACKNOWLEDGEMENTS

Appendix 4A. The total dry weight (g) of the plants of the final harvest for *D. tenuifolia* and *C. officinalis* in response to increased (0-400 mM NaCl) root zone salinity treatments. Plants were finally harvested 20 days (*D. tenuifolia*) or 37 days (*C. officinalis*) after salt addition started. Values are means of eight (*D. tenuifolia*) or five (*C. officinalis*) replicates ± s.e.. Different letters indicate a significant difference at P<0.05 (Tukey's test, One-way ANOVA).

Treatment (in mM NaCl)	*D. tenuifolia*	*C. officinalis*
0	1.52 ± 0.11^a	5.47 ± 0.69^a
50	1.72 ± 0.04^a	6.15 ± 0.58^a
100	1.71 ± 0.13^a	3.06 ± 0.17^b
200	0.60 ± 0.03^b	1.63 ± 0.16^c
300	0.35 ± 0.02^c	nd
400	nd	0.42 ± 0.05^d

nd=not determined

Chapter 5.

The effect of seawater irrigation on the biomass production and mineral composition of two potential halophyte crops, a feasibility study under field conditions

Arjen C. de Vos[1]
Willem Ursem[1]
Rob Broekman[1]
Marc van Rijsselberghe[2]
Jelte Rozema[1]

[1] *Institute of Ecological Science, Department of Systems Ecology, VU University Amsterdam De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

[2] *Texelse Milieuvriendelijke NatuurProducten BV*

- *Background and Aims* There is a need to test the growth performance of potential halophyte crops under field conditions. The aim of this research was first to assess the feasibility of crop cultivation on a seawater drip irrigated experimental field. There was particular interest in the spatio-temporal variation of soil salinity as a result of the joint impact of seawater irrigation, evapotranspiration and natural precipitation. Secondly, it was aimed at testing the biomass production and growth of the two potential saline crops under saline field conditions.
- *Methods* An experimental saline field site (10 by 15 m) was created on the Waddenisland of Texel, The Netherlands, where seawater drip irrigation was applied to cultivate two crop species under saline conditions. Individual irrigation tubes were spaced 50 cm apart and seawater was applied regularly.
- *Key Results* The effects of seawater drip irrigation on soil salinity, soil mineral composition and the growth performance of three varieties of the sea beet, *Beta vulgaris* ssp. *maritima* and *Plantago coronopus* were evaluated. The chosen irrigation regime resulted in a relatively constant soil salt concentration of about 200 mM NaCl, or 40 % seawater salinity, during the course of the experiment (54 days). Soil salinity was spatially found to be homogeneously distributed: no differences in soil salt concentration or other soil characteristics occurred between the different blocks in which the plants were cultivated. Differences in salt tolerance were found between the varieties of *Beta vulgaris* ssp. *maritima*.
- *Conclusions* The feasibility of the chosen experimental setup to conduct reliable field experiments with regard to soil salinity was demonstrated. The three varieties of *Beta vulgaris* ssp. *maritima*, as well as *P. coronopus*, all showed considerable and continued growth under field conditions at 40 % seawater salinity.

Keywords seawater drip irrigation, soil salinity, halophyte crop, *Beta vulgaris* ssp. *maritima*, *Plantago coronopus*, growth, mineral composition

INTRODUCTION

Much literature has pointed out the potential of halophytes to be used as crops cultivated under saline conditions (Glenn *et al*, 1999; Rozema and Flowers, 2008 among others). These potential crops, however, were mainly tested under greenhouse conditions with the level of salt tolerance usually as the main focus. Although this is a crucial step for selecting potential halophyte crops, the evaluation of the growth performance under field conditions is the imperative next step. This, together with an assessment of the market potential and the development of an agricultural cultivation method, is necessary before these halophytes can be successfully introduced as new agricultural crops (Pasternak *et al*., 1985). To screen various potential halophyte crops under saline field conditions, an experimental saline field site was created on Texel, The Netherlands. Here, *Crambe maritima, Plantago coronopus, Beta vulgaris* ssp. *maritima, Limonium vulgare, Aster tripolium, Diplotaxis tenuifolia, Atriplex hortensis 'rubra', Salsola soda, Salicornia europea, Crithmum maritimum, Inula crithmoides, Mertensia maritima, Tripleurospermum maritimum* and *Cochlearia sp* were grown with seawater drip irrigation. Most of these species were cultivated for a qualitative analysis and to be tested for their market potential. For three different populations

(referred to as 'varieties' in the rest of this chapter) of *Beta vulgaris* ssp. *maritima* and for *Plantago coronopus* the growth performance was more extensively analysed. Sea beet, *Beta vulgaris* ssp. *maritima*, the ancestor of the modern cultivated sugar beet, red beet and fodder beet is commonly recognized as a halophyte with a natural occurrence at the upper part of salt marshes along the Atlantic and Mediterranean coast. Based on the growth performance of *Beta vulgaris* ssp. *maritima* under saline conditions can be classified as salt tolerant with potential for saline agriculture (Rozema *et al.*, 1993; Daoud *et al.*, 2008). *Plantago coronopus* also occurs in coastal saline habitats and has been recognized as a potential crop which can be cultivated under saline conditions (Aronson, 1989; Koyro, 2006).

The aim of this study was to investigate the effect of seawater irrigation on the spatio-temporal variation of soil salinity and the growth of *Plantago coronopus* and *Beta vulgaris* ssp. *maritima* under field conditions and to compare this with previously conducted greenhouse experiments. The salt tolerance, based on the growth performance under saline conditions, of the varieties of *Beta vulgaris* ssp. *maritima* was also evaluated. For this, various plant and soil parameters were monitored and measured during May, June and July 2009.

MATERIAL AND METHODS

Experimental field site
The experimental field site (10 by 15 m) was located on the Waddenisland of Texel, The Netherlands (53°01'N and 4°46'E). To prevent salt water from leaching to the groundwater, the first 50 cm of soil was removed and plastic foil was placed in the excavated area. The original soil (loam) was than replaced, after it was mixed with coarse (grain size 0.5-1 mm) sand (loam:coarse sand 3:1, v/v) to enhance the drainage capacity of the soil. Also, a total of 700 kg of organic compost was mixed into the removed soil to enhance the fertility. A layer of 10 cm of drainage sand was placed on top of the plastic foil together with a drainage pipe, which was connected to a well. From here, the excess water could be removed. Blocks of 2 by 2 m were created on the surface, which were used to cultivate the different plant species.

Seawater drip irrigation and precipitation
The field site was irrigated with seawater collected from the nearby Waddensea at the NIOZ harbour (53°00'N and 4°47'E). The irrigation water was distributed evenly over the field site by means of drip irrigation. For this, a centrifugal pump (DAB Jetinox) was connected to a main pipe (running along the side of the field site) from which the irrigation tubes (Uniram) branched every 50 cm. In total, 20 tubes of 15 m were placed in this manner, with the actual drippers located every 20 cm, capable of releasing 1.6 l per hour. Seawater irrigation was supplied weekly, especially after periods of rainfall. In periods of drought a minimal amount of seawater was supplied for irrigation. A maximum of 1000 l of seawater was applied during a single irrigation event. Precipitation was measured (mm) in a rain gauge on location and listed as total amount (l) within the 10 by 15 m field site (Table 5.1).

Plant material and growth conditions
In total, three varieties of *Beta vulgaris* ssp. *maritima* and one variety of *Plantago coronopus* were evaluated for different aspects related to the growth performance under saline conditions. The seeds of the first variety of *B. vulgaris* ssp. *maritima*

were obtained from Magic Garden Seeds (Regensburg, Germany), the second from a wild population in along the coastline of the harbour of Den Helder, The Netherlands and the third from a wild population of the Somme estuary, Le Crotoy, France. These three varieties are referred to as 'Magic', 'Den Helder' and 'Crotoy'. Seeds from the wild populations were collected in autumn 2008 and stored dry at 6 °C. Seeds of *P. coronopus* were obtained from Vreeken Zaden (Dordrecht, The Netherlands).

In February 2009 all seeds of *B. vulgaris* ssp. *maritima* were placed in plastic bags on moist vermiculite at 6 °C to break dormancy. Seeds of *P. coronopus* were treated similarly, but stratification started in March 2009. After 9, 16, 43 and 7 days, germination was observed inside the plastic bags for Magic, Den Helder, Crotoy and *P. coronopus*, respectively. Due to early germination, the varieties Magic, Den Helder and *P. coronopus* were transplanted into peat soil in the greenhouse before transplantion into the field site. After germination, *P. coronopus* was raised for 6 days, Den Helder for 14 days and Magic for 24 days before transplantation to the field site, whereas the germinating seeds of Crotoy were sown in the field directly. *Plantago coronopus,* Magic and Crotoy were allowed to establish and grow for 8 weeks at the field site before the start of seawater irrigation, for variety Den Helder this period was 10 weeks. At the start of seawater irrigation treatment, the seedlings of the three varieties of *B. vulgaris* ssp. *maritima* were in a comparable stage of development. Each plant species and variety was assigned to a random block within the field site. Seawater irrigation started on the 13th of May 2009 and sampling continued until the beginning of July. During the 54 days of salt exposure, plants were harvested on 7 occasions. At each harvest five plants per block were collected. Only the above-ground parts of plants were used for analysis.

Measurements of morphological parameters and relative growth rate
Directly after harvesting, plants were wrapped in wet tissues, sealed in a plastic bag and transported to the laboratory in a cool box. Here, plants were submersed in tap water for 24h after which the water saturated weight was recorded (based on Garnier *et al.*, 2001). All collected plants were tested for leaf fresh, leaf surface, leaf dry weight and leaf dry matter content (LDMC), total above ground weight and relative growth rate (RGR). To obtain dry weights, plants were oven-dried for 48 hours at 70°C. The leaf area was recorded with a LI-COR 3100 area meter (Li-Cor Inc, Lincoln, Nebraska, USA).

The relative growth rate (RGR) and the components unit leaf rate (ULR), leaf weight fraction (LWF) and specific leaf area (SLA) were estimated according to Hunt *et al.* (2002) with

$$RGR = ULR*LWF*SLA \tag{1}$$

with $ULR = (\Delta W * \Delta \log_e L_A)/ (\Delta L_A * \Delta t)$, $LWF = L_{DW}/W$ and $SLA = L_A/L_{DW}$

where L_A is total leaf area per plant, ΔW is the difference between two harvests in dry weight per plant (in this case only the above-ground dry weight), $\Delta(\log_e)L_A$ is the (natural logarithmic) value of leaf area (cm^2), Δt is the difference in days between two harvests, L_{DW} is the total leaf dry weight per plant and L_{FW} is the total leaf fresh weight per plant. Calculations of RGR were performed using a mean value of the initial harvest (13/05/2009).

The SLA can be analysed in more detail by:

$$1/SLA = LDMC * \text{leaf succulence} \tag{2}$$

with leaf dry matter content (LDMC) $=L_{DW}/L_{FW}$ and leaf succulence$= L_{FW}/L_A$ (De Vos *et al.*, 2010b).

Soil samples
Soil samples were collected with a gouge auger on a weekly basis. Two samples per block were collected and each sample consisted out of eight subsamples taken from the top 20 cm. All soil samples were analysed for soil moisture, electrical conductivity (EC) and pH. The EC and pH were determined by using the extract of the saturated paste. The salt concentration of the soil is commonly measured as electrical conductivity in the extract of the soil saturated paste (ECe) and expressed in dS m^{-1} (for more details see Rhoades *et al.*, 1999). Total P, Ca^{2+}, Mg^{2+}, Na^+ and K^+ were also measured in this extract (see section 'mineral composition' for details). The soil organic matter content was based on loss on ignition at 550 ^{o}C and was determined for samples collected on three dates (13/05/2009, 29/06/2009 and 06/07/2009). Soil samples were dried at 70 ^{o}C for 24 hours. Soil moisture was expressed as the ratio of water (g) to dry soil (g).

Mineral composition
The concentrations of total P, Ca^{2+}, Mg^{2+}, Na^+ and K^+, total C and N were determined as described in Chapter 2. The net K^+ / Na^+ selective uptake was estimated using the common formula

$$S = (K^+ / Na^+)_{leaves} / (K^+ / Na^+)_{soil} \tag{3}$$

Statistical analysis
Statistical data analysis was performed using SPSS (version 15.0 for Windows, SPSS Inc. 2000, Chicago, IL, USA). A one-factor within subjects ANOVA (repeated measures) was used to test for possible differences in EC, soil moisture, plant dry weight biomass production and soil mineral composition in time (temporal variation within separate blocks). The between-subjects effects (between blocks) and the interaction between time and blocks/species were also evaluated. Bonferroni pairwise comparisons were used to evaluate possible differences between separate blocks/species. Greenhouse-Geisser degrees of freedom corrections were used when assumptions about homogeneity of covariance were violated. To test for potential differences in RGR and its components and plant mineral composition a one way ANOVA was used with Tukey's pair-wise comparisons as a post hoc test to evaluate differences ($P<0.05$) between individual species/varieties.

RESULTS

Irrigation
To increase the soil salinity and to prevent any diluting effect of the relative high amount of precipitation, the largest amount (5000 l) of irrigation water was supplied in the first month of the experiment (Table 5.1). In June and July 2750 l and 500 l was used for irrigation and precipitation amounted 6900 l and 0 l in that period,

respectively. In total, 8250 l Waddensea water was used for irrigation of the 15*10 m field site. It should be pointed out that the seawater used for irrigation was collected in the Waddensea, which has a lower salt concentration (EC 32.5 ± 0.5 dS m^{-1}, $n=8$) than average North Sea water (approximately 41 dS m^{-1}, Van Aken, 2001).

Table 5.1. The amount of precipitation (l) and irrigation water (l) during the 54 days of salt exposure in May, June and July 2009.

period	13-18 May	18-26 May	26 May- 02 Jun	02 Jun- 08 Jun	08 Jun- 15 Jun	15 Jun- 22 Jun	22 Jun- 29 Jun	29 Jun- 06 Jul
precipitation	5100	3150	600	2700	3600	0	0	0
irrigation	3000	2000	1000	250	500	500	500	500

The listed values represent the total amount (l) within the 10 by 15 m field site.

Soil salinity and soil moisture

Figure 5.1 shows the effect of seawater (32.5 ± 0.5 dS m^{-1}, $n=8$) drip irrigation, in combination with precipitation and evapotranspiration, on the soil salinity and soil moisture percentage. The soil moisture content (Fig. 5.1A) showed a difference in time, so moisture content changed under the influence of precipitation, evapotranspiration and/or irrigation during the experiment within the separate blocks (the four blocks in Fig. 5.1 are named after the plant species/variety that was cultivated in that specific block). The repeated measures analysis also demonstrated a differences between blocks ($P=0.012$). The Bonferroni pairwise comparisons revealed that only the blocks *P. coronopus*-Magic and Magic-Den Helder showed significant difference between blocks.

The soil salinity level (Fig. 5.1B) also showed a difference in time, but when the first 2 dates of sampling were ignored, no further differences within the blocks occurred. So after two weeks of irrigation, no changes in soil salinity were observed within the blocks. The soil salinity level was relatively constant at a level around 21 dS m^{-1}. Between the different blocks, no differences occurred after seawater irrigation started ($P=0.185$).
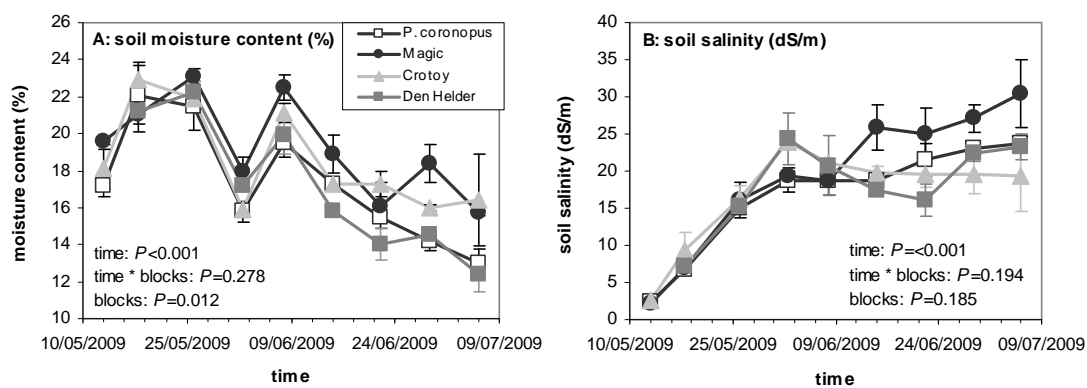

Figure 5.1. Time course (54 days) of the variation of soil moisture (A) and soil salinity (B) in relation to seawater irrigation, and precipitation. Measurements were performed within the blocks of the four different plant species/varieties and the results are listed per block. Possible differences within blocks (listed as 'time'), between blocks (listed as 'blocks') and the interaction (time * blocks) were analysed with a within subjects ANOVA (repeated measures). Values are means of two replicates ± s.e. The salinity of the irrigation water was 32.5± 0.5 dS m^{-1} ($n=8$).

Soil saturated paste percentage, organic matter and pH
There were no differences between the blocks regarding soil saturation percentage (which can be used to classify soil texture) and pH, although both parameters did differ in time (Table 5.2). However, the Bonferroni pairwise comparisons showed that these differences in time were minimal (only pH on the first date of sampling differed from the rest and ranged from 7 to 6.1, for saturation percentage only the first date differed from the 4th and the 8th date of sampling and ranged from 23.9 to 30.0 %). Because of the minimal range, means are presented for pH and SP in Table 5.2. The organic matter content of the soil showed a difference between the blocks 'Crotoy' and 'Den Helder', but both blocks did not differ from the other two blocks (Table 5.2).

Table 5.2. The soil saturated paste percentage (SP), the organic matter content and pH of the different blocks.

blocks	SP (%)	organic matter (%)	pH
P. coronopus	25.8±0.4 ^{A}	2.70±0.08 ^{AB}	6.34±0.08 ^{A}
Magic	26.3±0.4 ^{A}	2.87±0.14 ^{AB}	6.64±0.09 ^{A}
Crotoy	28.2±0.5 ^{A}	3.40±0.21 ^{A}	6.56±0.10 ^{A}
Den Helder	25.5±0.4 ^{A}	1.57±0.10 ^{B}	6.61±0.06 ^{A}
time	$P=0.011$	$P=0.277$	$P<0.001$
time*blocks	$P=0.194$	$P=0.944$	$P=0.292$
blocks	$P=0.170$	$P=0.020$	$P=0.198$

Possible differences were evaluated by means of repeated measures analysis using data collected on 8 different dates (SP, pH) or 3 different dates (organic matter) with $n=2$ per date per block. Values are means of the different dates ± s.e. Different letters indicate a significant difference at $P<0.05$ (Bonferroni pairwise comparisons).

Soil mineral composition
The mineral composition of the soil of the different blocks was determined at the start (18/05/2009), in the middle (08/06/2009) and at the end of the period of irrigation with Waddenseawater (06/07/2009). The soil Na^+ concentration, as well as the K^+, Ca^{2+} and Mg^{2+} concentration increased during the time of seawater irrigation (Fig. 5.2). This increase was comparable between the different blocks and no significant differences occurred between blocks. No difference in soil P concentration (overall mean was 0.049±0.007 µmol P/ml) was found between blocks or within blocks during the time of salt exposure (data not shown). Also, no difference was found in soil N concentration (overall mean was 680±45 µmol N/g DW soil) between or within blocks (data not shown).

Plant growth
The dry weight of the above ground biomass of the different plant species was monitored during the 54 days of salt exposure and the results are listed in Figure 5.3. Of the dry weight biomass production of the different plant species and varieties during the 54 days of salt exposure, only Crotoy differed from the others. The first three harvests (13/05/2009, 18/05/2009 and 26/05/2009) between Crotoy and Magic did not significantly differ (analysed with one-way ANOVAs, data not shown), indicating that no initial differences in biomass occurred.
The RGR showed similar results as biomass production, of which the RGR of 51 mg g^{-1} day^{-1} of Crotoy was larger than the rest (Table 5.3). The components LWF and SLA showed some differences among Magic, Crotoy and Den Helder, but the component ULR showed the greatest differences between varieties.
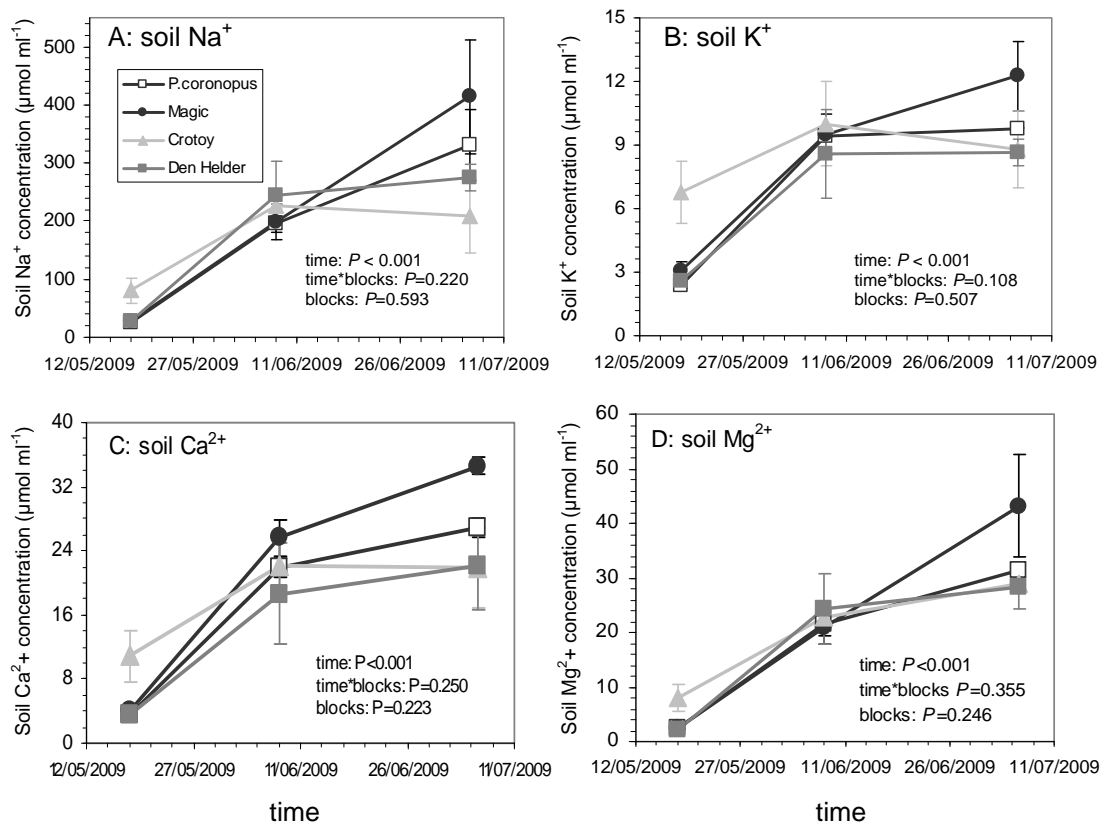
Figure 5.2. Time course (54 days) of the variation of soil mineral concentrations of Na^+ (A), K^+ (B), Ca^{2+} (C) and Mg^{2+} (D) in relation to seawater irrigation, and precipitation. Measurements were performed within the blocks of the four different plant species/varieties and the results are listed per block. Possible differences within blocks (listed as 'time'), between blocks (listed as 'blocks') and the interaction (time * blocks) were analysed with a within subjects ANOVA (repeated measures). Values are means of two replicates ± s.e.

Plant mineral composition, Na^+ / K^+ ratio and K^+-selectivity

The K^+, Ca^{2+}, Mg^{2+}, and Na^+ concentrations, the Na^+-K^+ ratio and net K^+ / Na^+ selectivity of the leaves of the different species/varieties are listed in Table 5.4. Between the different *B. vulgaris* ssp. *maritima* varieties, differences occurred in the K^+, Ca^{2+} and Mg^{2+} concentrations. The variety Crotoy showed greater concentrations of K^+ and Ca^{2+} compared to Magic and the variety Den Helder showed a greater Mg^{2+} concentration than Magic. The Na^+ concentration was greatest in Magic. The relative low concentration of K^+ and relative high concentration of Na^+ in Magic resulted in a high Na^+ / K^+ ratio. Also, the K^+ selectivity of Magic was lower compared to Crotoy and Den Helder. No differences in leaf N concentrations between the species/varieties occurred (1.58±0.18, 1.95±0.13, 2.03±0.20 and 1.75±0.12 mmol g^{-1} for *P. coronopus*, Magic, Crotoy and Den Helder, respectively). The leaf P concentration of Den Helder (0.16±0.02) was significantly greater than *P. coronopus* (0.07±0.01) and Crotoy (0.09±0.01), but not compared to Magic (0.12±0.01). No other differences in P concentrations occurred between the species/varieties.
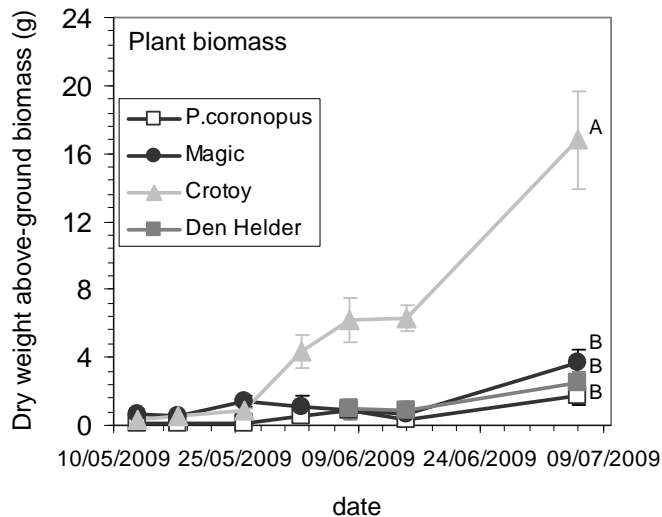
Figure 5.3. Increase of above-ground dry weight production of the different plant species during the 54 days of drip-irrigation with seawater and ambient precipitation. Values are means ± s.e. with *n*=5. Differences between the different species/varieties were analysed with a within subjects ANOVA (repeated measures) and the different letters indicate a significant difference (*P*<0.05) in biomass production.

DISCUSSION

Before the differences in growth performance between the varieties of *B. vulgaris* ssp. *maritima* can be ascribed to actual differences between these varieties, it should be ruled out that soil conditions could be responsible for the observed growth differences. For this, the joint impact of seawater irrigation, evapotranspiration and natural precipitation on the spatio-temporal variation of soil moisture and soil salinity will be discussed first, as well as the other soil characteristics which were determined.

Soil moisture
The soil moisture percentage showed a temporal and spatial variation (Fig. 5.1A), but this likely did not influence plant growth. The saturation percentage can be used to indicate the permanent wilting point (approximately 1/4 of SP) and field capacity (approximately 1/2 of SP) (Richards, 1954). With a mean saturation percentage of 26.5 %±0.2 (*n*=64) this would imply that the permanent wilting point is approximately 7 % and the field capacity approximately 13 %. The soil moisture percentage during the 54 days of salt exposure showed a maximum of 23.1 % and a minimum of 12.4 %. In this regard, the soil moisture percentage remained above or close to the field capacity throughout the time of salt exposure. Also, the soil moisture percentage was never close to the permanent wilting point, so the variable soil moisture percentage in time likely did not influence plant growth between the different blocks. The differences in soil moisture percentage between blocks were minimal. Statistical analysis revealed that only the blocks *P. coronopus*-Magic and Magic-Den Helder were significantly different. Leaching was observed in the first weeks of salt exposure (visible as water collecting in the well), demonstrating that water was supplied in excess of evapotranspiration, which is necessary to prevent excess salt accumulation in the root zone (Rhoades *et al.*, 1992). Although leaching is

required to prevent accumulation of salts when saline irrigation water is used, but this does not necessarily imply an increase in irrigation frequency (Shalhevet, 1994). The intensity of irrigation in this field study was based on the weekly measurements of soil salinity. Although irrigation water was applied weekly, it was especially applied after rainfall to prevent any diluting effects of precipitation and it was applied only minimally during periods of drought. The chosen irrigation frequency was focussed on preventing a temporal variation in soil salinity, rather than preventing a temporal variation in soil moisture.

Table 5.3. The relative growth rate and its components of the four plant species / varieties during the 54 days of seawater irrigation.

	RGR $(mg\ g^{-1}\ d^{-1})$	ULR $(mg\ cm^{-2}\ d^{-1})$	LWF $(g\ g^{-1})$	SLA $(cm^2\ g^{-1})$	Succulence $(g\ cm^{-2})$	LDMC $(mg\ g^{-1})$
P.coronopus	32.5 ± 2.9^a	0.55 ± 0.08^a	0.44 ± 0.04^a	139 ± 7^{ab}	0.093 ± 0.004^{ab}	0.078 ± 0.006^a
Magic	31.9 ± 2.7^a	0.27 ± 0.03^b	0.72 ± 0.02^b	167 ± 7^a	0.084 ± 0.002^a	0.072 ± 0.004^a
Crotoy	51.4 ± 3.2^b	0.67 ± 0.08^a	0.55 ± 0.05^a	146 ± 7^{ab}	0.081 ± 0.003^a	0.086 ± 0.006^a
Den Helder	22.2 ± 2.1^a	0.27 ± 0.04^b	0.70 ± 0.01^b	123 ± 7^b	0.102 ± 0.004^b	0.081 ± 0.004^a

Values are means ± s.e. (*n*=5). Means in the same column that have the same letter are not significantly different at P<0.05 (one way ANOVA, Tukey's Post hoc test). RGR=ULR*LWF*SLA, 1/SLA=leaf succulence*LDMC.

Table 5.4. The K^+, Ca^{2+}, Mg^{2+}, and Na^+ concentrations, the Na^+ / K^+ ratio and net K^+ / Na^+ selectivity of the leaves of the different species/varieties after 54 days of salt exposure

Species / varieties	K^+ mmol g^{-1}	Ca^{2+} mmol g^{-1}	Mg^{2+} mmol g^{-1}	Na^+ mmol g^{-1}	Na^+ / K^+ ratio	K^+ selectivity
P.coronopus	0.35 ± 0.02^A	0.28 ± 0.04^{AB}	0.23 ± 0.02^A	2.33 ± 0.16^A	6.76 ± 0.68^B	3.97 ± 0.45^A
Magic	0.51 ± 0.05^{AB}	0.24 ± 0.01^A	0.29 ± 0.03^A	3.19 ± 0.06^B	6.48 ± 0.67^B	4.15 ± 0.48^A
Crotoy	0.90 ± 0.11^C	0.38 ± 0.03^B	0.32 ± 0.02^{AB}	2.13 ± 0.27^A	2.50 ± 0.44^A	11.36 ± 1.60^B
Den Helder	0.76 ± 0.11^{BC}	0.33 ± 0.05^{AB}	0.43 ± 0.05^B	2.47 ± 0.15^A	3.64 ± 0.70^A	8.26 ± 1.63^B

Values are means ± s.e. (*n*=5) and are expressed per gram dry weight of the leaves. Means in the same column that have the same letter are not significantly different at *P*<0.05 (one way ANOVA, Tukey's test).

Soil salinity
Soil salinity under field conditions often shows spatial and temporal variation (Yaron *et al.*, 1972; Rhoades *et al.*, 1992; Shalhevet, 1994). In general, salts accumulate where soil is allowed to dry. For drip irrigation this is in the middle of two drip irrigation lines (Yaron *et al.*, 1972). The space between two irrigation lines should be small enough to allow an overlap between the two areas of water infiltration, by which no drying of the soil occurs (Yaron *et al.*, 1972). Christen *et al.* (2007) showed that at a distance of 60 cm between two drip lines, no difference in salt spatial distribution occurred. It should be noted that most studies on the effect of saline irrigation on soil salinity were performed in arid or semi-arid areas, whereas the study described here was performed in a temperate region with relative low evapotranspiration and high precipitation. Although the uniform distribution of salts also depends on the evapotranspiration and precipitation, it seems likely that the space of 50 cm used in the field study was sufficient to ensure a uniform salt distribution between two drippers. In fact, the chosen irrigation regime (design and frequency) of this field study resulted in a relatively constant soil salt concentration (21 ± 0.6 dS m^{-1}, *n*=56) during the experiment. No differences in soil salinity occurred between the different blocks (Fig. 5.1B), so no spatial variation was observed within the research site. Also, the drip irrigation regime resulted in a constant salinity level during the experiment (no temporal variation), with the exception of the first two weeks of

irrigation. The soil salt concentration, based on the conductivity of the soil saturated paste extract, appeared to be independent of the soil moisture percentage.

Effects of saline irrigation on physical soil properties
Irrigation with saline water can influence the soil structure, resulting in a poor physical condition of the root zone. Certain physico-chemical changes in the soil can occur with saline irrigation, resulting in poor permeability and waterlogging (Abrol *et al.*, 1988; Rhoades *et al.*, 1992, among others). Soils containing a high concentration of Na^+ in relation to other salts, commonly characterized as SAR>13 (SAR= Na^+ / $\sqrt{[(Ca^{2+} + Mg^{2+})/2]}$), as well as a pH>8 often have a poor physical condition (Abrol *et al.*, 1988). The values of SAR of the different blocks were in the range of 40-60 after irrigation started. However, the pH values were in the range of 6.6 and soil solutions composed of high solute concentrations (salinity) are conducive to good soil physical properties (Abrol *et al.*, 1988; Rhoades *et al.*, 1992). Because of the high salinity of the irrigation water and pH<8, little or no negative effects of saline irrigation on the soil structure is expected. Also, topsoil properties largely control the water entry rate of the soil (Rhoades *et al.*, 1992) and no effects of waterlogging or reduced permeability were observed here.

Mineral composition soil
Seawater contains many of the macro- and micronutrients that are essential for plant growth and function (Rozema and Flowers, 2008). Here, seawater irrigation increased the concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} in the soil. More importantly, no difference occurred between blocks so no difference in plant growth between blocks was expected based on these soil mineral concentrations. There was a trend that the concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were greater in block 'Magic' compared to the other blocks.

pH, SP and organic matter content soil
Based on the pH and saturation percentage, which can be used to classify soil texture (Richards, 1954; Rhoades *et al.*, 1999), no differences occurred between the different blocks and only minimal differences occurred in organic matter content (Table 5.2). Based on the saturation percentage and organic matter content, the soil of all blocks can roughly be classified as peaty sand or sandy loam.

Plant growth
One of the requirements for conducting reliable evaluations of plant salt tolerance is to provide stable soil salinity levels during the period of rapid growth. As was mentioned above, the irrigation regime did result in a constant soil salt concentration during the experiment, with the exception of the first two weeks. However, in these first two weeks only minimal plant growth occurred. So during the period of rapid plant growth, the plants experienced a constant level of soil salinity of about 200 mM NaCl.

Growth of B. vulgaris ssp. *maritima*
To compare the growth of the three varieties of *B. vulgaris* ssp. *maritima* it should be ruled out that initial differences in biomass caused the differences in biomass at the final harvest. The first three harvests of Crotoy and Magic did not significantly differ and no difference occurred on 15/06/2009 between Magic and Den Helder. In this regard, it is likely that the observed differences in biomass production were due to

differences in growth performances and not due to initial differences at the beginning of the salt exposure. The analysis of RGR and its components showed that some differences did occur between the morphological components LWF and SLA of the different varieties, but both components were not the greatest for Crotoy. So the greater RGR of Crotoy did not result from greater values of LWF or SLA and it appeared that only the physiological component ULR was responsible for the greater RGR of Crotoy compared to Magic and Den Helder. The ULR is a parameter that integrates various aspects of plant functioning (photosynthesis, respiration, chemical composition) and therefore can not be related directly to a specific physiological process (Poorter and Garnier, 2007).

Although the three varieties showed a difference in growth performance in the field study, this can not be directly linked with a difference in salt tolerance. To evaluate the salt tolerance, plants are generally exposed to increasing salt concentrations whereas in this field study only one salt concentration was used. The growth performance of under various salinity levels has been evaluated in literature (Rozema *et al.*, 1993; Koyro, 2000; Daoud *et al.*, 2008) and more insight in the salt tolerance can possibly be obtained by the comparison between the different results. First of all, the seedlings used in the field experiment were allowed to grow in the field for a period 8 (Crotoy, Magic), 10 (Den Helder) or 11 weeks (*P. coronopus*) before the start of the salt treatment, which is considerably longer than the period used in many greenhouse experiments (Rozema *et al.*, 1993; Koyro, 2000; Daoud *et al.*, 2008). This could have resulted in differences in the stage of development of the seedlings between greenhouse experiments and the field experiment. However, the seedlings in the field experiment were allowed to grow for this relative long period (before irrigation started) due to the relatively low temperatures during which little plant growth occurred. The irrigation treatment was started when obvious growth of seedlings was observed. Also, it could be argued that the RGR based on above ground biomass, which is reported in this field study, is difficult to compare with a RGR based on whole-plant biomass. However, the biomass of *Beta vulgaris* ssp. *maritima* forms about 84 % (Rozema *et al.*, 1993) to 89 % (Daoud *et al.*, 2008) of the total biomass, so some comparison with the RGR reported by Rozema *et al.* (1993) can be made. In a greenhouse experiment, the reported RGR of *Beta vulgaris* ssp. *maritima* was 151, 141 and 119 mg g^{-1} day^{-1} at 0, 200 and 400 mM NaCl, respectively (Rozema *et al.*, 1993). This data was based on the interval growth between 20 and 30 days after salt exposure. The maximum recorded RGR under field conditions in this study was 51 mg g^{-1} day^{-1} for Crotoy at a mean soil salinity level of approximately 200 mM NaCl, which was considerably less than the values reported by Rozema *et al.* (1993). When the results of the field study are compared with Koyro (2000), the growth of both Magic and Den Helder was also considerably less, but the growth of Crotoy was possibly comparable. In the greenhouse experiment of Koyro (2000), six-week-old seedlings were exposed to 400 mM NaCl for 6 weeks in which the shoot fresh weight dropped by about 50% to 93 g compared to the control (Koyro, 2000). The mean above-ground fresh weights of the final harvest of Magic, Crotoy and Den Helder were 48, 174 and 28 g, respectively. Daoud *et al.* (2008) exposed four-week-old *Beta vulgaris* ssp. *maritima* seedlings to salinity for 7 weeks and reported 30, 37, 17, 14, and 9 g fresh weight for plants exposed to 0, 125, 250, 375 and 500 mM NaCl. When the data is compared with the results of Daoud *et al.* (2008) it appeared that the growth of Magic and Den Helder were somewhat comparable and Crotoy performed considerable better. Based on the continued growth of the three varieties at 200 mM NaCl they can be classified as halophytes (Flowers and Colmer, 2008). However the

growth performance and salt tolerance under field conditions is not easily comparable with that measured under greenhouse conditions.

Mineral composition B. vulgaris ssp. maritima
Possibly, the mineral composition of the three varieties can be used to obtain more insight in the growth performance under saline conditions. Plants growing in a saline environment must have a high K^+ selectivity and a subsequent low Na^+ / K^+ leaf ratio to maintain adequate growth (Flowers *et al*., 1986; Flowers and Colmer, 2008). Since no differences occurred between soil samples, the K^+ selectivity was calculated by using the mean Na^+ / K^+ ratio of the soil samples of 08/06/2009 and 06/07/2009 of all blocks. The concentration of K^+ (as well as Ca^{2+} and Mg^{2+}) were lower in Magic than the other two *B. vulgaris* ssp. *maritima* varieties, whereas the Na^+ concentration was greatest in Magic. This resulted in a relative high Na^+ / K^+ ratio compared to Crotoy and Den Helder. Possibly, the relative low K^+ selectivity of Magic was partially responsible for the observed differences in growth between the three varieties. The Na^+ / K^+ ratio of *B. vulgaris* ssp. *maritima* leaves reported by Rozema *et al*. (1993) was 6.4 for plants exposed to 200 mM NaCl and 7.3 at 400 mM NaCl. Daoud *et al*. (2008) reported a Na^+ / K^+ ratio of about 4.5 at 250 mM NaCl. These reported values are greater than or comparable to the values of the Na^+ / K^+ ratio of the three varieties under field conditions (Table 4). In this regard it seems likely that the Na^+ / K^+ ratio did not cause the lower growth rates under field conditions. Daoud *et al*. (2008) reported a shoot Na^+ concentration of about 2.5 mmol g^{-1} DW at 250 mM NaCl, which is comparable with the reported concentrations of the leaves of Crotoy and Den Helder. Possibly, the greater shoot Na^+ concentration of Magic influenced the growth. More importantly, the shoot Na^+ concentration of the three varieties under field conditions was clearly increased, demonstrating that the three varieties had taken up considerable amounts of Na^+.

Growth of P. coronopus
The continued growth of *P. coronopus* in this study (about 200 mM NaCl or 40 % seawater) demonstrated that it can, just as *B. vulgaris* ssp. *maritima*, be cultivated under the highly saline field conditions. After 54 days of salt exposure, plant fresh weight reached 15 g. However, this growth under field conditions was not comparable with the growth reported under greenhouse conditions. Koyro (2006) evaluated the growth of 6 week-old *P. coronopus* seedlings exposed to various salinity levels for 6 weeks. Here, the above-ground fresh weight dropped to 60 g at full strength seawater salinity (500 mM NaCl) compared to the 200 g of the control. Despite the longer growth period and lower salinity level in the field study, the final biomass was lower. In another greenhouse experiment, *P. coronopus* seedlings were exposed to 180 mM NaCl for 21 days (Zurayk *et al*., 2001). Here, it was concluded that the above-ground dry weight of the 180 mM NaCl treatment did not differ from the control and reached about 0.17 g. The mean dry weight of *P. coronopus* of the field experiment reached 0.16 g after 14 days and 0.55 g after 21 days of salt exposure. So the absolute growth of *P. coronopus* under saline conditions reported by literature varies widely and the growth under field conditions showed intermediate results.

Mineral composition P. coronopus
The shoot Na^+ concentration of *P. coronopus* was comparable with that of Crotoy and Den Helder. So similar to the *B. vulgaris* ssp. *maritima* varieties, the Na^+ uptake was considerable and the shoot Na^+ concentration of *P. coronopus* was clearly increased.

At comparable salinity levels, the shoot Na^+ concentration for other halophytes are commonly in the range of 4-5 mmol g^{-1} DW (Glenn *et al.*, 1999). In this regard, the shoot Na^+ concentration of *P. coronopus*, as well as the *B. vulgaris* ssp. *maritima* varieties, were relatively low. However, the reported shoot Na^+ concentration in this field study should be compared with other studies that focussed on *P. coronopus*. In the greenhouse experiment of Zurayk *et al.* (2001), the shoot Na^+ concentration reached 6.96 mmol g^{-1} DW, whereas under field conditions the shoot Na^+ concentration reached 2.33 mmol g^{-1} DW (Table 5.4). The shoot Na^+ concentration reported by Zurayk *et al.* (2001) was reached after 21 days of salt exposure (180 mM NaCl). Although this salt concentration is comparable with the salt concentration used in the field experiment, the shoot concentration was considerable greater, whereas the exposure time was considerable shorter. The Na^+ concentration of the leaves in the greenhouse experiment of Koyro (2006) reached about 7.5 mmol g^{-1} DW (based on 90 % water content and the reported concentration of 0.75 mmol g^{-1} FW). The salinity used by Koyro (2006) was about twice the salinity used in the field and the duration of the salt exposure in the field was 12 days longer. This makes it difficult to compare the results. It may be assumed that the Na^+ concentration of *P. coronopus* in the field experiment would be about half of the reported concentrations in the greenhouse experiment. In this regard, the Na^+ concentrations of the leaves under field conditions were lower than expected.

Concluding remarks
Between the different blocks in the experimental field, no differences occurred in the spatial and temporal distribution of salts after seawater irrigation started. The irrigation regime, in combination with natural ambient precipitation and drainage, were sufficient to prevent salt accumulation in the soil. Since the determined soil characteristics showed no or minimal differences between the different blocks, it seems likely that the plant species and varieties examined were all exposed to comparable conditions. This demonstrated the feasibility of the chosen experimental setup to conduct relevant field experiments with regard to soil salinity. The three varieties of *Beta vulgaris* ssp. *maritima*, as well as *P. coronopus*, all showed continued growth under field conditions at 40 % seawater salinity. This demonstrated the feasibility of crop cultivation on the seawater drip irrigated experimental field, as well as the potential of these halophyte crops under field conditions. Since no differences in initial biomass occurred between the three varieties in the first few weeks, it is likely that the observed differences in biomass at the final harvest were due to differences in growth performances under saline conditions. Of the three varieties of *Beta vulgaris* ssp. *maritima* tested under saline conditions, variety Crotoy showed greater growth than the other two varieties. Due to variable results presented in literature, it is difficult to compare the growth performance of the tested species under field conditions with experiments performed under greenhouse conditions.

Chapter 6.

General discussion

Sustainable exploitation of saline resources from an agronomic point of view
The sustainable exploitation of saline resources by means of saline agriculture has great potential. The amount of seawater is virtually inexhaustible and seawater itself contains many of the nutrients needed for (optimal) plant growth. Consequently, saline agriculture will need less fertilizers than conventional agriculture and due to the saline conditions the need of pest control is probably limited. Also, it seems likely that saline agriculture can be integrated into nature conservation and development. For example, a seawater farm in Eritrea, realised by the Seawater Foundation, sheltered innumerable plant and animal species. Large scale saline agriculture can only be realised when sufficient knowledge about the ecophysiology and (small scale) cultivation of salt tolerant plant species, among others, is available. This thesis focussed on different aspects of the eco(physio)logy and cultivation of halophytes. The ecology, ecophysiology and cultivation will be discussed separately. Following this, the implications, the perspectives of saline agriculture and the final conclusions are discussed.

Ecology, ecophysiology and cultivation of halophytes
Although the concept of saline agriculture is not new, little research has been undertaken to make large scale saline agriculture a reality. Therefore, the general aim of this thesis (see Chapter 1) was to contribute to the scientific knowledge of the ecology of (strandline) halophytes, to evaluate their potential as halophyte crops, their ecophysiological response to salinity, and their cultivation under field conditions. A case study was performed to elucidate the spatial and temporal variation in strandline salt exposure and the effects on some plant characteristics. Following this, greenhouse experiments were performed to elucidate the effects of increasing salinity on different aspects of growth. Finally, the feasibility of performing reliable field experiments with regard to soil salinity was evaluated. The different research questions and results of this thesis are summarized in Table 6.1. In Fig. 6.1 a general overview is presented of different aspects of salt stress and salt tolerance, based on Munns (1993), Zhu (2001), Flowers and Colmer (2008) and Munns and Tester (2008). As was pointed out by Munns (1993), the growth response to salinity stress occurs in two phases: a rapid response to the increase in external osmotic pressure (the osmotic phase), and a slower response due to the accumulation of Na^+ in leaves (the ionic phase). This ionic phase can take weeks or even months to develop, so the eventual 'continued growth' in Fig 6.1 can only be observed by long-term observations. Based on the definition of halophytes by Flowers and Colmer (2008) the duration of this 'continued growth' should comprise the entire life cycle and a salinity level of at least 200 mM NaCl before a given plant species can be classified as salt tolerant or halophytic.

Ecology
This above mentioned continued growth during the life cycle is generally based on continuous exposure to increased salinity, which illustrates the difficulty of answering one of the research questions considered in this thesis. In Chapter 2 an attempt was made to answer the research question *"Can strandline plant species be recognized as salt tolerant based on their habitat?"*. It was shown in Chapter 2 that the influence of soil salinity and especially inundation can not be neglected in determining the vegetation coverage and species abundance of the strandline habitat, but it appeared that the influence of salt spray is most important. Also, the root zone salt exposure of species occurring in the strandline habitat is only occasionally in the range of ≥ 200 mM NaCl. However, salt spray above a certain level which is known to affect plant

growth, was recorded in the strandline habitat during a total of 11 and 6 weeks for two consecutive years during the growing season of 26 weeks. In this regard, strandline plant species have to be adapted to salt spray, rather than to root zone salinity, to be able to survive in this habitat. It was concluded in Chapter 2 that "strandline plant species are exposed to considerable levels of salt spray and occasional seawater inundation and appear to be moderately salt tolerant and able to survive short periods of high salinity". Nevertheless the strandline soil salinity can be classified as non to moderately saline and only occasionally very strongly saline. Commonly, plant species are classified as salt tolerant based on their ability to grow under saline root zone conditions and not on their ability to withstand salt spray. Since the salt exposure in the root zone appears to be elevated during a relative short period, the ability of strandline species to grow in a saline soil throughout the growing season needs to be evaluated in greater detail. This has been considered in Chapters 3 and 4.
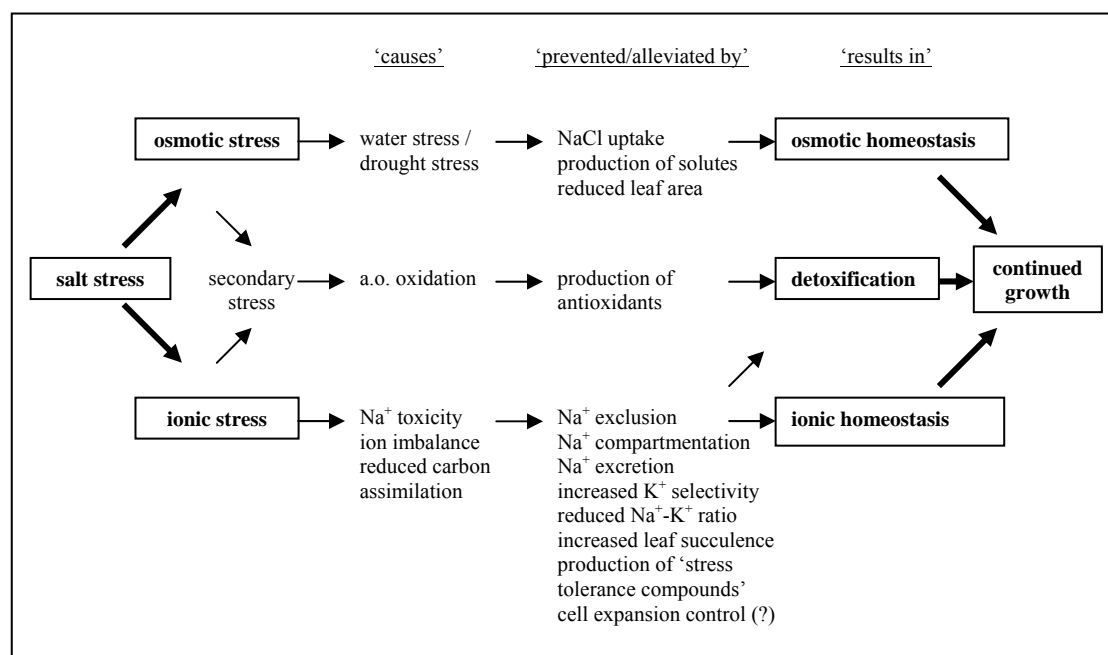

Figure 6.1. General overview of different aspects of salt stress and salt tolerance, based on Munns (1993), Zhu (2001), Flowers and Colmer (2008) and Munns and Tester (2008).

Ecophysiology

The greenhouse experiments performed with *Crambe maritima* (Chapter 3) and *Diplotaxis tenuifolia* and *Cochlearia officinalis* (Chapter 4) showed the effects of increasing (long-term root zone) salinity on different aspects of growth. For the strandline species *Crambe maritima* the effect of salt spray was also studied, which demonstrated that *C. maritima* is salt spray tolerant and can endure the salt spray levels which it encounters in its natural habitat. Salt spray caused only minor physiological changes in the plants compared to the root zone salinity treatments and the salt spray tolerance of *C. maritima* seemed to be based on preventing salt from entering the leaves. Also, an attempt was made to elucidate the underlying mechanisms of salt tolerance of *C. maritima*, *D. tenuifolia* and *C. officinalis*. These findings are summarized in Fig 6.2 (focused on the effects and adaptations of the 200 mM NaCl treatment) and discussed in the following section.

As was mentioned before, salt stress can induce both osmotic and ionic stress (see Fig 6.1). Osmotic stress is caused by salts in the root zone which results in a decrease in the soil water potential and this affects the water absorption of plant

species. This stress is comparable with a water-stress effect or drought stress (Zhu, 2001). The osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure (Munns and Tester, 2008). A common tolerance mechanism to alleviate osmotic stress includes the uptake of NaCl and production of compatible organic solutes, which results in a lower (more negative) leaf osmotic potential and sustained water absorption. It is commonly believed that Na^+ is sequestered in the vacuole of a cell and compatible organic solutes must accumulate in the cytosol to balance the osmotic pressure of the ions in the vacuole. Also, leaf growth is often reduced, probably to decrease the water use by the plant (Munns and Tester, 2008).

The ionic stress starts when plant species actually take up the salts and especially when Na^+ accumulates to toxic concentrations. The plants' transport system (xylem) is a very sophisticated system which ensures controlled loading/unloading of ions and water and their effective translocation to the required sinks (De Boer and Volkov, 2003). The main site of Na^+ toxicity for most plants is the leaf blade, where Na^+ accumulates after being deposited in the transpiration stream, rather than in the roots (Munns and Tester, 2008). How the salts exactly cause their toxicity remains unknown, but salts may build up in the apoplast and dehydrate the cell, they may build up in the cytoplasm and inhibit enzymes involved in carbohydrate metabolism, or they may build up in the chloroplast and exert a direct toxic effect on photosynthetic processes (Munns and Tester, 2008). Toxic Na^+ concentrations can result in the senescence or death of (old) leaves and if the rate at which they die is greater than the rate at which new leaves are produced, the photosynthetic capacity of the plant will no longer be able to supply the carbohydrate requirement of the young leaves, thereby further reducing the growth rate (Munns and Tester, 2008). Consequently, the carbon assimilation of the plant species is reduced. By controlled Na^+ uptake by the roots, by the compartmentalization in the vacuole, or excretion of Na^+ by salt glands or bladders, dicotyledonous salt tolerant plant species are able to prevent toxic Na^+ concentrations from occurring in the cytosol (Tester and Davenport, 2003; Flowers and Colmer, 2008; Munns and Tester, 2008). It is clear that elevated Na^+ inhibits the uptake of other nutrients, but the effects of nutrient deficiencies are probably not as severe as the direct effects of Na^+ toxicity (Tester and Davenport, 2003). However, an increased net K^+ / Na^+ selectivity and the ability to maintain a low Na^+ / K^+ ratio in the cytosol are important mechanisms in the salt tolerance of halophytes (Tester and Davenport, 2003; Flowers and Colmer, 2008).

As was pointed out by Zhu (2001), both the osmotic stress and the ionic stress can cause secondary stress such as oxidative stress, which might be an important cause of damage under salt stress. A salt tolerant plant species must be able to prevent or alleviate this damage.

Increasing root zone salinity up to 20 % seawater salinity showed no or a moderate effect on the growth performance of the three tested species (*Crambe maritima*, Chapter 3; *Diplotaxis tenuifolia* and *Cochlearia officinalis*, Chapter 4) and all were able to survive at 60-80 % seawater salinity. However, for *C. maritima*, *D. tenuifolia* and *C. officinalis*, the 200 mM NaCl treatment resulted in a sharp decrease in growth compared to the 0 mM NaCl treatment and this reduction was apparently caused by the reduction in SLA. So, this reduction was largely due to changes of leaf morphology (decrease of SLA, increase of leaf succulence) rather than toxic leaf Na^+ concentrations. This lowering of the SLA (increased leaf succulence), as well as the consequent lowering of the relative growth rate, has likely resulted in reduced water uptake and thus reduced salt uptake. As was pointed out in Chapter 4, increased leaf

thickness or succulence with increased salinity results in conservation of internal water, efficient water storage and dilution of accumulated salts (Flowers *et al*., 1986; Munns and Tester, 2008, among others). This increased leaf succulence with increasing salinity has also been reported for the Brassicaceae species *Cakile maritima* (Debez *et al*., 2004), *Crambe maritima* (De Vos *et al*., 2010b) and *Thellungiella halophila* (M'rah *et al*., 2006) and indeed appears to be a common adaptation among Brassicaceae species. Smaller, thicker leaves have a higher chloroplast density per unit leaf area and, as a result, the rates of photosynthesis per unit leaf area in salt-treated plants are often unchanged (Munns and Tester, 2008).
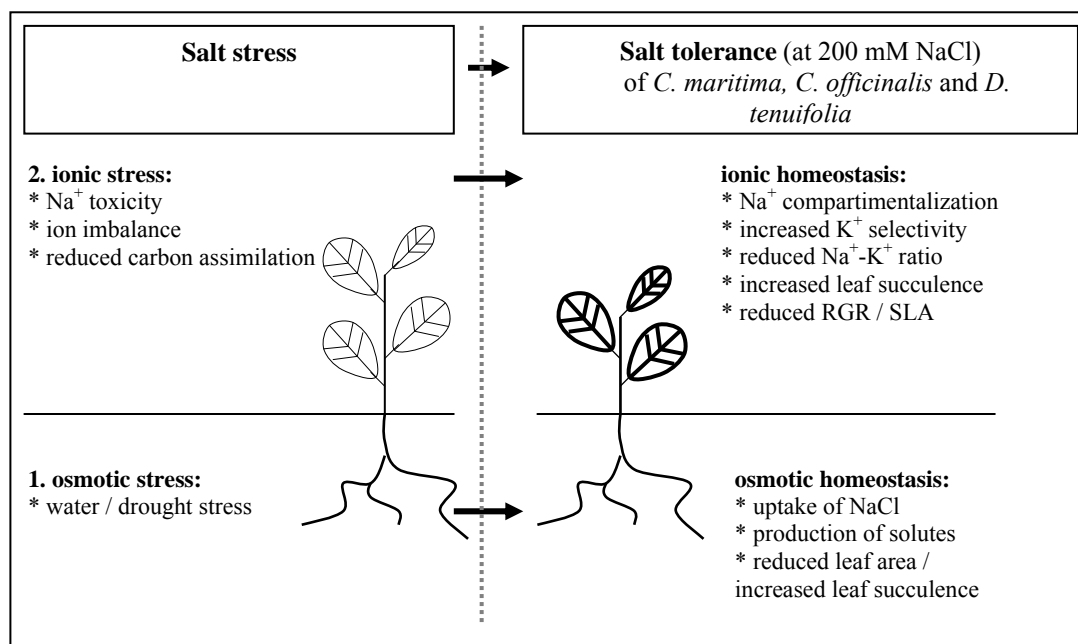

Figure 6.2. The general aspects of salt stress and the adaptations of *Crambe maritima, Diplotaxis tenuifolia* and *Cochlearia officinalis* supporting the salt tolerance of these species at 200 mM NaCl. These adaptations were evaluated in Chapters 3 (*C. maritima*) and 4 (*D. tenuifolia* and *C. officinalis*) of this thesis.

However, when photosynthesis is expressed on a unit chlorophyll basis, rather than on a leaf area basis, a reduction due to salinity can usually be measured and the reduction in leaf area due to salinity means that photosynthesis per plant is always reduced (Munns and Tester, 2008). Also, as was pointed out in Chapter 3, the increased internal surface for CO_2 absorption does not necessarily lead to higher CO_2 uptake rates because the CO_2 resistance as well as stomatal resistance can increase even more with salinity, resulting in a decrease in photosynthesis (Longstretch and Nobel, 1979; Flowers, 1985; Geissler *et al*., 2009). So an increase in leaf succulence can compensate for the negative effects of salinity on leaf cell metabolism to some extent, but at high(er) salinities growth is nonetheless reduced. Reduced growth is an adaptive feature of plant survival under stress because it allows plants to rely on multiple resources (e.g. building blocks and energy) to avoid or reduce the stress (Zhu, 2001). The ability of the three tested species to reduce the SLA can be interpreted as a high level of plasticity. Without this plasticity, water uptake and thus salt uptake likely would have been higher and salts could have accumulated to even greater concentrations. Increased osmotic tolerance will be mainly evident by an increased ability to continue production of new leaves (Munns and Tester, 2008), which was clearly demonstrated by the continued growth for *C. maritima* (Fig

3.1/Table 3.2) and *D. tenuifolia* and *C. officinalis* (Fig 4.1). So for the evaluated species, the adaptations to the osmotic effect of increased salinity caused the largest growth reductions. Although these adaptations resulted in a significant growth reduction, all three species appear to be tolerant to the osmotic effects of increased salinity.

Preventing salt accumulation to a level that will affect plant growth is, is an important aspect of salt tolerance. For the Brassicaceae species which were evaluated in this thesis the values of the net K^+ / Na^+ selectivity and Na^+ / K^+ ratio appeared to influence the growth performance at salinities $\geq$ 200 mM NaCl. For all three species, the net K^+ / Na^+ selectivity of the highest salinity treatment decreased compared to the 100 mM NaCl treatment and the Na^+ / K^+ ratio showed a great increase. In this regard it appeared that the species 'lost control' over the Na^+-K^+ balance and the high leaf Na^+ concentration possibly influenced the growth. However, as was discussed in Chapter 3, this ionic stress would likely reduce the ULR and this was only observed for *C. officinalis* at the highest salinity treatment. For *C. maritima* there was only a trend that the ULR was negatively affected at the highest salinity treatment and for *D. tenuifolia* no effect was observed. Also, the ionic stress starts when salt (Na^+) accumulates to toxic concentrations resulting in the death of old leaves (Tester and Davenport, 2003; Munns and Tester, 2008), but no such senescence or necrosis of leaves was observed for *C. maritima*, *D. tenuifolia* or *C. officinalis*. Since no or only little effects on the ULR of the three tested species were observed with increasing salinity, it can be argued that the lowering of the SLA was sufficient to prevent (major) negative effects on the ULR from occurring.

Although the general overview presented in Fig. 6.1 includes the different aspects of salt stress and salt tolerance which were presented by Munns (1993), Zhu (2001), Flowers and Colmer (2008) and Munns and Tester (2008), the main cause of growth reduction often remains unclear. First of all, for halophytes salinity is part of the natural habitat and it does not need not be stressful *per se* (Flowers and Colmer, 2008), although most halophytes show growth reductions at high salinity levels (see Fig. 1.2). Possibly, inadequate photosynthesis and direct inhibition of cell division and expansion cause the growth rate reduction under salt stress (Zhu, 2001). Munns (1993) argued that plant growth under saline conditions is mostly affected by the reduction in leaf area (affecting the supply of assimilates or hormones to the growing regions) and not by the direct effect of salts on turgor, photosynthesis or another important metabolic process. It is commonly accepted that especially Na^+ can reach toxic concentrations in the leaves, but as was pointed out before it remains unknown how the salts exert their toxicity exactly. Also, the relative importance of the various tolerance mechanisms varies with species, exposure time and salt concentration, among others (Munns and Tester, 2008). Although the nature of the damage which high salt concentrations inflict on plants is not entirely clear (Zhu, 2001) and the mechanism that downregulates leaf growth and shoot development under stress is not precisely known (Munns and Tester, 2008), plant species can only grow and survive under saline conditions if salt damage is prevented or alleviated and homeostatic conditions can be re-established or maintained (Munns, 1993; Zhu, 2001; Flowers and Colmer, 2008; Munns and Tester, 2008).

Cultivation

The work presented in Chapter 5 was mainly performed for the project 'Saline Agriculture Texel' (BSIK-Transforum Agro&Groen-Living with Salt Water research program) and this thesis was carried out against the background of this project. Field

experiments with varying but controlled soil salinity are needed to test the plants' suitability as a new saline vegetable crop and to evaluate the practice of cultivation of halophytes under saline field conditions. Soil salinity under field conditions often shows spatial and temporal variation and the field study described in Chapter 5 mainly focussed on the feasibility of performing reliable field experiments with regard to soil salinity. The chosen experimental setup demonstrated that no differences occurred in the spatial and temporal distribution of salts. It was shown that differences in salt

Table 6.1. An overview of the research questions which were considered in the different chapters of this thesis, together with the main results.

Area of research	Research questions
Ecology	*What is the effect of salt spray and soil salinity on the overall salt exposure of the strandline and the subsequent plant growth?* The soil salinity of the strandline can be classified as non-saline to moderately saline and only incidentally (after seawater inundation) as saline. Although it seems likely that salt spray is most important in determining the vegetation coverage and abundance, the influence of soil salinity and especially inundation can not be neglected. (**Chapter 2**) *Can strandline plant species be recognized as salt tolerant based on their habitat?* Strandline plant species are exposed to considerable levels of salt spray and occasional seawater inundation and appear to be moderately salt tolerant and able to survive short periods of high salinity. (**Chapter 2**)
Ecophysiology	*Does salt spray affect the growth of the strandline species* Crambe maritima*?* *C. maritima* is salt spray tolerant and can endure the salt spray levels encountered in its natural habitat. Salt spray caused only minor physiological changes in the plants as compared to the root zone salinity treatments, although leaf thickness and leaf succulence significantly increased with salt spray. (**Chapter 3**) *Does increased soil salinity affect the growth of the strandline species* Crambe maritima*?* Root zone salinities up to 100 mM NaCl or 20 % seawater salinity did not result in a decrease in growth after 40 days of exposure, but a sharp decrease in growth was observed at 200 mM NaCl (40 % seawater). (**Chapter 3**) *What are the morphological and physiological adaptations underlying these growth responses at higher salinities?* *Crambe maritima* responded to increasing soil salinity by lowering the relative growth rate. The observed growth reduction at 200 mM NaCl root zone salinity (RZS) was mainly caused by the reduction in specific leaf area (SLA). This was caused by increased leaf succulence as well as by increased leaf dry matter content (LDMC). Many other determined parameters changed with increasing salinity, but it appeared that no single parameter

	was clearly linked with the observed growth reduction between 100 and 200 mM NaCl RZS. (**Chapter 3**) *What is the level of salt tolerance of* Diplotaxis tenuifolia *and* Cochlearia officinalis *and do both species have potential as halophyte crops?* Both species were able to survive the highest salinity treatment (300 mM NaCl or 60 % seawater salinity for *D. tenuifolia* and 400 mM NaCl for *C. officinalis*) and can be classified as moderately salt tolerant with potential as vegetable crops for saline agriculture. (**Chapter 4**) *What are the morphological and physiological adaptations underlying these growth responses at higher salinities?* The RGR at higher salinity levels ($\geq$ 200 mM NaCl) showed reductions of around 20 % for both species compared to growth at 0 mM NaCl. The reductions in RGR were mainly due to reduction of SLA, which were similar for the two species. The reduction of SLA was caused by an increase in leaf succulence which showed a maximum increase of 63 % at the 300 mM NaCl level for *D. tenuifolia* and 72 % at the 400 mM NaCl level for *C. officinalis*. (**Chapter 4**) *Are there common adaptations within the Brassicaceae family concerning the response to increased salinity?* Although the mechanisms of salt tolerance appeared to be comparable among the different Brassicaceae species, growth performance under saline conditions varied widely. In comparison with seven salt sensitive and highly salt tolerant Brassicaceae species, *D. tenuifolia* and *C. officinalis* showed an intermediate response to increasing salinity concerning growth and mineral composition. (**Chapter 4**)
Cultivation	*What is the effect of the chosen irrigation regime, in combination with the evapotranspiration and precipitation, on the spatio-temporal variation of soil salinity?* The irrigation regime, in combination with precipitation and drainage, were sufficient to prevent salt accumulation in the soil. No differences occurred in the spatial and temporal distribution of salts after seawater drip irrigation. The different plant species and varieties were all exposed to comparable soil conditions. This demonstrated the feasibility of the chosen experimental setup to conduct relevant field experiments with regard to soil salinity. (**Chapter 5**) *Are there differences in salt tolerance between the tested varieties of* Beta vulgaris *ssp*. maritima*?* Based on the growth performance under saline conditions it appeared that differences in salt tolerance did occur between the three tested varieties of *Beta vulgaris* ssp. *maritima*. (**Chapter 5**)

tolerance did occur between the populations and varieties of *Beta vulgaris* ssp. *maritima*, demonstrating the importance of selection before domestication. Also, due to variable results presented in literature, it is difficult to compare the growth performance of the tested species under field conditions with experiments performed under greenhouse conditions. This highlighted the importance of conducting field experiments before saline agriculture can be introduced on a larger scale.

Implications

The ecology of a given species can help to elucidate its potential and the development into a crop. It can provide insight in the optimal agronomic conditions for cultivation. Since the strandline habitat generally consists of well drained soils and seawater inundation is a rare event, it is reasonable to assume that these plant species will not tolerate waterlogged conditions. Also, due to the mineralization of organic matter the strandline is relatively rich in nutrients, and strandline plant species will be able to benefit from such conditions. Strandline plant species in general appear to have crop potential for cultivation under moderate saline conditions (Aronson, 1989; Niazi, 2007; De Vos *et al.*, 2010b, among others). Based on the growth performance in the greenhouse experiments, the tested species can be recognized as a potential crop for saline agriculture. However, at 40 % seawater salinity the growth performance of the species we examined was negatively affected and the level of salt tolerance can not be compared with that of typical salt marsh halophytes as *Aster tripolium* (Geissler *et al.*, 2008) or *Salicornia spp.* (Ayala and O'Leary, 1995, among others), which show little or no growth reductions at this salinity level.

Also, research on plant salt tolerance often focuses on the aspect of physiological effects of increasing salinity and much of the research focussed on three topics: water relations, photosynthesis, or the accumulation of a particular metabolite (Munns, 1993). This thesis showed that in the case of *C. maritima*, *D. tenuifolia* and *C. officinalis*, the growth reduction at higher salinities was mainly caused by reduction in the morphological component, i.e. the specific leaf area. In this regard, it seems advisable to analyse the relative growth rate to obtain insight in the effects of increasing salinity. By analysing the components of the relative growth rate it is possible to separate the morphological and physiological effects of increasing salinity and identify the cause of decreased growth. Based on the results of this thesis, possible morphological effects of increasing salinity should not be ignored in the research on the mechanisms of salt tolerance.

Field experiments should be performed to validate the results obtained under greenhouse conditions. However, due to variable results found in the literature, it was difficult to compare the growth performance of the tested species under field conditions with experiments performed under greenhouse conditions. This could imply that field studies are not only important for the development of agronomic production systems, but also to evaluate the salt tolerance of a given species under field conditions. It should also be pointed out that even if the initial field experiments were satisfactory, this does not guaranty the success of sustainable large scale cultivation of halophyte crops. For instance, with the upscaling of cultivation of *Crambe maritima*, which was part of the research project 'Biosaline Agriculture Texel', variable results during three consecutive growing seasons were obtained and the tap root was not able to survive the winter season in the wet clay soil. Before saline agriculture can really contribute to the sustainable exploitation of saline resources, agronomic issues related to such large scale cultivation still need to be addressed. Besides the cultivation method and crop rotation, among others, the

sustainability has to be evaluated. Besides addressing the agronomic issues, other 'non-agronomic' activities need to be undertaken. Further development of sustainable exploitation of saline resources relate to market development, costs and benefits of saline crop products and imply upscaling of outdoor or indoor saline crop cultivation.

Perspective

Saline agriculture can facilitate the adaptation to the increasing global and regional salinization and decreasing fresh water availability. Around one billion hectares of the world's total land area is salt affected and these soils are often considered to be unsuitable for conventional agriculture. However, this arable land lost to salinization and also coastal areas or areas with saline water resources can be turned into production again. In this way, salinization can be regarded as an opportunity for sustainable exploitation of saline resources, in contrast with the negative association which is often linked with salinization. In practice saline agriculture often uses 'marginal soils' and water resources not suitable for most conventional crops. Therefore it does not compete with (conventional) food production supplied with freshwater on arable land which is not salt-affected. The identification of suitable halophyte species for crop production is just getting started. Numerous potential halophyte crop species with various applications exist worldwide. The success of the process of selecting suitable crop species to the eventual market introduction can depend on many factors such as salt tolerance, environmental conditions, yield, harvestability and taste. The increasing demand for food, fodder, crops with an industrial application and biofuels can possibly be met by saline agriculture production systems. The use of halophyte crops for human consumption can be restricted by the salt content of leaf vegetables. In this regard, these food crops should consist of seed or tuber crops, since these parts are relatively salt free. Most conventional crops are relatively sensitive to soil salinity and the bioengineering approach has not delivered salt-tolerant cultivars of conventional crops. However, this does not necessarily imply that only salt tolerant halophyte crops are suitable for cultivation under saline conditions. Many of the traditional crops used today have a coastal ancestor and, despite breeding and selection, may have retained salt tolerance at least to some extent. Salt tolerance has never been part of the selection criteria for breeders and it seems likely that above average salt tolerance is present among cultivars of crops with a coastal ancestor. Especially beet and cabbage cultivars and different grain crops are likely candidates for this screening. Based on the (potential) salt tolerance of these traditional crops it seems likely that these crops can be cultivated at up to 20 % seawater salinity. The screening of conventional crops for increased salt tolerance, the development of the eventual salt tolerant crops and the domestication of halophytes is a process that will take 15 to 20 years (Brandenburg; Keizer, personal communication). In this regard, this process should start as soon as possible. By identifying plant traits which are related to salt tolerance, the breeding and selection of suitable varieties for saline agriculture can possibly be enhanced. The identification of such traits is difficult and the only sure method is to measure growth rates. It is well known that plants have evolved various mechanisms to cope with salinity. In this regard, species specific traits for salt tolerance, which can be used for breeding and selection, should be identified. As was discussed before, differences in salt tolerance between different varieties take time to show up and rapid screening methods would only test for tolerance of high external salt concentration, i.e. for tolerance of low water potential (Munns, 1993). Daily measurements of the length of a growing leaf, or spot measurements of stomatal conductance with a porometer, are

good indicators of growth rate in general (Munns and Tester, 2008) and are quick and easy to perform. When these measurements are carried out for a sufficient amount of time (i.e. also during ionic phase; weeks or months), possible differences in salt tolerance between varieties can be indentified. Ion transporters are responsible for the translocation of Na^+ and K^+ and play an important role in the salt tolerance of plant species (Zhu, 2001; Flowers and Colmer, 2008) and screening for their activity or abundance can possibly indentify varieties with increased salt tolerance. The results presented in Chapters 3 and 4 of this thesis, indicated that low leaf Na^+ concentrations, high K^+ selectivity and a low Na^+-K^+ ratio are important aspects of the salt tolerance of the three tested Brassicaeae species. Possibly these parameters can be useful in the screening for more salt tolerant varieties. For some species, the low rates of salt accumulation can be used in selecting for salt tolerance (Munns, 1993). Also, combining rates of senescence in older leaves with measurements of leaf Na^+ concentration provides an estimate of tolerance to Na^+ that has accumulated (tissue tolerance) (Munns and Tester, 2008).

Final conclusions

By studying different aspects of the ecology, ecophysiology and cultivation of halophytes, this thesis contributes to the insight of the salt tolerance mechanisms (in particular of Brassicaceae species) and the development of saline agriculture. It was shown that strandline plant species are exposed to considerable levels of salt spray and occasional seawater inundation. These strandline plant species appear to be at least moderately salt tolerant and are able to survive short periods of high salinity. For *Crambe maritima* it was demonstrated that this typical strandline species is salt spray tolerant. However a sharp decrease in growth was observed of Seakale plants experiencing 40 days of 40 % seawater salinity in the root zone. Similar results were found for two other coastal species, *Diplotaxis tenuifolia* and *Cochlearia officinalis*. The growth reductions at even higher salinity levels (>40 % seawater) of all three species seemed to be caused by reductions of the specific leaf area. Many other determined physiological and morphological growth parameters responded to increasing salinity, but no single parameter appeared to be clearly linked with the observed growth reduction at these higher salinity levels. The feasibility of reliable field seawater irrigation experiments to assess halophyte crop growth under saline conditions was demonstrated in this thesis. Before saline agriculture can really contribute to the sustainable exploitation of saline resources, various agronomic issues related to large scale cultivation of saline crops still need to be addressed. One of these issues is concerned with domestication of halophytes which should start as soon as possible.

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Publications

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Curriculum vitae

Arjen de Vos was born on September 18th 1975 in Koudekerk aan den Rijn, The Netherlands. After secondary school (Bonaventura College, Leiden), which he finished in 1994, he started to study Tropical Agriculture at the University of Applied Sciences, Van Hall Larenstein, Deventer (The Netherlands). After obtaining his propedeuse in Tropical Agriculture (1996), he started to study Biology at the Vrije Universiteit Amsterdam and graduated in 2001. During his masters program he specialised in ecological risk assessments and biosaline agriculture for which he performed an internship in Tunisia. After working for an environmental consultancy agency for several years, he 'returned to science' in 2006 to start his PhD candidacy at the Vrije Universiteit Amsterdam. His PhD was carried out against the background of the project 'Saline Agriculture Texel' with was part of the BSIK-Transforum program 'Living with Water'. After this, he conducted research for the Small Business Innovation Research Program (SBIR), which was focussed on the setup and start-up of a saline research field site. Here, conventional and potential halophyte crops can be screened for their salt tolerance, among others. Recently, he was invited to participate in the project '*Saline Perspectives*' which has been funded by the 'Waddenfonds'. This project is focussed on the opportunities and the development of saline agriculture in the Waddensea region in The Netherlands and has an educational and scientific approach. Also, he was invited to participate in the project *'Zeekraal, slib en zout, een mogelijke win-win situatie'*, funded by the Visserij Innovatie Platform (VIP). This research is focussed on using different seed treatments to reduce weed growth in the upscaling of *Salicornia* cropping systems and also using mud/sludge in controlling this weed growth.