

Effects of temperature, salinity and cold stratification on seed germination in halophytes

Batoul N. Al-Hawija, Monika Partzsch and Isabell Hensen

Batoul N. Al-Hawija, Monika Partzsch (monika.partzsch@botanik.uni-halle.de) and Isabell Hensen, Inst. of Biology/Geobotany and Botanical Garden, Martin-Luther-Univ. Halle-Wittenberg, Am Kirchtor 1, DE-06108 Halle, Germany.

Salt tolerance of halophytes corresponds with the habitat requirement of the species. It is an important factor during the germination phase and it can determine successful establishment. This paper presents the effects of alternating temperature–light regimes (4/8°C, 10/20°C, 20/32°C; 12 h dark: 12 h light) and different salinity levels (0, 200, 400, 600 mmol l⁻¹ NaCl) on seed germination of five halophytes, *Halimione pedunculata*, *Bupleurum tenuissimum*, *Aster tripolium*, *Triglochin maritimum* and *Armeria maritima*. The five species differ with respect to family and life-form and spatially correspond to a decreasing salt gradient (i.e. distance from salt water, with *H. pedunculata* being the most tolerant and *A. maritima* being the least). *Armeria maritima*, *A. tripolium* and *T. maritimum* seeds were additionally subjected to a cold stratification experiment. The results showed that *Halimione pedunculata*, an annual therophyte of year-round heavily saline habitats, was dormant under all experimental conditions. *Bupleurum tenuissimum*, a species typical to sites of varying salinity prone to leaching during spring and autumn rainfall, germinated best under cold and warm temperatures, but only under non-saline conditions. *Aster tripolium* and *T. maritimum*, close neighbours in salt marshes, showed very similar germination behaviour: seeds of both species tolerated high levels of salinity and germinated best in summer temperatures during periods of highest soil salinity, and germination was significantly promoted by cold. *Armeria maritima*, a species usually found on the marginal fringes of saline habitats, germinated only under low salt levels and maximum germination was under cold (spring) and warm (autumn) temperatures, with no significant effect of cold stratification.

Halophytes are highly specialized plants that germinate, establish, grow and reproduce successfully in saline conditions; conditions that otherwise inhibit growth of plants (glycophytes). Halophytes can be divided into two categories: 1) species that require salt for survival and/or maximum growth, and 2) species that tolerate salt (Waisel 1972). Most of these species are found in salt marsh systems along seashores or around landlocked inland lakes and flat plains with high evaporation rates (Khan and Weber 2006).

Several factors (water, temperature, light and salinity) interact in the soil interface and regulates seed germination. These factors may even interact with the seasonal variation in temperature to determine the temporal pattern of germination. Temperature variation under saline conditions has differing effects on the germination of halophytes (Ungar 1991, El-Keblawy and Al-Rawai 2005), probably due to differences in temperature regimes among the geographical regions where the halophyte species occur. Khan and Ungar (1997) pointed out that germination of halophytes under natural conditions is regulated by variations in soil salinity and ambient thermo-period.

For the purposes of experimentation, seeds need to be incubated at alternating temperatures simulating those of the natural habitat (Baskin and Baskin 2001). The soils

where halophytes normally grow become more saline following rapid evaporation of water, particularly during summer, resulting in high soil salinity and high water potential (Khan and Gul 1998, Khan and Ungar 1998). In inland regions, especially those with low rainfall and high evaporation, the maximum yearly salinity level can reach 8% (Waisel 1972). Seed germination in saline environments therefore usually occurs during spring or autumn and thus in seasons with high precipitation when soil salinity is reduced (Li and Zhang 2007).

Many studies have shown that halophyte germination is higher under non-saline conditions and that germination decreases with increased salinity (Khan and Gulzar 2003, Qu et al. 2008a, b). Salinity inhibits halophyte germination in two ways: 1) by causing a complete inhibition of the germination process at salinities beyond the tolerance limit of species, and 2) by delaying the germination of seeds within a certain salinity range that causes some stress to seeds without completely preventing germination (Khan et al. 2002a). In addition, many halophytes belong to families that are known to have physiological dormancy, in which germination-inhibiting substances in seeds decompose over time (Baskin and Baskin 2004). This type of dormancy can be broken by cold stratification (Baskin and Baskin

2001). Several authors have found that cold stratification can increase germination percentages and rates as well as temperature change and light requirements for germination (Bewley and Black 1982, Philipupillai and Ungar 1984, Huang et al. 2004a, b, Qu et al. 2008b).

In central Germany, many inland salt sites occur with a high diversity of halophytes. We selected five species from five different plant families with different life-forms, namely *Halimione pedunculata*, *Bupleurum tenuissimum*, *Aster tripolium*, *Triglochin maritimum* and *Armeria maritima*. In nature, these species follow a decreasing salt gradient (Ellenberg and Leuschner 2010). We hypothesize that the species show different germination strategies and reflect seasonal germination patterns in nature in accordance with their occurrence in different plant communities along a salt gradient. Therefore, the aims of this study were: 1) to investigate the germination behaviour under different temperature and light regimes in order to ascertain the optimal thermal germination conditions of the five species, 2) to compare the germination response of the species to different salt concentrations, and 3) to assess whether cold stratification influences the germination response to temperature–light regimes and salinity.

Material and methods

Study region and study species

Seeds of the five species (20–50 individuals per population) were collected from inland salt sites in central Germany. The climate of the region is characterized by a mean annual temperature of 9.2°C and a mean annual precipitation of 473 mm (Döring 2004).

The five species were ordered with decreasing salinity tolerance: 1) *Halimione pedunculata* L. Aellen (Chenopodiaceae) (syn: *Atriplex pedunculata* L., *Obione pedunculata* (L.) Moq.) is an annual therophyte which flowers in Jul–Oct (Clapham et al. 1987, Jäger 2011); 2) *Bupleurum tenuissimum* L. (Apiaceae) is an annual therophyte which flowers in Jun–Aug (Clapham et al. 1987, Jäger 2011), germination occurs between autumn and spring (Stewart et al. 1994); 3) *Aster tripolium* L. (Asteraceae) is an annual to perennial hemicytrophite which flowers in Jul–Oct (Hill et al. 2004, Jäger 2011), its fruits are achenes which disperse in autumn and germinate in spring and summer (Hutchings and Russell 1989, Davy and Bishop 1991); 4) *Triglochin maritimum* L. (Juncaginaceae) is a perennial hemicytrophite which flowers in Jun–Aug (Hill et al. 2004, Jäger 2011), seed dispersal occurs in autumn (Hutchings and Russell 1989) with germination taking place in spring following moderate temperature fluctuations (Davy and Bishop 1991); 5) *Armeria maritima* (Mill.) Willd. (Plumbaginaceae) (syn: *Statice maritima* Mill.) is a perennial hemicytrophite which flowers in Apr–Oct (Jäger 2011) its fruits are dispersed throughout the summer while germination occurs in autumn, winter and spring (Hutchings and Russell 1989, Woodell and Dale 1993).

Seed collection and germination experiment

Seeds of the five species were collected in Aug–Oct 2008 and stored in paper bags under lab conditions until the experiment commenced in Nov 2008. Seed viability varied between 85% and 98% at the onset of the experiment. Thirty seeds of each species were put on standard Petri dishes and kept permanently moist with de-ionized water or salt solution in programmed incubators (four replications per treatment). The effect of temperature on germination was determined for alternating temperature–light regimes of 4/8°C, 10/20°C and 20/32°C in 12 h darkness and 12 h warm and white light. The three temperature treatments simulated the study regions' natural conditions of cold, warm and hot for the seasons spring, autumn, and summer, respectively, and the light regime simulated the approximate day and night length during spring and autumn. The dishes were monitored every two or three days for the emergence of radicles – indicating germination and velocity – and germinated seeds were removed. In order to also assess late germinating seeds, the duration of the experiment lasted 45 days. After this time the observation was randomised.

In order to investigate the effect of different salt concentrations, the above procedures were carried out for seeds in 200, 400 and 600 mmol l⁻¹ NaCl solutions, with distilled water serving as a control. The 600 mmol l⁻¹ NaCl solution simulated the typical salt concentration of seawater (Woodell 1985).

To simulate the effect of winter temperature on germination, a cold stratification experiment was conducted in December 2008 on *A. maritima*, *A. tripolium* and *T. maritimum*. Unfortunately, there were not enough seeds to carry out the procedure on *B. tenuissimum* and *H. pedunculata*. Imbibed seeds were stored under refrigeration at 5°C for 4 weeks, after which time the stratified seeds were subjected to the same conditions (except the 600 mmol l⁻¹ concentration), with each treatment being replicated 3 or 4 times with 20 seeds each.

Before and after the experiments, seed viability was checked using the Tetrazolium-test (Hendry and Grime 1993) and by visual checks for intact embryos.

Data analysis

The final germination percentage relates to the number of seeds which germinated during the germination period (45 days). The germination velocity was estimated using a modified Timson's index (Timson 1965, Pérez-Fernández et al. 2006). The index was calculated as the cumulative sum of all germinated seeds during the study period divided by the number of days (45). The data were arcsine square-root transformed before statistical analysis to ensure homogeneity of variance tested by Bartlett's test. With this data transformation, we met the assumptions for ANOVA testing. The multiple comparisons were performed using a parametric one-way ANOVA and Tukey's post hoc test ($p < 0.05$) to evaluate the effect of temperature on germination percentage and Timson's index of all five species. Because

both variables (germination percentage and velocity) were highly correlated, a generalized linear model (GLM) was calculated to determine the relative weight of each fixed factor (temperature, salinity and cold stratification) for *A. tripolium*, *T. maritimum* and *A. maritima*. With the multi-variable response, including germination percentage and germination velocity, the dominant factor of each species was quantified. For the GLM, a Tukey's post hoc test ($p < 0.05$) was also performed and the results were illustrated by different letters. The data was analyzed using SPSS 19.0 (2010).

Results

Effects of temperature under non-saline conditions

Halimione pedunculata did not germinate at all during the course of the experiment (45 d), but the Tetrazolium test indicated that seeds were viable. They started to germinate three weeks after the end of the experiment. *Bupleurum tenuissimum* showed highest germination percentage under cold and warm conditions (Table 1). Timson's index was highest at 10/20°C and significantly lowest at hot conditions.

Aster tripolium and *T. maritimum* showed a similar response to alternating temperature–light regimes. Germination percentage and Timson's index were highest under hot conditions and significantly decreased with decreasing temperature (Table 1).

Germination of *A. maritima* did not significantly differ between the three temperature–light regimes (Table 1). Under hot conditions, Timson's index was significantly lower than under cold or warm conditions.

Aster tripolium started to germinate very early (day 2) under warm and hot conditions whereas *T. maritimum*, *A. maritima* and *B. tenuissimum* started between day 4 and day 7 (Table 3). Under cold conditions germination commenced on day 9 for *A. maritima* and *B. tenuissimum*, on day 18 for *A. tripolium* and on day 21 for *T. maritimum*.

Effects of salinity and temperature

Seeds of *B. tenuissimum* and *H. pedunculata* did not germinate under saline conditions during the experimental period, but they started to germinate three weeks after the end of the experiment. *Bupleurum tenuissimum* achieved approximately 20% germination under 200 mmol l⁻¹ NaCl after 66 days, and some seeds of *H. pedunculata* (20%) also germinated with 600 mmol l⁻¹ NaCl (data not shown).

Aster tripolium and *T. maritimum* showed similar temperature dependent germination in 200 and 400 mmol l⁻¹ NaCl to that in distilled water, but germination percentages decreased with increasing salinity (Fig. 1, left). At 600 mmol l⁻¹ NaCl, the germination of both species was very low under warm and hot conditions; under cold conditions no seeds germinated.

Armeria maritima germinated best under warm conditions at 200 mmol l⁻¹ NaCl (Fig. 1, left). Under cold conditions with 200 mmol l⁻¹ NaCl, germination was similar to that in distilled water. At 400 mmol l⁻¹ NaCl, germination was minor under warm conditions; under hot conditions *A. maritima* did not germinate after salt addition.

However, with increasing salinity, the commencements of germination of *A. tripolium*, *T. maritimum* and *A. maritima* were delayed in all temperature treatments (Table 3).

Effects of temperature and salinity after cold stratification

Germination of cold-stratified seeds of *A. tripolium* and *T. maritimum* was strongly promoted at all three temperature–light regimes and salinity levels (Fig. 1, right). In *A. tripolium*, stratification additionally increased germination under cold conditions. Germination of stratified seeds of *T. maritimum* under warm conditions was higher at all salinity levels than without stratification and similar to that under hot conditions. Under cold conditions germination was also minor after cold stratification. The commencement of germination

Table 1. Effects of temperature–light regime on germination percentage and Timson's index (germination velocity) of the five species (ordered by decreasing salt gradient) during the experiment of 45 days with distilled water. The arc sine square-root transformed data were calculated by factorial ANOVA. Test statistic F-value and p-value are shown. Different letters (A, B and C) distinguish between significant groups (n = 4; n. s. = not significant).

Parameters	Temperature–light regimes			ANOVA	
	4/8°C	10/20°C	20/32°C	F	p
<i>Halimione pedunculata</i>					
Germination (%)	0	0	0	–	–
Timson's index	0	0	0	–	–
<i>Bupleurum tenuissimum</i>					
Germination (%)	96.67 ^A	99.17 ^A	8.33 ^B	131.007	< 0.0001
Timson's index	61.52 ^A	77.70 ^B	3.26 ^C	431.423	< 0.0001
<i>Aster tripolium</i>					
Germination (%)	5.84 ^A	44.20 ^B	83.33 ^C	113.819	< 0.0001
Timson's index	2.04 ^A	38.02 ^B	75.31 ^C	167.243	< 0.0001
<i>Triglochin maritimum</i>					
Germination (%)	2.50 ^A	68.33 ^B	90.83 ^C	75.363	< 0.0001
Timson's index	0.66 ^A	45.76 ^B	82.02 ^C	162.964	< 0.0001
<i>Armeria maritima</i>					
Germination (%)	53.33	44.17	38.33	1.597	n. s.
Timson's index	37.57 ^A	37.69 ^A	15.46 ^B	8.139	< 0.05

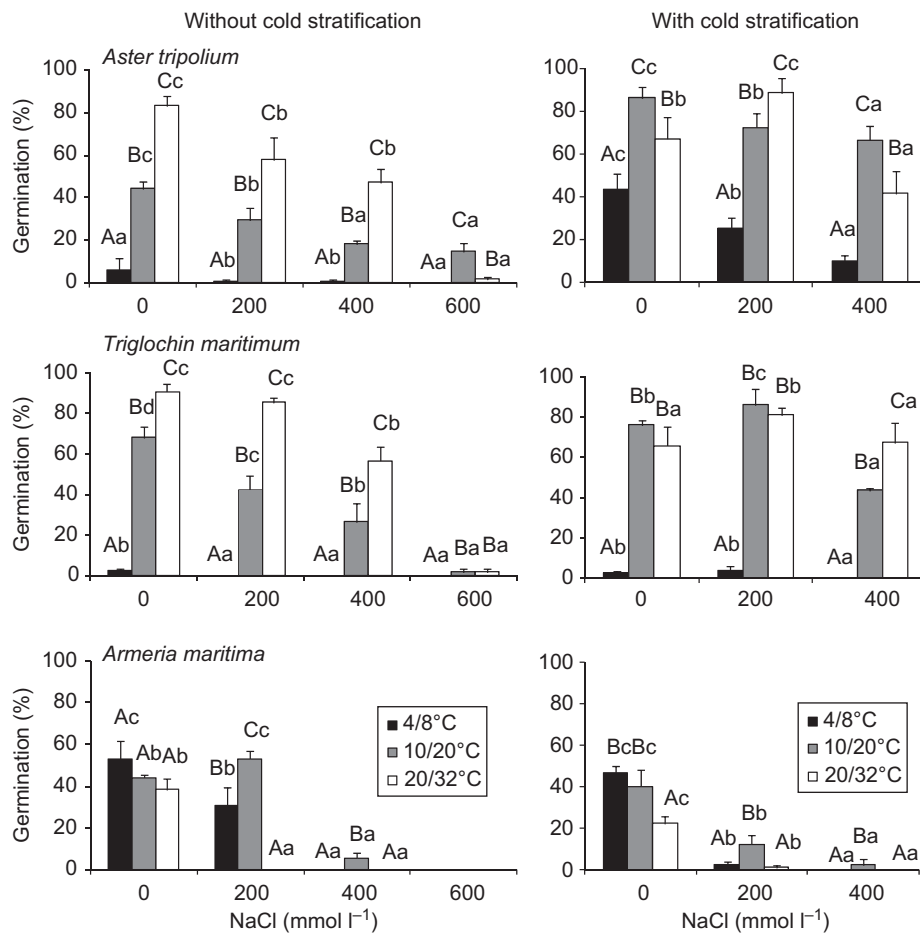


Figure 1. Germination percentage of *A. tripolium*, *T. maritimum* and *A. maritima* at four different salt concentrations without cold stratification (left side) and at three different salt concentrations with cold stratification (right side), each at three different temperature–light regimes. Bars indicate standard error (SE). Different upper case letters indicate significant differences in germination percentage at different temperatures. Different lower case letters indicate significant differences in germination percentage at different salinity levels at same temperature.

and germination velocity of cold-stratified seeds of *A. tripolium* and *T. maritimum* was accelerated at all salinity and temperature treatments (Table 2, 3).

Stratified seeds and non-stratified seeds of *A. maritima* showed similar temperature dependent germination in distilled water. At 200 mmol l⁻¹ and 400 mmol l⁻¹ NaCl, germination percentages of the stratified seeds were clearly

lower. Germination commencement was roughly the same under stratified and non-stratified conditions, and germination velocity was strongly reduced (Table 2, 3).

The GLM showed that cold stratification significantly promoted germination behaviour, including germination percentage and velocity for *A. tripolium* and *T. maritimum*, but not for *A. maritima*. In all other cases, the environmental

Table 2. Timson's index of *A. tripolium*, *T. maritimum* and *A. maritima* at four different salt concentrations (mmol l⁻¹ NaCl) without cold stratification and at three different salt concentrations with cold stratification, each with three different temperature–light regimes during 45 days. Different upper case letters indicate significant differences in germination velocity at different temperatures. Different lower case letters indicate significant differences in velocity at different salinity levels at same temperature.

Salinity	<i>Aster tripolium</i>			<i>Triglochin maritimum</i>			<i>Armeria maritima</i>		
	4/8°C	10/20°C	20/32°C	4/8°C	10/20°C	20/32°C	4/8°C	10/20°C	20/32°C
Without cold stratification									
0	2.04 ^A	38.02 ^B	75.31 ^C	0.67 ^A	45.76 ^B	82.02 ^C	37.57 ^B	37.69 ^B	15.46 ^A
200	0.46 ^A	27.59 ^B	50.40 ^C	0 ^A	26.85 ^B	72.65 ^C	18.91 ^B	39.98 ^C	0 ^A
400	0 ^A	10.95 ^B	35.87 ^C	0 ^A	11.17 ^B	13.61 ^B	0 ^A	2.89 ^B	0 ^A
600	0 ^A	8.48 ^B	1.19 ^C	0 ^A	0.37 ^B	0 ^A	0 ^A	0 ^A	0 ^A
With cold stratification									
0	17.36 ^A	69.64 ^B	63.03 ^B	1.08 ^A	56.69 ^B	60.8 ^B	34.11 ^B	36.40 ^B	20.11 ^A
200	6.83 ^A	52.64 ^B	81.28 ^C	1.69 ^A	65.14 ^B	72.97 ^B	1.14 ^A	5.75 ^B	1.22 ^A
400	1.30 ^A	42.18 ^C	29.30 ^B	0 ^A	25.72 ^B	46.19 ^C	0 ^A	2.34 ^B	0 ^A

Table 3. Commencement of germination (day) of *A. tripolium*, *T. maritimum* and *A. maritima* at four different salt concentrations (mmol l⁻¹ NaCl) without cold stratification and at three different salt concentrations with cold stratification, each with three different temperature–light regimes during 45 days.

Salinity	<i>Aster tripolium</i>			<i>Triglochin maritimum</i>			<i>Armeria maritima</i>		
	4/8°C	10/20°C	20/32°C	4/8°C	10/20°C	20/32°C	4/8°C	10/20°C	20/32°C
Without cold stratification									
0	18	2	2	21	7	4	9	7	7
200	23	4	2	–	9	2	14	7	–
400	42	7	3	–	11	11	–	14	–
600	–	4	14	–	35	42	–	–	–
With cold stratification									
0	2	2	2	14	2	2	4	2	2
200	9	2	2	14	4	2	14	7	2
400	21	11	4	–	4	7	–	7	–

factors of temperature and salinity had highly significant effects on all three species (Table 4). The germination behaviour of *A. maritima* was significantly affected by all interactions, while *A. tripolium* and *T. maritimum* was significantly affected by the interactions stratification \times temperature, temperature \times salinity, and stratification \times salinity (only for *T. maritimum*), but there was no significant interaction of stratification \times temperature \times salinity on either species.

Discussion

Effects of temperature

Our results show that the optimal thermal germination conditions of the five target species varied: while seeds of *H. pedunculata* were dormant, *B. tenuissimum* germinated well under cold and warm conditions, but not under hot conditions. Germination of *A. tripolium* and *T. maritimum* significantly increased with an increase in temperature while *A. maritima* germinated equally well under all temperature conditions. In accordance with our initial expectation, the germination response of our study species coincides with the typical seasonality of germination patterns in nature.

In our study, the annual therophyte *H. pedunculata* was dormant during the experiment period of 45 days, but after 66 days the seeds started to germinate, which can be an indication of non-deep physiological dormancy (sensu Baskin and Baskin 2001). Qu et al. (2008b) and Wei et al. (2007) described similar germination behaviour in other species of the Chenopodiaceae family (*Kalidium capsicum*, *Salsola affinis*). In contrast, *B. tenuissimum*, a winter annual therophyte that normally germinates in autumn and spring,

germinated better and faster under cold and warm conditions. The results of Partzsch (2009) indeed indicated that winter annual therophytes prefer cold conditions for germination. In contrast, Heard and Ancheta (2011) found that the annual halophyte *Symphyotrichum laurentianum* germinated best at 30°C and germination was strongly reduced with decreasing temperature, which indicates summer germination.

Baskin and Baskin (2001) reported a high variability of germination responses of halophytes, with species specific optimum temperatures for germination ranging between 5° and 25/35°C (mean optimum about 21°C). They recorded optimal germination temperatures for *A. tripolium* both at 10/15°C and 16/27°C; and for *T. maritimum* between 20° and 30°C. Our data showed the highest germination for both species under hot conditions, indicating seed germination in summer. Woodell and Dale (1993) found that 100% of *A. maritima* seeds germinated at room temperature (20 \pm 3°C) after 8 days, and that germination mainly took place in autumn but continued through the winter to the following spring. This agrees with our results where this species germinated at a broad range of temperatures but was most successful under cold conditions.

Effects of salinity, temperature and cold stratification

Our study species also responded differently to increasing salt concentrations, and the optimal temperature for seed germination was clearly reflected in their response to salinity. *Bupleurum tenuissimum* did not germinate in salt solution, but germinated very well under non-saline conditions. This is in line with the results of Houle et al. (2001), who found the highest germination under non-saline conditions and

Table 4. Generalized linear model analyses of germination behaviour of *A. tripolium*, *T. maritimum* and *A. maritima*.

	<i>Aster tripolium</i>			<i>Triglochin maritimum</i>			<i>Armeria maritima</i>		
	DF	F	p	DF	F	p	DF	F	p
Stratification	2,53	18.881	<0.0001	2,53	5.311	0.008	2,53	1.033	0.363
Temperature	4,108	24.998	<0.0001	4,108	48.198	<0.0001	4,108	10.510	<0.0001
Salinity	4,108	12.664	<0.0001	4,108	12.661	<0.0001	4,108	23.822	<0.0001
Strat. \times Temp.	4,108	6.913	<0.0001	4,108	5.291	0.001	4,108	6.342	<0.0001
Strat. \times Sali.	4,108	2.338	0.06	4,108	3.278	0.014	4,108	6.212	<0.0001
Temp. \times Sali.	4,108	3.532	0.001	4,108	5.329	<0.0001	4,108	7.540	<0.0001
Strat. \times Temp. \times Sali.	4,108	1.71	0.104	4,108	1.363	0.221	4,108	2.012	0.052

low or zero germination at higher salinities for the annual halophyte *Symphyotrichum laurentianum*. For the same species, Heard and Ancheta (2011) reported that the effect of temperature ($p < 0.001$) on germination was much stronger than the effect of salinity ($p = 0.035$).

Aster tripolium was the only species able to germinate under the highest concentration of 600 mmol l⁻¹ NaCl. *Triglochin maritimum* germinated well up to concentrations of 400 mmol l⁻¹ NaCl and *A. maritima* up to 200 mmol l⁻¹ NaCl. Our data are in line with Gulzar and Khan (2001), who stated that the overall salinity threshold for a significant reduction in germination ranges between 100–500 mmol l⁻¹ NaCl.

The seeds of *A. tripolium* germinated under all salinity levels, but germination decreased with increasing salinity and the species germinated best under hot conditions. Woodell (1985) pointed out that *A. tripolium* salt marshes exhibited a germination of 78% under non-saline conditions at room temperature, while germination rates of 58% and 24% were obtained at 300 mmol l⁻¹ and 600 mmol l⁻¹, respectively. Also, Yuichi et al. (1996) found that *A. tripolium* germinated best (31%) at 23°C in distilled water and less so (4%) at 150 mmol l⁻¹ (17%) and 300 mmol l⁻¹ NaCl.

Khan and Ungar (1997) reported that *T. maritimum* appears to be sensitive to changes in thermo-periods. Khan and Ungar (1999) found that the variation in alternating temperature–light regimes significantly affected seed germination of *T. maritimum* under both saline and non-saline conditions. Germination percentage was highest at 24% in distilled water under alternating temperature regimes of 5/25°C, and few seeds germinated at salinities higher than 300 mmol l⁻¹. In addition, Ungar (1978) reported that the ability of seeds to germinate at increased levels of salinity partly depended on the test temperature. Our results showed that *T. maritimum* germinated best under hot conditions at the given salinity levels.

Armeria maritima prefers 200 and 400 mmol l⁻¹ NaCl under cold and warm conditions for germination. This is in agreement with Woodell (1985), who reported that while nearly all seeds of *A. maritima* germinated in fresh water, the germination percentage strongly decreased with increasing salinity. Baskin and Baskin (2001) pointed out that dormancy of diaspores of the families Asteraceae, Juncaginaceae and Plumbaginaceae is physiological in nature and can be broken by cold stratification. Our results showed that germination behaviour of *A. maritima* was not significantly affected by cold stratification. However, there were significant interactions between stratification × temperature, stratification × salinity and temperature × salinity, and marginal significant interaction between stratification × temperature × salinity. In contrast, cold stratification affected the germination behaviour of *A. tripolium* and *T. maritimum* highly significantly or significantly, respectively. The germination behaviour of the two species was clearly promoted in non-saline and saline solutions, mostly under warm and hot conditions. These results agree with Baskin and Baskin (2001), who describe a physiological dormancy of *A. tripolium* and *T. maritimum*, which both need a dormancy-breaking treatment by cold stratification of 30 days. Also, Masuda et al. (1999) found an enhancement of germination

percentages of *T. maritimum* in 400 mmol l⁻¹ NaCl after 5 months moist-chilling pre-treatment at 4°C. Several authors point out that cold stratification can increase germination percentages and germination rates as well as change temperature and light requirements for germination (Bewley and Black 1982, Huang et al. 2004a, b, Qu et al. 2008b). Baskin and Baskin (2001) argued that seeds which disperse in autumn, such as *A. tripolium* and *T. maritimum*, require cold stratification and, with a loss of dormancy, the ability to germinate at increased levels of salinity increases.

Moreover, the germination behaviour of all three species was highly significantly affected by salinity, temperature and the interaction salinity × temperature. This implies that under different temperatures, the sensitivity of the species to different salinity levels changes. This is in line with Khan et al. (2002a), who pointed out that salinity and temperature interact in their effects on germination and that the greatest inhibition usually occurs at the maximum or minimum limits of temperature tolerance.

Germination behaviour and habitat preference

Our species show different affinities with the halophile plant communities. The rare species *H. pedunculata* occurs in the neighbourhood of *Salicornia europaea* in the *Salicornietum europaea* Christ. 1955 corr., whereas *B. tenuissimum* settles in the *Spergulario-Puccinellietum distantis* Feek. 1943 (Schubert et al. 2001). Both communities are characterised by their tolerance to high salt concentrations, levels which only therophytes can withstand. The habitats of *H. pedunculata* are characterised by continuous salinity, whereas the sites of *B. tenuissimum* vary in salinity with rainfall. In accordance, *H. pedunculata* was strongly dormant under all experimental conditions. Several authors (Khan et al. 2002b, Redondo et al. 2004, Gorai et al. 2006, Gorai and Neffati 2007) describe such behaviour as an adaptive strategy in hypersaline conditions. In contrast, *B. tenuissimum* germinated nearly completely under non-saline conditions, at cold (spring) and warm (autumn) temperatures. This is in line with Khan et al. (2002a), who found that species from inland locations germinate when soil salinity is diluted after long periods of rainfall (in our region in spring and autumn) and that an increase in salinity usually delays seed germination of annual halophytes, but that salinity usually does not prevent germination of halophytes. This strongly agrees with our data on the two therophytes *B. tenuissimum* and *H. pedunculata*, which started to germinate three weeks after the end of the experiment.

Aster tripolium, *T. maritimum* and *A. maritima* occur in communities of the *Armerion maritimae* alliance, in typical salt marshes. The salt concentration here is reduced to such a level that long-living hemicryptophytes are able to survive. Therefore, *A. tripolium* and *T. maritimum* are close neighbours that show nearly the same germination behaviour under all conditions, even though they belong to different plant families. In contrast, *A. maritima* settles in habitats with less salt, which reflects its sensitivity to higher salt concentrations (400 and 600 mmol l⁻¹). However, Breckle (2000) argued that reproduction under salt stress conditions

and consequent regeneration ability of the halophytes is important for the composition of plant communities. As such, the variation in response to the alternating temperature–light regimes and different salt concentrations may be correlated with the habitat to which each species belongs. In accordance, Woodell (1985) found that germination is stimulated by salinity in species that are permanently exposed to it.

Conclusions

In general, we can conclude that the five investigated halophytes, which follow an indicative ecological salt gradient in the field, show different germination behaviours: *H. pedunculata*, which settles on mostly salty sites, is fully dormant. *Aster tripolium* and *T. maritimum* are tolerant to higher levels of salinity and are adapted to germinating best at summer temperatures, which is the time when there is a higher salt concentration. *Bupleurum tenuissimum* and *A. maritima* differ in their salt gradient position and tolerate only low salt levels, which corresponds to periods of lower salt concentrations, due to higher rainfall or to greater distance to salt emergence, respectively. Both species reach maximum germination under spring and autumn temperatures.

Acknowledgements – We greatly appreciate the help of Daniel McCluskey for checking our English.

References

- Baskin, C. C. and Baskin, J. M. 2001. Seeds: ecology, biogeography and evolution of dormancy and germination. – Chapman and Hall.
- Baskin, C. C. and Baskin, J. M. 2004. A classification system for seed dormancy. – Seed Sci. Res. 14: 1–16.
- Bewley, J. and Black, M. 1982. Physiology and biochemistry of seeds in relation to germination. Vol. 2. Viability, dormancy and environmental control. – Springer.
- Breckle, S.-W. 2000. Wann ist eine Pflanze ein Halophyt? Untersuchungen an Salzpflanzen in Zentralasien und anderen Salzwüsten. – In: Breckle, S.-W. et al. (eds), Ergebnisse weltweiter ökologischer Forschung. – Verlag Günther Heimbach.
- Clapham, A. R. et al. 1987. Flora of the British Isles, 3rd ed. – Cambridge Univ. Press.
- Davy, A. J. and Bishop, G. F. 1991. Biological flora of the British isles, no. 172. *Triglochin maritima* L. (*Triglochin maritimum* L.). – J. Ecol. 79: 531–555.
- Döring, J. 2004. Zu den Klimaverhältnissen im östlichen Harzvorland. – Hercynia N. F. 37: 137–154.
- Ellenberg, H. and Leuschner, C. 2010. Vegetation Mitteleuropas mit den Alpen, 6nd ed. – Ulmer.
- El-Keblawy, A. and Al-Rawai, A. 2005. Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (SW). D. C. J. – J. Arid Environ. 61: 555–565.
- Gorai, M. and Neffati, M. 2007. Germination responses of *Reaumuria vermiculata* to salinity and temperature. – Ann. Appl. Biol. 151: 53–59.
- Gorai, M. et al. 2006. Seed germination characteristics of *Phragmites communis*: effects of temperature and salinity. – Belg. J. Bot. 139: 78–86.
- Gulzar, S. and Khan, M. A. 2001. Seed germination of a halophytic grass *Aeluropus lagopoides*. – Ann. Bot. 87: 319–324.
- Heard, S. B. and Ancheta, J. 2011. Effects of salinity and temperature on ex situ germination of the threatened Gulf of St Lawrence Aster, *Symphotrichum laurentianum* Fernald (Nesom). – Plant Species Biol. 26: 158–162.
- Hendry, G. A. and Grime, J. P. 1993. Methods in comparative plant ecology. – Chapman and Hall.
- Hill, M. O. et al. 2004. Attributes of British and Irish plants: status, size, life history, geography and habitats. – CEH Publication Sales.
- Houle, G. et al. 2001. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianum* (Asteraceae). – Am. J. Bot. 88: 62–67.
- Huang, Z. et al. 2004a. Caryopses dormancy, germination and seedling emergence in sand, of *Leymus racemosus* (Poaceae), a perennial sand dune grass inhabiting the Junggar Basin of Xinjiang, China. – Austr. J. Bot. 52: 519–528.
- Huang, Z. et al. 2004b. Factors influencing seed dormancy and germination in sand, and seedling survival under desiccation, of *Psammochloa villosa* (Poaceae), inhabiting the moving sand dunes of Ordos, China. – Plant Soil 259: 231–241.
- Hutchings, M. J. and Russell, P. J. 1989. The seed regeneration dynamics of an emergent salt marsh. – J. Ecol. 77: 615–637.
- Jäger, E. J. (ed.) 2011. Exkursionsflora von Deutschland. Rothmaler: Gefäßpflanzen: Grundband. – Spektrum Akad. Verlag.
- Khan, M. A. and Gul, B. 1998. High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. – Int. J. Plant Sci. 159: 826–832.
- Khan, M. A. and Gulzar, S. 2003. Light, salinity, and temperature effects on the seed germination of perennial grasses. – Am. J. Bot. 90: 131–134.
- Khan, M. A. and Ungar, I. A. 1997. Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. – Am. J. Bot. 84: 279–283.
- Khan, M. A. and Ungar, I. A. 1998. Seed germination and dormancy of *Polygonum aviculare* L. influenced by salinity, temperature and gibberellic acid. – Seed Sci. Technol. 26: 107–117.
- Khan, M. A. and Ungar, I. A. 1999. Effect of salinity on seed germination of *Triglochin maritima* under various temperature regimes. – Great Basin Nat. 59: 144–150.
- Khan, M. A. and Weber, D. J. 2006. Ecophysiology of high tolerant plants. Tasks for vegetation science 40. – Springer.
- Khan, M. A. et al. 2002a. Seed germination in the Great Basin halophyte *Salsola iberica*. – Can. J. Bot. 80: 650–655.
- Khan, M. A. et al. 2002b. Seed germination in relation to salinity and temperature in *Sarcobatus vermiculatus*. – Biol. Plant. 45: 133–135.
- Li, L. and Zhang, X. 2007. Germination strategies of two halophytes in salt desert of northwestern China. – Sci. China Ser. D Earth Sci.
- Masuda, M. et al. 1999. Effects of salinity and temperature on seed germination in a Japanese endangered halophyte *Triglochin maritimum* (Juncaginaceae). – J. Plant Res. 112: 457–461.
- Partzsch, M. 2009. Zur Keimungsbiologie acht ausgewählter ephemerer Xerothermrasenarten. – Hercynia N. F. 42: 239–254.
- Pérez-Fernández, M. A. et al. 2006. Simulation of germination of pioneer species along an experimental drought gradient. – J. Environ. Biol. 27: 679–685.
- Philippoullai, J. and Ungar, I. A. 1984. The effect of seed dimorphism on the germination and survival of *Salicornia europaea* L. populations. – Am. J. Bot. 71: 542–549.
- Qu, X. et al. 2008a. Effects of temperature, light and salinity on seed germination and radicle growth of the geographically widespread halophyte scrub *Halocnemum strobilaceum*. – Ann. Bot. 101: 293–299.
- Qu, X. et al. 2008b. Effects of cold stratification, temperature, light and salinity on seed germination and radicle growth of the desert halophyte shrub, *Kalidium caspicum* (Chenopodiaceae). – Plant Growth Reg. 54: 241–248.

- Redondo, S. et al. 2004. Influences of salinity and light on germination of three *Sarcocornia* taxa with contrasted habitats. – *Aquat. Bot.* 78: 255–264.
- Schubert, R. et al. 2001. Bestimmungsbuch der Pflanzengesellschaften Deutschlands. – Spektrum Akad. Verlag.
- Stewart, A. et al. 1994. Scarce plants in Britain. – Joint Nat. Conserv. Committee, Peterborough.
- Timson, J. 1965. New method of recording germination data. – *Nature* 207: 216–217.
- Ungar, J. 1978. Halophyte seed germination. – *Bot. Rev.* 44: 233–264.
- Ungar, J. 1991. *Ecophysiology of vascular halophytes*. – CRC Press.
- Waisel, Y. 1972. *Biology of halophytes*. – Academic Press.
- Wei, Y. et al. 2007. Seed polymorphism, dormancy and germination of *Salsola affinis* (Chenopodiaceae), a dominant desert annual inhabiting the Junggar Basin of Xinjiang, China. – *Austr. J. Bot.* 55: 464–470.
- Woodell, S. R. J. 1985. Salinity and seed germination patterns in coastal plants. – *Vegetatio* 61: 223–229.
- Woodell, S. R. J. and Dale, A. 1993. Biological flora of the British isles *Armeria maritima* (Mill.) Willd. – *J. Ecol.* 81: 573–588.
- Yuichi, U. et al. 1996. The evaluation of salt tolerance during germination and vegetative growth of *Asparagus*, table beet and sea aster. – *J. Japanese Soc. Horticult. Sci.* 65: 579–585.