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Tolerance of *Holcus lanatus* and *Agrostis stolonifera* to sodium chloride in soil solution and saline spray

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Summary Inland and sea cliff populations of both *Agrostis stolonifera* L. and *Holcus lanatus* L. were subjected to soil NaCl treatments, of 100 and 200 mol m^{-3} NaCl, and tolerance examined using plant dry weight data. A parallel experiment subjected them to salt spray treatments of 2.5%, 5% and 10% NaCl in distilled water, and tolerance assessed from leaf damage.

Both populations of each species were equally sensitive to soil NaCl. When subjected to salt spray the sea cliff populations however showed marked resistance to leaf damage. Soil salinity resistance and salt spray resistance thus appear to be independent characteristics in these two species.

Introduction

A study to assess the degree of salt tolerance of coastal zone populations of *Holcus lanatus* L. and *Agrostis stolonifera* L, from an exposed sea cliff on Anglesey, North Wales, using the rooting technique (Ashraf *et al.*, unpublished data) failed to detect any differences between such populations and control populations from a non saline inland habitat. This was surprising in view of the findings of Ahmad and Wainwright[1] who, also using the rooting technique with *A. stolonifera* populations from a different coastal site, found a spray zone population to be intermediate in tolerance to NaCl between a salt marsh, and an inland population. The same order of salt tolerance was found in three species of the genus Elytrigia[10] from a salt marsh, a salt spray zone, and an inland site, in response to salt applied via the rooting medium.

It was therefore surprising that neither *A. stolonifera* nor *H. lanatus* from this exposed west facing sea cliff, which regularly receives considerable inputs of sea spray[4] exhibited any NaCl tolerance when tested in solution culture using the rooting technique.

The present work was therefore carried out to assess separately tolerance to salt spray and to soil salinity in samples of these two populations.

Materials and methods

At least 20 plants, as tillers, of *Holcus lanatus* L. and of *Agrostis stolonifera* L. were collected in July 1984 from the steep west facing sea cliff at Abraham's Bosom, Anglesey, North Wales, together with 10 soil samples per site. Because most of the plants were growing in rock crevices, soil samples had to be taken as close as possible to them wherever soil was present in sufficient quantity. The same number of plants and soil samples of the two species was collected from wild material found in the University of Liverpool Botanic Gardens, Ness, Cheshire. Single tillers were transplanted into standard potting compost in 11 cm plastic pots and grown in a glasshouse at 18°C with a 16 h photoperiod for eight months. Two experiments were then set up to run concurrently using this plant material.

1. Soil salinity experiment

In March 1985, 10 genotypes of each population were randomly selected and six tillers, selected for uniformity of size, were taken from each genotype. One tiller of each genotype was transplanted into 1600 g normal potting compost in 18 cm plastic pots. Each pot thus contained 5 tillers from 5 different genotypes of the same population. The experiment consisted of two randomized blocks, each block comprising 10 genotypes (2 pots) of each of the two populations of two species, subjected to 0, 100 or 200 mol m^{-3} of NaCl.

The plants were cut to 3 cm after seven weeks' growth and after one week, stepwise initiation of the treatments was begun by firstly adding 50 mol m^{-3} NaCl in 1/2 strength Rorison nutrient solutions with stepwise increments of 50 mol m^{-3} every other day until concentrations of 100 or 200 mol m^{-3} NaCl were reached.

The pots were placed in plastic saucers and treatment solutions applied once per week at a rate of 2 l per pot. This solution was flushed through until the electrical conductivity of the leachate which collected in the saucers was equivalent to that of the added solution. Saucers were then emptied. Because the plants were of considerable size, transpiration losses were made good by twice weekly sub-irrigation of each pot with 150 ml distilled water.

Two harvests of shoot biomass were taken after four and eight weeks. Individual plants were cut to a height of 3 cm. All the samples were oven dried at 70°C for three days, and dry weight measured. Data were transformed to percentages of control dry weight for each population of each species. For statistical treatment, these percentage data were transformed to arcsine $\sqrt{\text{percentage}}$ normalising the distribution, since percentage data tend to be distributed binomially. Analysis of variance was carried out separately for each species and each harvest.

2. Salt spray experiment

In an exactly similar experimental design, four salt spray treatments of 0, 2.5%, 5%, and 10% NaCl solution in deionised distilled water were applied to the same populations. Plants were allowed to grow for seven weeks at which time they were cut to a height of 3 cm, allowed to grow for a further week, and spray treatments begun. Spray was applied once weekly with an atomiser at a rate of 430.5 ml m^{-2}, following Humphreys[6], for three weeks. One week after this three week period, plants were clipped to a height of 3 cm. Plant leaf material was washed, dried at 70°C for three days, sorted into green and dead leaves, dried for a further 24 h, and weighed.

Plants were treated individually by turning pots onto their side, and gently spraying the foliage from the side, whilst rotating the pot. In this way contamination of the soil was minimized. During the experiment the humidity in the greenhouse was maintained between 70% and 80% to reduce evaporation of the saline solution, and hence maximise penetration.

Plants were allowed to grow untreated for a further seven days, and the treatments reapplied as before, but the frequency of spraying increased to twice per week, and treatments continued for three further weeks. After seven days, plants were cut to a height of 3 cm, washed, dried, and green and dead leaves separated as for harvest I.

Dead leaf material was expressed as a percentage of total leaf weight (living + dead) at harvests I and II. Percentage data were again transformed to arcsine values.

3. Soil chemical analysis

Electrical conductivity (EC) of the soil samples taken from the sites of origin was measured after shaking the samples for 30 min. with deionised distilled water (ratio, 1 part soil : 2.5 parts H_2O). Exchangeable Na was estimated after extraction with 1*N* ammonium acetate for 30 minutes. Na in the filtrate was estimated by flame photometry.

Results

1. Soil salinity effects

Dry weight for the different treatments expressed as percent of control treatment for each harvest are given in Fig. 1. Increasing NaCl treatments markedly inhibited growth of both of the populations of each species. At harvest I, after four weeks of soil treatments there was a

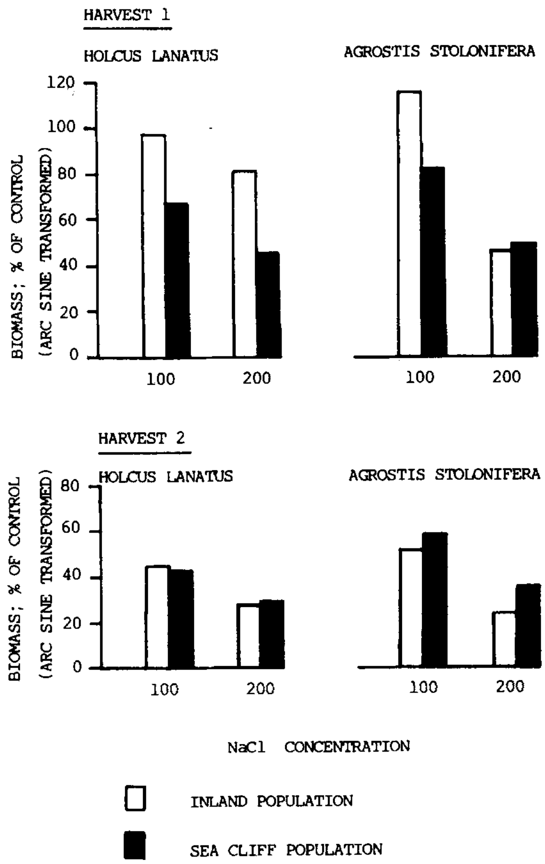

Fig. 1. The response of an inland and a sea cliff population of *Agrostis stolonifera* and *Holcus lanatus* to increasing soil NaCl concentrations (mol m^{-3}). Data presented as arcsine transformation of shoot dry weights expressed as percentage of control dry weight.

Table 1. Analyses of variance of shoot dry weight at 100 and 200 mol m^{-3} NaCl (applied to the rooting medium) of contrasting populations of *Holcus lanatus* L. and *Agrostis stolonifera* L. Data expressed as percent of dry weight of controls (grown without NaCl) and then transformed to arc sine values

Source of Variation	*Holcus lanatus* L.		*Agrostis stolonifera* L.	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
a) First harvest				
Blocks	1	2509.1 NS	1	3314.0 NS
Populations (P)	1	220700.4***	1	463.3 NS
Concentrations (C)	1	6478.7**	1	52957.0***
P × C	1	164.4 NS	1	7420.0 NS
Residual	75	791.1	75	1883.0
Total	78	1128.8	78	2644.0
b) Second harvest				
Blocks	1	81.4 NS	1	153.3 NS
Populations (P)	1	2.5 NS	1	1786.4 NS
Concentrations (C)	1	4745.0**	1	12548.0***
P × C	1	103.1 NS	1	319.3 NS
Residual	75	537.5	75	510.3
Total	78	579.1	78	678.5

**Significant at 1% level; ***significant at 0.1% level; NS not significantly different.

significant difference between populations ($p \leqslant 0.001$) in *H. lanatus*. Surprisingly the sea cliff population showed significantly greater reduction in dry weight than the inland population in both the treatments when assessed as percentage of the control treatment. Yet the soil samples from which it had been sampled had higher salinity values ($EC_{(2.5)}$ 4.7 dS m^{-1}, Na 1470 $\mu g\ g^{-1}$) than those of the control site ($EC_{(2.5)}$ 0.33 dS m^{-1}, Na 80 $\mu g\ g^{-1}$). This difference in their response to salt was not, however, maintained at harvest II taken four weeks after harvest I.

In *A. stolonifera* the populations did not differ significantly in response to salt at either harvest. There was, however, a significant ($p \leqslant 0.001$) effect of increasing NaCl concentrations.

In general there was therefore no difference between the sea cliff and inland populations of the two species when grown in soil irrigated with 100 and 200 mol m^{-3} NaCl despite the fact that this method is considered to be a good test for salt tolerance.[10]

2. Salt spray effects

There was as expected no leaf damage in the control spray treatment, and these data are therefore not considered further. The percent mortality data for the NaCl spray treatments are given in Fig. 2 and show that there was an adverse effect of salt spray treatments on both populations.

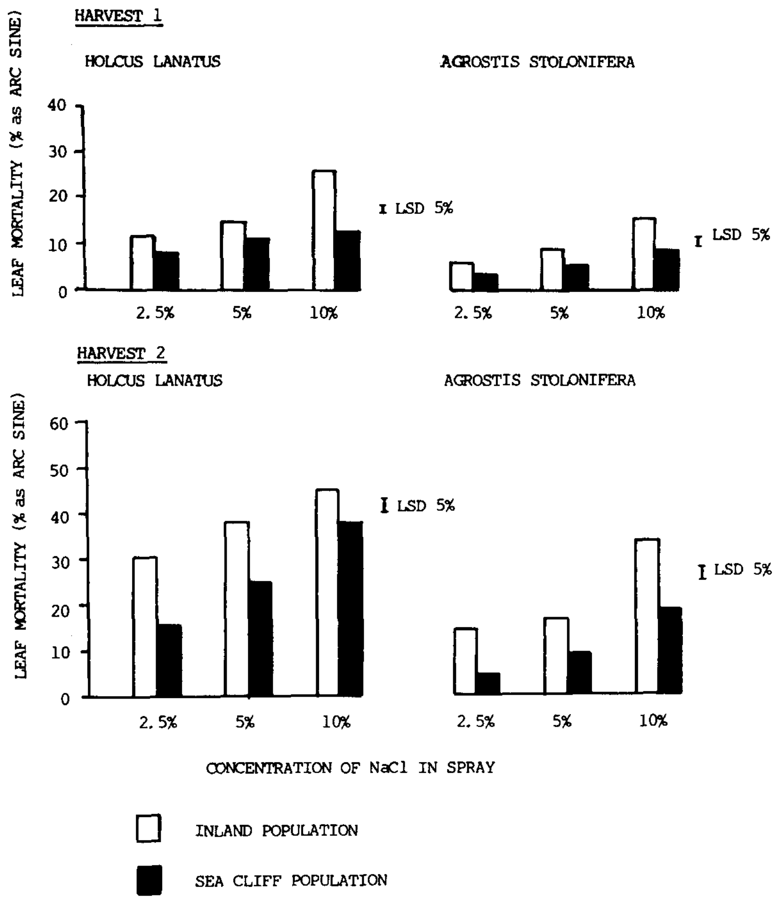

Fig. 2. Percentage leaf mortality (arcsine transformed) of one inland and one sea cliff population of *Agrostis stolonifera* and *Holcus lanatus* to three concentrations of NaCl spray, after 3 (Harvest I) and 6 weeks (Harvest II) spraying.

However the sea cliff populations of both species showed markedly less damage ($p \leqslant 0.001$) due to salt spray than the inland populations. The treatment x population interaction was significant ($p \leqslant 0.01$) in both species at both harvests, and the percent leaf mortality at harvest II was markedly higher than at harvest I in both species.

In *H. lanatus* at harvest I the percent leaf mortality of the inland population was significantly ($p \leqslant 0.05$) higher than that of the sea cliff population with all three salt spray treatments. At 10% salt spray the difference in leaf mortality between the two populations was almost 50%.

In *A. stolonifera* at harvest I there was no significant difference in percent leaf mortality between control and sea cliff populations at 2.5%

salt spray. However the 5% and 10% salt spray treatments caused a clear difference in percentage leaf mortality in both the populations, the inland population having a significantly higher leaf mortality ($p \leqslant 0.05$) than the sea cliff population.

The increased frequency of the second set of spraying treatments considerably increased the percentage leaf damage in the two population of both species. In *H. lanatus* at Harvest II, overall leaf damage in the inland popution was significantly greater than in the sea cliff population ($p \leqslant 0.05$). The data however show that as the concentration of NaCl in the treatments increased, the differences in leaf mortality between the populations became less. An almost 50% difference was observed at 2.5% salt spray between the two populations, but at 5% and 10% treatments the differences were reduced to 26% and 14% respectively.

In *A. stolonifera* at harvest II the difference in percentage leaf mortality between the inland and sea cliff populations was significant ($p \leqslant 0.05$) and consistent across all treatments, the sea cliff population having lower percentage leaf mortality values.

The data (Fig. 2) also clearly show that there was a considerable difference between the species in salt spray tolerance. The sea cliff populations of *A. stolonifera* showed a higher resistance to salt spray than *H. lanatus* from the same site.

Nonetheless both the spray zone populations of *Holcus lanatus* and

Table 2. Analyses of variance of percent leaf mortality (arcsine values) in *Holcus lanatus* L. and *Agrostis stolonifera* L. sprayed with three levels of NaCl

Source of variation	*Holcus lanatus* L.		*Agrostis stolonifera* L.	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
a) First harvest				
Blocks	1	548.65***	1	22.33 NS
Populations (P)	1	5883.49***	1	2209.52***
Concentrations (C)	2	3812.89***	2	2266.35***
P × C	2	1234.77***	2	300.33**
Residual	113	47.71	113	32.39
Total	118	181.10	118	93.25
b) Second harvest				
Blocks	1	67.8 NS	1	596.48*
Populations (P)	1	16146.8***	1	13851.59***
Concentrations (C)	2	13284.6***	2	12086.43***
P × C	2	675.0**	2	533.07**
Residual	113	124.7	113	90.47
Total	118	429.9	118	417.92

*Significant at 5% level; **Significant at 1% level; ***Significant at 0.1% level; NS not significantly different.

Agrostis stolonifera were markedly more resistant to salt spray damage than the inland populations of these species.

Discussion

The coastal populations of *A. stolonifera* and *H. lanatus* did not show any significant difference in response compared to their inland populations when grown in soil salinized with NaCl. These similar responses were confirmed by their root growth in solution culture where they again did not differ (Ashraf, unpublished data). These results are however in contrast to those of Ahmad and Wainwright[1] and Rozema *et al.*[10]. Yet the coastal populations had remarkably greater resistance to salt spray. Spray resistance has been reported for populations of other species from salt spray affected habitats[2,6,7,8].

It is therefore amply clear that tolerance to salt spray and tolerance to salt in the rooting zone are completely distinct characters. The lack of tolerance to salt in the rooting zone of these cliff populations must however be explained. It seems most likely that, despite receiving considerable amounts of salt spray throughout the year[6] the cliff face is so steep[2] and there is so little soil cover, that incoming salt could readily be removed from the soil by percolating or surface water runoff. There is in fact a great deal of fresh water percolating down the cliff face coming from further inland. It is therefore likely that the populations are rooting into effectively non saline soil in crevices on the cliff face despite the salinity of the surface soil samples collected from the sea cliff. Since this is a particular characteristic of the site it may well be possible to find populations elsewhere combining both spray and soil salt tolerance, especially since tolerance to salt in solution culture has already been shown in *Agrostis stolonifera*[1].

The difference in salt spray tolerance between the cliff populations of the two species is interesting. *A. stolonifera* is markedly more tolerant of the high NaCl concentrations than *H. lanatus*. The two species grow intermixed at the site, and so have similar exposure to salt spray. The cliff face populations of *A. stolonifera* at Abraham's Bosom have very small leaves[2] and populations from a similar spray zone habitat have pronounced epicuticular wax depositions[1] . By contrast *H. lanatus* plants have considerably longer and broader leaves, and hence may retain considerably greater amounts of salt spray than the *A. stolonifera* plants, leading to greater salt spray damage. But this does not explain the reason for their coexistence in the salt spray zone. Further work is necessary to determine the reasons for this difference between the two species.

Since salt spray tolerance and soil salinity tolerance are two independent characters, the term aero-halophytes, defined by Rozema *et al.*[9], would seem to be the appropriate term for describing the coastal populations which have been described in this paper.

References

1 Ahmad I and Wainwright S J 1977 Tolerance to salt, partial anaerobiosis, and osmotic stress in *Agrostis stolonifera*. New Phytol. 79, 605–612.
2 Aston J L and Bradshaw A D 1966 Evolution in closely adjacent plant populations. II. *Agrostis stolonifera* in maritime habitats.Heredity 21, 649–664.
3 Boyce S G 1954 The salt spray community. Ecol. Monograph 24, 29–67.
4 Goldsmith F B 1973 The vegetation of exposed sea cliffs at South Stack Anglesey. II. Experimental studies. J. Ecol. 61, 819–829.
5 Hewitt E J 1966 Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureau. Technical Communication No. 22. 2nd Edition.
6 Humphreys M O 1981 Response to salt spray in red fescue and perennial ryegrass. *In* Proc. Intl. Turfgrass Res. Conf. Ed. R W Sheard. American Soc. of Agron., Madison, WI.
7 Humphreys M O 1982 The genetic basis of tolerance to salt spray in populations of *Festuca rubra* L. New Phytol. 91, 287–296.
8 Oosting H J and Billings W D 1942 Factors affecting vegetational zonation on coastal dunes. Ecology 23, 131–142.
9 Rozema J, Bijl F, Dueck T and Wesselman H 1982 Salt-spray stimulated growth in strand line species. Physiol. Plant. 56, 204–210.
10 Rozema J, Vanmanen Y, Vugts H F and Leusink A 1983 Airborne and soilborne salinity and the distribution of coastal and inland species of the genus Elytrigia. Acta Bot. Neerl. 32, 447–456.