Effect of Bracteoles on Seed Germination and Dispersal of Two Species of Atriplex

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The influence of bracteoles on the germination response of a salt marsh annual, Atriplex prostrata, and a salt desert perennial, A. griffithii, was determined. Attached bracteoles did not inhibit germination of A. prostrata but completely inhibited germination of A. griffithii seeds. Germination of seeds of A. griffithii was also somewhat inhibited in the presence of detached bracteoles. The osmotic potential of dissolved substances in water-saturated bracteoles was $-4.8 \pm 0.05$ MPa for A. griffithii and $-2.4 \pm 0.15$ MPa for A. prostrata. Ash content of bracteoles on a dry weight basis was $28.7 \pm 0.37$% for A. prostrata and $30.0 \pm 0.29$% for A. griffithii. The presence of relatively high concentrations of dissolved salts in bracteoles may explain the reduced germination of A. griffithii. Germination of seeds of the perennial A. griffithii may also be inhibited mechanically by the presence of persistent bracteoles, whereas the annual A. prostrata has ephemeral bracteoles that may serve to limit germination in the autumn but are not present during the normal spring germination period. Bracteoles could affect the dispersal of seeds by water since some fruiting structures of A. griffithii and A. prostrata are able to float in 1.0% NaCl solutions for 5 and > 30 d, respectively, compared with a maximum of 4 and 3 d for seeds without bracteoles. Fruiting structures of A. prostrata floated for longer over a range of salinities than did those of A. griffithii; this may play a significant role in the dispersal of this salt marsh species.

Key words: Atriplex, bracteoles, dispersal, flotation, germination, salinity, temperature.

INTRODUCTION

Bracteoles on fruiting structures in Atriplex species may serve to control the timing of seed germination and also aid the wind or water dispersal of seeds (Osmond et al., 1980). Inhibition of seed germination of Atriplex spp. by bracteoles has been reported in the literature (Beadle, 1952; Kadman-Zahavi, 1953; Osman and Ghassali, 1997). Inhibition of germination could occur through a number of different pathways: induction of a light requirement for germination, mechanical inhibition, osmotic stress, specific ion effects, negative effects of growth regulator substances, and the production of allelopathic compounds (Beadle, 1952; Koller, 1957; Cresswell and Grime, 1981; Giusti and Grau, 1983; Aiazzi and Argüello, 1992). Bracteoles of Atriplex inflata Mull. contained as much as 31% NaCl on a dry weight basis or 0.9 M NaCl in saturated bracteoles (Beadle, 1952). Beadle (1952) concluded that the high ionic content of bracteoles inhibited germination and therefore protected seeds from unsuccessful establishment during periods when they were exposed to drought stress.

Sankary and Barbour (1972) reported that bracteoles probably did not contain inhibitors, and their presence was not responsible for a reduction in seed germination in Atriplex polycarpa S. Wats. In contrast, Osman and Ghassali (1997) determined that removal of the fruiting bracteoles of Atriplex halimus L. increased germination from 35 to 98%, but no significant inhibition of germination was found when soluble bracteole leachates were tested. Giusti and Grau (1983) reported that bracteoles produced a water-soluble substance that inhibited germination of A. cordobensis Gandoger and Stuckert seeds. Fruiting structures of Atriplex repanda Phil. accumulated 10% saponins and these substances could cause a significant reduction in germination percentages of both A. repanda and A. semibaccata R. Br. seeds (Fernandez et al., 1985, 1986). Another possibility reported by Beadle (1952) is that ion accumulation in bracteoles can retard seed germination, since soaking bracteoles for 24 h and removing excess salts increased germination in A. spongiosa Muell. from 6% in uns soaked to 100% in soaked and leached fruiting structures. Soaking of bracteoles and leaching did not increase germination of Atriplex patula var. hastata L. seeds, but this treatment did stimulate germination in two other species: A. lentiformis (Torn.) S. Wats. and A. semibaccata (Young et al., 1980). Binet (1965) and Aiazzi and Argüello (1992) concluded that the chief inhibitory action of bracteoles in A. tornabeni Gandoger and Stucker and A. cordobensis was mechanical. In contrast, Springfield (1970) determined that the bracteoles of Atriplex canescens (Pursh.) Nutt. did not inhibit germination.

In this investigation we compare the effects of bracteoles on germination of two species: Atriplex prostrata Bouchier, an annual salt marsh species, and Atriplex griffithii Moq. var. stocksii Boiss, a perennial salt desert species. It is possible that differences in germination response in the presence of bracteoles of Atriplex spp. is related to the habit...
and longevity of the species. Bracteoles are not usually present during the spring germination period of salt marsh Atriplex species, having decayed in the period between seed dispersal in the autumn and seed germination the following spring. We hypothesize that salt marsh species may be less affected by bracteoles, which are often ephemeral and not fused in these species, than are salt desert species that tend to have more persistent, fused bracteoles that may inhibit germination by some mechanical, allelopathic, or osmotic mechanism. The significance of bracteoles in the dispersal of seeds will be considered in flotation experiments. Because of its broad distribution in coastal marshes of North America and Europe, we hypothesize that seeds of the salt marsh species Atriplex prostrata will have a longer flotation period than those of the salt desert species Atriplex griffithii.

**MATERIALS AND METHODS**

Seeds (utricles) of Atriplex griffithii were collected during autumn 1994 from salt flats situated on the University of Karachi campus, Karachi, Pakistan. Atriplex prostrata seeds were collected in autumn 1998 at the Rittman salt marsh, Rittman, Ohio, USA. Germination tests were carried out in 50 × 9 mm (Gelman No. 7232) tight-fitting Petri dishes with 5 ml of test solution, and each dish was placed in a 10 cm diameter plastic Petri dish as an added precaution to avoid water loss from dishes by evaporation. Four replicates of 25 seeds each were used for each of the treatments and we considered seeds to have germinated after radicle emergence.

**Germination**

Seeds of A. griffithii and A. prostrata were germinated for 30 d in incubators with a 12 h photoperiod (Sylvania cool white fluorescent lamps, 25 µmol m⁻² s⁻¹, 400–700 nm) and 12 h thermoperiods (dark : light) of 5 : 15, 5 : 25 and 15 : 25°C. The three seed treatments were a control with seeds and attached bracteoles, seeds in the presence of detached bracteoles, and seeds with bracteoles removed. To determine whether bracteoles of A. griffithii limit germination, seeds with attached bracteoles were germinated for 20 d at 15 : 25°C (dark : light) before bracteoles were removed and seeds were left for another 20 d. To test the possibility that leachable soluble inhibitors present in bracteoles of A. griffithii prevent seeds from germinating, seeds with attached bracteoles were soaked in 100 ml of distilled water for 1, 24 and 48 h. Germination was then monitored for a 30 d period. The aqueous extracts were diluted from full strength 1.5 g bracteoles per 100 ml distilled water to 0.50, 0.25 and 0.125 strength, and seeds were germinated in these solutions.

**Flotation**

The floating capacity of seeds and dispersal units (seeds + bracteoles) of A. griffithii and A. prostrata was determined by using five replicates containing five seeds of each species. Five seeds or dispersal units were placed in individual beakers containing 100 ml 1-0 % NaCl solution to mimic inland salt marsh water and shaken on a platform shaker for 20 d to determine how many seeds or dispersal units remained afloat. Another flotation test was run using the above procedure to determine the effect of salt treatment (1.5, 3.0, 6.0 and 12.0 % NaCl) on the flotation of fruiting structures, because salinities higher than that of seawater may occur in salt marshes and salt deserts.

**Bracteole water and salt status**

The ash content of bracteoles was determined by placing dried bracteoles in a crucible and heating them in a muffle furnace for 24 h at 450°C. Aqueous extracts were made by placing the bracteoles in boiling water (100°C) for 1 h. Aqueous extracts were cooled at room temperature, filtered and centrifuged, and an estimate of the osmotic potential of soluble substances in the extracts was determined with a Wescor model 5520 vapour pressure osmometer, which measures the osmolarity of solutions.

**Statistical analysis**

A two-way analysis of variance (ANOVA) was used to determine if significant differences occurred among treatment means in the bract, soaking, and water leachate experiments. One-way ANOVA was used to identify significant differences among treatment means for the bracteole water, ash, and salt status data. If significant differences occurred among treatments, a Bonferroni post hoc test was used to determine if there were significant differences between individual treatments (Sokal and Rohlf, 1995).

**RESULTS**

**Germination**

A two-way ANOVA indicated that both the presence of bracteoles (F = 14.18, P < 0.001) and temperature treatment (F = 212.78, P < 0.001) significantly affected germination; there was a significant interaction (F = 20.2, P < 0.001) because of reduced germination in the bracteole treatment at low temperature. Seeds of Atriplex prostrata were not inhibited by the presence of bracteoles in the 5 : 25°C and 15 : 25°C treatments, and over 90 % of seeds germinated in all three bracteole treatments (i.e. bracteoles attached, bracteoles present, bracteoles absent) (Fig. 1B). At the suboptimal temperature 5 : 15°C, germination was generally lower and inhibited by the presence of bracteoles. There was no significant effect (F = 1.28, P = 0.29) of soaking time on the germination of A. prostrata seeds. Soaking seeds with attached bracteoles for 1, 24 and 48 h completely alleviated the inhibitory effect of bracteoles on germination of A. prostrata seeds at the low temperature regime (F = 0.68, P = 0.51) and there was no significant interaction between temperature and soaking time (F = 0.44, P = 0.78) (Fig. 1A).

Germination of A. griffithii seeds with attached bracteoles was not significantly affected (F = 1.61, P = 0.219) by soaking for 1, 24, or 48 h, or by temperature (F = 0.68, P = 0.51) and there was no significant interaction between
temperature and soaking time ($F = 0.44, P = 0.78$) (Fig. 2A). Full strength aqueous extracts were inhibitory to seed germination at the three temperature regimes, averaging 7% germination compared to 32% germination in the quarter-strength treatment at 15 × 25°C (Fig. 3). A two-way ANOVA indicated a significant ($F = 15.38, P < 0.001$) effect of extract strength, but the effect of temperature ($F = 0.61, P = 0.55$) and the interaction
between extract strength and temperature were not significant ($F = 1.17, P = 0.34$).

Two-way ANOVA showed that both the presence of bracteoles ($F = 105.80, P < 0.001$) and temperature treatment ($F = 6.92, P = 0.004$) significantly affected germination of *A. griffithii* seeds, but there was no significant interaction between these factors ($F = 2.02, P = 0.12$). Germination was strongly inhibited in all temperature regimes when bracteoles were attached (Fig. 2B). Temperature did not significantly affect germination of *A. griffithii* seeds in treatments when bracteoles were removed.

After a 20 d germination period at $15 \times 25^\circ C$, only $3.0 \pm 1.9\%$ of the *A. griffithii* seeds with attached bracteoles had germinated. Removal of the bracteoles released the seeds from dormancy and after 1 d $19.5 \pm 5.1\%$ of the seeds had germinated; at the end of the 20 d germination period this percentage had increased to $76.0 \pm 9.1\%$ (Fig. 4), which was significantly different ($F = 125.875, P = < 0.001$) to the percentage germination in the 20 d pretreatment with bracteoles.

**Flotation**

Seeds of both species with bracteoles removed floated for less than 5 d in 1.0 % NaCl, whereas fruiting structures of *A. griffithii* (76.5 ± 15.0 %) and *A. prostrata* (92 ± 5.5 %) remained afloat for 5 d. After 10 d, 0.0 % of *A. griffithii* and 24 ± 7.0 % of *A. prostrata* fruiting structures were still floating in 1.0 % NaCl. Fruiting structures of *A. prostrata* remained afloat for longer than did those of *A. griffithii* at higher salinities. This was especially apparent in the 3.0 % NaCl treatment in which 35 % of *A. prostrata* and 0 % of *A. griffithii* fruiting structures remained afloat after 30 d (Table 1). Fewer fruiting structures remained afloat at lower salinities than at higher salinities (Table 1). All of the *A. prostrata* and 50 % of *A. griffithii* fruiting structures were still floating after 30 d in the 10 % NaCl treatment in comparison to 25 and 0 %, respectively, in the 1.5 % NaCl treatment (Table 1).

**Bracteole water and salt status**

Mean dry weights of bracteoles were significantly different for *A. griffithii* (3.08 ± 0.05 mg) and *A. prostrata* (0.68 ± 0.05 mg) ($F = 1326.7, P = < 0.001$) (Table 2). The percentage water content of saturated bracteoles was 70 % for *Atriplex griffithii* and 80 % for *Atriplex prostrata*. Ash content of bracteoles was significantly different for *A. griffithii* (30.0 ± 0.58 %) and *A. prostrata* (28.7 ± 0.37 %) ($F = 7.31, P = 0.035$). The osmotic potential of dissolved substances in water-saturated bracteoles was $-4.8 \pm 0.05$ MPa for *A. griffithii* and $-2.4 \pm 0.15$ MPa for *A. prostrata*; these values were significantly different ($F = 16.37, P = 0.007$) (Table 2).

**DISCUSSION**

The two *Atriplex* spp., *A. prostrata* (a salt marsh species with ephemeral bracteoles) and *A. griffithii* (a salt desert species with persistent bracteoles), showed different germination responses when their bracteoles were attached to seeds. Germination of *Atriplex prostrata* seeds was not inhibited by the presence of attached or detached bracteoles at higher temperatures but showed some bracteole x temperature inhibitory interaction at the lowest temperature regime. It has been suggested that bracteoles inhibit germination via a number of different pathways, which include mechanical inhibition of germination, allelopathy, osmotic effects, specific ion effects, and change in the red:far-red ratio of incident radiation (Koller, 1957; Young et al., 1980; Cresswell and Grime, 1981; Giusti and Grau, 1983; Aiazzi and Argüello, 1992).

Intact fruits of *A. griffithii* are dormant, and their bracteoles inhibited germination in this investigation: less
Bracteoles, followed by their removal, stimulated germina-
tion; this may be because the seeds are mechani-

cally inhibited from germinating by fused bracteoles. The

germation of seeds when bracteoles were removed, whereas the more dilute leachate solutions were

inhibited germination, and Giusti and Grau (1983) reported that an organic inhibitor, probably ABA, was present in the bracteoles. However, Aiazzi and Argüello (1992) reported that soaking fruiting structures in water did not promote seed germination, that bracteoles of *A. cordobensis* formed a mechanical barrier to water uptake, and that they required scarification to enhance germination. A similar mechanical effect was shown by Binet (1965) for *A. tornabeni* Tin. He concluded that only in less humid environments would an osmotic effect be significant, since salts in the bracteoles (12.7 %) on a dry weight basis) would be leached out in high rainfall habitats. Ash content of bracteoles of *A. griethii* was 30.0 % and that of *A. prostrata* was 28.7 ± 0.37 %, which was in the same range found for the salt desert species *A. dimorphostegia* (27–6 %) (Koller, 1957).

**Table 1. Percentage (+ s.e.) of fruiting structures of *Atriplex griethii* (Ag) and *A. prostrata* (Ap) that remained afloat in a range of saline solutions over a 30 d period.**

<table>
<thead>
<tr>
<th>Days</th>
<th>NaCl (%)</th>
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<tbody>
<tr>
<td>5</td>
<td>5 100 5 100</td>
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<tr>
<td>10</td>
<td>5 100 5 100</td>
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<tr>
<td>15</td>
<td>5 100 5 100</td>
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<tr>
<td>20</td>
<td>5 100 5 100</td>
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<td>25</td>
<td>5 100 5 100</td>
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<td>30</td>
<td>5 100 5 100</td>
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**Table 2. Mean dry weight, ash content, saturated water content and osmolarity of extracted sap in bracteoles of *Atriplex griethii* and *A. prostrata.*

<table>
<thead>
<tr>
<th>Atriplex griethii</th>
<th>Atriplex prostrata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight (mg)</td>
<td>3.08 ± 0.05</td>
</tr>
<tr>
<td>Ash content (%)</td>
<td>30.0 ± 0.58</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>68.4 ± 0.94</td>
</tr>
<tr>
<td>Osmotic potential (MPa)</td>
<td>-4.8 ± 0.05*</td>
</tr>
</tbody>
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Data are means ± s.e. *P < 0.05.

Seeds of *A. dimorphostegia* Kar. et Kir. in intact dispersal units did not germinate (Koller, 1957). The ash content of these bracteoles was 27.6 % of their air dry weight. Osmotic potentials of extracts from bracteoles of *A. dimorphostegia* averaged −0.6 MPa, which was sufficiently high to inhibit germination. Koller (1957) concluded that salts or inhibitory substances would be leached by precipitation, allowing seeds to germinate and providing sufficient moisture for plant growth. Soluble salt content of bracteoles of *A. cordobensis* was not considered sufficiently high to inhibit germination, and Giusti and Grau (1983) reported that an organic inhibitor, probably ABA, was present in the bracteoles. However, Aiazzi and Argüello (1992) reported that soaking fruiting structures in water did not promote seed germination, that bracteoles of *A. cordobensis* formed a mechanical barrier to water uptake, and that they required scarification to enhance germination. A similar mechanical effect was shown by Binet (1965) for *A. tornabeni* Tin. He concluded that only in less humid environments would an osmotic effect be significant, since salts in the bracteoles (12.7 % on a dry weight basis) would be leached out in high rainfall habitats. Ash content of bracteoles of *A. griethii* was 30.0 % and that of *A. prostrata* was 28.7 ± 0.37 %, which was in the same range found for the salt desert species *A. dimorphostegia* (27–6 %) (Koller, 1957). The osmotic potential of saturated bracteole extracts was higher in *A. prostrata* (−2.4 MPa) than in *A. griethii* (−4.8 MPa) because the former species imbied a greater amount of water when their bracteoles were saturated with water.

Beadle (1952) found that three *Atriplex* spp. (*vesicaria* Heward, *inflata* Muell., and *spongiosa*) showed 95–100 % germination when bracteoles were attached. He showed that bracteoles contained high concentrations of NaCl but that soaking dispersal units for 24 h removed the inhibitory effect of bracteoles on germination. It has been suggested that the bracteoles maintain dormancy in seeds until conditions are favourable for germination (Beadle, 1952). Bracteoles may serve to maintain a bet-hedging strategy in some *Atriplex* spp. such as *A. prostrata* and *A. griethii*, determining the season and controlling the timing of germination. If all of the seeds germinated simultaneously in the unpredictable salt marsh or salt desert environments,
populations could go locally extinct whenever flooding occurred or salt stress increased during the period of seedling development (Ungar, 1987; Egan and Ungar, 1999).

Results for A. prostrata indicate that the presence of bracteoles may inhibit germination of seeds under the lower temperatures in late autumn and winter but they would not be inhibitory under warmer temperature regimes. Since A. prostrata is a summer annual and its seedlings are not frost tolerant, the presence of bracteoles in the autumn may serve to maintain dormancy in seeds before they are exposed to the freezing temperatures of winter. We have not observed bracteoles on seeds during the spring germination period, indicating that they probably begin decaying sometime between October–December (Ungar and Khan, unpubl. obs.). Binet (1965) also reported that the bracteoles of A. tornabeni were not present on seeds during the spring germination period in French coastal salt marshes.

Cresswell and Grime (1981) suggested that the presence of green bracteoles around seeds in A. hastata or photosynthetic fruiting structures in other species can alter the red: far-red ratio perceived by seeds and may be significant in inducing a light requirement for germination. In a seed burial experiment, Khan and Ungar (1986) showed that small black seeds of A. prostrata are more dormant in the soil than are the larger brown seeds. Since both seed types are produced with attached bracteoles, the influence of bracteoles may be differential, or exposure to cold stratification in the winter may have replaced the light requirement more readily in the large seed morph than in the small seed morph of A. prostrata. Baskin and Baskin (1998) reported that stratification can sometimes replace the light requirement in dormant seeds of some plant species. Bracteoles may play a significant role in the dispersal of Atriplex seeds. Osmond et al. (1980) indicated that, in arid shrubland, Atriplex spp. bracteoles could possibly aid wind dispersal. Bracteoles may also be significant in determining the distribution of species through the flotation properties of dispersal units of salt marsh populations of Atriplex spp. (Gustafsson, 1973), especially if narrowly-limited species have short flotation times and widely-distributed species such as A. glabriscula and A. triangularis have long flotation times. Gustafsson (1973) showed that flotation times of Atriplex species varied from up to 24 d for 71% of the dispersal units in A. glabriscula Edmonston to 0% in 10 d for the more narrowly-distributed A. calotheca (Rafn.) Rafn. and Fries. However, he concluded that even though there was a correlation between the breadth of distribution and flotation time of dispersal units, this factor alone could not explain the cosmopolitan distribution of A. triangularis Willd. He suggested that human factors could have played a significant role in the widespread distribution of this species. Atriplex griffithii dispersal units are relatively heavy (5-6 mg) compared to those of A. prostrata (3-0 mg) and remained floating in 1% NaCl for only 5 d compared to >30 d for the latter species. The fruiting structures of A. griffithii may play a more significant role in determining the timing of germination than in the dispersal of diaspores. Thirty-five percent of the fruiting structures of A. prostrata remained floating in 3% NaCl after 30 d compared to 0% for fruiting structures of A. griffithii, and their capacity to float for an extended period of time could play a significant role in the dispersal of diaspores in water as was suggested by Gustafsson (1973). Seeds of both species with their bracteoles removed remained floating for a shorter period of time in 10% NaCl than did seeds enclosed in bracteoles (4 and 3 d for seeds and 5 and >30 d for fruiting structures of A. griffithii and A. prostrata, respectively).

Beadle (1952) indicated that bracteoles of salt desert species in Australia had high salt content, which could osmotically inhibit the germination of seeds contained in the bracteoles because of the low water potentials produced. This would inhibit germination until sufficient precipitation occurred to leach the soluble salts from bracteoles. Bracteoles of both A. griffithii and A. prostrata may play a significant role in determining the timing of seed germination. Only under low temperature conditions were bracteole-enclosed seeds of A. prostrata inhibited from germinating, and leaching of fruiting structures completely alleviated the effect of bracteoles. The inhibition of germination by the persistent bracteoles of A. griffithii is related to a combination of morphological and physiological factors. A soaking pretreatment of the dormant A. griffithii fruiting structures could have removed an osmotic or allelopathic inhibitor substance, which then permitted seeds to germinate when bracteoles were removed.

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LITERATURE CITED


