

Seed Germination of Rigid Ryegrass (*Lolium rigidum*) and Sterile Oat (*Avena sterilis*) under Water Salinity Conditions at Constant or Alternating Temperatures

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RIGID ryegrass (*Lolium rigidum*) and sterile oat (*Avena sterilis*) are well known grasses in many parts of the world. They grow in both grasslands and arable habitats and both are recorded as serious weeds of arable lands in many countries worldwide. Seed germination of both species was tested against four levels of water salinity (0, 100, 300, 500mM/L of NaCl) and five different temperature regimes, constant at 8°C or alternating temperatures at 6/14, 8/16, 10/18 and 10/20°C, all in dark/light photoperiods of 8/16h, respectively. *A. sterilis* seeds generally showed higher germination rates than *L. rigidum* in all tested temperature regimes. Germination was very high in both species at alternating 10/20°C, and in *A. sterilis* also at 10/18°C, with nearly 40% in both. *A. sterilis* seeds also showed higher germination than *L. rigidum* at a constant temperature of 8°C, the latter favoring alternating temperatures. *A. sterilis* seeds resisted water salinity as high as 100 mM/L of NaCl and germinated at about 25%, compared to *L. rigidum* seed, which, while also showing some resistance to 100mM/L of NaCl only germinated at a rate of about 20%. Both species gave poor germination at higher levels of water salinity of 300 and 500mM/L of NaCl. Results confirmed that germination of seeds of both species favoured moderate alternating temperatures and showed some resistance to water salinity, which may indicate how both species can invade and persist in arable lands especially in Mediterranean climates and subtropics conditions.

Keywords: Germination, Temperature, Water salinity, *Avena sterilis*, *Lolium rigidum*.

Introduction

Avena sterilis L (Sterile oat) is one the most widespread, common and harmful to arable crop weed species of Mediterranean climates (Damanakis, 1983 and Castellanos-Frias, 2014). It is also widespread across the whole of Europe (Tutin et al., 1980), India (Balyn et al., 1991), North America (Carlson & Hill, 1985) and Australia (Torner et al., 1984). Chaudhary (1989) confirmed the presence of this species as a serious weed of cereals in Saudi Arabia. *Lolium rigidum* Gaudin (Annual ryegrass) is also considered a major weed in Mediterranean climatic regions worldwide (Monaghan, 1980; Gracia Baudin, 1982; Jauzein & Montegut, 1983 and Recasens et al., 1997). Chaudhary (1989) confirmed that *L. rigidum* is the most serious narrow-leaf weed in cereal fields in Saudi Arabia. It has been also recorded in a check list of weed flora in Saudi Arabia (Elghazali & Alsoqeer, 2013). Germination is a crucial stage in the life cycle of the plant (Khan & Gulzar, 2003). Temperature affects the percentage and rate of germination, a crucial stage in the plant life cycle,

through its effect on seed deterioration, loss of dormancy and the germination process itself (Roberts, 1988), moreover, temperature fluctuations can stimulate seed germination (Thompson & Grime, 1983). Temperature, light, water and salinity are all thought to interact at the soil-atmosphere interface to regulate seed germination (Xue et al., 2012), but soil temperature and salinity are the most important factors controlling germination in the saline soils of arid and semi-arid regions (Khan & Unger, 1999). Salinity generally causes a reduction in germination rates and delays germination and it is both in soil or water a major stress factor limiting seed germination (Shannon, 1998 and Ozdener & Kutbay, 2008). This paper investigates the effect of constant and fluctuating temperatures and a range of salinity concentrations on seed germination on two very well-known and destructive grass weed species, *Avena sativa* and *Lolium rigidum*. Since germination determines abundance, understanding the biological factors which affect it could contribute to both the prediction and control of expected populations.

Materials and Methods

Seeds of sterile oat (*Avena sterilis*) and Annual ryegrass (*Lolium rigidum*) were collected in the late spring of 2014 from a wheat farm in the Aljouf Area of northern Saudi Arabia. Seeds were kept in sealed glass jars and refrigerated at 4°C to maintain viability. Experiments were carried out in five incubators maintained at the five temperature regimes under investigation (Fig. 1). Temperatures were: Constant at 8°C and alternating at 8/16, 10/18, 6/14 and 10/20°C. The lower temperature period lasted 8h and seeds were kept in the dark, the higher lasted 16h during which seeds were in the light. The same photo period regime (8/16h dark/light) was also applied to the constant temperature regime. Salt solution was prepared at four concentrations: 0, 100, 300, 500mM/L NaCl. Seeds were placed in a double layers of filter paper in sterilized petri dishes, with 20 seeds/petri dish. 10ml of GA₃ solution at 200ppm was added to the seeds of both species at the beginning of the experiment to stimulate germination and break seed dormancy where it existed. Samples were monitored daily for 60 days to maintain moisture levels and to record germination. The experiment followed a block randomized design with four replicates of each treatment. Total germination was analyzed using GenStat (GENSTAT Version 18, VSN International, Hemel Hempstead, UK), germination responses to temperature and salinity were assessed with an ANOVA. Means were compared *post hoc* by a least significant difference (LSD) multiple comparison test.



Fig. 1. Germination test procedure where (A) Seeds dispersal within petri dishes, (B) Allocation of petri dishes in the incubators.

Results

Results are based upon the total number, cumulatively, of germinated seeds at the end of the trial period.

Overall germination by species

Across all trial conditions combined, significantly higher ($P \leq 0.01$) percentages of *A. sterilis* seeds germinated than of *L. rigidum*, as shown in Fig. 2, suggesting a better response from *A. sterilis* to the various factors under investigation. However, overall, germination in both species was low, likely because of an absence of response to some water salinity concentrations and some temperatures regimes under investigation, in both species.

Effect of temperatures on seeds germination

A. sterilis showed higher germination at all temperatures compared to *L. rigidum*, with maximum germination at alternating temperatures of 10/18°C and 10/20°C of about 40%. *L. rigidum* showed its highest germination at 10/20°C but only at about 25% and this was the only germination rate above that achieved at constant 8°C. Both species favoured higher temperatures, of 10/20°C, for germination, with lower and not significantly different responses at 8, 6/14 and 8/18°C. Both species showed higher germination at constant 8°C than at 8/18°C. Figure 3 shows germination responses of both species to the different temperatures tested.

Effect of water salinity on seed germination

Water salinity inhibited germination of both species significantly, even at the lowest tested NaCl concentration of 100mM/L. Germination at 100mM/L NaCl was low, but neither species germinated at 300 or 500mM/L. Figure 4 illustrates the germination responses of both species at different concentrations of water salinity, approximately 20% of *L. rigidum* seeds germinated at 100mM/L of NaCl, compared to 25% of *A. sterilis* and in the control (NaCl absent) approx. 30% of *L. rigidum* germinated compared to 100% of *A. sterilis*.

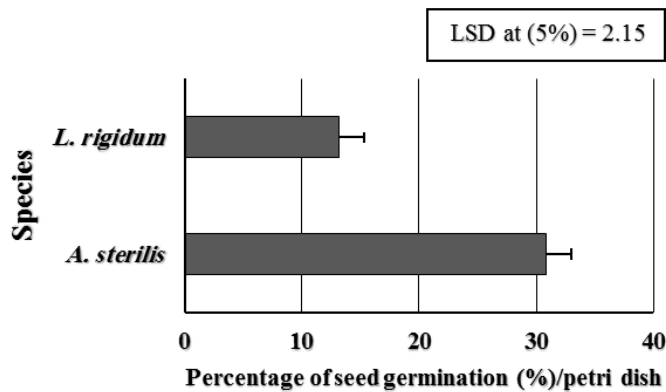


Fig. 2. Overall seed germination responses (%) of the two tested species. LSD: Least significant difference at 5 %.

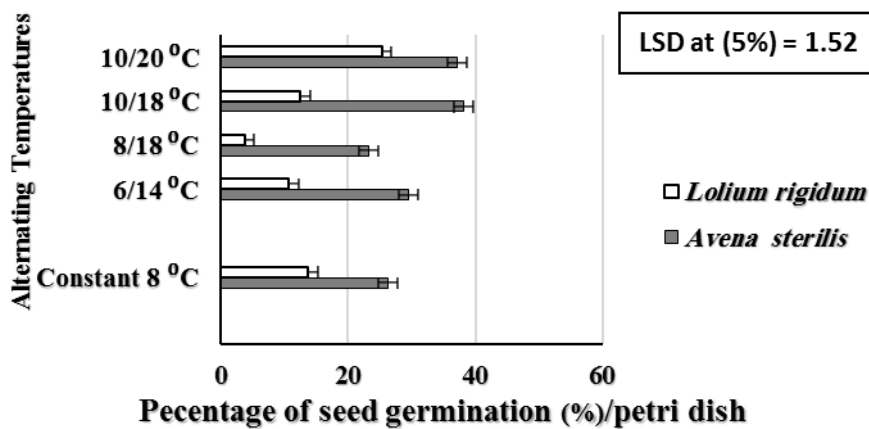


Fig. 3. Seed germination responses of the two species to different temperatures. LSD: Least significant difference at 5 %.

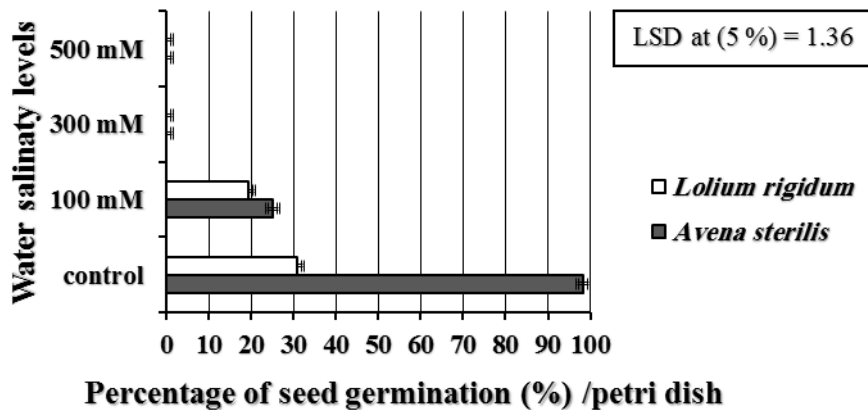


Fig. 4. Germination responses of the two species to different water salinity levels. LSD: Least significant difference at 5 %.

Effect of interactions between temperature and water salinity on seed germination

Statistical analysis showed limited interaction between temperature and salinity. However, while salt reduced germination significantly in both species at all temperatures, compared to no salt,

A. sterilis showed moderate germination with 100mM/L of salt at both higher temperatures of 10/18 and 10/20°C, and *L. rigidum* also responded, even with 100mM/L NaCl, at 10/20°C. Table 1 shows the percentage of germination at each temperature/salinity interaction.

TABLE 1. Effect of interaction between temperature and water salinity level on the mean percentage (%) of germinated seeds of *A. sterilis* and *L. rigidum*/petri dish.

Temperature Regimes (°C for 8/16h dark/light periods)	<i>A. sterilis</i>		<i>L. rigidum</i>	
	Water salinity (mM/L NaCl)		Water salinity (mM/L NaCl)	
	0	100	0	100
8	20.00	1.00	6.00	5.00
6/14	20.00	3.50	5.75	2.75
8/16	18.25	0.25	2.50	0.00
10/18	20.00	10.50	5.00	4.25
10/20	20.00	9.75	11.50	7.25

LSD: Least significant difference at 5% (0.05) 3.04

Discussion

Higher germination occurred in *Avena sterilis* than *Lolium rigidum*, although both seed samples were collected simultaneously from the same location and kept in the same storage conditions. Variation in germination, under the experimental conditions, can, therefore, be attributed to natural variation between the two species in seed structure and dormancy characteristics. Both species germinated at all the experimental temperatures, demonstrating their ability to germinate in a wide range of natural climatic conditions. However, alternating temperatures appeared to have a more significant effect in warmer conditions in both species, though constant temperatures were only trialled at a single temperature in this study. Germination was consistently higher at higher temperature regimes, also confirming an adaptation to germination at higher temperatures in these species. Seeds of many species have respond differently to a variety of amplitudes of temperature fluctuation (Thompson & Grime, 1983). Castellanos-Frias et al. (2014) reported *A. sterilis* germination to be highly adapted to a wide range of temperatures, related to its worldwide distribution. *L. rigidum* is also reported to be adapted to a broad range of temperatures between 12 and 30°C for germination (Turner et al., 2001). The germination responses to temperature of both species are in line with the pattern of winter climate in Saudi Arabia, which can, therefore, be used to predict both the amount and time of seed germination in these species.

However, lower germination rates of both species at some temperatures were found to occur in saline conditions, especially at high concentrations of 300 and 500mM/L of NaCl. Results confirmed the sensitivity of both species to

water salinity, with low germination at 100mM/L of NaCl and poor to absent germination at higher concentrations. Reduced germination attributed to high salinity has been described by numerous authors (Al-Taisan,, 2010). The findings in this study are confirmed by Chauhan et al. (2006), who reported a decrease in germination of *L. rigidum* seeds by 50% above 40mM/L of NaCl and Watt (1983) who reported low germination of *Lolium perenne* with increasing salinity in soil or water. Several authors have reported the changes in osmotic potentials caused by NaCl to be implicated in lower imbibition by seeds and, therefore, lower germination (Almansouri et al., 2001). Welbaum et al. (1990) and Faheed et al. (2005) report generally that salinity stress limits seed germination through osmotic effects. The inhibitory effects of NaCl on seed germination have been attributed to its direct effect on embryo growth (Al-Taisan,, 2010) and Poljakoff-Mayber et al. (1994) also reported that embryo elongation was strongly inhibited by high levels of NaCl in irrigation solutions. Seedling growth was inhibited by NaCl in wheat seedlings germinated *in vivo*, with development of the coleoptile most affected through a reduction in the rate of respiration with increasing NaCl concentrations (Moud & Maghsoudi, 2008).

The ability of both species to resist moderately salty conditions, could reflect their adaptation to a wider range of temperatures. This correlation is supported by Chauhan et al. (2006) who reported some germination of *L. rigidum* seeds even when watered with a solution at 160mM/L of NaCl. A high degree of adaptation in both species to a wide range of temperatures and a degree of water salinity explains their presence in a wide range of natural Saudi climatic conditions. The favoured

temperatures for the seed germination of both species dominate in Aljouf region northern Saudi Arabia in the winter; 4.2/18.6°C, especially from mid-November to mid-March (Climate report for winter, 2017). Results confirmed that germination of seeds of both species favoured moderate alternating temperatures and showed some resistance to water salinity, which may indicate how both species can be invasive and persistent in arable croplands, and why they are reported as serious weedy species especially in Mediterranean climates and under subtropical conditions. This should be considered in strategies for their control, which should aim to limit the ability of these species to produce large volumes of seeds resulting in large populations.

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انبات بذور الراي الصلب و الشوفان البري في ظروف ملوحة الماء عند درجات الحرارة الثابتة والمتغيرة

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اعشاب الراي الصلب والشوفان البري هي اعشاب تنمو في اجزاء مختلفة من العالم سواء في البيئات النباتية البرية أو المزروعة وقد سجلت كأعشاب ضارة في المناصق الزراعية في العديد من دول العالم. تم في هذه الدراسة اختبار قدرة بذور الراي الصلب والشوفان البري بعد ترطيبها بمحلول حامض الجلبرين بتركيز 200 جزء في المليون لمدة 48 ساعة على الانبات عند ترطيبها لمدة 60 يوم باحدى تراكيز ملوحة ماء (صفر - 100 - 300 - 500) ملمول/لتر من محلول كلوريد الصوديوم وذلك في خمس مستويات من درجات الحرارة (ثابتة عند 8 درجات مئوية او متغيرة خلال الأربع وعشرين ساعة: 14/6 و 16/8 و 18/10 و 20/10) مع فترة ظلام لمدة 8 ساعات خلال فترة درجة الحرارة الدنيا و 16 ساعة إضاءة خلال فترة درجة الحرارة العليا وبنفس النظام 8 درجات ظلام و 16 إضاءة مع درجة الحرارة الثابتة.

بشكل عام كان انبات بذور الشوفان أفضل بمستوى معنوية عالية ($P < 0.001$) من بذور الراي في مختلف المعاملات التي تم تطبيقها في الدراسة حيث كان متوسط نسبة انبات بذور الشوفان البري 30% بينما كان متوسط نسبة انبات بذور الراي الصلب 15%. كذلك كان انبات بذور الشوفان أعلى معنويا من انبات بذور الراي في كل مستويات درجة الحرارة الثابتة أو المتغيرة. كلا بذور النوعين أنبتت بأعلى مستوى انبات لها عند درجة حرارة تبادلية 20/10 درجة مئوية حيث كان متوسط نسبة انبات بذور الشوفان البري 40% (8 بذور من 20 بذرة لكل طبق بتري) بينما كان متوسط انبات بذور الراي الصلب 25% (5 بذور من 20 بذرة لكل طبق بتري) ما يعني تكيفها مع درجة الحرارة المعتدلة نسبيا. كما اظهرت بذور الشوفان البرية قدرة أفضل من بذور الراي الصلب على الانبات في ظروف درجة الحرارة الثابتة 8 درجات مئوية حيث أنبتت بنسبة 25% لكن بذور الراي الصلب أنبتت بنسبة أقل مما يعني حاجتها للتغير في درجات الحرارة كأحد عوامل تحفيز الانبات. في ظروف ملوحة الماء أظهرت بذور الشوفان تحمل جيد للملوحة حيث أنبتت بنسبة 50% عند ترطيبها بمحلول كلوريد الصوديوم بتركيز 100ملمول/لتر في درجة حرارة تبادلية 20/10 درجة مئوية بينما أنبتت بذور الراي الصلب بنسبة 35% عند ترطيبها بمحلول كلوريد الصوديوم بتركيز 100ملمول/لتر في درجة حرارة تبادلية 20/10 درجة مئوية. انبات بذور النوعين كان ضعيفا جدا عند ترطيبها بمحلول كلوريد الصوديوم بتركيز 300ملمول/لتر أو 500ملمول/لتر وذلك في مختلف درجات الحرارة التي طبقت. هذه النتائج تؤكد قدرة جيدة لبذور النوعين على التكيف مع مدى واسع من درجات الحرارة المعتدلة و على قدرة انبات جيدة في ظروف ملوحة المياه. كما تفسر النتائج أسباب إنتشار هذين النوعين من الأعشاب الضارة في المناطق المزروعة في مناطق مناخ البحر الأبيض المتوسط والمناطق تحت الإستوائية وكذلك إنتشارها في مناطق بيئات المياه المالحة. كما تساعد هذه النتائج على فهم الخصائص البيولوجية لبذور هذين النوعين مما يساعد على مكافحتها وأخذ ذلك في الإعتبار عند إختيار طرق المكافحة.