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Al Farsi, K., Cameron, R.W., Hitchmough, J. et al. (2016) Assessing the effects of drought and temperature on the establishment of *Juniperus seravschanica* saplings in Northern Oman. *Sibbaldia: the Journal of Botanic Garden Horticulture*, 14. pp. 37-53. ISSN: 2513-9231

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STUDENT PROJECT: ASSESSING THE EFFECTS OF DROUGHT AND TEMPERATURE ON THE ESTABLISHMENT OF *JUNIPERUS SERAVSCHANICA* SAPLINGS IN NORTHERN OMAN

Khalid Al Farsi,¹ Ross Cameron,² James Hitchmough³ & Darach Lupton⁴

ABSTRACT

Climate change poses a serious threat to the survival and distribution of *Juniperus seravschanica* in the northern mountains of Oman. A better understanding of this species' responses to environmental changes is essential if the potentially harmful effects of climate change are to be mitigated. One such step is to understand how changes in climate may influence the growth of juniper saplings. Two- and five-year-old saplings were grown under different temperature and watering regimes to determine effects on establishment and growth. Under an optimum growing temperature, reducing water to 50 per cent and 25 per cent of the optimal irrigation regime significantly decreased the growth of juniper saplings. In field studies, saplings re-introduced to three different altitudinal locations showed varying rates in establishment success and growth. Both two-year-old and five-year-old saplings established better at higher altitude. Overall, survival rates were considerably better with the transplantation of five-year-old rather than two-year-old saplings. Applying irrigation improved the survival of two-year-old stock when grown at the lowest altitude, but rates were not always significantly different from other treatments. Apical extension growth was found to be reduced at higher altitude, indicating that temperature influences the growth of juniper saplings. However, it was the combination of drought and high temperatures that reduced the growth of non-irrigated saplings at lower altitudes. These preliminary results suggest there is potential to artificially re-introduce juniper saplings to their natural habitat as part of a conservation programme, but more time is required to judge the success of the transplanting initiative when dealing with slow-growing trees such as juniper.

INTRODUCTION

Juniperus seravschanica (synonym *Juniperus excelsa* subsp. *polycarpus*) is a key component of woodland above 2,000m above sea level (a.s.l.) in the northern mountains of Oman. It is a vital element of Oman's endemic flora and the only coniferous species found in Oman. *Juniperus* trees are restricted to the highest areas of the central massif of Jabal al Akhdar and the outlying mountains of Jabal Qubal and Jabal Kawr. Cool climate and higher precipitation at the higher altitudes encourage the presence of a range

1. Khalid Al Farsi is a Senior Horticulturist at Oman Botanic Garden and a PhD student at the Department of Landscape in the University of Sheffield.

Address: Oman Botanic Garden, Diwan of Royal Court, PO Box 808, PC 122, Muscat, Sultanate of Oman.

Email: khalid.alfarsi@omanbotanicgarden.om, kaaalfarsi1@sheffield.ac.uk

2. Dr Ross Cameron is a Senior Lecturer at the Department of Landscape in the University of Sheffield.

Address: Department of Landscape, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK.

3. Prof. James Hitchmough is Head of Department at the Department of Landscape in the University of Sheffield.

Address: as above.

4. Dr Darach Lupton is a Senior Botanist at Oman Botanic Garden.

Address: Oman Botanic Garden, Diwan of Royal Court, PO Box 808, PC 122, Muscat, Sultanate of Oman.

of native species not found elsewhere in Arabia, including *J. seravschanica* (Ghazanfar, 1991). Within the Hajar mountains, juniper trees can be found from 2,000m a.s.l. upwards to the peak of Jabal Shams at 3,009m a.s.l. (Gardner and Fisher, 1996). As it is the dominant species at higher altitude, this species provides shade and a source of food for birds, mammals and invertebrates. It is important in the region's ethnobotany, and local people use juniper foliage as a remedy for stomach complaints and diabetes. Resins collected from the trees are usually burned as incense (Matwani, 2011). Previous studies have indicated a decline in *Juniperus* populations below 2,400m, whereas the populations above this critical line appear to have a healthier status (Fisher & Gardner, 1995; Gardner & Fisher, 1996; MacLaren, 2016). The decline of existing juniper woodlands is being exacerbated by low regeneration, with only a few young trees being evident in these locations (Fig. 1). Climate change has been reported as a potential reason for this decline, especially at lower altitudes, although the precise effects of changing temperature and precipitation patterns on plant viability are not clear. Recently, MacLaren (2016) stated that healthy juniper trees predominate only above 2,600m above sea level; below this point, healthy trees are restricted to sheltered, shallow depressions where, presumably, trees are less stressed.

On Jabal Al Akhdar, Al Sarmi and Washington (2011) noted an increase in mean winter temperatures of 0.85°C per decade and a decrease in annual precipitation of 67mm per decade between 1980 and 2008. Al-Kalbani *et al.* (2015) described an increase in annual average temperature of 0.27°C per decade from 1979 to 2012, and a reduction in annual rain of 9.42mm per decade for the same period. These indicate the area is undergoing a significant shift in climate patterns, with lower rainfall and higher mean temperature becoming typical. Forest mortality triggered by drought and warm temperature as a consequence of climate change has been reported in many areas and appears to be increasing across the globe in recent decades (Allen *et al.*, 2010).

The shortage of research combining both field and controlled environment studies has tended to lead to insufficient information on the actual mechanisms underpinning drought-induced mortality of young trees in *in vivo* conditions. In addition, there is a degree of uncertainty when trying to predict how much moisture loss young seedling trees will tolerate, when cross-referencing information from controlled environments to more complex field situations (McDowell *et al.*, 2013). Factors such as wind, solar intensity and capillary action of soil are likely to have a large influence on plant establishment in the field; however their actions are not always easy to replicate or predict under controlled environmental conditions. Often, in reality, a combination of controlled studies and field evaluations helps provide more confidence in determining appropriate plant management programmes.

In situ habitats are the optimum locations for the natural regeneration of wild plant species and populations (McNaughton, 1989). However, it is vital that the habitat is protected from disturbance. Where required, control of grazing and human disturbance should be the first step in a conservation programme to ensure that the habitat is suitable



Fig. 1 Health condition of *J. seravschanica* at different altitudes in the northern mountains of Oman. Top: completely dead tree at the lower altitude (2,100m a.s.l.). Middle: stressed tree with signs of dieback symptoms (2,300m a.s.l.). Bottom: healthy tree growing at a higher altitude (2,600m a.s.l.). Photos: Khalid Al Farsi.

for re-introduction. For some species such as *Juniperus*, the natural germination and establishment of new plants is very low (Menges, 2008). Al Haddabi and Victor (2016) indicated lower natural regeneration of *J. seravschanica* in the northern mountains of Oman, with small seedlings (aged between six months and one year) representing only 9 per cent of juniper woodland. They also indicated lower germination success, 31 per cent maximum, of juniper seeds under laboratory conditions. Supplemental planting is essential to establishing or re-establishing plant populations, especially when the limitation is due to the lack or absence of regeneration (Godefroid *et al.*, 2011). Re-introducing threatened plants to their natural habitat has been widely used as a method to restore habitat loss and increase plant population in distributed areas (Maunder, 1992; Dalrymple *et al.*, 2011). Understanding why regeneration in a given population has stopped is the key to effective conservation efforts.

The study presented here aims to investigate the influence of climate change through a better understanding of the effects of temperature and water availability on the growth and establishment of *J. seravschanica* saplings. Experiments were carried out in both controlled and field conditions to assess the factors that affected plant viability. Finally, the research aims to determine the potential for re-establishment of *Juniperus* populations in the northern mountains of Oman, through the re-introduction of nursery-raised plants.

MATERIALS AND METHODS

Experiment 1: heat and drought tolerance assessment of five-year-old saplings under controlled environment conditions

A controlled environment experiment was conducted at Oman Botanic Garden (OBG) nursery. Juniper seeds used to germinate these saplings were collected from the middle altitudinal range of the Oman mountains, 2,339m a.s.l. (N 23°06'31.24, E 57°39'06.96). Seeds were propagated in 2008 and seedlings were grown under cooled glasshouse conditions at OBG. Five-year-old juniper saplings grown at OBG were placed in three environmentally controlled glasshouses. Glasshouses were set at three different temperatures: low (18–20°C), medium (22–25°C) and high (28–30°C). Preliminary assessments indicated that the weekly irrigation requirements for the juniper saplings was found to be 2.5L of water per plant per week, i.e. replacing all the water lost from evapotranspiration. Saplings at the different temperature conditions were subjected to three irrigation regimes based on a proportion of this optimal irrigation volume per plant, i.e. 100 per cent (2.5L wk⁻¹), 50 per cent (1.4L wk⁻¹) and 25 per cent (0.7L wk⁻¹) irrigation. Saplings were placed in each glasshouse to acclimatise to the different temperatures for one month before commencement of the various irrigation regimes. The experiment started in December 2014 and continued for 12 months. Plant height (apical growth) increments were measured every two months.

FIELD ESTABLISHMENT OF TWO- AND FIVE-YEAR-OLD JUNIPER SAPLINGS

Study site

These experiments were conducted in Jabal Shams (sun mountain) which is part of the Western Hajar mountain range in northern Oman. At 3,009m a.s.l., Jabal Shams is the highest mountain in Oman. The climate is classified as arid to semi-arid, with the temperature decreasing with increasing altitude. The annual mean temperature at the top of the mountain is c. 11°C. The temperature drops to below 0°C during winter and the average temperature during summer is c. 20°C. Rain is scattered throughout the year with two main seasons, February to April and August to September. The annual precipitation is between 250 and 350mm yr⁻¹. The catchment of water after rain is considered to be low, due to both the steep topography of the mountain and the relatively sparse vegetation cover. Most of the water flows rapidly down the mountain through deep wadis and ravines. The geology and soil of the mountain consists of very hard tertiary limestone covered in some parts with a thin layer of gravel. Deep gravels and sandy soils can be found in bowls, deep valleys and runnels.

Site selection and pre-planting practices

Seed used in these experiments were selected from plant populations in the middle altitudinal zone of the Oman mountains. Juniper cones used for the propagation of five-year-old saplings were collected in April 2007 from Saiq Plateau in Jabal Al Akhdar at 2,339m a.s.l. (N 23°06'31.24, E 57°39'06.96). Seeds were cleaned at Oman Botanic Garden and stored in the seed bank until propagation in 2008. For the new (two-year-old) stock seeds were collected in May 2013 from Hayl Al Jwari in Jabal Shams at 2,300m a.s.l. (N 23°18'25.23, E 57°06'22.97).

In January 2014, three planting sites were selected in Jabal Shams (Fig. 2). Sites were selected based on altitudinal range (low, mid and high), site security and suitability for planting. Site 1 is located at 2,220m a.s.l. (N 23°17'33.4 and E 57°09'07.76); this site has public access. Site 2 is located at 2,300m a.s.l. (N 23°18'08.19 and E 57°06'14.25); site entry is restricted to locals and is controlled by guards. Site 3 is located at 2,570m a.s.l. (N 23°19'13.26 and E 57°06'16.02); site entry is prohibited and special permission to enter the site is required.

In each site, three plots of 100m² (10x10m) were enclosed using steel poles and wire fence. Large rocks and scrubby grasses were removed from each plot. At the time the fence was constructed, 15 planting holes (50cm in diameter and 50cm deep) were dug in each plot (Fig. 3). All three sites are located in areas with no electrical outlets or access to water. Water was delivered by truck and stored in tanks placed close to the plots. Irrigation was applied manually and water rates were measured using a hose-mounted water meter.

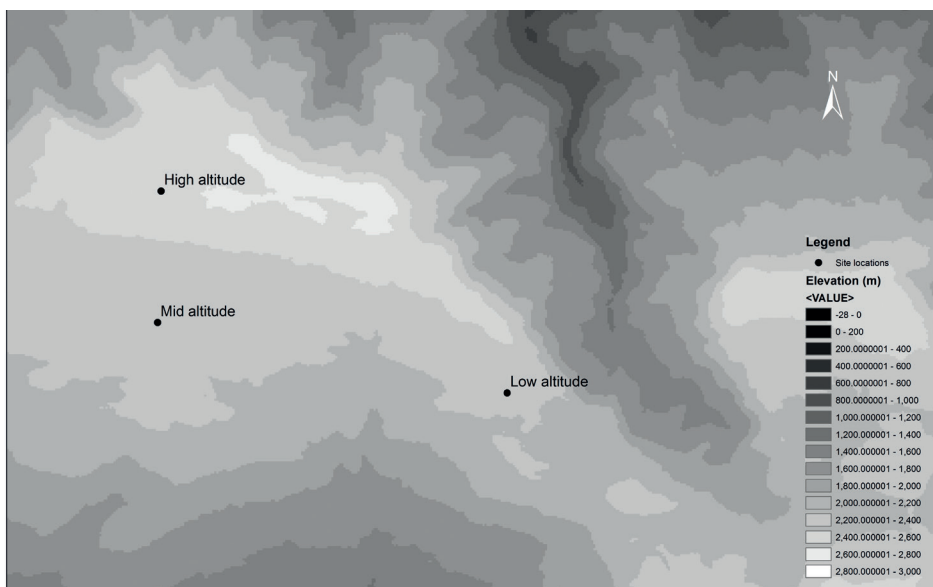


Fig. 2 The locations of the three sites selected for *J. seravschanica* re-establishment in the northern mountains.

Experiment 2: the planting and establishment of five-year-old juniper saplings in the field

On 25 March 2014, five-year-old juniper saplings were transported to the experimental plots on the mountain. In order to provide a transition zone between nursery soil and natural soil, coarse peat was mixed with habitat soil at 1:1 ratio and then used to fill in the holes. A total of 135 five-year-old juniper saplings were planted across the three altitudinal sites (Fig. 3). A raised soil rim was prepared around the saplings to keep irrigation water close to the roots and avoid run-off down the slope. During the first five months after planting, saplings were given uniform water across treatments/altitudes to establish them in the ground, although the volume applied was progressively reduced to encourage deeper rooting into the soil profile and less reliance on the artificial irrigation. Five saplings died after planting, and these were replaced with new saplings in May 2014.

From September 2014, juniper saplings at the three different altitudes were subjected to three different water regimes in each location. These were zero irrigation, irrigation (10L) added every 15 days and irrigation (10L) every 30 days. At each of the three altitudes, saplings were planted in three blocks with five replications per water treatment. Increments in height were measured once every two months.

Experiment 3: planting and establishment of two-year-old saplings in the field

In April 2015, two-year-old juniper saplings grown from seed in OBG were planted in the three altitude sites. Again, a total of 135 individual saplings were planted in all sites; saplings



Fig. 3 Planting of five-year-old juniper saplings. Top: sapling placed near the planting hole prior to planting. Bottom: juniper saplings planted at lower altitude in a fenced plot. Photos: Khalid Al Farsi.

were covered with shade net to protect them from direct sun (Fig. 4). The establishment procedures employed with the five-year-old saplings were repeated for this experiment. Starting from September 2015, saplings were subjected to the three water regimes as described for the five-year-old saplings experiment although in this case saplings in the two irrigation regimes were given 2L water each, at the different time intervals (i.e. a smaller volume of water compared to the five-year-old specimens, but a volume proportional to their smaller size). At each of the three altitudes, saplings were planted in three blocks with five replications per water treatment. Survival percentages of living saplings were calculated.

STATISTICAL ANALYSIS

The nursery experiment tested the effects of two factors on the growth of saplings, with temperature as the main factor and irrigation as the secondary factor, and five replications per treatment. Saplings were simply randomised; no blocking was used as the plants were grown under homogeneous conditions. Latin square arrangement type was used to eliminate any edge effect. Randomised complete block design (RCBD) was used in the field establishment experiment.

Using SPSS software (version 21), a general linear model (GLM) was used to indicate the effect of independent factors in sapling growth. Data were tested for normal distribution and equality of variance; where these assumptions were not met, data were transformed to the square root value before being analysed. Fisher's least significant difference (LSD) was used to show the differences between means. The LSD value was calculated according to Williams & Abdi (2010) as follows:

$$\text{LSD} = t_{v,\alpha} \sqrt{2 MS/n}$$

where $t_{v,\alpha}$ is the t critical distribution associated with degree of freedom at α level, MS is the mean square of the error and n is number of observations used to calculate the mean at each level.

RESULTS

Experiment 1: heat and drought tolerance assessment of five-year-old saplings under controlled environment conditions

Plants survived under the glasshouse conditions, irrespective of the treatments imposed. The growth of *Juniperus* saplings, however, were strongly affected by irrigation regime, with lower volumes of water corresponding to significantly less shoot growth (Table 1, Fig. 5). In most cases the growth in the 100 per cent watering regime resulted in twice as much shoot extension as either of the two lower (drought) regimes. There was no clear effect of temperature, with plants in the middle temperature range having marginally (and non-significantly) better growth than either the low or the high temperature regimes.



Fig. 4 Planting of two-year-old saplings. Top: 15 juniper saplings were planted in each plot. Bottom: planting plots were covered by green shade to protect saplings from direct sun. Photos: Khalid Al Farsi.

Temperature	Low			Mid			High		
	100%	50%	25%	100%	50%	25%	100%	50%	25%
December to March	45.4	8.8	20.4	36.4	16.8	17.6	35.4	5.6	4.7
March to May	14.4	4.2	1.6	25.4	7.0	2.4	16.0	3.2	1.7
May to July	10.0	4.2	1.2	28.2	6.6	2.8	8.6	2.2	0.3
July to September	27.6	3.4	1.8	17.6	7.6	3.2	10.4	3.0	0
September to November	10.2	4.0	0	15.0	3.0	2.6	16.2	0.4	0
One year	107.6	24.6	25	122.6	41	28.6	86.6	14.4	6.7

Table 1 Juniper saplings height increment (mm) under low, medium and high temperature; three water regimes (100 per cent, 50 per cent or 25 per cent of ET_p) were applied.

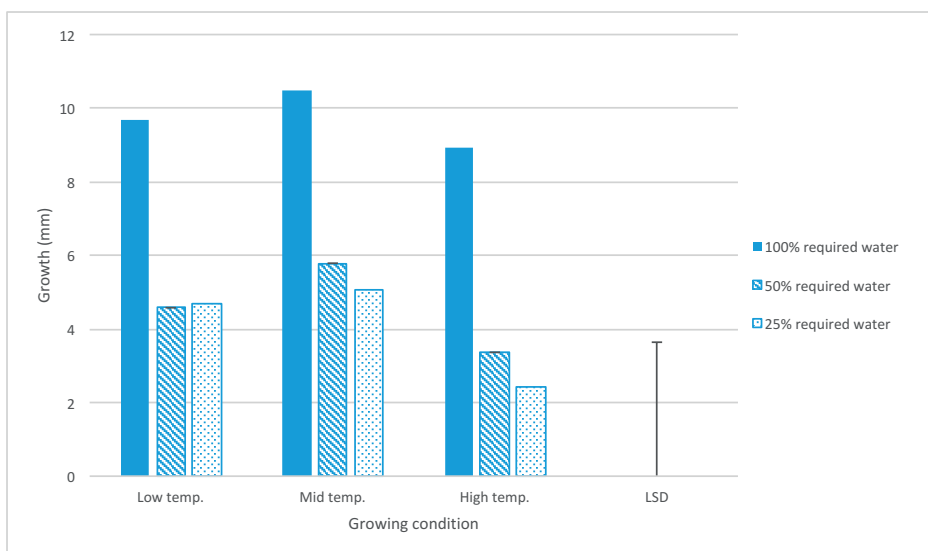


Fig. 5 Height increase in five-year-old juniper saplings growing under different temperature and water regimes. Data were square root transformed for statistical analysis ($n=5$, $LSD_{(0.05, DF 34)} = 3.64$).

Experiment 2: planting and establishment of five-year-old juniper saplings in the field

The first year's data showed a high survival rate of the five-year-old juniper saplings planted in all field sites (Fig. 6). Saplings planted in the high-altitude plots had a higher survival rate (not significant) than those at the lower and middle altitudes.

For plant height, saplings planted above 2,500m a.s.l. had lower apical growth than those planted at 2,200m and 2,300m a.s.l, but differences due to altitude were not significant overall (Fig. 7). A significant effect of no watering (drought) was evident on sapling growth but only at the lower altitude. In this location, saplings that had not

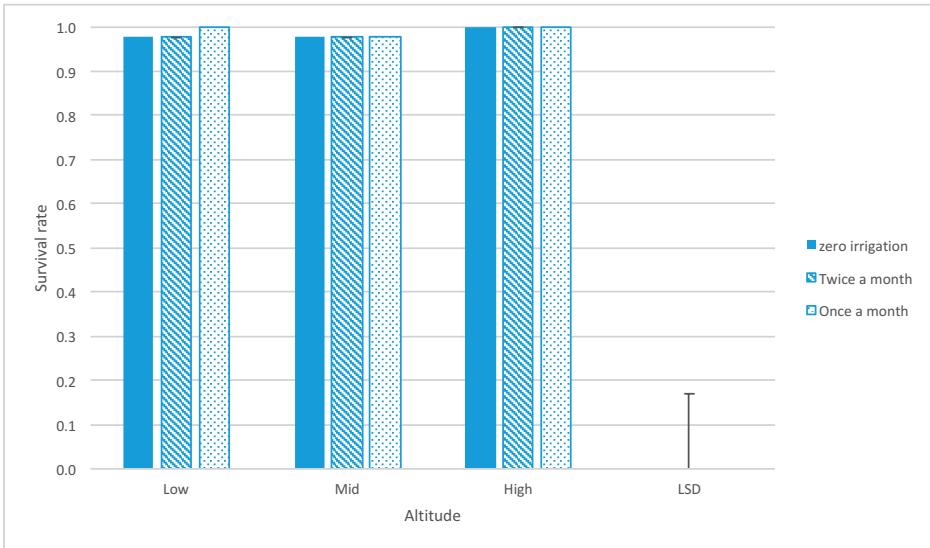


Fig. 6 Survival rate of five-year-old saplings re-established in the northern mountains of Oman at different altitude and water regimes for statistical analysis (n=3, $LSD_{(0.05, DF 18)} = 0.17$).

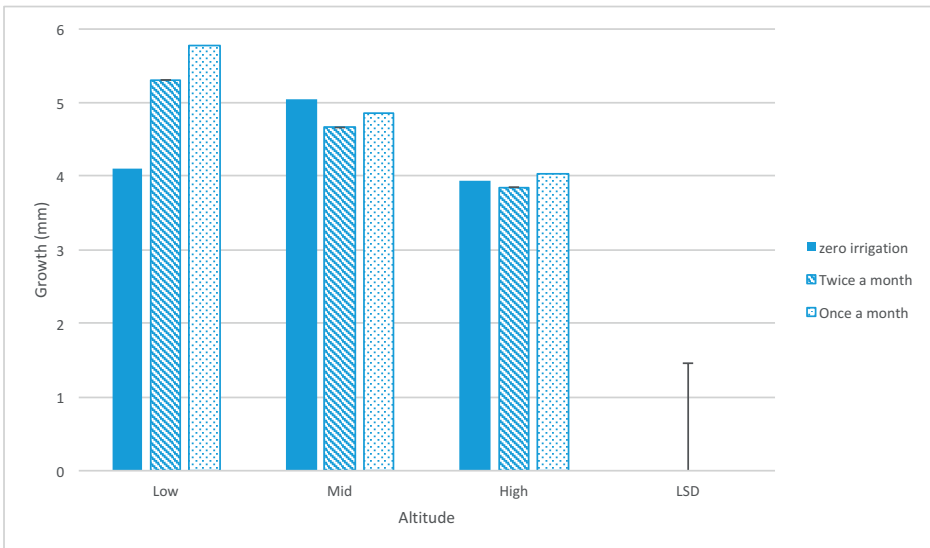


Fig. 7 Height increase in five-year-old saplings re-established in the northern mountains of Oman at different altitude and water regimes. Data were square root transformed for statistical analysis (n=15, $LSD_{(0.01, DF 120)} = 1.46$).

received irrigation for one year showed significantly lower growth compared to the other irrigation treatments ($p < 0.01$). However, there was no significant effect of water treatment on sapling growth at the middle and higher altitudes.

Experiment 3: planting and establishment of two-year-old saplings in the field

The survival rate of the two-year-old saplings was significantly greater overall at higher altitude ($p < 0.05$). The mortality of saplings at lower and middle altitudes was high, reaching 40 per cent in some instances (Fig. 8). The effect of water treatment on survival was observed at the lower altitude, where those saplings that were irrigated twice a month had a higher survival rate than both other irrigation treatments, with differences with the non-watered control verging on significant (Fig. 8).

DISCUSSION

Heat and drought effect in five-year-old juniper saplings under controlled conditions

Abiotic stress such as heat and prolonged drought are the main factors that affect tree growth and establishment (Wang *et al.*, 2003). Results from this study indicate that water shortage (drought stress) reduced the growth of juniper saplings under controlled conditions, irrespective of varying temperature. Reduction in shoot extension can be seen as one of a number of adaptation responses to limited water availability. Water shortage in the soil reduces water absorption by the roots, which affects water potential, cell

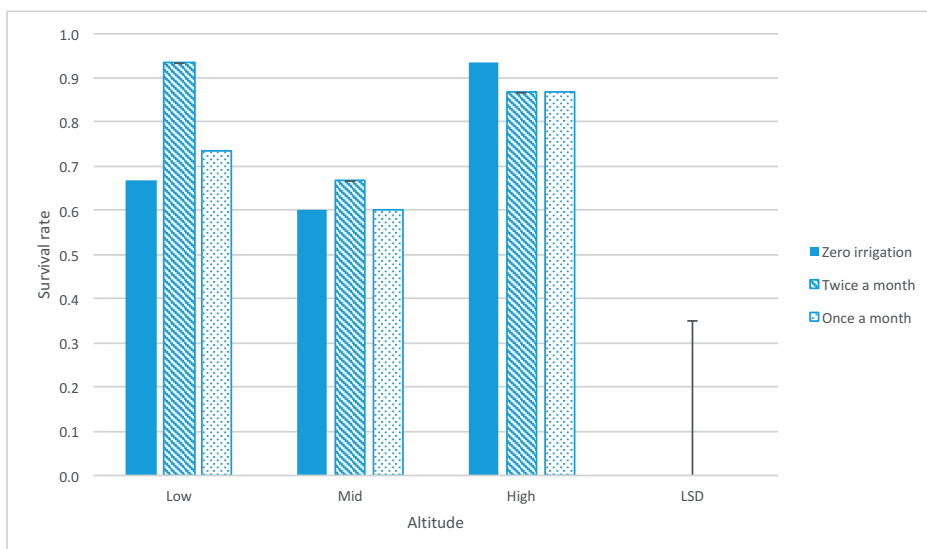


Fig. 8 Survival rate of two-year-old saplings established in the northern mountains of Oman at different altitude and water regimes for statistical analysis ($n=3$, $LSD_{(0.05, DF 18)} = 0.35$).

turgidity and stomatal behaviour (Clark *et al.*, 2005), and leads to a reduction in stem elongation (Lipiec *et al.*, 2013). Zhao *et al.* (2013) found that temperature can affect the carbon balance even under moderate drought stress. The data presented here is in line with previous studies in conifers. Under field experiments, Borghetti *et al.* (1998) concluded that drought influenced the growth of *Pinus halepensis* by reducing apical stem growth. The current study indicates that increasing water availability to a plant can ameliorate the potentially negative effects of high temperature.

Field establishment of two-year-old and five-year-old juniper saplings

This study evaluated how the age of transplant could affect survival and establishment in field situations. Native species normally have higher survival rates than exotic species when planted in their natural habitats (Alrababah *et al.*, 2008). The result of this study overall indicates a relatively high survival rate of juniper saplings planted in their natural habitat. In general, establishment success of juniper saplings was high for five-year-old saplings compared to two-year-old saplings. The reasons for this are unclear, in that the smaller rootball with the two-year-old saplings may have affected their ability to access water within the soil profile, or the actual volumes of irrigation supplied had some effect on survival (the larger five-year-old plants had higher volumes of water applied). Analysed data across re-introduction experiments indicate a positive correlation between survival rate and age of propagule (Albrecht & Maschinski, 2012). The results showed that the survival percentage of five-year-old saplings was high even for the non-irrigated saplings (93 per cent at lower and middle altitudes and 100 per cent at higher altitude). Tabari and Shirzad (2012), in a plantation of *J. excelsa*, found that survival percentage for the first year was 93 per cent for irrigated saplings and 69 per cent for rain-fed saplings. They concluded that adding irrigation to re-introduced saplings enhanced survival rate. Alrababah *et al.* (2008) found the survival of rain-fed one-year-old *J. phoenicea* for the first year was 42 per cent but decreased to 32 per cent in the second year, whereas it reached 82 per cent for the irrigated saplings in the first and second year. The lower survival percentage in both studies can be attributed to establishment practices after planting. Watering plants during establishment period for the first months is critical when moving plants to their natural habitat (Maschinski *et al.*, 2012).

The slow growth rate of juniper trees is well known. In the current field study, the apical growth of juniper saplings did not exceed more than 6mm per year for the first year. Root disturbance and environmental stress for the first year after planting affects the apical growth of saplings when transferred to its natural habitat (Tabari & Shirzad, 2012). When comparing the growth of re-introduced juniper saplings with other conifer species, Alrababah *et al.* (2008) found that *J. phoenicea* had the lowest growth rate.

Curiously, the height increment of juniper saplings in this study was greater at lower and middle altitudes than at higher altitude. The decrease of temperature at

higher altitude may be a factor reducing growth. Swidrak *et al.* (2011), working on *Pinus sylvestris* under drought treatments, stated that air temperature influences apical growth more than precipitation, and Gimeno *et al.* (2012) noted that increasing annual temperature increased apical growth of *J. thurifera*. In this latter case it was stated that this is because juniper has the ability to maintain carbon gain during periods of drought. It has also been stated that survival in the field may relate to the capability of juniper roots to penetrate hard soil, thus accessing moisture further down the soil profile and hence overcoming drought stress (Weaver & Jurena, 2009).

In this study, by providing plants with water at the lower altitudes the results suggest that the higher temperature encountered there may actually be promoting growth. This would tend to indicate that the problems *Juniperus* are facing in this region are related to a lack of water rather than higher temperatures *per se*, but further studies will be required to confirm this. Certainly at lower altitude, the drought treatment reduced survival of young plants and reduced the height growth of juniper saplings compared to the irrigated saplings.

CONCLUSIONS

The present study highlights the effect of temperature and drought stress in growth and survival of *J. seravschanica* saplings in both controlled conditions and natural habitats. Under controlled conditions, drought reduced the growth of juniper saplings even under optimum growing temperature. The first year data from the reintroduction sites showed the potential success of juniper re-establishment in its natural habitat even at the lower altitude (Fig. 9), where supplementary irrigation aided establishment. Planting larger saplings rather than small ones increased survival rates. Saplings planted above 2,500m a.s.l. had overall better survival rates, showing that establishment is favoured by lower temperature and higher precipitation, typical of the higher altitudes. Supporting the conservation of this species through re-introduction of nursery-grown specimens seems feasible, but will require careful selection of appropriate sites and careful management of plants during the establishment phase.

This study is part of a PhD project that is investigating altitudinal effect on regeneration, recruitment and establishment of *J. seravschanica* in the northern mountains of Oman.

ACKNOWLEDGMENT

This study and the project are funded by Diwan of Royal Court, Sultanate of Oman. The authors would like to thank all staff in Oman Botanic Garden for their great support. Many thanks are also due to Salim Al Hinai, Khalid Al Hinai and Abdullah Al Rahbi for their support during the initiation of this project.



Fig. 9 Re-introduced juniper saplings after one year. Left: non-irrigated five-year-old juniper saplings growing at the lower altitude (2,200m a.s.l.). Right: non-irrigated two-year-old juniper saplings growing at the higher altitude (2,600m a.s.l.). Photos: Khalid Al Farsi.

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