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1 **How fast can conifers climb mountains? Investigating the effects of a**  
2 **changing climate on the viability of *Juniperus seravschanica* within the**  
3 **mountains of Oman, and developing a conservation strategy for this tree**  
4 **species.**

5 **Khalid A. A. Y. Al Farsi<sup>1,2</sup>, Darach Lupton<sup>2</sup>, James D. Hitchmough<sup>1</sup> and Ross W. F.**  
6 **Cameron<sup>1\*</sup>**

7 <sup>1</sup> **Department of Landscape, University of Sheffield, Western Bank, Sheffield, S10 2TN,**  
8 **UK.**

9 <sup>2</sup> **Oman Botanic Garden B. O. Box 808, P.C. 122, Muscat, Sultanate of Oman.**

10 **\*Corresponding author [r.w.cameron@sheffield.ac.uk](mailto:r.w.cameron@sheffield.ac.uk)**

11 **Abstract**

12 The conifer, *Juniperus seravschanica* is a keystone species within Oman, yet its decline is  
13 typical of other arid-adapted, montane tree species. This research aimed to identify causes of  
14 decline and subsequent viable conservation strategies; strategies that may have wider  
15 application for tree conservation. Decline in *J. seravschanica* is typified by foliar dieback and  
16 little regeneration via seed; traits most apparent at lower altitudes. The research evaluated the  
17 viability of seeds collected at three different altitudes: 2100-2220m (Low), 2300-2400m  
18 (Mid) and 2500-2570m above sea level (High). In addition, seeds and young trees were  
19 planted at these altitudes and maintained under differential irrigation. Results showed that  
20 trees grown at Low altitude produced fewer, less-viable seed. Transplanting young trees  
21 proved more successful than seed sowing in re-establishing plants in the wild. Age of  
22 transplant had an effect, however, with 5-year-old stock showing greater survival (> 97%)  
23 than 2-year-old trees. The younger trees only established well when planted at High altitude,  
24 or provided with irrigation at Mid/Low altitudes. Water availability did not entirely explain  
25 survival, and in some locations direct heat stress too may be limiting viability. Practical  
26 conservation measures include identifying genotypes with greater drought/heat tolerances and  
27 planting only more mature nursery trees.

28 **Highlights**

- 29 • **Climate change is thought responsible for the decline of the conifer *Juniperus***  
30 ***seravschanica***  
31 • **Trees from lower altitude have greatest decline, and reduced reproductive**  
32 **potential**  
33 • **Conservation strategies are promoted by the planting of nursery-raised trees**  
34 • **Older specimens had greater establishment success than younger trees**  
35 • **Water stress and possibly heat stress are limiting the viability of *J. seravschanica***

36  
37 **Key Words: Climate change, seed, young trees, drought, heat stress, plant**  
38 **establishment.**

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40 for funding this research.

41

## 42 1. Introduction

43 The coniferous tree, *Juniperus seravschanica* (Zeravschan juniper) is a keystone species  
44 within the montane habitat of Oman. This juniper was formally classified as *J. excelsa* subsp.  
45 *polycarpos*, but recent DNA analysis (Adams et al., 2014) has identified it as *J.*  
46 *seravschanica*, allying it to other populations of this species found further north in  
47 mountainous regions of Iran, Afghanistan, Pakistan, India, Turkmenistan, Tajikistan,  
48 Kyrgyzstan, Uzbekistan and Kazakhstan (Gardner and Fisher 1996; Breckle and Wucherer,  
49 2006; Adams, 2014). It is not yet clear if the Oman population is a relic from the Pleistocene  
50 epoch when the species may have been more widespread in the region or is a more recent  
51 population, established by long distance dispersal from Iran. The extent to which it is  
52 genetically distinct from other populations further north still requires clarification (Adams et  
53 al., 2014). As with other *Juniperus* species it is relatively slow growing and individual  
54 specimens of *J. seravschanica* are thought to be able to reach 1,500 years of age (Sass-  
55 Klaasen et al., 2006). In Oman it is found at altitudes  $\geq 2100$  m above sea level (asl) and is  
56 the only native conifer (Fisher and Gardner, 1995), being restricted to the highest areas of the  
57 central massif of Al Jabal Al Akhdar and the outlying mountains of Jabal Qubal and Jabal  
58 Kawr. In such locations *Juniperus* trees not only play a vital ecological role, providing habitat  
59 and food for bird species, but also shade for endemic mammals and reptiles, including the  
60 nationally endemic geckos: *Asaccus montanus*, *A. platyrhynchus* and *Pristurus gallagheri*  
61 (Gardner, 1999). The trees also largely define the landscape character, and provide important  
62 ecosystem services ranging from land stabilization, wood fuel, shade for livestock, through to  
63 aspects of cultural symbolism with plant components being used in traditional ethnobotany  
64 (MacLaren, 2016).

65

66 *Juniperus seravschanica* populations (Fisher and Gardner, 1995, Al Haddabi and Victor,  
67 2016; MacLaren, 2016), along with numerous other *Juniperus* species globally, are under  
68 pressure and often represent fragile ecosystems (Gauquelin et. al., 1999; Ciesla, 2002; Long  
69 and Williams, 2007). Degradation of other *Juniperus* species in arid / semi-arid environments  
70 has been cited for *J. procera* (Ethiopia – Aynekulu et al., 2009; Saudi Arabia (El-Juhany,  
71 2009), *J. thurifera* (Morocco and Spain - Gauquelin et al., 1999), *J. communis* (Spain -  
72 Garcia et al., 1999) and *J. excelsa* (Greece – Milios et al., 2007). Throughout history,  
73 *Juniperus* forests have been exploited for human use, especially for wood collection and  
74 animal grazing, but with rising human populations such activities are becoming  
75 unsustainable, corresponding with rapid forest degradation. Problems are exacerbated by the  
76 fact that natural *Juniperus* regeneration rates are very low, and more consideration needs to  
77 be given to the management and conservation of these forests.

78

79 In Oman a number of studies have examined the status of *Juniperus seravschanica* woodland,  
80 (Fisher and Gardner 1995; Gardner and Fisher 1996; Matwani, 2011; Al Haddabi and Victor,  
81 2016; MacLaren, 2016). Degradation of woodlands within Oman have been associated with  
82 an increase in access to the mountains by tourists and other visitors, who use the wood for  
83 campfires, and through over-exploitation by local people, who may also use branches for fuel  
84 or medicinal purposes, as well as letting their goats and donkeys browse the foliage. In  
85 addition, woodland soils are sometimes mechanically stripped off for agricultural use or road  
86 construction, with negative consequences for tree stability, root viability and moisture

87 retention in the remaining soil. Removal of the top layer also denudes the existing seed bank  
88 held within the soil (Al Haddabi and Victor, 2016; MacLaren, 2016). Although significant,  
89 the extent to which these pressures are impacting on *J. seravschanica* depends on local  
90 conditions and the degree to which the forests are protected, and this protection being  
91 enforced, as destruction is often greater outside nature reserves (Matwani 2011; Al Haddabi  
92 and Victor, 2016). Nevertheless, even tree populations within protected areas are declining  
93 and a number of researchers have suggested that climate change is a primary factor  
94 undermining the viability of *J. seravschanica* in Oman (Al Haddabi and Victor, 2016;  
95 MacLaren, 2016).

96  
97 In recent surveys it was found that trees growing in xeric habitats showed poor health with  
98 low reproductive ability and fewer seedlings compared to junipers growing in mesic habitats.  
99 These mesic habitats included dry river valleys or ravines (wedian – plural or wadi - single)  
100 (Fisher and Gardner, 1995) or lower lying depressions, with deeper, and perhaps more moist,  
101 soils (MacLaren, 2016). MacLaren (2016) found that trees with less foliar dieback were  
102 associated with higher altitudes, or with locations towards the east of the mountain ranges  
103 (where precipitation is thought to be greater) and sites experiencing less direct solar  
104 irradiance. Surveys indicated that there was a measurable increase in tree height with  
105 increasing altitude or when trees were found growing in a wadi, compared to those located on  
106 open hillsides, where water is less likely to accumulate (Al Haddabi and Victor, 2016). Such  
107 data implies that soil moisture availability is a key factor affecting the distribution, growth  
108 potential and health status of *J. seravschanica*, but reports of direct correlations between  
109 growth and actual soil moisture availability remain rare.

110  
111 Reductions in tree health within *J. seravschanica* and the loss of population viabilities at  
112 lower altitudes is now a major concern (Gardner and Fisher 1996; Al Haddabi and Victor,  
113 2016, MacLaren, 2016). In 1996, Gardner and Fisher noted that trees found above 2400m  
114 were generally in better health and possessed a markedly better reproductive status than those  
115 living below this altitude. A similar trend was noted in the closely related *J. procera* in the  
116 Rayadh Reserve in Saudi Arabia, where unhealthy trees, relatively poor production of cones,  
117 and widespread tree death below 2400-2500m were reported (Fisher, 1997). More recent  
118 surveys confirm these trends for *J. seravschanica* (Matwani 2011; Al Haddabi and Victor,  
119 2016) with MacLaren (2016) concluding that the optimal habitat for *J. seravschanica* has  
120 shifted upwards by 250 m in the last 20 years. Such shifts are being noted in a wide range of  
121 other tree species where ranges are being restricted to higher altitudes as temperatures rise  
122 and the incidence of drought in montane locations increases (Allen et al., 2010).

123  
124 An active conservation plan is now in place for *J. seravschanica* in the mountains of Oman,  
125 but more information is required on precisely what is causing the decline of the native forest  
126 stands. Over-exploitation is one aspect and those areas that are protected from human use and  
127 livestock grazing show signs of improvement, but other areas without direct anthropogenic  
128 impact are also declining. This raises the question as to what other biotic and abiotic factors  
129 may be inducing decline of native stands of *J. seravschanica*. Trees under stress from climate  
130 change influences may be more susceptible to pest and disease pressures, but as yet, no  
131 pathogens or pests have been correlated with tree decline. The trend for trees located at the  
132 lower altitudes to show greater decline than those higher up indicates that direct climatic  
133 factors may be to blame for some of the forest degradation. Which climatic factors are  
134 primarily responsible, however, is not clear with 1. drought stress due to a lack of water and  
135 increasing evapo-transpirational demand, and 2. heat stress due to supra-optimal  
136 temperatures, both being likely candidates. Changes in temperature or water availability may

137 be affecting reproduction potential, by reducing the chances of any viable seeds being  
138 produced, and preventing those that are viable from germinating and establishing. In her  
139 study, MacLaren (2016) only found four seedlings across 86 survey sites. Such  
140 environmental changes also seem to be inducing higher mortality rates in mature trees. The  
141 aim of this research therefore, is to investigate how temperature and water availability affects  
142 development in *J. seravschanica*, and to test the primary hypothesis that altitudinal  
143 differences in plant viability relate to a lack of soil water availability and high evapo-  
144 transpirational demand inducing drought stress. The research presented here focusses on seed  
145 viability and the early development / establishment success of young plants. Secondly, the  
146 research aims to identify practical ways of reviving the populations of *J. seravschanica* in the  
147 wild, and specific opportunities for enhancing the number of young progeny through nursery  
148 cultivation and subsequent transplantation. It is anticipated that information generated from  
149 this research will have application to the conservation of other tree species, showing similar  
150 trends in population viability across climatic and altitudinal ranges.

151

## 152 **2. Materials and Methods**

153

### 154 **2.1. Plant Material**

155 Three natural populations of *Juniperus seravschanica* were identified, based on their  
156 altitudinal range within the Jabal Shams, part of the Al Jabal Al Akhdar mountain range in  
157 Oman. These populations were used to monitor plant viability at different altitudes within a  
158 wider project remit and as a source of seed (and derived seedling plants) for the individual  
159 experiments described here. The populations were selected were:

160 Population 1 (Low altitude) at 2100-2220 m asl (N 23°17'07.76 and E 57°09'07.76).

161 Population 2 (Mid altitude) at 2300-2400 m asl (N 23°18'06.22 and E 57°06'15.55) and

162 Population 3 (High altitude) at 2500-2570 m asl (N 23°19'13.26 and E 57°06'16.02).

163

164 Cones were collected by hand from each location and transferred to the seed bank in the  
165 Oman Botanic Garden (OBG). Cones were ground by hand in 2.24 mm steel sieves, air dried  
166 and a Vacuum separator (Kimseed, Wangara, Perth, Australia) used to separate seeds from  
167 the debris. Seeds were soaked in warm water and those that floated were retained (Scianna,  
168 2001). Seed was dried in the shade outdoors, counted and stored in paper envelopes at 12 ±  
169 4°C in the OBG seed bank.

170

171 For trees propagated in the nursery (i.e. used in Experiments 4 and 5), seeds were collected  
172 from the one locational source, i.e. Al Jabal Al Akhdar region (at 2300-2350 m asl 'Mid  
173 altitude') both in 2007 and 2013. These were soaked in warm water for 24 h to help  
174 overcome dormancy and then sown in propagation trays (380 x 240 x 50 mm) using fine peat  
175 (Kekkila propagation peat, 0-6 mm). Trays were placed in air-conditioned glasshouses at 18-  
176 25°C and irrigated from the base using shallow water baths every two weeks. After  
177 germination, seedlings were potted in 1 L air pots with a 1:1 peat / soil mix for approximately  
178 one year. Subsequently they were potted on into 10 L pots filled with 3:1 soil / peat mix. In  
179 October 2011 the older batch of saplings ('5-year-old stock') were potted in 45 L pots, using  
180 the same growing medium. Plants were maintained in controlled environment glasshouses at  
181 25-28°C throughout the growing-on period.

182

### 183 **2.2. Experiment 1. The effect of population location (altitude) on seed production and** 184 **viability.**

185 For each altitude range, cones were collected from a population of 10 different trees and  
186 grouped as a replicate for a given altitude. This was done 3 times for each location during

187 spring 2015. A population of 9000 cones was selected in total (3 altitudes x 10 trees x 100  
188 cones x 3 reps). Seeds were extracted and cleaned (see above). Seeds with signs of insect  
189 damage were separated and counted. The total number of undamaged 'healthy' seeds per 100  
190 cones was counted and their viability determined by the presence of the white fleshy intact  
191 embryo (i.e. 'filled' seeds). Fresh weight was also measured by selecting batches of 50 seeds  
192 from each rep and weighing these.

193

### 194 **2.3. Experiment 2. The effect of seed source (altitude), stratification and temperature** 195 **during germination, on germination percentage and rate under controlled conditions.**

196 Additional seed collected from Experiment 1 were used to determine germination percentage  
197 and rate. Three hundred seeds from individual trees at each altitudinal location were used and  
198 divided into two sub-batches: these were either placed in a refrigerator at 4°C for three  
199 months (Stratified) or stored in paper bags at an ambient room temperature of approximately  
200 20°C (Control). After three months, seeds were removed from their storage conditions and  
201 soaked in warm water for 24 h to help remove any inhibitors to germination. Batches of seed  
202 from each treatment combination were then planted in 90 mm dia. plastic pots filled with fine  
203 peat; 25 seeds being sown in each pot. Pots were placed in incubators at either 15°C or 25°C  
204 to determine how temperature interacted with the other factors to influence germination.

205 These temperatures were selected to cover the spectrum of potentially optimum temperatures  
206 for the 3 altitudinal populations (Al Farsi, unpublished). Photoperiod was set at 12:12 h light /  
207 dark and chambers illuminated with white fluorescent lamps (Osram L20W / 640sa). Each  
208 treatment combination was represented by 3 pots (3 altitudes x 2 stratification x 2 growing  
209 temperature x 10 trees x 3 replications) giving a total population of 360 pots or 9000 seeds,  
210 mean values per 3 replications were used for analysis. Pots were sub-irrigated once every two  
211 weeks, with germination rates monitored weekly over 12 weeks. Germination date, number  
212 of days to germination and number of seeds germinating per week were recorded. At the end,  
213 germination percentages and mean germination time were calculated. The mean germination  
214 time was calculated using the following equation:

$$215 \text{MGT} = \Sigma (n * t) / \Sigma n$$

216 Where n is the number of germinated seed at each recording time. t is the recording time in  
217 days.  $\Sigma n$  is the sum of total germinated seeds (Tigabu et al. 2007).

218

### 219 **2.4. Experiment 3. Establishment of *Juniperus seravschanica* plants through seed sowing** 220 ***in situ*.**

221 Secure sites suitable for seed and tree planting were selected within each of the altitudinal  
222 ranges under study and used to assess seed performance *in situ*. These were defined as Low  
223 altitude at 2220 m asl (N 23°17'33.4 and E 57°09'07.76); Mid altitude at 2300 m asl (N  
224 23°18'08.19 and E 57°06'14.25) and High altitude located at 2570 m asl (N 23°19'13.26 and  
225 E 57°06'16.02). Sowing plots at each altitude were replicated 3 times. In each of the 9  
226 selected field plots an area of 12 m<sup>2</sup> was cleared of grasses and rocks and used to evaluate  
227 tree regeneration from field-sown seed. The cleared areas were divided into sub-plots (45 in  
228 total each 0.3 x 0.3 m and watered prior to sowing). The same source of seeds used for stock  
229 germination was used for field seed germination, i.e. from trees growing naturally at the Mid  
230 altitude (2300 m asl). A piece of flat wood was used to ensure seeds were sown at consistent  
231 spacing (50 mm apart) and depth (10 mm). Twenty-five seeds were sowed in each sub-plot at  
232 3 different times of year to increase the chances of successful germination and establishment,  
233 namely April (just after cone ripening on site and before onset of warmer, drier conditions of

234 summer), June (warmest period and before onset of July-August rain) and September 2014  
235 (end of summer and after July-August rain). Plots were irrigated once every week for one  
236 month and then 3 irrigation treatments were applied with 5 replicates per irrigation treatment.  
237 The 3 irrigation treatments applied were; 'Irrig.15d' (2 L applied every 15 days), Irrig.30d (2  
238 L irrigation every 30 days) or Control with no artificial irrigation and where seeds were  
239 reliant on natural precipitation. Plots were irrigated via watering can with a 'fine spray' rose,  
240 ensuring good distribution of water over each plot.

241 In addition to seed being placed in the soil, seed were also sown in pots (130 mm dia.) using  
242 either the parent soil from each location or a 1:1 peat:soil mix. Each growing media was  
243 represented by 5 pots per location and hosted 25 seeds per pot. The base of each pot was  
244 inserted into the ground (for stability and to encourage drainage / capillary movement of  
245 water between the pot and surrounding soil). Pots were watered at the same frequency as the  
246 highest rate of irrigation on the plots, i.e. every 15 days.

247

#### 248 **2.5. Experiment 4. Establishment of 5-year-old trees in the field.**

249 Five-year-old trees previously grown-on at the OBG, seed were collected in April 2007 from  
250 Jabal Shams at 2,300-2,400 m asl, were transplanted to experimental field plots in each of 3  
251 altitudinal locations (see Exp. 3 above for locations and site preparation) in March 2014  
252 (Figure 1A), with 45 specimens being planted at each location. To help plants with their  
253 initial establishment, planting holes dug out from the soil were 'back-filled' with a medium  
254 comprising a 1:1 ratio of peat and parent soil; this helping roots to proliferate out from the  
255 established root ball they had previously been restricted to. After planting, the young trees  
256 were uniformly irrigated irrespective of altitude or designated treatment in an attempt to aid  
257 root establishment in the parent soil. The volume applied, however, was reduced  
258 progressively over time to encourage deeper rooting into the soil profile. Once plants were  
259 deemed to have become established with new root development into the parent soil (plants  
260 were gently pulled to ensure they were 'secured' to the ground) differential irrigation was  
261 imposed (November 2014). Treatments imposed were a higher irrigation regime (10 L  
262 applied every 15 days – Irrig.15d), or lower regime (10 L irrigation every 30 days – Irrig.30d)  
263 or a Control with no artificial irrigation and where plants were reliant on natural precipitation  
264 post establishment. At each of the three altitudes, trees were planted in 3 blocks with 5  
265 replications per water treatment. Plants were monitored for survival and growth until July  
266 2016. Physiological stress was determined by sampling foliage for chlorophyll fluorescence  
267 parameters at monthly intervals (data for Fv/Fm being presented). Chlorophyll fluorescence  
268 was measured on days with low cloud cover with a 'pocket plant efficiency analyser', PEA  
269 (Hansatech Instrument, King's Lynn, UK); leaves were dark adapted for 20 minutes with leaf  
270 clips before measurement. Soil moisture levels in plots were monitored throughout via  
271 capacitance soil moisture probes (Waterscout SM100, Spectrum Technology, Fort Worth,  
272 Texas, USA) and moisture conditions related to meteorological data from a weather station at  
273 each altitudinal site (WatchDog 2900ET, Spectrum Technologies, Fort Worth, Texas, USA).  
274 Moisture probes were installed vertically, 20 mm deep, around a sapling's root ball. Data  
275 from the research sites were also correlated with longer-term climatic data from the Saiq  
276 Meteorological Station at 1993 m asl (N 23°04'26.58 and E 57°39'59.56); approximately 40  
277 km from the planting locations.

278

#### 279 **2.6. Experiment 5. Establishment of 2-year-old trees in the field.**

280 Seed used in this experiment were collected in May 2013 from Jabal Shams at 2,300-2,400 m  
281 asl. Procedures were similar to those of Experiment 4, with the exception that planting of 2-  
282 year-old stock took place one year later (March 2015), with differential irrigation treatments  
283 commencing in November 2015 (ending July 2016). Planting was delayed by a year  
284 compared to 5-year-old specimens to allow the young trees to acquire sufficient biomass  
285 (Figure 1B) before transplanting; i.e. as 1-year-old stock in March 2014 plants were variable  
286 in size and some specimens were very small (< 100 mm high). These 2-year-old stock (2 L  
287 pots) were given a reduced volume of water (2 L on each occasion) compared to their older  
288 counterparts, due to their much reduced canopy size (Figure 1); the volume being applied  
289 being proportional to the water use ratios observed for the potted plants on the nursery site.  
290 As before, treatments imposed were a higher irrigation regime (2 L applied every 15 days –  
291 Irrig.15d), or lower regime (2 L irrigation every 30 days – Irrig.30d) or a Control with no  
292 artificial irrigation and where plants were reliant on natural precipitation post establishment.  
293 At each altitude, trees were planted in 3 blocks (adjacent to the 5-year-old blocks) with 5  
294 replications per water treatment.

295

## 296 **2.7. Data handling and statistics.**

297 Analysis of variance (ANOVA) was used to determine the significance of different  
298 experimental factors. Fisher's protected least significant difference PLSD (Genstat) was used  
299 to denote significance between two means within multiple comparison post-hoc tests. If  
300 variance within data sets were non-homogenous, then data was transformed by square root,  
301 before commencing an ANOVA. Where data sets were non-parametric and ANOVA was not  
302 valid, then a Kruskal Wallis test was employed, with pairwise comparisons used to test  
303 significance between two means. Mean data are depicted with associated standard errors  
304 (S.E.), and letters denoting significant differences between means where appropriate.

305

## 306 **3. Results**

307

### 308 **3.1. Experiment 1 The effect of population location (altitude) on seed production and** 309 **viability.**

310 There was a significant difference in seed number per 100 cones between altitudes ( $P < 0.01$ ).  
311 Trees at the High altitude produced significantly more seeds per 100 cones (approx. 462) than  
312 trees growing at Low (368) or Mid (358) altitudes (Table 1). The proportion of seeds  
313 damaged by insects was relatively small throughout (< 5%, Table 1) and a non-parametric,  
314 Kruskal-Wallis test, indicated no significant influence of altitude in the proportion of  
315 damaged seed ( $P = 0.83$ ). There were no overall significant effects of altitude on percentage  
316 of viable seeds or seed weight ( $P = 0.1$  and  $P = 0.06$ ) (Table 1).

317

### 318 **3.2. Experiment 2. The effect of seed source (altitude), stratification and temperature** 319 **during germination, on germination percentage and rate.**

320 Multi-factorial ANOVA indicated that germination was strongly influenced by altitude ( $P <$   
321  $0.01$ ) and germination temperature ( $P < 0.01$ ) but not stratification treatment ( $P = 0.20$ )  
322 (Figure 2); there were no significant interactions between factors (all  $P > 0.05$ ). Seeds  
323 sourced from a High altitude had significantly higher germination percentage at 15°C than  
324 those sourced from Low and Mid altitudes ( $P < 0.01$ ). With stratified seed derived from the

325 High altitude, 25°C suppressed germination significantly compared to the lower temperature  
326 of 15°C (Figure 2).

327 Results indicated that there was no significant effect of altitude in mean germination rate ( $P=$   
328 0.39), although the temperature seeds were exposed to during germination and pre-treatment  
329 were significant ( $P < 0.01$ , Figure 3). Seeds germinated under the lower temperature of 15°C,  
330 taking longer to germinate than those under 25°C and in both cases cold stratification delayed  
331 germination.

332

### 333 **3.3. Experiment 3. Establishment of *Juniperus seravschanica* plants through seed** 334 **sowing *in situ*.**

335 No seeds germinated after either direct sowing in the ground or into pots. This was true of all  
336 3 altitudinal locations. Some, but not all field plots showed evidence of soil erosion (after  
337 periods of heavy precipitation) resulting in some seed being buried deeper than originally  
338 envisaged. There was no evidence of damage to the seeds from rodents or invertebrates.

339

### 340 **3.4. Experiment 4. Establishment of 5-year-old trees in the field.**

341 Tree survival of 5-year-old stock was high throughout with no significant differences  
342 observed between treatments ( $\geq 97\%$  survival in all treatments and field sites, over the 21  
343 months of the study, data not shown). Plant height (Table 2), as assessed on transformed data  
344 was significantly affected by altitude ( $P < 0.01$ ) and irrigation treatment ( $P < 0.05$ ) but also by  
345 interactions ( $P < 0.05$ ) between these two factors (Figure 4). These factors also influenced  
346 side branch growth ( $P < 0.01$  for both), but in this case without significant interaction ( $P=$   
347 0.27) (Table 2). Growth (height and branch increments) tended to be less at the High altitude  
348 (above 2500 m asl) than the Low altitude (Table 2, Figure 4). Irrigation at the Low altitude,  
349 however, further enhanced plant height (Figure 4) and branch growth (Table 2) significantly  
350 ( $P < 0.05$ ). There was a positive effect of supplementary irrigation on branch growth at the  
351 Mid and High altitudes too ( $P < 0.05$ , Table 2) but there was no clear advantage, (i.e. non-  
352 significant), in terms of plant height *per se* at these altitudes (Figure 4).

353 Values for chlorophyll fluorescence Fv/Fm showed strong seasonal patterns, with most  
354 notable reductions associated with some treatments in mid-summer (selected data sets for  
355 2016 are depicted in Figure 5). For example, there were significant reductions in Fv/Fm  
356 during July 2016 based on altitude ( $P < 0.01$ ), with plants grown at the Mid and Low altitudes  
357 showing suppressed photosynthetic capacity (Figure 5). Irrigation had no overall effect on  
358 plant stress levels at each of the altitudes.

359 Soil moisture levels throughout the experimental period were strongly associated with rainfall  
360 events (Figure 6), with greatest moisture retention being associated with Mid altitudinal  
361 location. Applying supplementary irrigation generally enhanced the moisture availability to  
362 plants, especially under Irrig.15d treatment (Figure 7), although actual recorded values could  
363 be low on occasions (e.g. December 2015). Irrigation was particularly important in  
364 maintaining higher moisture levels during the summer periods, e.g. July 2015 and July 2016  
365 (Figure 7). For example in July 2016, recorded moisture levels at the Low altitude plots were  
366 13.8 (Irrig.15d), 7.8 (Irrig.30d) and 4.2  $\text{m}^2 \text{m}^{-2}$  (Control).

367 Meteorological data also tended to suggest that the High altitude plots experienced the lowest  
368 annual temperatures (data for maximum recorded temperatures for each altitude is depicted in

369 Figure 8, and comparison made to both the mean and actual maximum temperatures  
370 experience over the last two decades at the nearby Saiq Meteorological Station). This data  
371 indicates that the maximum monthly recorded temperatures tended to be lower at the High  
372 altitude than either the Mid or Low altitudes.

373

### 374 **3.5. Experiment 5. Establishment of 2-year-old trees in the field.**

375 Data for plant survival was non-parametric so a Kruskal-Wallis test was employed to  
376 determine significance levels. Altitude affected plant survival of 2-year old stock ( $P= 0.02$ ,  
377 with mean values for 87% High, 63% Mid and 76% Low altitudes; the pairwise comparison  
378 showing a significant difference between High and Mid values. There was no overall  
379 significant effect ( $P= 0.52$ ) associated with irrigation from Kruskal-Wallis, but restriction of  
380 the data to the Low altitude alone, showed that irrigation level here was significant ( $P= 0.05$ ),  
381 with more frequent irrigation improving survival i.e. 60% for control, 73% for Irrig. 30d and  
382 93% for Irrig. 15d (Figure 9). Overall survival during the 16 months post-planting appeared  
383 to be favoured at High altitude, more variable at Mid altitude (note the relatively large S.E.  
384 values indicating plot differences) and strongly dependent on irrigation at the Low altitude.  
385 Branch extension was not measured on 2-year-old stock, due to the limited number of  
386 branches available per plant. There was no overall effect of altitude ( $P= 0.61$ ) or irrigation  
387 ( $P= 0.32$ ) on plant height extension (interaction  $P= 0.06$ ) (Figure 10).

388 Chlorophyll fluorescence values were generally lower throughout with 2-year-old plants  
389 (Figure 11) than 5-year-old plants (Figure 5). Values were significantly affected by altitude,  
390 but varied depending on season. Low altitude had higher values during winter and spring ( $P<$   
391  $0.01$ ); whereas there were sharp reductions ( $P< 0.01$ ) in values associated with the July  
392 readings for plants in the Low altitude (all values  $< 0.3$ ) At this point, additional irrigation  
393 had no effect on relieving the stress being experienced by these plants. Values were greater  
394 for trees grown at High altitudes, although still below 0.7, which is often considered the  
395 threshold for when plants start experiencing stress (Fang-yuan and Guy, 2004). Of note was  
396 the fact that plants under the Irrig.15d regime at the High altitude had relatively low values  
397 compared to equivalent plants at other altitudes throughout the winter and spring periods,  
398 suggesting that these plants may have been experiencing other forms of abiotic stress (e.g.  
399 chilling), rather than just high temperature or water shortage.

400

## 401 **4. Discussion**

402 This research demonstrates that factors associated with altitude are influencing the viability  
403 of *Juniperus seravschanica* populations in the mountains of Oman. This confirms and re-  
404 enforces previous observations on foliar die-back noted on mature trees and a lack of natural  
405 regeneration due to an absence of young trees and seedlings within the mature stands (Fisher  
406 and Gardner, 1995, Al Haddabi and Victor, 2016; MacLaren, 2016). The data presented here  
407 illustrates that trees grown at lower altitudes are producing less seed with reduced  
408 germination rates than seed derived from trees growing at a higher altitude. This has  
409 implications for the natural regeneration of tree stands at lower altitudes, but also is  
410 symptomatic of the health status of existing trees growing at these lower elevations in Oman.  
411 Such results are consistent with recent reports on other arid and semi-arid tree species. Here  
412 too, abiotic stress has been linked with negative effects on tree abundance and seed  
413 production, thus limiting new seedlings recruitment, e.g. *Pinus edulis* in southwestern USA

414 (Redmond, et al., 2015), *Moringa peregrine* in the mountains around the Red Sea (Hegazy, et  
415 al. 2008) and *J. procera* and *Olea europaea* subsp. *cuspidata* in northern Ethiopia (Aynekulu,  
416 et al., 2011).

417 The systematic approach taken here to observe plant development under semi-protected  
418 conditions confirms that loss of viability in some populations of *J. seravschanica* is climate  
419 related. Seed and tree material planted in the field was protected from human and livestock  
420 interference in these studies, thereby eliminating browsing pressure and biomass loss  
421 (collection of branches for fuel) as factors reducing the viability of these young trees. We  
422 therefore conclude that abiotic factors related to climatic conditions and perhaps regional  
423 climatic shifts are adding to the pressure on native populations of *J. seravschanica*.

424

#### 425 **4.1. Environmental stress on young trees**

426 The ability to cope with these abiotic stress factors was influenced markedly by plant age,  
427 with older (5-year-old) pot-grown trees being more resilient and establishing better than  
428 younger, 2-year-old stock when planted out in the natural environment ('field'). These  
429 plantings were carried out in different years (2014 for 5-year-old and 2015 for 2-year-old  
430 trees) and plants given different volumes of water (in proportion to their canopy sizes) so  
431 experience of stress factors may have varied slightly between the two age groups; hence some  
432 caution may be required in making direct comparisons. Nevertheless, the period after planting  
433 of the 5-year-old trees (Jan 2015-Jul 2015) was generally drier than the equivalent period  
434 after planting of the 2-year-old material (Jan 2016-Jul 2016; Figure 6), yet it was the older 5  
435 year old plants that established more successfully. Indeed, the 5-year-old plants had relatively  
436 high survival rates irrespective of altitudinal location or the irrigation regimes they were  
437 exposed to. Even plants that were not given any irrigation after initial establishment retained  
438 greater than 97% survival rates. In contrast, significant plant losses were experienced in 2-  
439 year-old stock, especially when planted in the Mid altitude, or planted in the Low altitude and  
440 not provided with supplementary irrigation. The fact that survival improved for those young  
441 specimens grown at the Low altitude when supplementary irrigation was provided suggests  
442 strongly that moisture availability is a critical factor in improving tree viability, at least for  
443 the warmer, lower altitudes where evapo-transpirational demand may be greatest. This is re-  
444 enforced by evidence from previous studies that least injury in the-lower altitude natural  
445 populations corresponds with more mesic soils, where drought may be less common  
446 (MacLaren, 2016).

447 Growth was also enhanced in some cases when artificial irrigation was supplied to trees. At  
448 the Low altitude, supplementary irrigation increased tree height and side-branch extension of  
449 the 5-year-old stock. This is likely to result in a greater foliar canopy area, and hence greater  
450 capacity to generate photosynthates. This in turn will promote subsequent root growth and  
451 can encourage root proliferation deeper down the soil profile (Grossnickle, 2005). Thus the  
452 development of roots growing vertically downwards can capitalise on natural moisture  
453 reserves found deeper in the soil profile, and is a common survival strategy adopted by plants  
454 in arid environments (Jackson et al., 1996; Canadell et al., 1996; Peek et al., 2006). The risk  
455 of artificial irrigation, however, is that if only the upper profiles of the soil are wetted, then  
456 root proliferation may only occur at the surface (Fernández et al., 1991; Sokalska et al.,  
457 2009), rendering the tree more susceptible to drought stress should the artificial supply of  
458 irrigation ever cease (Gilman et al., 2003; Cameron and Hitchmough, 2016). Regular  
459 irrigation also limits the capacity of the tree to condition itself against any subsequent severe

460 drought it might experience after irrigation is withdrawn. This is because acclimation to  
461 severe drought is itself elicited by exposure to drying soil, or short periods of moderate, sub-  
462 lethal, water stress (Cameron et al., 2008). If irrigation is sufficient and regular, then plants  
463 may never experience the moderate levels of stress that naturally induce the acclimation  
464 response.

465 Access to water, however, may not entirely explain viability of *Juniperus seravschanica*  
466 populations. The Mid altitude was associated with greater rainfall and higher soil moisture  
467 content than other locations, but still experienced significant loss of 2-year-old trees. At this  
468 altitude, however air temperatures tended to mirror those of the Low altitudes (i.e. trees  
469 experienced greater maximum temperatures than those at High altitude). This raises the  
470 hypothesis that heat stress is acting as an independent limiting factor at these Mid and Low  
471 altitudes, and future research should aim to verify this. Low Fv/Fm values during summer (<  
472 0.7) despite supplementary irrigation, provides some support for this argument.

473 If excessive heat is inducing a secondary stress on plant material, then the implication for the  
474 long term viability of the *J. seravschanica* populations is not positive, at least for those  
475 located at the Mid and Low altitudes. Our data collected over the last three years, suggests  
476 that the high temperatures experienced during this period are typically less than those  
477 documented for nearby locations over the last two decades (Figure 8). It is unclear how the  
478 trees planted here would respond to the higher temperatures that can occur in the region, and  
479 which may become more frequent as climate change impacts are realised. Despite our finding  
480 that tree viability is improved considerably if older stock is field-planted and given  
481 supplementary irrigation during establishment, this does not take account of the fact that  
482 temperatures higher than those experienced over the three years of the study period may still  
483 limit their potential in future; especially as an increase in a mean maximum temperature of  
484 2°C between 2011 to 2040 is projected for this area (Al-Charaabi and Al-Yahyai, 2013).  
485 Indeed, the dieback observed in natural populations of *Juniperus* at lower elevations could be  
486 caused by high temperature stress. Current research is underway to examine these  
487 populations more closely to determine if trees growing in cooler, shadier locations are  
488 proving more resilient than those in open areas exposed to greater solar radiation, and that  
489 this is not due solely to moisture availability (MacLaren, 2016). The reality is that although  
490 the 5-year-old trees have established well, they have not yet experienced the very high  
491 thermal pressures that could be typical of the region in future.

492 Although the key stresses identified by the research were due to water deficits and high  
493 temperatures in summer, there was also some evidence that lower growth rates and some  
494 relatively low Fv/Fm values (see Figure 11- Feb.2016 High and Mid altitudes) could relate to  
495 lower winter temperatures in these locations (data not shown). The Mid location, for example  
496 was surrounded by montane ridges and could trap cold air as it flowed down the slopes of the  
497 mountain. There was no evidence of long term or significant damage, however, associated  
498 with colder winter temperatures.

499 Previous research suggests that greater moisture availability and cooler temperatures (and  
500 hence, in practice, longer growing periods in summer) favour the development of taller trees  
501 further up the mountain profiles (Al Haddabi and Victor, 2016). The highest study plots  
502 (2350 m asl) in the Al Haddabi and Victor (2016) survey, however, are equivalent to our Mid  
503 altitude treatment, and these authors did not determine tree growth at altitudes represented by  
504 our High treatment (i.e. at 2570 m asl). In this present study, however, we generally found the  
505 opposite trend, in that extension growth was less in those trees planted at higher altitudes. It  
506 should be noted of course that our timeframe was very short relative to the species entire

507 lifespan and caution is required in interpreting such short-term growth trends. Nevertheless,  
508 our growth data combined with the winter chlorophyll fluorescence results suggest that  
509 growth potential at the top of the mountains may be impaired by cold, as well as by drought  
510 and heat at the lower altitudes during summer. Other factors, however, cannot be eliminated  
511 from explaining the variations in growth between the plot sites in this study, including factors  
512 due to soil nutrient levels or the rapidity by which the young plants became established (i.e.  
513 the degree to which they experienced a transplant shock at the different sites).

514

#### 515 **4.2. Environmental stress on seeds**

516 Abiotic stress may have affected the ability of seeds to germinate and grow in the natural  
517 environment. This could be through the direct influence of climatic factors in reducing seed  
518 number and viability, e.g. drought decreased the proportion of filled seed in *J. thurifera*  
519 (Mezquida et al., 2016) and higher temperatures decreased seed production by 40% in *Pinus*  
520 *edulis* (Redmond et al., 2012). There may also be indirect effects, such as stress reducing the  
521 canopy density of adjacent mature trees, thereby altering the micro-climatic conditions that  
522 normally favour seedling development. (Redmond et al., 2015). An attempt to re-generate  
523 tree populations via direct sowing of seed into the ground (at all three altitudes) was  
524 unsuccessful. This may have been due to a variety of reasons: poor viability (although  
525 batches of the same seeds had up to 22% germination under controlled conditions), abiotic  
526 stress in the field, including periodic water stress (despite regular irrigation in some  
527 treatments) but also paradoxically, over-wetting during heavy precipitation and the allied  
528 difficulty for the hypocotyls to break through soils that had become capped after rain (i.e.  
529 where a crust forms on the soil surface and this impedes the movement of moisture and air to  
530 the seed, but also induces a physical barrier to the developing shoot and leaves). Sowing  
531 seeds in peat / soil media in pots placed in the natural environment proved no more  
532 successful, however, although again this could relate to oscillations in moisture availability  
533 within the pots. In contrast to the failure to germinate seeds in the field *per se*, germinating  
534 seeds and growing-on seedlings in a nursery before transplanting out in the field was more  
535 successful. In controlled conditions within the nursery, germination percentage of seed was  
536 promoted by lower temperatures (15°C compared to 25°C) for seed collected at High altitude.  
537 This trend was less evident, however, for seed from the Low altitude, perhaps suggesting that  
538 seed derived from this warmer zone has some adaptation to higher temperatures during  
539 germination.

540 Germinating seed on a nursery and raising seedlings under cultivation appeared to be a more  
541 effective approach to ensuring good numbers of young trees are made available to support  
542 existing populations. The procedures associated with protected cultivation, however, are  
543 likely to be more resource intensive in practice, especially in terms of labour, water, growing  
544 media and transport costs, but based on this research at least, they are a more viable way to  
545 re-establish tree populations in the wild.

546

#### 547 **4.3. Procedures and recommendations to conserve *J. seravschanica* within Oman**

548 Collectively, our results support the hypothesis that the populations of *J. seravschanica*  
549 growing at the lower altitudinal ranges within the mountains of Oman are under stress, and  
550 that this stress is at least partially explained by climatic factors driving drier soils and higher  
551 temperatures. This is augmented by further pressure on tree populations (at all altitudes, but

552 again worst at the lower elevations) due to over-browsing by livestock and physical damage  
553 to trees through human activities. There was no evidence from this and allied studies to date  
554 that tree stocks are being impacted by plant pathogens *per se*, or regeneration limited by  
555 rodent activity or other pest species. Research is on-going with respect to the long-term  
556 viability of *J. seravschanica* and its associated ecosystem. Nevertheless, based on the  
557 research presented here and from recent literature, preliminary recommendations to conserve  
558 *J. seravschanica* in the mountains of Oman include:

- 559 • Continue to minimise human and livestock activity in the remaining stands of *J.*  
560 *seravschanica* through effective fencing and sign-posting; priority being given to  
561 those trees currently growing in damper mesic zones (such as wadi or depression  
562 sites) or shaded areas.
  - 563 • Identify ‘superior’ trees growing in the wild which are demonstrating some degree of  
564 tolerance to water and heat stress, and harvest seeds from these on the basis that these  
565 may inherit some of these tolerance traits.
  - 566 • Re-introduce young trees through a cultivation programme based on nursery  
567 production, with a *proportion* of the trees being derived from parent trees designated  
568 as having superior stress tolerance. Plant older, larger trees (e.g. 5-year-old material)  
569 as these appear to establish better than younger stock.
  - 570 • Plant the young trees in a variety of sites and locations, but ensuring good numbers  
571 are planted in wadis, shaded north facing slopes, and within existing stands of mature  
572 trees (so called refugia sites, MacLaren, 2016).
  - 573 • Provide irrigation until young trees become established. A practical consequence of  
574 this is that irrigation needs to be managed to ensure trees are ‘weaned-off’ the  
575 artificial supplies of water. For example, progressively reducing the volume applied  
576 on each occasion, or increasing the periods between subsequent watering events.
  - 577 • Monitor the development of young trees across a range of contrasting sites and  
578 altitudes to help further identify potential problems or verify procedures that are  
579 aiding the recovery of the species.
  - 580 • Continue controlled studies to investigate more-fully heat stress and tolerance to it  
581 within *J. seravschanica*. Investigate the extent to which heat and water stress interact  
582 to affect the viability of young trees.
  - 583 • Establish additional protected sites for Juniper, particularly at high altitude.
  - 584 • Provide studies to assess the genetic status and variability of *Juniperus* trees to  
585 consider its viability in this geographically isolated location, and to avoid localised in-  
586 breeding.
- 587

#### 588 **4.4. Implications for the conservation and management of montane tree species within** 589 **the context of a changing climate**

590 It is anticipated that information from this research will aid the practical conservation of *J.*  
591 *seravschanica*, but also highlights the influence of climate change on other montane plant  
592 species, and what practical measures should be considered in aiding their conservation. The  
593 research raises the controversial dilemma facing conservationists, in that some native plant  
594 populations may in the future only survive through active management (in this case, selecting  
595 stress-adapted superior progeny, growing trees in nurseries and irrigating them after  
596 planting). As well as the financial implications, this raises a range of ethical issues not least in  
597 that attempting to deal with anthropogenic climate change; humans will interfere with  
598 ‘ecological processes’ such as natural vegetation succession through their desire to conserve

599 notable, iconic species. In the case of the Oman juniper, on the assumption it is sufficiently  
600 genetically distinct from other populations of *J. seravschanica*, then perhaps even more  
601 controversially the concept of assisted migration to other mountain ranges (out-with its  
602 natural distribution, e.g. western parts of the Zagros mountain range in Iran) where  
603 temperature and rainfall patterns are more conducive for survival should be considered. This  
604 species is unlikely to reach these areas through natural means, so an introductory programme  
605 would be required. The pros and cons of assisted migration are well documented (Ricciardi  
606 and Simberloff, 2009; Vitt et al., 2010; Williams and Dumroese, 2013; Koralewski et al.,  
607 2015; Sansilvestri et al., 2015 ) and include ; the ability to conserve a plant species (and  
608 potentially a component of its linked ecosystem, as is the case with a keystone species such  
609 as *J. seravschanica*) balanced against the potential to introduce invasive alien plants to a new  
610 site, or damaging fauna or micro-organisms associated with the translocated species. Such  
611 factors can radically disrupt the ecosystem composition, development and functioning of the  
612 'host' site. So the potential impacts on the ecosystem processes and services of any host site,  
613 e.g. primary and secondary production, hydrology, nutrient cycles and existing food-webs  
614 need to be carefully considered before an assisted migration is undertaken. In light of this, we  
615 conclude that every attempt should be made to assist the retention of viable population of *J.*  
616 *seravschanica* in the mountains of Oman through the active management processes outlined  
617 here, whilst in the longer term evaluating whether assisted migration is appropriate / feasible  
618 for this species. Moreover, such active management processes are likely to aid the  
619 conservation of other montane tree species too, similarly under threat from increasingly arid  
620 soils and raised aerial temperatures.

621

#### 622 **4.5. Conclusions**

623 This research demonstrates that abiotic stress, particularly drought stress and potentially heat  
624 stress affect the viability, growth potential and photochemical efficiency of young *J.*  
625 *seravschanica* trees in the field. Increases in these stress factors are compatible with the  
626 impacts of climate change in the region, with trends recorded here being demonstrated in  
627 other montane tree species (Allen et al., 2010). This is typified by a reduction in tree viability  
628 at lower altitudes and overall loss of habitat as tree populations shrink due to a lack of new  
629 land to colonise. Data here indicated that trees located at the Low altitudes experienced  
630 higher temperatures and presumably greater evapo-transpirational demand than those placed  
631 at the High altitude. Older pot-grown stock showed more resilience than younger trees, post  
632 transplanting. Within the current study, supplementary irrigation was required at the Low  
633 altitude to ensure the youngest (2-year-old) trees survived, although older specimens could  
634 survive without irrigation after the initial establishment phase. It is unclear, however, how  
635 such plants will tolerate more extreme moisture deficits and higher temperatures that could be  
636 experienced in future. It is prudent that current conservation efforts concentrate on reducing  
637 the anthropological impact on wild populations of *J. seravschanica* and that the wild  
638 populations are supplemented with young trees derived from nursery grown stock. The  
639 planting of these should be concentrated in the cooler and damper locations throughout the  
640 mountain landscape, as well as at suitable habitat at higher altitudes, where natural  
641 colonisation is slow or inhibited by other factors (for example soil capping or erosion  
642 restricting the establishment of seedlings). Longer term strategies for this, and indeed other  
643 montane species / sub-populations within the context of a changing climate, is to consider  
644 assisted migration to more conducive environments, despite the risks this brings.

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765 Figure 1. Five-year-old (A - left) compared to 2-year-old (B - right) specimens of *Juniperus*  
766 *seravschanica* planted in the field.

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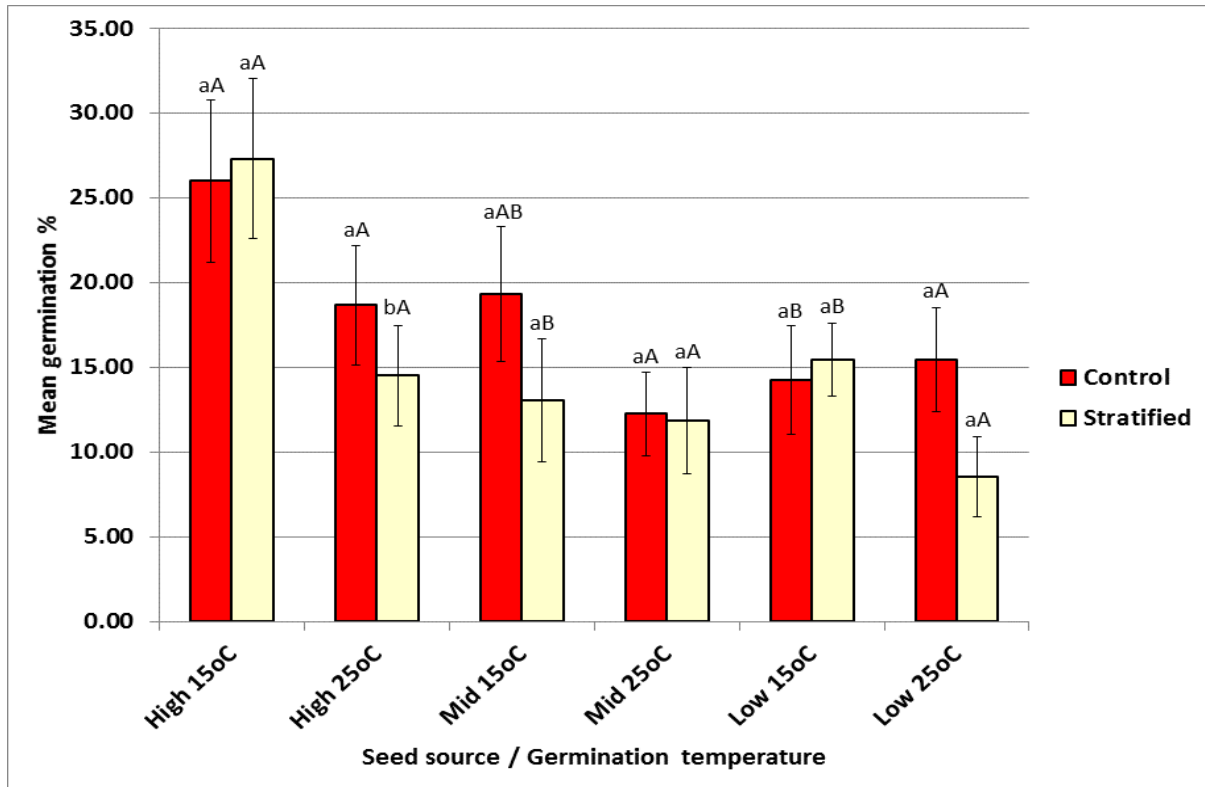
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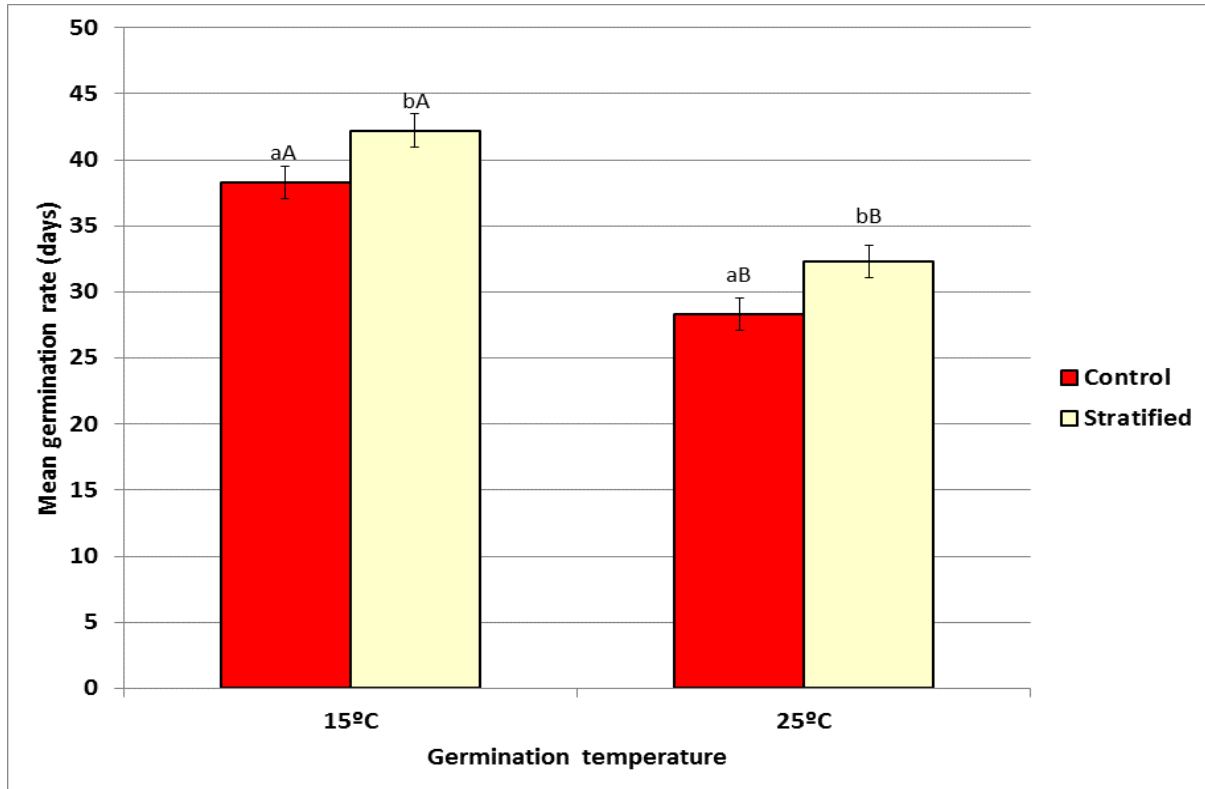
782 Figure 2. Germination percentage of seed collected from different altitudes (High, Mid and  
 783 Low) with two pre-treatments (Control, Stratified) and germinated under two temperature  
 784 regimes (15 and 25°C). Data are mean  $\pm$  SE. Bars with different lowercase letters indicate  
 785 significant effect of germination temperature at same altitude whereas uppercase letters  
 786 indicate significant effect of seed source (altitude) at same pre-treatment and growing  
 787 temperature. The effect of pre-treatment is not indicated due to a non-significant effect.



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790 Figure 3. Germination rate of seed as affected by two pre-treatments (Control, Stratified) and  
791 growing temperature (15 and 25°C). Data is pooled from three altitudes and represents means  
792  $\pm$  SE. Bars with different lowercase letters indicate significant effect of pre-treatment at same  
793 germination temperature whereas uppercase letters indicate significant effect of growing  
794 temperature.

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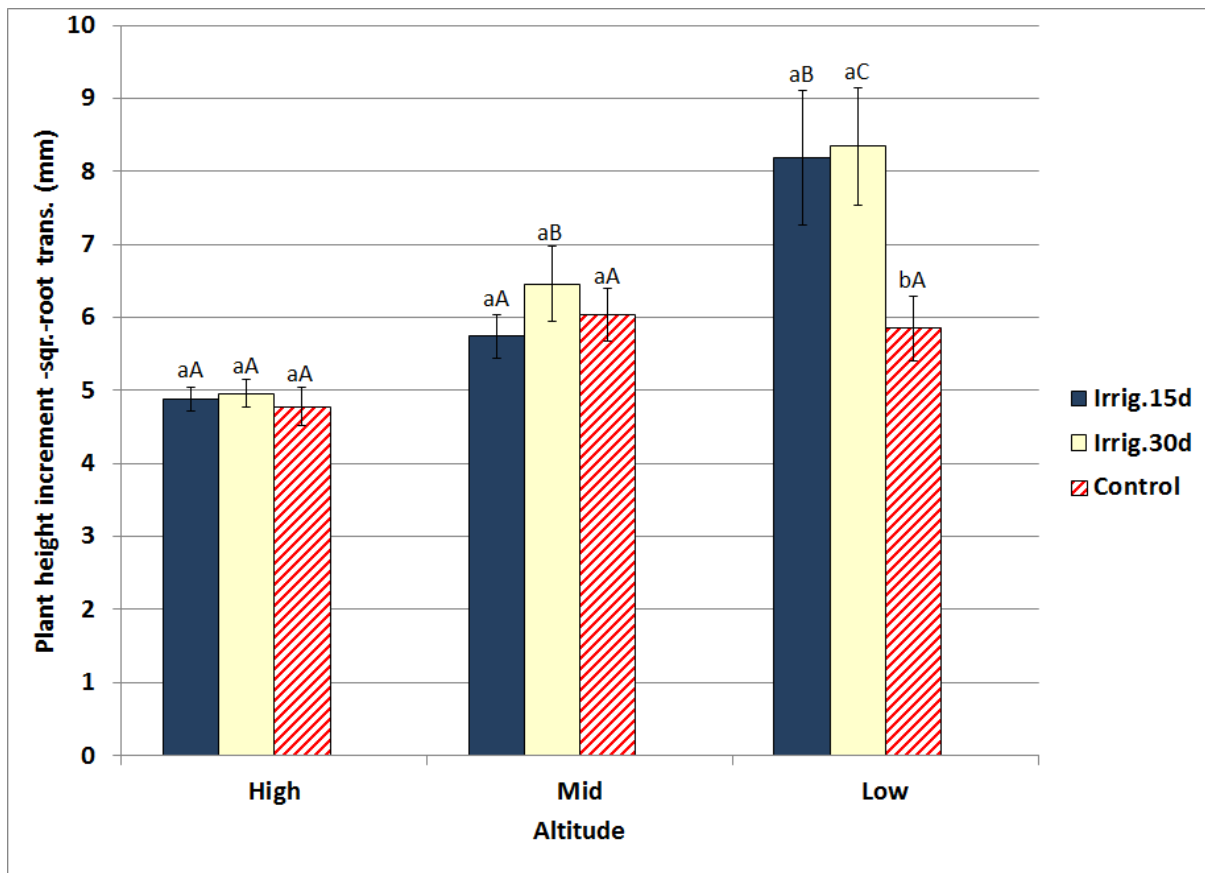
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798 Figure 4. Plant height increment (mm. data square root transformed) in 5-year-old trees  
 799 planted at different altitudes (High, Mid and Low) and watered under different irrigation  
 800 regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation =  
 801 Control). Data are mean  $\pm$  SE (n=15). Bars with different lowercase letters indicate  
 802 significant effect of irrigation regimes at same altitude whereas uppercase letters indicate  
 803 significant effect in different altitudes at same water treatment.

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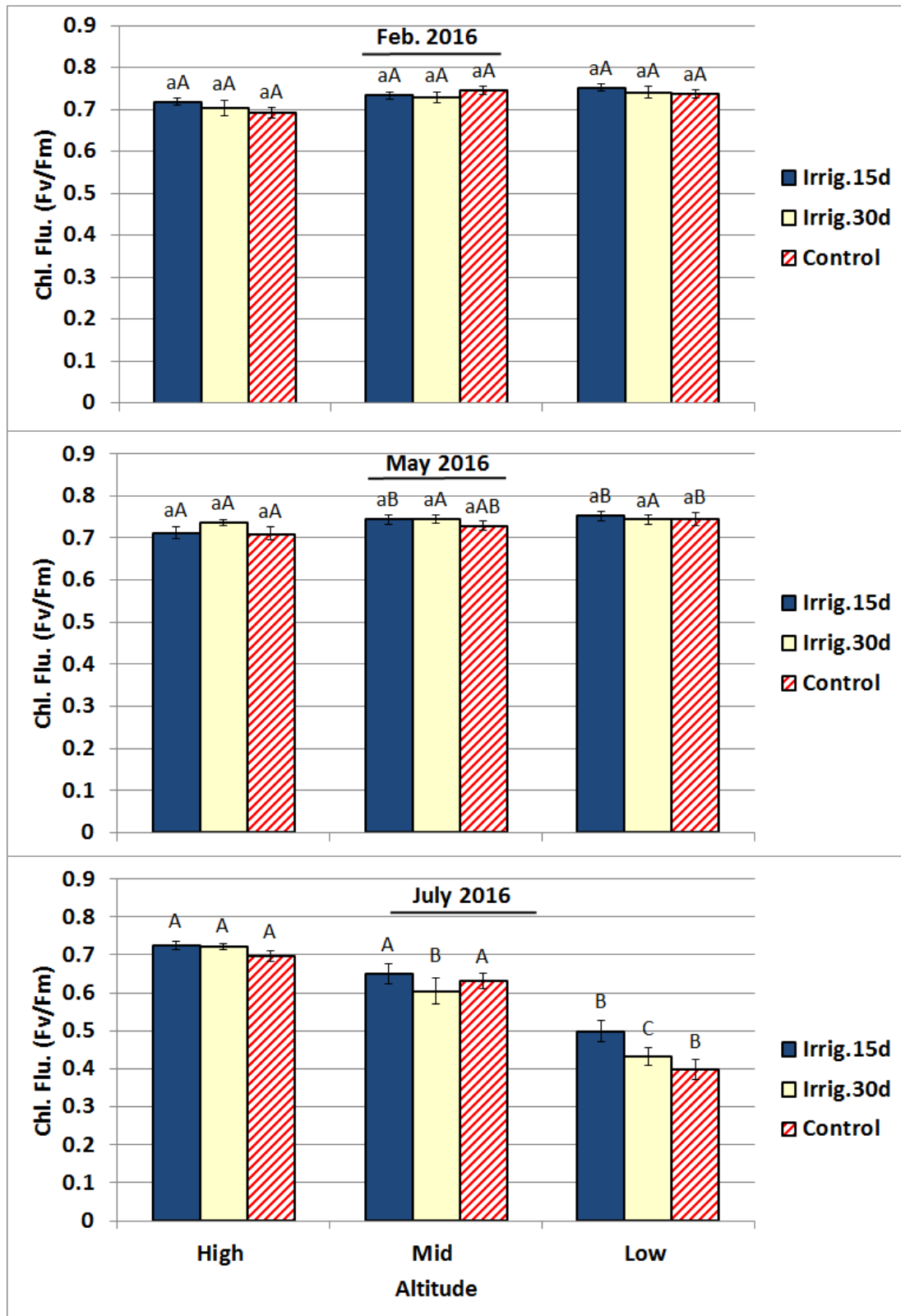
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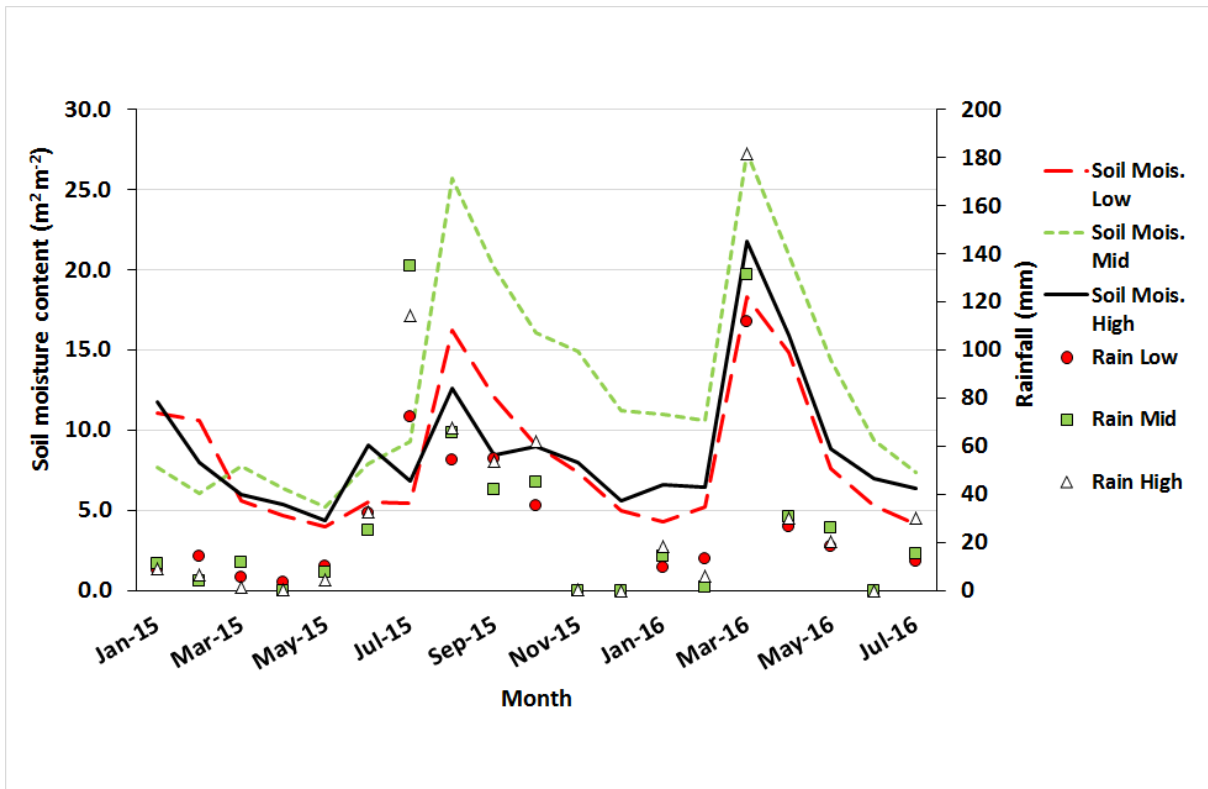
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808 Figure 5. Chlorophyll fluorescence values (Fv/Fm) of 5-year-old trees during winter  
 809 (February 2016), spring (May 2016) and summer (July 2016) when grown at different  
 810 altitudes (High, Mid and Low) and under different irrigation regimes (every 15 days =  
 811 Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation = Control). Data are mean  $\pm$   
 812 SE (n=15). Bars with different letters indicate significant effect of altitudes at each irrigation  
 813 treatment.



815 Figure 6. Rainfall events over time and soil moisture content ( $m^2 m^{-2}$ ) in non-irrigated plots  
 816 containing 5-year-old-trees. Plots located at Low, Mid and High altitudes.

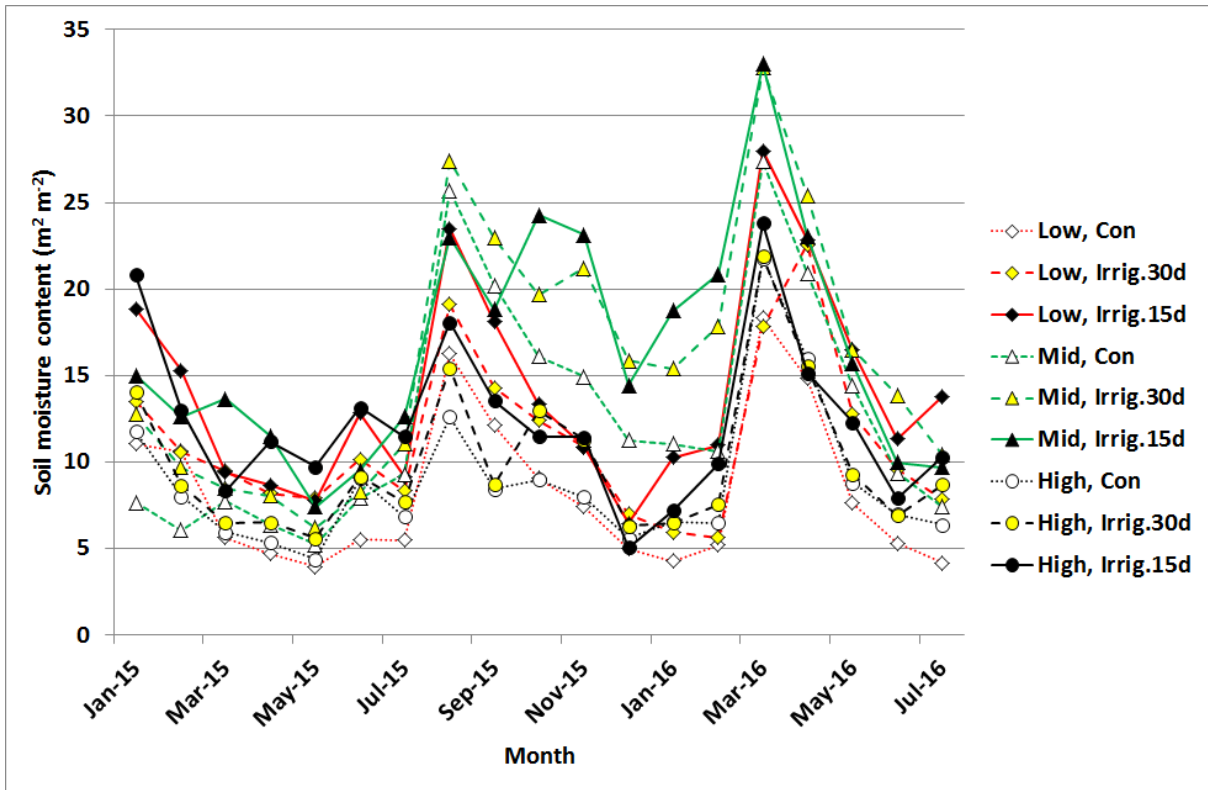


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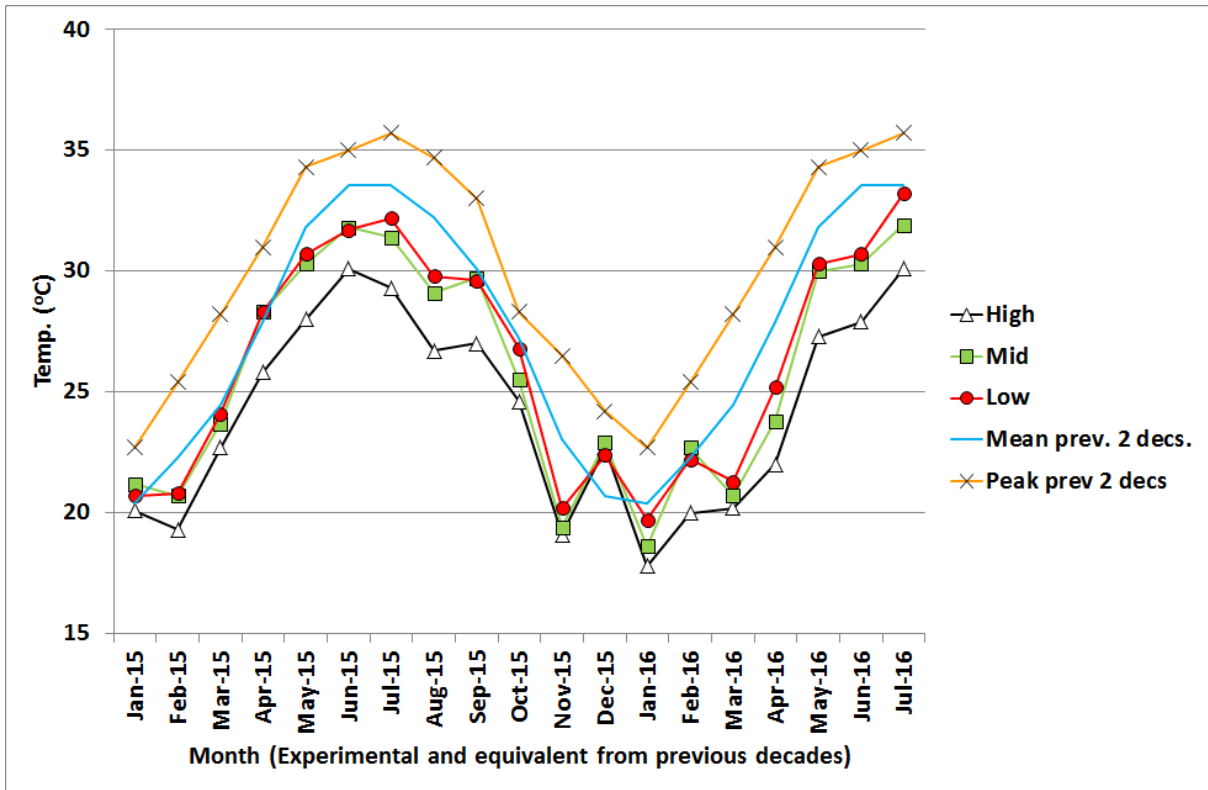
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820 Figure 7. Soil moisture ( $m^2 m^{-2}$ ) availability over time in the plots containing 5-year-old trees.  
 821 Plots located at Low, Mid and High altitudes and irrigated at every 15 days = Irrig.15d, every  
 822 30 days = Irrig.30d or with no artificial irrigation = Control.



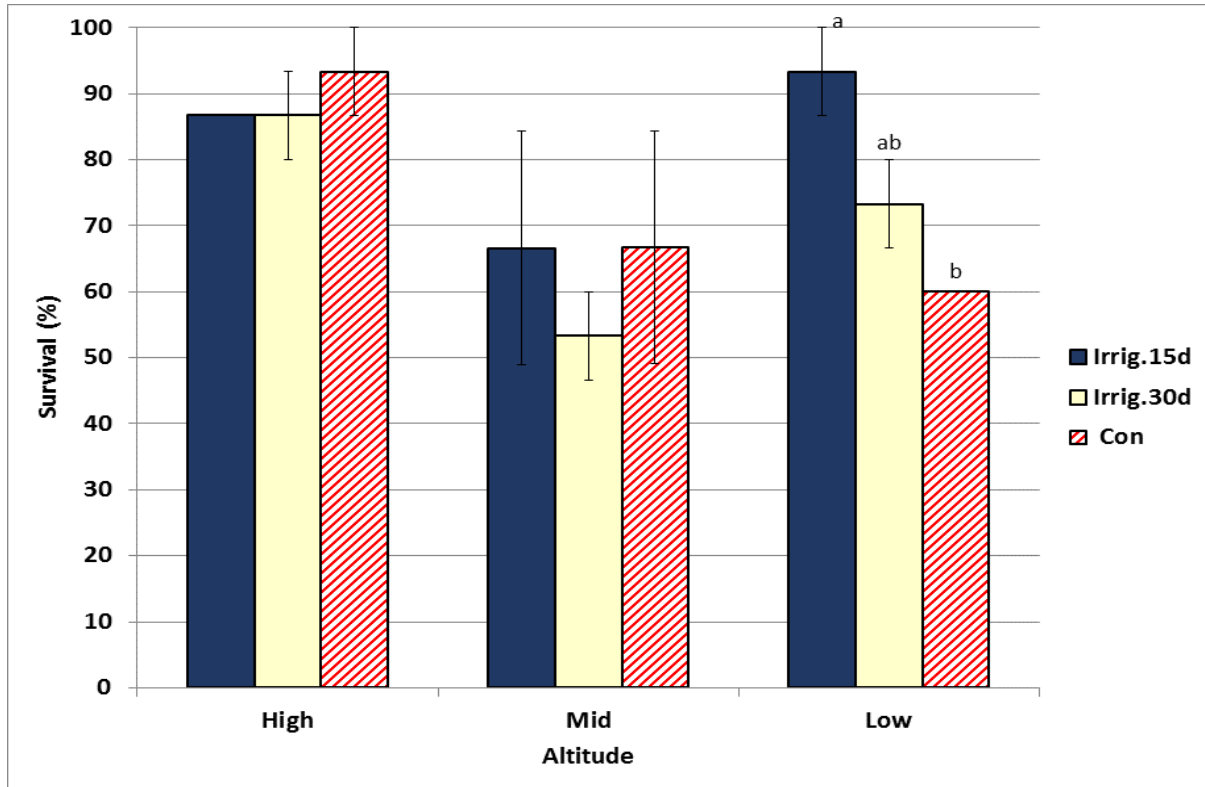
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826 Figure 8. Maximum monthly temperatures recorded at the three altitudes (High, Mid and  
 827 Low) and data compared to that from weather station (depicting mean maximum  
 828 temperatures and peak recorded temperature for the last two decades).



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833 Figure 9. Survival rate of 2-year-old trees planted at different altitudes (High, Mid and Low)  
834 and watered under different irrigation regimes (every 15 days = Irrig.15d, every 30 days =  
835 Irrig.30d and no artificial irrigation = Control). Data are mean  $\pm$  SE (n=3). Bars with different  
836 letters represent significant pairwise differences resulted from Kruskal-Wallis test of  
837 irrigation treatment at Low altitude.

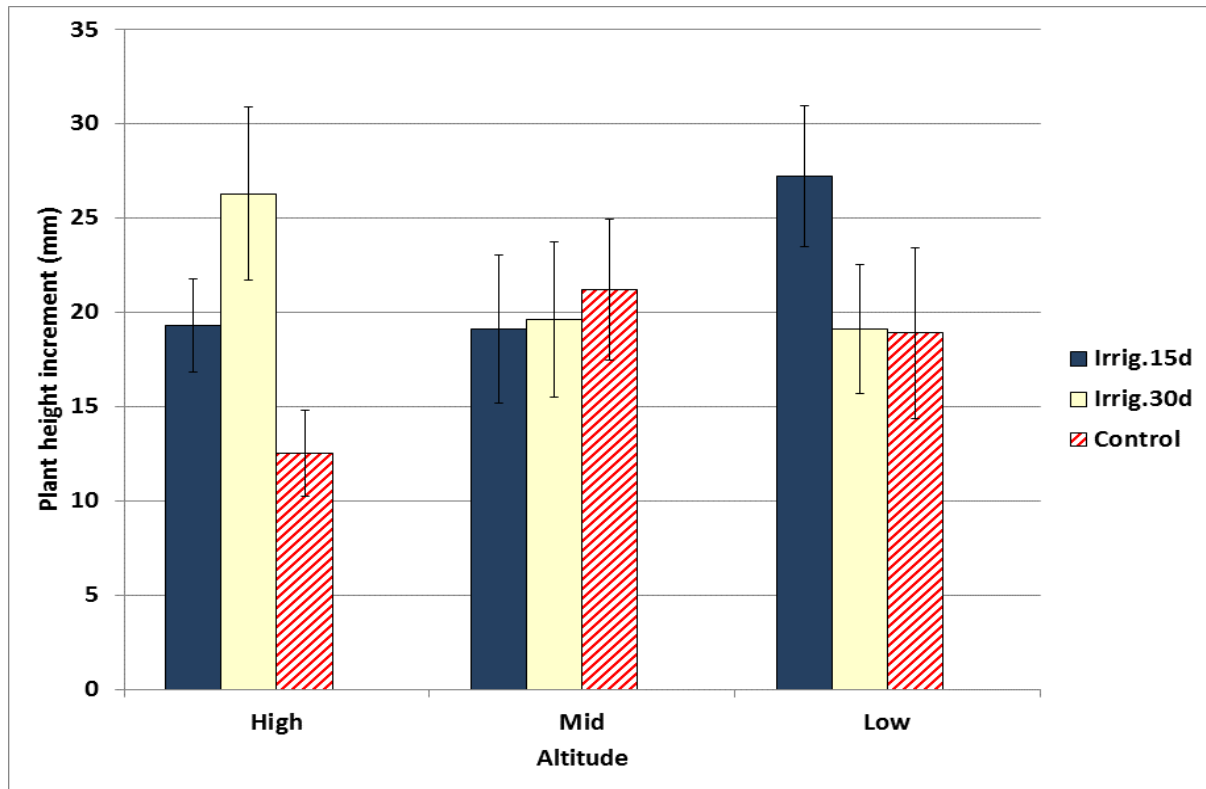


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840 Figure 10. Plant height increment (mm) in 2-year-old trees planted at different altitudes  
841 (High, Mid and Low) and watered under different irrigation regimes (every 15 days =  
842 Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation = Control). Data are mean  $\pm$   
843 SE (n=14).

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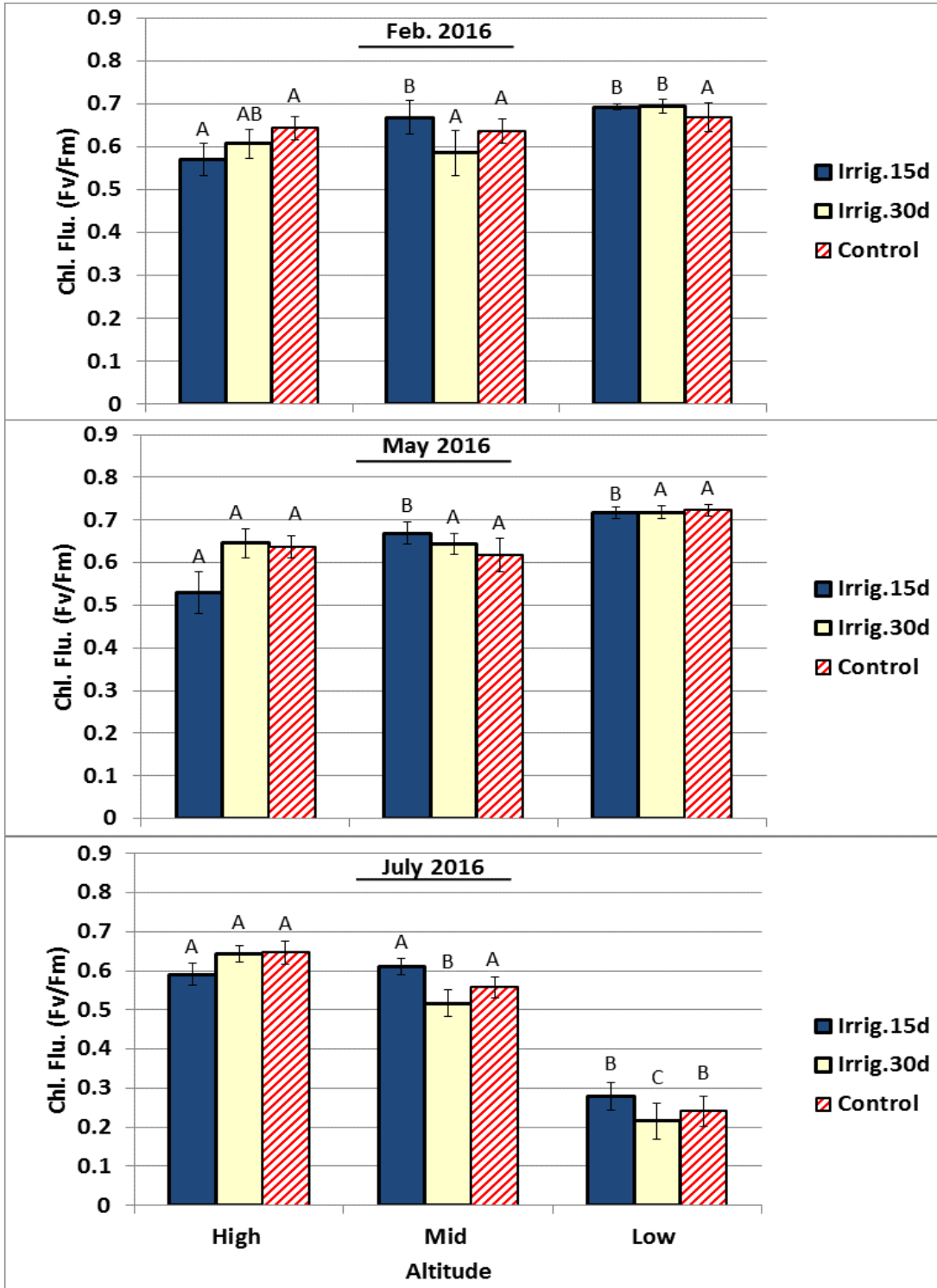
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849 Figure 11. Chlorophyll fluorescence values (Fv/Fm) of 2-year-old trees during winter  
 850 (February 2016), spring (May 2016) and summer (July 2016) when grown at different  
 851 altitudes (High, Mid and Low) and under different irrigation regimes (every 15 days =  
 852 Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation = Control). Data are mean  $\pm$   
 853 SE (n=14). Bars with different letters indicate significant effect of altitudes at same water  
 854 treatment.

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857 Table 1. Total seed number, percentage insect damaged and filled (viable) seed per 100  
 858 cones; and fresh weight of 50 seeds from *Juniperus* cones collected from three different  
 859 altitudes (High, Mid and Low). Data are mean  $\pm$  SE (n=10). Different letters indicate  
 860 significant effect of seed source (altitude).

	Seed number / 100 cones	Insect damaged seed (%)	Filled (viable) seed (%)	Fresh weight / 50 seeds (g)
High altitude	462 $\pm$ 16.4A	4.6 $\pm$ 2.0*	15.3 $\pm$ 1.8*	0.70 $\pm$ 0.07*
Mid altitude	358 $\pm$ 23.2B	2.6 $\pm$ 0.5*	13.3 $\pm$ 2.1*	0.88 $\pm$ 0.05*
Low altitude	368 $\pm$ 16.1B	4.9 $\pm$ 1.8*	9.5 $\pm$ 1.7*	0.89 $\pm$ 0.05*

861 \* no multiple comparison was applied due to non-significant effect.

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865 Table 2. Growth increments (plant height and branch extension [mm]) in 5- year-old trees  
 866 planted at different altitudes (High, Mid and Low) and watered under different irrigation  
 867 regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation =  
 868 Control). Data are mean  $\pm$  SE (n=15). Lower-case letter indicates effect of irrigation  
 869 treatment within altitude and upper-case letter indicates differences between altitudes.

	Altitude	Growth increment (mm)		
		Irrig.15d	Irrig.30d	Control
Plant height*	High	24 $\pm$ 1.7	25 $\pm$ 2.0	24 $\pm$ 2.6
	Mid	34 $\pm$ 3.7	45 $\pm$ 7.5	38 $\pm$ 4.3
	Low	78 $\pm$ 20.1	78 $\pm$ 16.3	37 $\pm$ 6.0
Branch length	High	23 $\pm$ 2.5aA	19 $\pm$ 1.3abA	15 $\pm$ 1.1bA
	Mid	29 $\pm$ 2.7aB	29 $\pm$ 2.7aB	22 $\pm$ 1.5bB
	Low	35 $\pm$ 4.5aC	39 $\pm$ 3.8aC	23 $\pm$ 3.0bB

870 \*Significance tests performed on transformed data for plant height.

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