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

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Abstract

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

Highlights

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

Key Words: Climate change, seed, young trees, drought, heat stress, plant establishment.

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1. Introduction

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

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

In Oman a number of studies have examined the status of *Juniperus seravschanica* woodland, (Fisher and Gardner 1995; Gardner and Fisher 1996; Matwani, 2011; Al Haddabi and Victor, 2016; MacLaren, 2016). Degradation of woodlands within Oman have been associated with an increase in access to the mountains by tourists and other visitors, who use the wood for campfires, and through over-exploitation by local people, who may also use branches for fuel or medicinal purposes, as well as letting their goats and donkeys browse the foliage. In addition, woodland soils are sometimes mechanically stripped off for agricultural use or road construction, with negative consequences for tree stability, root viability and moisture retention in the remaining soil. Removal of the top layer also denudes the existing seed bank held within the soil (Al Haddabi and Victor, 2016; MacLaren, 2016). Although significant, the extent to which these pressures are impacting on *J. seravschanica* depends on local conditions and the degree to which the forests are protected, and this protection being enforced, as destruction is often greater outside nature reserves (Matwani 2011; Al Haddabi and Victor, 2016). Nevertheless, even tree populations within protected areas are declining and a number of researchers have suggested that climate change is a primary factor undermining the viability of *J. seravschanica* in Oman (Al Haddabi and Victor, 2016; MacLaren, 2016).

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

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

An active conservation plan is now in place for *J. seravschanica* in the mountains of Oman, but more information is required on precisely what is causing the decline of the native forest stands. Over-exploitation is one aspect and those areas that are protected from human use and livestock grazing show signs of improvement, but other areas without direct anthropogenic impact are also declining. This raises the question as to what other biotic and abiotic factors may be inducing decline of native stands of *J. seravschanica.* Trees under stress from climate change influences may be more susceptible to pest and disease pressures, but as yet, no pathogens or pests have been correlated with tree decline. The trend for trees located at the lower altitudes to show greater decline than those higher up indicates that direct climatic factors may be to blame for some of the forest degradation. Which climatic factors are primarily responsible, however, is not clear with 1. drought stress due to a lack of water and increasing evapo-transpirational demand, and 2. heat stress due to supra-optimal temperatures, both being likely candidates. Changes in temperature or water availability may be affecting reproduction potential, by reducing the chances of any viable seeds being produced, and preventing those that are viable from germinating and establishing. In her study, MacLaren (2016) only found four seedlings across 86 survey sites. Such environmental changes also seem to be inducing higher mortality rates in mature trees. The aim of this research therefore, is to investigate how temperature and water availability affects development in *J. seravschanica,* and to test the primary hypothesis that altitudinal differences in plant viability relate to a lack of soil water availability and high evapo-transpirational demand inducing drought stress. The research presented here focusses on seed viability and the early development / establishment success of young plants. Secondly, the research aims to identify practical ways of reviving the populations of *J. seravschanica* in the wild, and specific opportunities for enhancing the number of young progeny through nursery cultivation and subsequent transplantation. It is anticipated that information generated from this research will have application to the conservation of other tree species, showing similar trends in population viability across climatic and altitudinal ranges.

2. Materials and Methods

2.1. Plant Material

Three natural populations of *Juniperus seravschanica* were identified, based on their altitudinal range within the Jabal Shams, part of the Al Jabal Al Akhdar mountain range in Oman. These populations were used to monitor plant viability at different altitudes within a wider project remit and as a source of seed (and derived seedling plants) for the individual experiments described here. The populations were selected were:
Population 1 (Low altitude) at 2100-2220 m asl (N 23°17’07.76 and E 57°09’07.76).
Population 2 (Mid altitude) at 2300-2400 m asl (N 23°18’06.22 and E 57°06’15.55) and
Population 3 (High altitude) at 2500-2570 m asl (N 23°19’13.26 and E 57°06’16.02).

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

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

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

For each altitude range, cones were collected from a population of 10 different trees and grouped as a replicate for a given altitude. This was done 3 times for each location during spring 2015. A population of 9000 cones was selected in total (3 altitudes x 10 trees x 100 cones x 3 reps). Seeds were extracted and cleaned (see above). Seeds with signs of insect damage were separated and counted. The total number of undamaged 'healthy' seeds per 100 cones was counted and their viability determined by the presence of the white fleshy intact embryo (i.e. 'filled' seeds). Fresh weight was also measured by selecting batches of 50 seeds from each rep and weighing these.

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

Additional seed collected from Experiment 1 were used to determine germination percentage and rate. Three hundred seeds from individual trees at each altitudinal location were used and divided into two sub-batches: these were either placed in a refrigerator at 4°C for three months (Stratified) or stored in paper bags at an ambient room temperature of approximately 20°C (Control). After three months, seeds were removed from their storage conditions and soaked in warm water for 24 h to help remove any inhibitors to germination. Batches of seed from each treatment combination were then planted in 90 mm dia. plastic pots filled with fine peat; 25 seeds being sown in each pot. Pots were placed in incubators at either 15°C or 25°C to determine how temperature interacted with the other factors to influence germination. These temperatures were selected to cover the spectrum of potentially optimum temperatures for the 3 altitudinal populations (Al Farsi, unpublished). Photoperiod was set at 12:12 h light / dark and chambers illuminated with white fluorescent lamps (Osram L20W / 640sa). Each treatment combination was represented by 3 pots (3 altitudes x 2 stratification x 2 growing temperature x 10 trees x 3 replications) giving a total population of 360 pots or 9000 seeds, mean values per 3 replications were used for analysis. Pots were sub-irrigated once every two weeks, with germination rates monitored weekly over 12 weeks. Germination date, number of days to germination and number of seeds germinating per week were recorded. At the end, germination percentages and mean germination time were calculated. The mean germination time was calculated using the following equation:

$$MGT = \Sigma (n * t) / \Sigma n$$

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

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

Secure sites suitable for seed and tree planting were selected within each of the altitudinal ranges under study and used to assess seed performance *in situ*. These were defined as Low altitude at 2220 m asl (N 23°17'33.4 and E 57°09'07.76); Mid altitude at 2300 m asl (N 23°18'08.19 and E 57°06'14.25) and High altitude located at 2570 m asl (N 23°19'13.26 and E 57°06'16.02). Sowing plots at each altitude were replicated 3 times. In each of the 9 selected field plots an area of 12 m^2 was cleared of grasses and rocks and used to evaluate tree regeneration from field-sown seed. The cleared areas were divided into sub-plots (45 in total each 0.3 x 0.3 m and watered prior to sowing). The same source of seeds used for stock germination was used for field seed germination, i.e. from trees growing naturally at the Mid altitude (2300 m asl). A piece of flat wood was used to ensure seeds were sown at consistent spacing (50 mm apart) and depth (10 mm). Twenty-five seeds were sowed in each sub-plot at 3 different times of year to increase the chances of successful germination and establishment, namely April (just after cone ripening on site and before onset of warmer, drier conditions of summer), June (warmest period and before onset of July-August rain) and September 2014 (end of summer and after July-August rain). Plots were irrigated once every week for one month and then 3 irrigation treatments were applied with 5 replicates per irrigation treatment. The 3 irrigation treatments applied were; ‘Irrig.15d’ (2 L applied every 15 days), Irrig.30d (2 L irrigation every 30 days) or Control with no artificial irrigation and where seeds were reliant on natural precipitation. Plots were irrigated via watering can with a ‘fine spray’ rose, ensuring good distribution of water over each plot.

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

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

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

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

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

2.7. Data handing and statistics.

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

3. Results

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

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

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

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

High altitude, 25^{o}C suppressed germination significantly compared to the lower temperature of 15^{o}C (Figure 2).

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

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

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

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

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

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

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

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

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

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

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

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

4. Discussion

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

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

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

4.1. Environmental stress on young trees

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

Growth was also enhanced in some cases when artificial irrigation was supplied to trees. At the Low altitude, supplementary irrigation increased tree height and side-branch extension of the 5-year-old stock. This is likely to result in a greater foliar canopy area, and hence greater capacity to generate photosynthates. This in turn will promote subsequent root growth and can encourage root proliferation deeper down the soil profile (Grossnickle, 2005). Thus the development of roots growing vertically downwards can capitalise on natural moisture reserves found deeper in the soil profile, and is a common survival strategy adopted by plants in arid environments (Jackson et al., 1996; Canadell et al., 1996; Peek et al., 2006). The risk of artificial irrigation, however, is that if only the upper profiles of the soil are wetted, then root proliferation may only occur at the surface (Fernández et al., 1991; Sokalska et al., 2009), rendering the tree more susceptible to drought stress should the artificial supply of irrigation ever cease (Gilman et al., 2003; Cameron and Hitchmough, 2016). Regular irrigation also limits the capacity of the tree to condition itself against any subsequent severe drought it might experience after irrigation is withdrawn. This is because acclimation to severe drought is itself elicited by exposure to drying soil, or short periods of moderate, sub-lethal, water stress (Cameron et al., 2008). If irrigation is sufficient and regular, then plants may never experience the moderate levels of stress that naturally induce the acclimation response.

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

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

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

Previous research suggests that greater moisture availability and cooler temperatures (and hence, in practice, longer growing periods in summer) favour the development of taller trees further up the mountain profiles (Al Haddabi and Victor, 2016). The highest study plots (2350 m asl) in the Al Haddabi and Victor (2016) survey, however, are equivalent to our Mid altitude treatment, and these authors did not determine tree growth at altitudes represented by our High treatment (i.e. at 2570 m asl). In this present study, however, we generally found the opposite trend, in that extension growth was less in those trees planted at higher altitudes. It should be noted of course that our timeframe was very short relative to the species entire lifespan and caution is required in interpreting such short-term growth trends. Nevertheless, our growth data combined with the winter chlorophyll fluorescence results suggest that growth potential at the top of the mountains may be impaired by cold, as well as by drought and heat at the lower altitudes during summer. Other factors, however, cannot be eliminated from explaining the variations in growth between the plot sites in this study, including factors due to soil nutrient levels or the rapidity by which the young plants became established (i.e. the degree to which they experienced a transplant shock at the different sites).

4.2. Environmental stress on seeds

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

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

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

Collectively, our results support the hypothesis that the populations of *J. seravschanica* growing at the lower altitudinal ranges within the mountains of Oman are under stress, and that this stress is at least partially explained by climatic factors driving drier soils and higher temperatures. This is augmented by further pressure on tree populations (at all altitudes, but again worst at the lower elevations) due to over-browsing by livestock and physical damage to trees through human activities. There was no evidence from this and allied studies to date that tree stocks are being impacted by plant pathogens *per se*, or regeneration limited by rodent activity or other pest species. Research is on-going with respect to the long-term viability of *J. seravschanica* and its associated ecosystem. Nevertheless, based on the research presented here and from recent literature, preliminary recommendations to conserve *J. seravschanica* in the mountains of Oman include:

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

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

It is anticipated that information from this research will aid the practical conservation of *J. seravschanica,* but also highlights the influence of climate change on other montane plant species, and what practical measures should be considered in aiding their conservation. The research raises the controversial dilemma facing conservationists, in that some native plant populations may in the future only survive through active management (in this case, selecting stress-adapted superior progeny, growing trees in nurseries and irrigating them after planting). As well as the financial implications, this raises a range of ethical issues not least in that attempting to deal with anthropogenic climate change; humans will interfere with 'ecological processes' such as natural vegetation succession through their desire to conserve notable, iconic species. In the case of the Oman juniper, on the assumption it is sufficiently genetically distinct from other populations of *J. seravschanica*, then perhaps even more controversially the concept of assisted migration to other mountain ranges (out-with its natural distribution, e.g. western parts of the Zagros mountain range in Iran) where temperature and rainfall patterns are more conducive for survival should be considered. This species is unlikely to reach these areas through natural means, so an introductory programme would be required. The pros and cons of assisted migration are well documented (Ricciardi and Simberloff, 2009; Vitt et al., 2010; Williams and Dumroese, 2013; Koralewski et al., 2015; Sansilvestri et al., 2015) and include ; the ability to conserve a plant species (and potentially a component of its linked ecosystem, as is the case with a keystone species such as *J. seravschanica*) balanced against the potential to introduce invasive alien plants to a new site, or damaging fauna or micro-organisms associated with the translocated species. Such factors can radically disrupt the ecosystem composition, development and functioning of the 'host' site. So the potential impacts on the ecosystem processes and services of any host site, e.g. primary and secondary production, hydrology, nutrient cycles and existing food-webs need to be carefully considered before an assisted migration is undertaken. In light of this, we conclude that every attempt should be made to assist the retention of viable population of *J. seravschanica* in the mountains of Oman through the active management processes outlined here, whilst in the longer term evaluating whether assisted migration is appropriate / feasible for this species. Moreover, such active management processes are likely to aid the conservation of other montane tree species too, similarly under threat from increasingly arid soils and raised aerial temperatures.

4.5. Conclusions

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

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

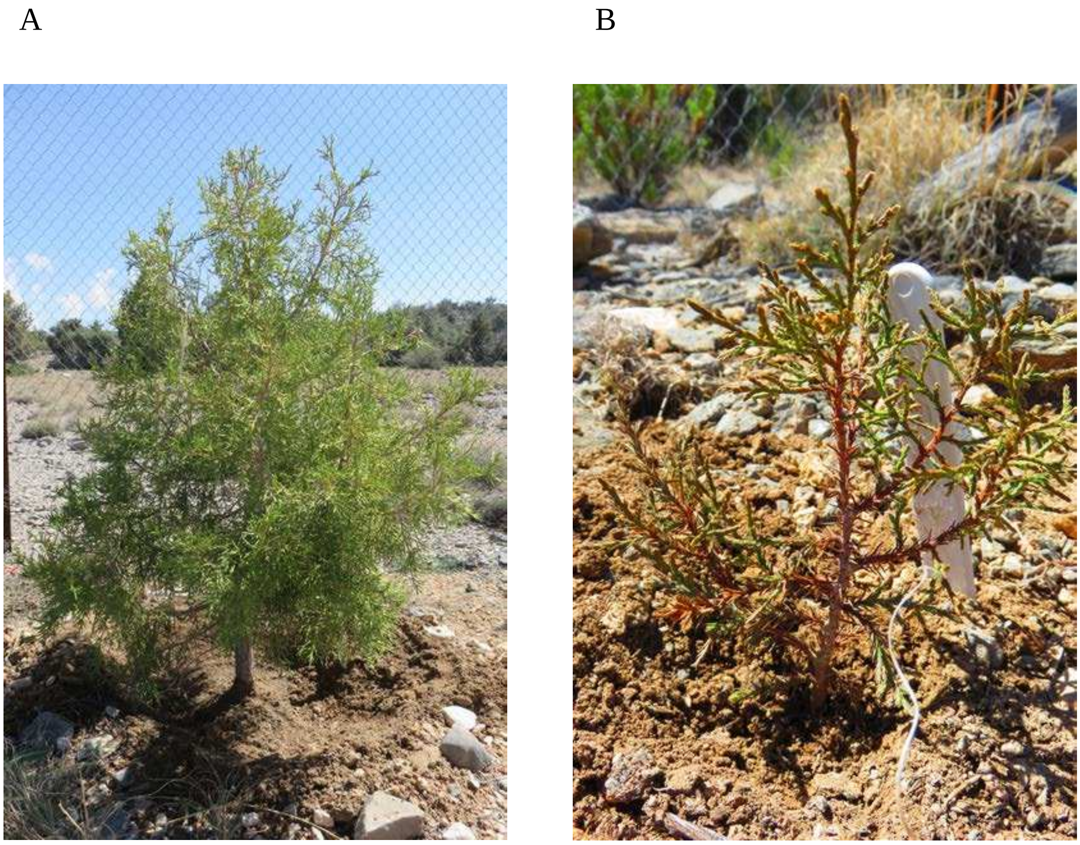

Figure 2. Germination percentage of seed collected from different altitudes (High, Mid and Low) with two pre-treatments (Control, Stratified) and germinated under two temperature regimes (15 and 25°C). Data are mean ± SE. Bars with different lowercase letters indicate significant effect of germination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

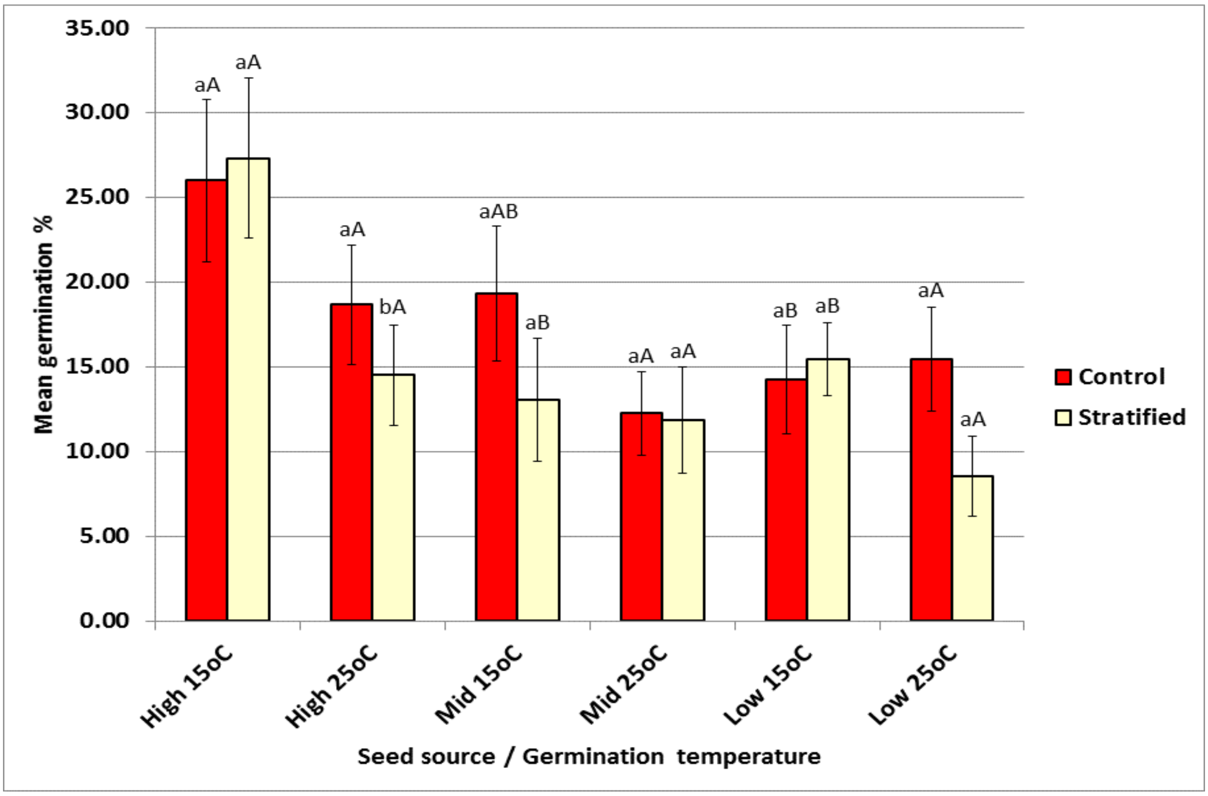

Figure 3. Germination rate of seed as affected by two pre-treatments (Control, Stratified) and growing temperature (15 and 25°C). Data is pooled from three altitudes and represents means ± SE. Bars with different lowercase letters indicate significant effect of pre-treatment at same germination temperature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

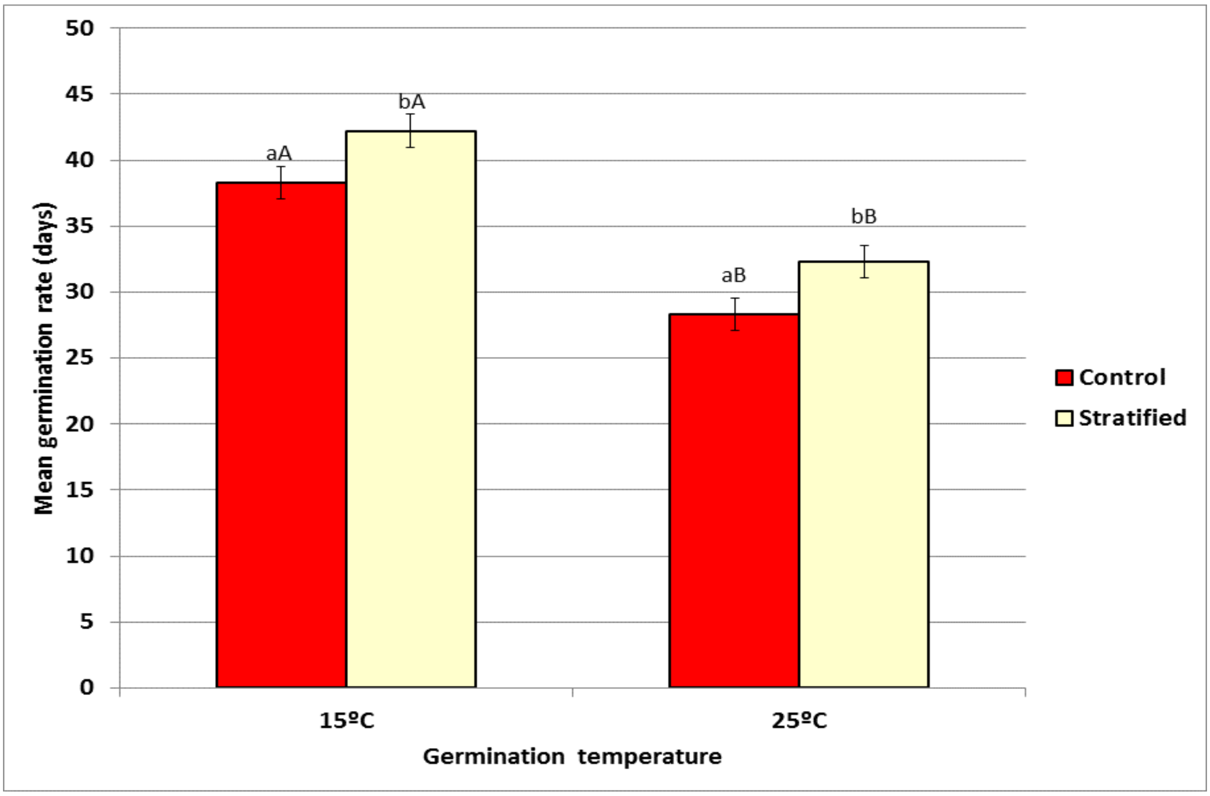

Figure 4. Plant height increment (mm. data square root transformed) in 5-year-old trees planted at different altitudes (High, Mid and Low) and watered under different irrigation regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation = Control). Data are mean ± SE (n=15).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

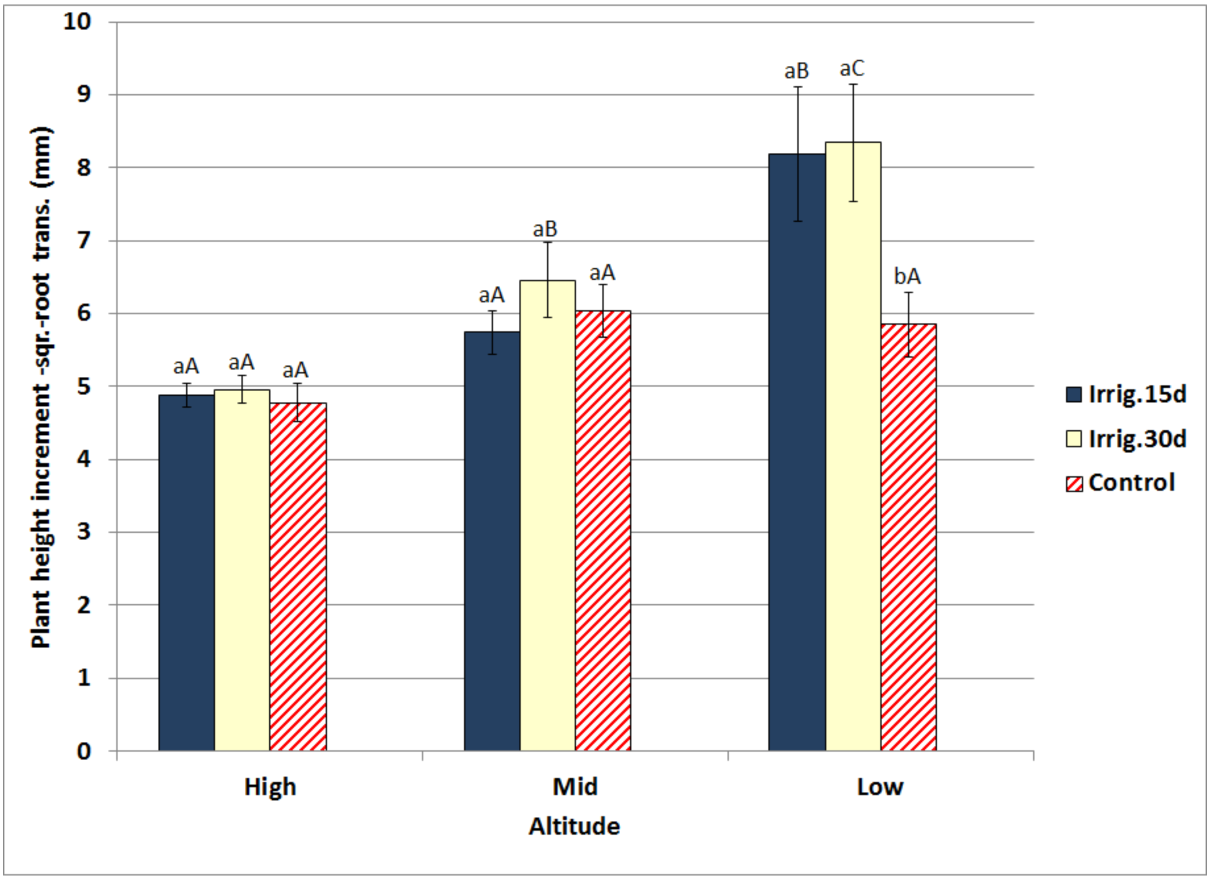

Figure 5. Chlorophyll fluorescence values (Fv/Fm) of 5-year-old trees during winter (February 2016), spring (May 2016) and summer (July 2016) when grown at different altitudes (High, Mid and Low) and under different irrigation regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial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

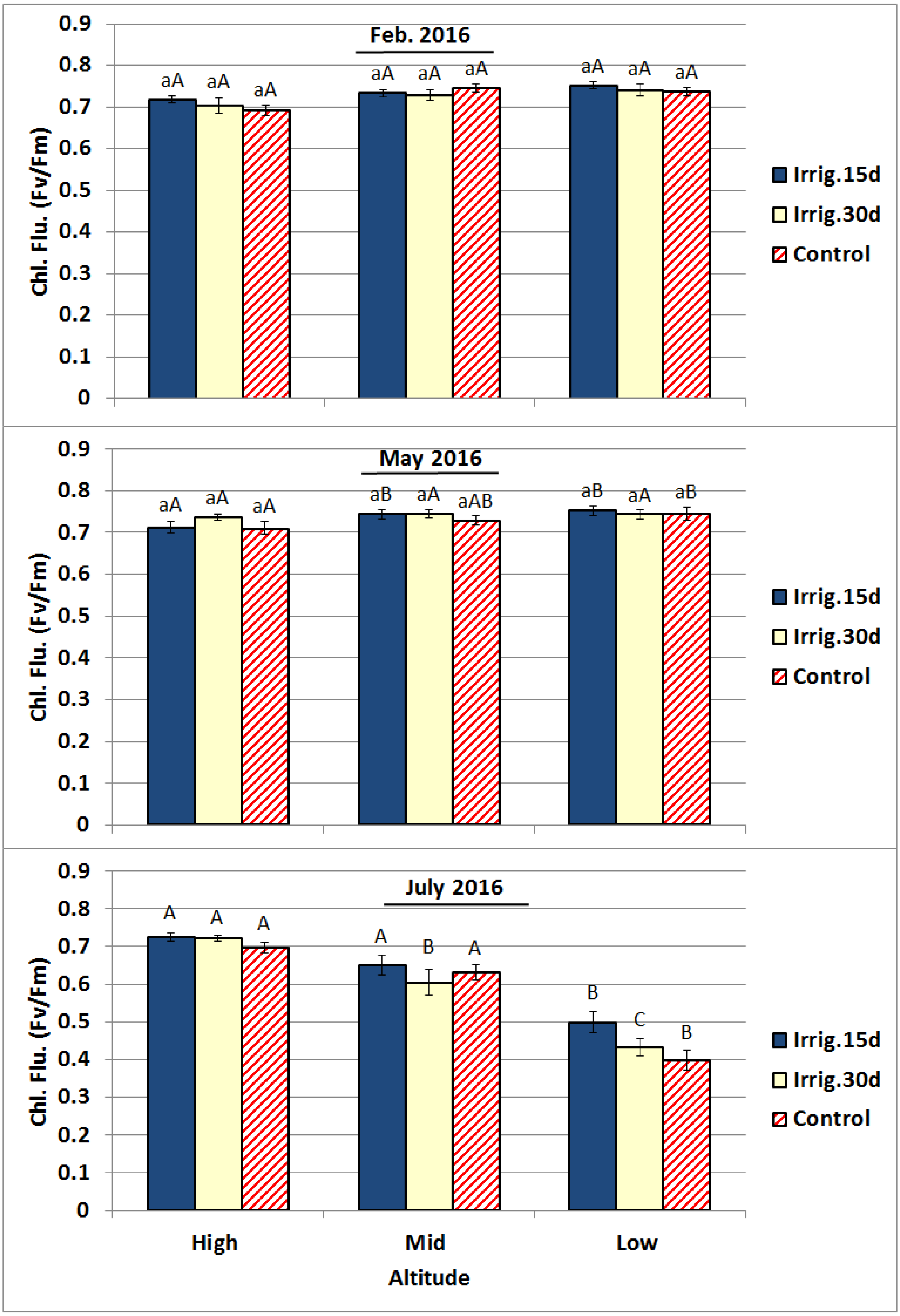

Figure 6. Rainfall events over time and soil moisture content ($m^2\ m^{-2}$) in non-irrigated plots containing 5-year-old-trees. Plots located at Low, Mid and High altitudes.

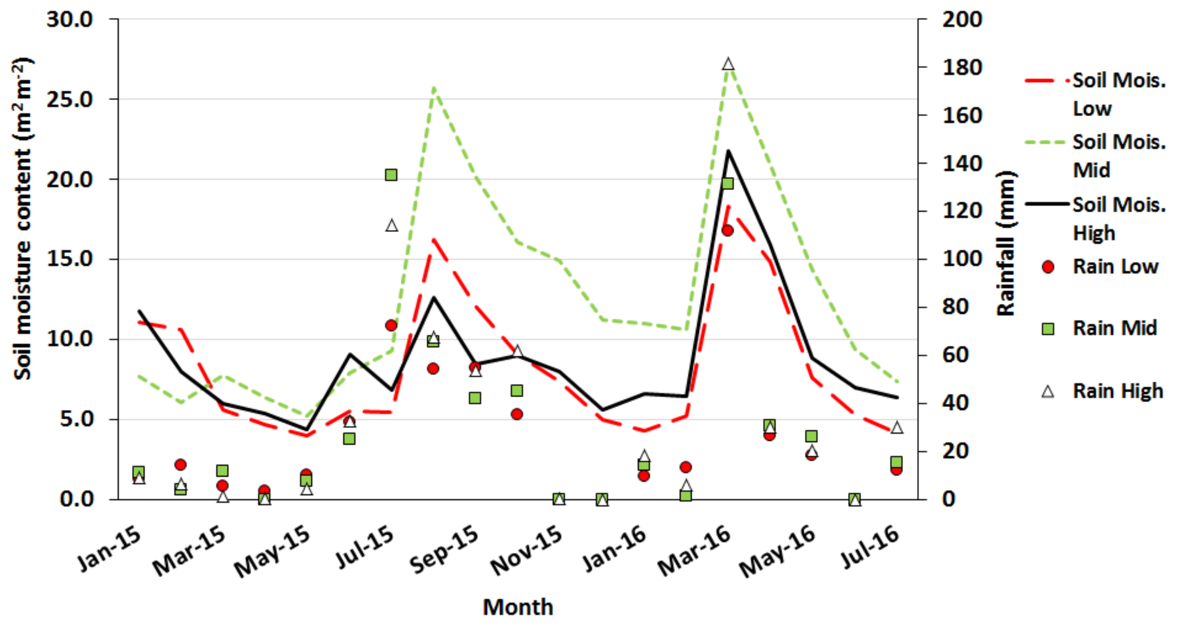

Figure 7. Soil moisture (m^2 m^{-2}) availability over time in the plots containing 5-year-old trees. Plots located at Low, Mid and High altitudes and irrigated at every 15 days = Irrig.15d, every 30 days = Irrig.30d or with no artificial irrigation = Control.

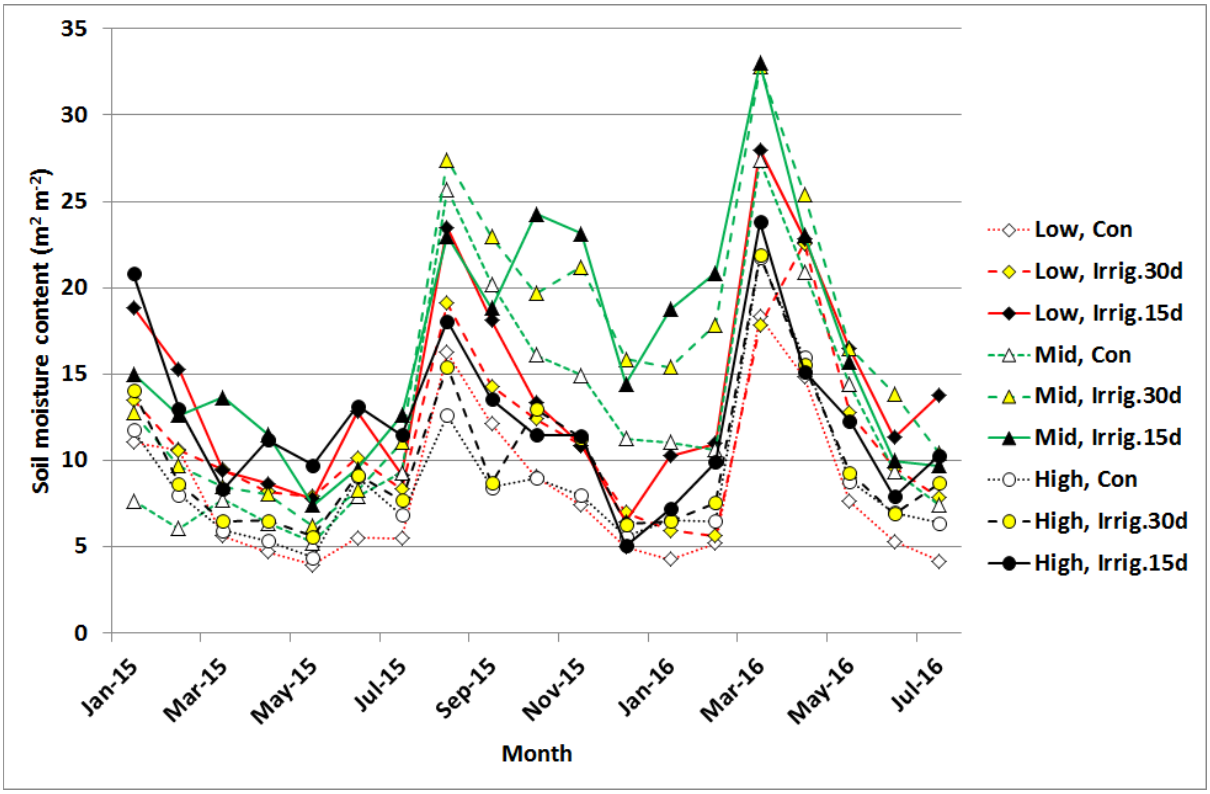

Figure 8. Maximum monthly temperatures recorded at the three altitudes (High, Mid and Low) and data compared to that from weather station (depicting mean maximum temperatures and peak recorded temperature for the last two decades).

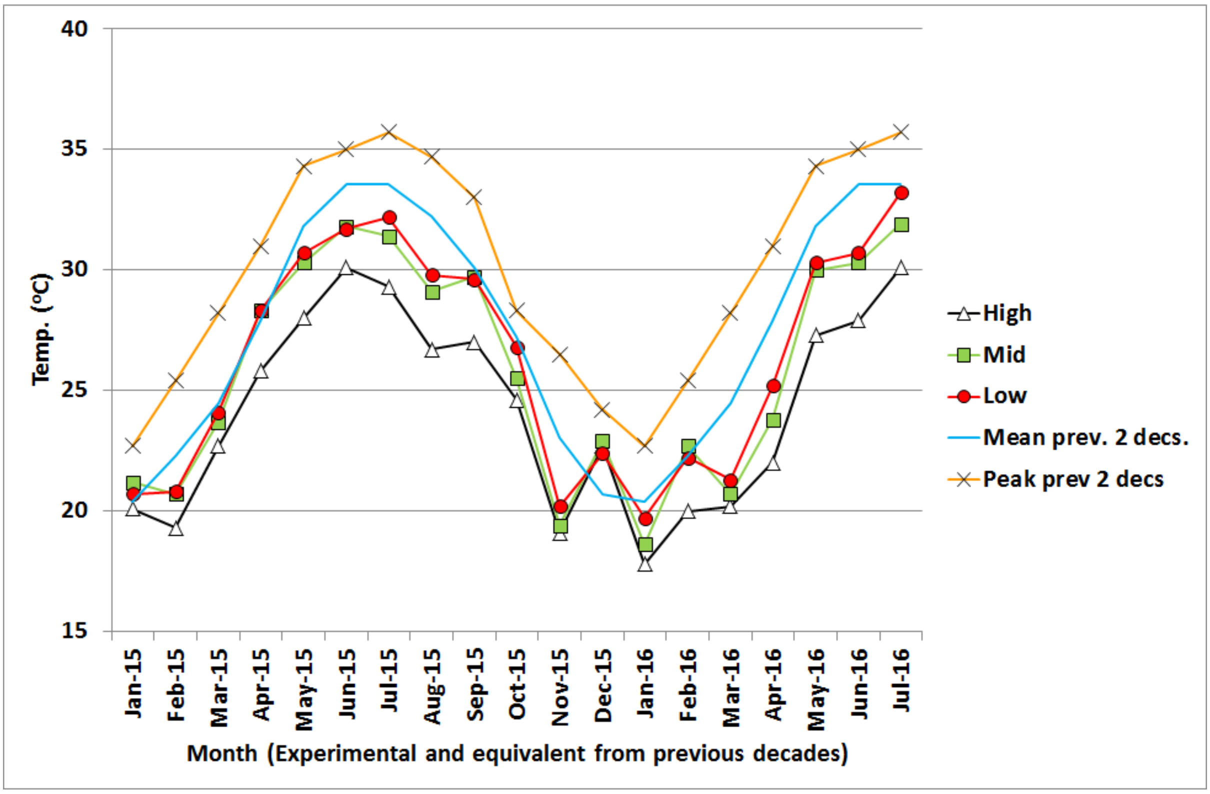

Figure 9. Survival rate of 2-year-old trees planted at different altitudes (High, Mid and Low) and watered under different irrigation regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation = Control). Data are mean ± SE (n=3). Bars with different letters represent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

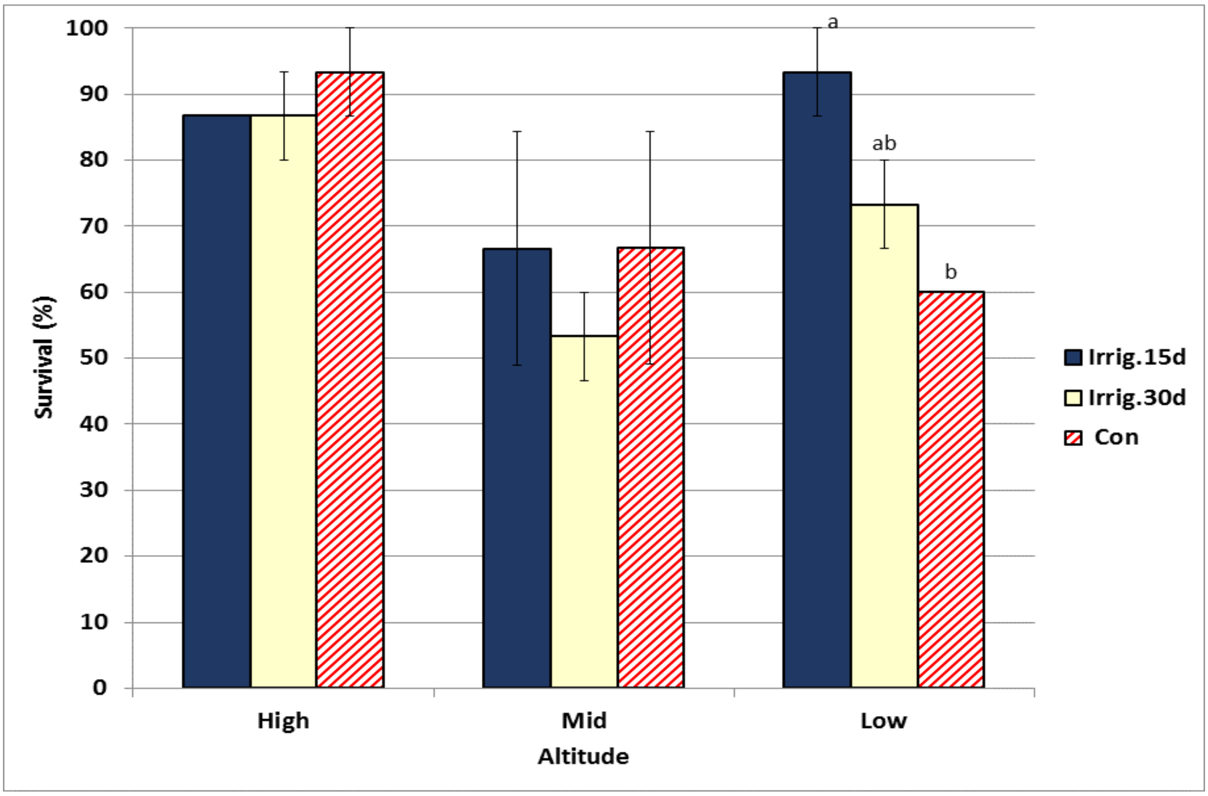

Figure 10. Plant height increment (mm) in 2-year-old trees planted at different altitudes (High, Mid and Low) and watered under different irrigation regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial irrigation = Control). Data are mean ± SE (n=14).

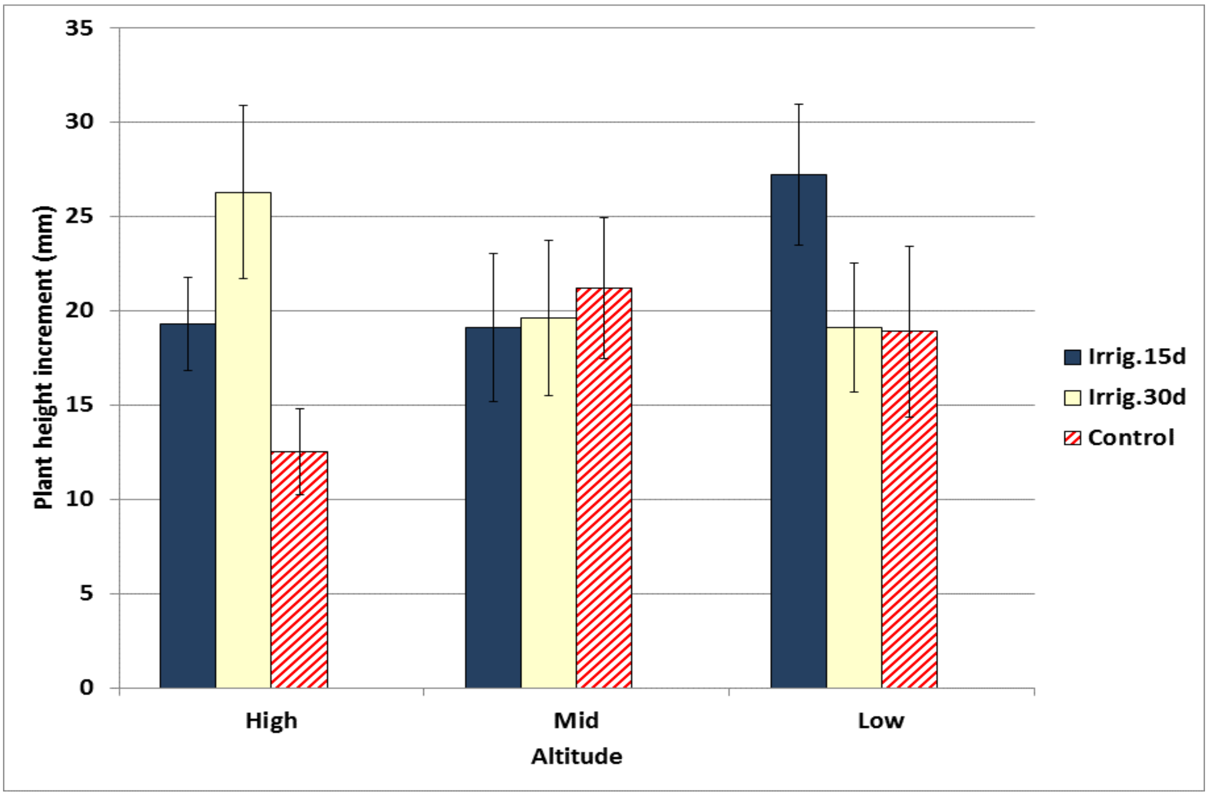

Figure 11. Chlorophyll fluorescence values (Fv/Fm) of 2-year-old trees during winter (February 2016), spring (May 2016) and summer (July 2016) when grown at different altitudes (High, Mid and Low) and under different irrigation regimes (every 15 days = Irrig.15d, every 30 days = Irrig.30d and no artificial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

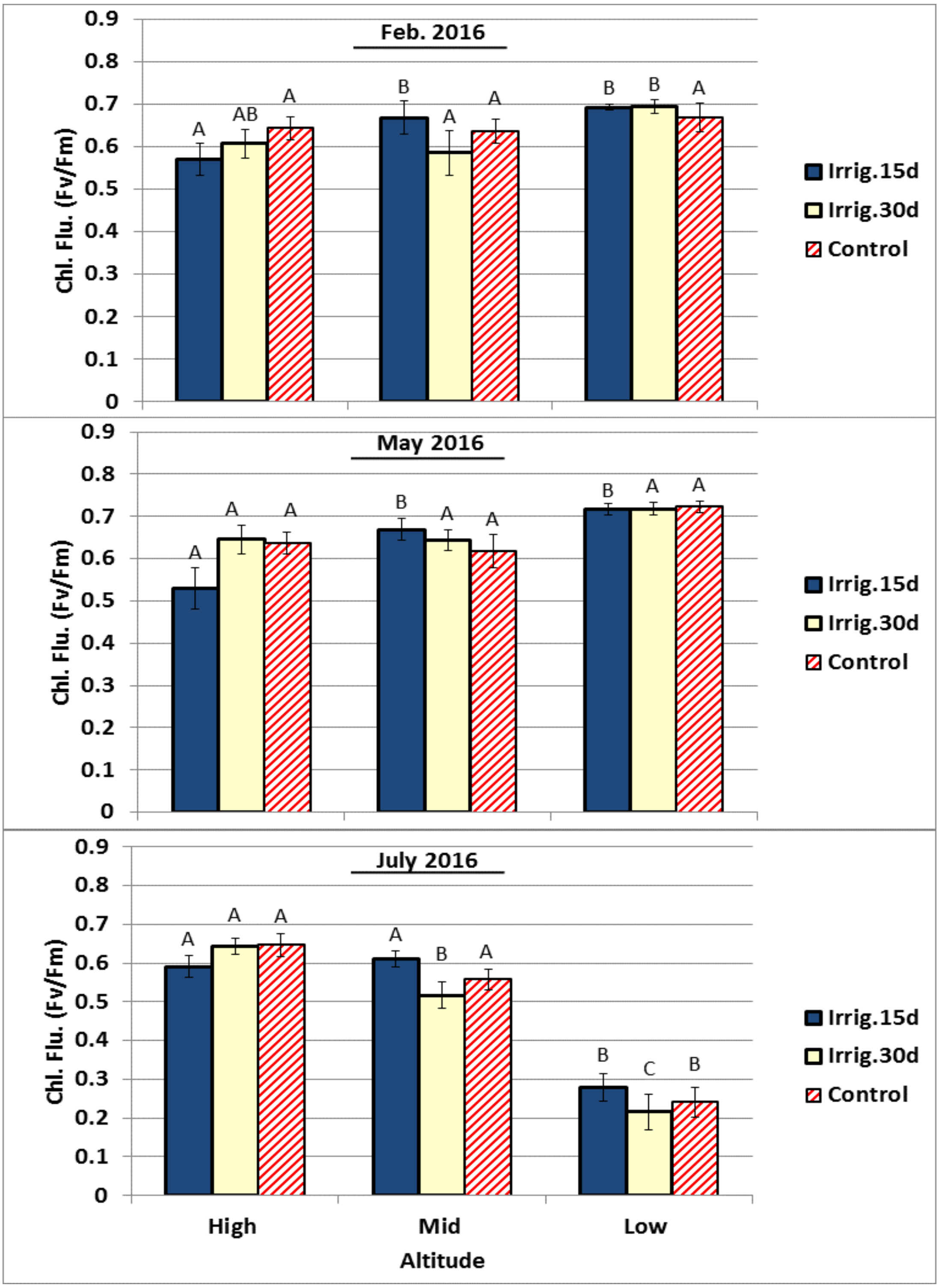

Table 1. Total seed number, percentage insect damaged and filled (viable) seed per 100 cones; and fresh weight of 50 seeds from *Juniperus* cones collected from three different altitudes (High, Mid and Low). Data are mean ± SE (n=10). Different letters indicate significant effect of seed source (altitude).

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

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

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

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

*Significance tests performed on transformed data for plant height.