Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees

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| Abstract: | Higher temperatures associated with climate change are anticipated to trigger an earlier start to the growing season, which could increase the terrestrial C sink strength. Yet greater variability in the amount and timing of precipitation are also expected with higher temperatures, bringing increased drought stress to many ecosystems. We experimentally assessed the effects of higher temperature and drought on the foliar phenology and growth of mature trees of two semi-arid conifer species. We exposed field-grown trees to a ~45% reduction in precipitation with a rain-out structure, a 4.8°C temperature increase with open-top chambers, and a combination of both simultaneously (heat+drought). Over the 2013 growing season, heat, drought, and heat+drought treatments reduced shoot and needle growth in piñon pine (Pinus edulis) by ≥39%, while juniper (Juniperus monosperma) had low growth and little response to these treatments. Needle emergence on primary axis branches of piñon pine was delayed in heat, drought, and heat+drought treatments by 19 to 57 days, while secondary axis branches were less likely to produce needles in the heat treatment, and produced no needles at all in the heat+drought treatment. Growth of shoots and needles, and the timing of needle emergence correlated inversely with xylem water tension and positively with non-structural carbohydrate |

concentrations. Our findings demonstrate the potential for delayed phenological development and reduced growth with higher temperatures and drought in tree species that are vulnerable to drought and reveal potential mechanistic links to physiological stress responses. Climate change projections of an earlier and longer growing season with higher temperatures, and consequent increases in terrestrial C sink strength, may be incorrect for regions where plants will face increased drought stress with climate change.

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1   **Experimental drought and heat can delay phenological development and reduce growth in**

2   **semiarid trees.**

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4   Running Head: Drought and heat affect phenology and growth

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22   **Abstract.**

23   Higher temperatures associated with climate change are anticipated to trigger an earlier

24   start to the growing season, which could increase the terrestrial C sink strength. Yet greater

25   variability in the amount and timing of precipitation are also expected with higher temperatures,

26   bringing increased drought stress to many ecosystems. We experimentally assessed the effects

27   of higher temperature and drought on the foliar phenology and growth of mature trees of two

28   semi-arid conifer species. We exposed field-grown trees to a ~45% reduction in precipitation

29   with a rain-out structure, a 4.8°C temperature increase with open-top chambers, and a

30   combination of both simultaneously (heat+drought). Over the 2013 growing season, heat,

31   drought, and heat+drought treatments reduced shoot and needle growth in piñon pine (*Pinus*

32   *edulis*) by ≥39%, while juniper (*Juniperus monosperma*) had low growth and little response to

33   these treatments. Needle emergence on primary axis branches of piñon pine was delayed in heat,

34   drought, and heat+drought treatments by 19 to 57 days, while secondary axis branches were less

35   likely to produce needles in the heat treatment, and produced no needles at all in the

36   heat+drought treatment. Growth of shoots and needles, and the timing of needle emergence

37   correlated inversely with xylem water tension and positively with non-structural carbohydrate

38   concentrations. Our findings demonstrate the potential for delayed phenological development

39   and reduced growth with higher temperatures and drought in tree species that are vulnerable to

40   drought and reveal potential mechanistic links to physiological stress responses. Climate change

41   projections of an earlier and longer growing season with higher temperatures, and consequent

42   increases in terrestrial C sink strength, may be incorrect for regions where plants will face

43   increased drought stress with climate change.

44

Introduction.

The phenology of plants, i.e. the timing of development and growth, has a strong

influence on the atmospheric concentration of CO_2 at both short and long time scales.

Interannual fluctuations of atmospheric CO_2 concentrations illustrate the dominance of terrestrial

phenology on earth's atmosphere (Keeling 1960, Keeling et al. 1996). At longer-time scales

changes in the phenology of the terrestrial biosphere could affect planetary climate regulation, as

changes in growing season length and associated increases in terrestrial primary production are

an important feedback to climate change (Richardson et al. 2010, 2012, 2013, Keenan et al.

2014). The potential effects of climate change on growing season shifts, typically estimated as a

negative feedback on warming, are represented in the global vegetation components of earth

system models used in climate change projection (Richardson et al. 2012, Oleoson et al. 2013,

Keenan et al. 2014).

Studies of long-term records have consistently found that increased temperatures are

linked to an earlier and longer growing season in temperate (Aono and Kazui 2008, Miller-

Rushing and Primack 2008, Primack et al. 2009) and Mediterranean ecosystems (Peñuelas et al

2002, Ogaya and Peñuelas 2004, Gordo and Sanz 2009). Warming experiments have often

shown the same effect of earlier growth and an extended growing season in trees (Norby et al.

2003, Morin et al. 2010, Han et al. 2014). There is also evidence from experiments that

increased temperatures do not always produce the typical response but can have no effect on

phenological timing (Guak et al. 1998, Kuster et al. 2014). A potential explanation for such

atypical responses could be that temperature increases can be associated with an increase in

drought stress (Overpeck and Udall 2010, Williams et al. 2013). Observations of reduced

growth, decreased branching rate, and delayed leaf development have been reported in trees

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68   subjected to rainfall exclusion experiments (Borghetti et al. 1998, Ogaya and Peñuelas 2004,

69   Peñuelas et al. 2004, Girard et al. 2011, 2012, Limousin et al. 2012).

70   Investigation of the physiological causes of phenological responses may be a useful

71   approach for improving climate change predictions, given the variation in temperature and

72   drought effects on tree growth and development, (Schaber and Badeck 2003). Growth and

73   development are highly sensitive to stress from water availability (Körner 2003, Fatichi et al.

74   2014). Following the sink limitation hypothesis, reduced growth from drought is thought to be

75   caused by the direct effects on turgor necessary for cell wall expansion at active meristems, not

76   through any source limitation of C assimilation or availability of stored C resources (Körner

77   2003, Würth et al. 2005, Fatichi et al. 2014, Palacio et al. 2014). This hypothesis is supported by

78   much work demonstrating that drought, reflected in high xylem water tensions, limits growth

79   more than photosynthesis, and that non-structural carbohydrates (NSC) tend to increase in

80   response to stress and are reduced when environmental conditions favor growth, consistent with

81   change in C sink demand (Tissue and Wright 1995, Körner 2003, Würth et al. 2005, Oberhuber

82   et al. 2011, Gruber et al. 2012, Deslauriers et al. 2014, Fatichi et al. 2014, Dickman et al. 2015).

83   Changes in carbon supply through reduced photosynthesis serve to exacerbate these impacts

84   (McDowell 2011, Adams et al. 2013, Sevanto et al. 2014, Dickman et al. 2015, Hartmann et al.

85   2015).

86   Few studies have examined the combined effects of increased temperature and drought

87   on phenological responses in mature trees, despite the need for improved phenology models in

88   global change assessments (Richardson et al. 2012). Even fewer studies have investigated the

89   physiological processes behind the phenological responses to these environmental stresses. In

90   this study, we measured phenological, growth, and physiological responses to an experimental

91   field manipulation of temperature and drought in mature trees of two semi-arid conifer species,

92   *Juniperus monosperma* and *Pinus edulis*. These tree species are widespread across the

93   Southwest USA, a semi-arid region where tree phenological responses to climate have not been

94   well studied. Our objectives were to determine the effect of temperature and drought, separately

95   and in combination, on tree phenology, and to investigate the causal links between physiological

96   and phenological responses to these treatments. We tested the following hypotheses:

97   1. Increased temperature will cause earlier phenological development and increased growth.

98   2. Drought will delay development and reduce growth.

99   3. In combination, the effects of drought will counteract any heat effects, resulting in no

100   change in development and growth relative to ambient conditions.

101   4. Growth and development will be constrained primarily by xylem tension and will be

102   either unrelated or negatively correlated with NSC concentration.

103

104   **Methods.**

105   *Site description and experimental design.*

106   The Los Alamos Survival-Mortality experiment (SUMO) is located on Frijoles Mesa near

107   Los Alamos, New Mexico, USA, at an elevation of 2150 m (Garcia-Forner et al. 2015). The

108   experiment is located in a piñon-juniper woodland near the ponderosa pine (*Pinus ponderosa*)

109   forest ecotone. The tree community at SUMO is dominated by piñon pine (*Pinus edulis*

110   Engelm.) and one-seed juniper (*Juniperus monosperma* (Engelm.) Sarg.) with Gambel oak

111   (*Quercus gambelli* Nutt.) and the occasional ponderosa pine (*Pinus ponderosa* Douglas ex

112   C.Lawson), alligator juniper (*Juniperus deppeana* Steud.) and rocky mountain juniper (*Juniperus*

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113   *scopulorum* Sarg.) are also found. Soils are Hackroy clay loam and range in depth from 40 to 80

114   cm above a parent material of volcanic tuff.

115   The SUMO experiment includes a below canopy rain-out structure that diverts ~45% of

116   precipitation and 18 transparent open-top chambers (OTCs) regulated by heating and cooling

117   units (RJPL Package Heat Pump and RLPL Package Air Conditioner, Rheem Manufacturing

118   Company, Atlanta GA, USA) to enable temperature control (Garcia-Forner et al. 2015). The

119   rainout structure is similar in design to that described by Pangle et al (2012), but with troughs at

120   ~1.3 m above the ground. Piñon pine and one-seed juniper trees were assigned to 5 treatments,

121   drought (~45% rain-out), heat (~+5°C OTC), heat+drought (~45% rain-out and ~+5°C OTC),

122   ambient (ambient precipitation and temperature) and ambient chamber control (ambient

123   precipitation and ~+0°C OTC; Fig S1). OTCs maintained at ambient temperatures allowed

124   testing for any chamber effects independent of heating. Temperature was monitored by two

125   weather stations at the site and in each OTC at two height locations (1 m and 2/3 tree height;

126   CS215 Temperature and Relative Humidity Probe and CR1000 datalogger, Campbell Scientific,

127   Logan, UT, USA). This system was used as a thermostat to set desired temperature conditions in

128   chambers. Over the course of this study, the mean effect in the heated treatments (heat and

129   heat+drought) was +4.77°C (Fig S2) and in the ambient chamber control was -0.13°C, relative to

130   ambient site conditions.

131

132   *Environmental Conditions.*

133   Treatments were initiated in June 2012 (Garcia-Forner et al. 2015), approximately nine

134   months prior to initial phenology and growth measurements in March of 2013. From 1987-2012,

135   mean annual temperature at SUMO was 10.5°C, varying on average from -0.6°C in December to

136   21.6°C in July, with a mean annual precipitation total of 414 mm

137   (http://environweb.lanl.gov/weathermachine/). Precipitation at the site is influenced by the

138   North American Monsoon season and is highest from July to September with a relatively even

139   distribution throughout the rest of the year. Mean annual temperature in 2012, the year prior to

140   the study, was 12.2°C and total precipitation was 198 mm. Mean annual temperature in 2013,

141   the year of this study, was 9.7°C and total precipitation was 426 mm. However, 45% of annual

142   precipitation in 2013 (194 mm) fell in September (Fig S2), such that the majority of the 2013

143   growing season and study measurements occurred during a period of drought continuing from

144   2011 (Williams et al. 2014).

145

146   *Phenology and Growth.*

147   For each treatment, eleven branches were selected among four piñon pine and juniper

148   trees (2-3 per tree) for phenology and growth measurements. On each branch, we made

149   measurements on the primary axis of growth, and also on a secondary axis that had 3-5 years of

150   growth (measured in piñon pine, estimated in juniper) following the methods of Girard et al.

151   (2011, 2012). After branch selection, initial measurements were made on March 16, 2013 (day

152   of year (DOY) 75) and measurements were repeated periodically (every 9 to 29 days, mean

153   sampling interval was ~18 days) throughout the 2013 growing season until November 19, 2013

154   (DOY 323). In branches of piñon pine, we noted phenophase (developmental stage) and

155   measured the length of any new needles with a digital caliper. We described shoot phenophase

156   using a numerical classification scheme adapted for piñon pine from the methods of Girard et al.

157   (2011, 2012; Table 1, Fig S3). This scheme included the following stages: 1) bud dormant and

158   unchanged in size, 2) bud swelling or growth observed, 3) needle scales open (budbreak), 4) new

159   needle emergence and growth, 5) needle pairs separate. From these measurements we

160   determined the day of needle emergence for each branch (phenophase 4), and calculated the

161   proportion of branches which had needle growth for each piñon pine tree for each sampling date.

162   Concurrently, measurements of shoot length were also made with a digital caliper. For

163   piñon pine, we measured shoot length from the visible boundary that can be easily discerned in

164   this species between the previous year's growth and the base of the bud. As prior annual shoot

165   growth cannot be discerned visually in juniper, we marked the transition between green and

166   brown shoot tissue with ink, and measured from this mark to the tip of the growing shoot. In

167   both species we also noted the appearance of male and female reproductive structures and

168   calculated the percentage of measured branches with these. In juniper, male cones grow in late

169   summer for pollen release the following early spring, and we quantified the presence of both

170   2013 and 2014 pollen cones in 2013. Branches of both species were also photographed

171   approximately each month.

172

173   *Water potential*

174   Plant water potential on each tree (four per treatment) was measured monthly during the

175   2013 growing season to assess xylem water tension. Two twig samples were excised from the

176   south side of each tree before dawn and kept in a refrigerator at the site until measurement within

177   2 hours of collection. Water potential was measured with a Scholander pressure chamber (PMS

178   Instruments, Albany, OR) and determined as the mean of the two samples for each tree. A

179   growing season mean in pre-dawn plant water potential was calculated from monthly

180   measurements taken in March through October 2013.

181

9

182   *Non-structural carbohydrates.*

183   On June, 19 2013 (DOY 170), approximately 1 year after experimental treatments were

184   initiated, tissue samples were collected from all trees in the study for non-structural carbohydrate

185   (NSC) analysis. Foliar and shoot (twig) samples were collected by clipping recent canopy tissue.

186   Bole and large root samples were collected with an increment borer. Roots samples were not

187   obtained for some trees when roots of sufficient size for coring could not be found. All NSC

188   samples were placed in liquid N_2 after collection, transported to the laboratory on dry ice, stored

189   at -70°C, microwaved for 5 minutes at 800 W, and then dried at 65°C for 48 hours. All samples

190   were ground into a fine powder using a ball mill (VWR, Radnor, PA), and woody samples were

191   pre-ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ). NSC, defined as soluble

192   sugars (glucose, fructose, and sucrose) and starch, were assayed with water extraction and

193   enzymatic quantification following a protocol modified from Hoch et al. (2002). Complete

194   details of our NSC protocol were described by Dickman et al. (2015). Extraction was performed

195   on ~12 mg of sample with 1.6 mL of deionized water for 1 hour in a 100°C water bath.

196   Quantification was achieved via NAD-linked enzymatic assay and spectral assessment at 340 nm

197   (Varian Cary 50 UV-Vis spectrophotometer, Palo Alto, CA).

198

199   *Data analysis.*

200   Prior to data analysis, time series of phenology and growth data for each branch were

201   checked and inconsistent or missing data were corrected from branch photographs if possible, or

202   removed. All statistical analyses were performed in SPSS Statistic 22.0 (IBM, Amonk, NY)

203   with an α of 0.05. Before analysis, missing data were imputed conservatively by substituting the

204   measurement recorded at the previous sampling date on the same branch. Repeated measures

10

205   ANOVA was used in time series analysis of proportion of branches with needles, needle growth,

206   and shoot growth, with treatment (5 categories) and time as main fixed factors, and with Fisher's

207   LSD for post-hoc analysis. We used the Huynh-Feldt correction (Huynh and Feldt 1976) to

208   determine significance of results when unequal variance assumptions were violated (as

209   determined by Mauchly's test for sphericity). For day of needle emergence and percent of

210   branches with reproductive structures, data were analyzed with ANOVA using treatments as

211   factors and Fisher's LSD for post-hoc analysis. Data were analyzed using Kruskal-Wallis with

212   Dunn's test for post-hoc analysis if a Levene's test for unequal variance was significant. For

213   analysis of the relationships of NSC and water potential with phenology and growth metrics, we

214   calculated tree means of multiple branch data separately for the primary and secondary axes and

215   used linear regression.

216

217   **Results.**

218   *Phenophase and needle emergence timing in piñon pine.*

219   The proportion of piñon trees that had needle emergence was highest in ambient and

220   chamber control treatments for both primary and secondary branch axes ($p < 0.001$, Fig 1). For

221   the primary axis, this proportion was lower than ambient in the drought and drought+heat

222   treatments ($p < 0.05$), but not in the heat treatment ($p > 0.05$, Fig 1). For the secondary axis

223   branches, the proportion of trees that had needle emergence was lower in the heat, drought, and

224   drought+heat treatments than in ambient or chamber controls ($p < 0.05$). No needle emergence

225   was observed in selected secondary axis branches on trees in the drought+heat treatment (Fig 1).

226   Needle emergence on primary axis branches occurred earliest in ambient and control chamber

227   treatments, on average at DOY 196 (July 15) and 205 (July 24), respectively (Fig 2). Relative to

228   ambient, needle emergence in primary axis branches in the drought, heat, and drought+heat

229   treatments was delayed on average by 19, 39, and 57 days, respectively ($p < 0.05$, Kruskal-

230   Wallis and Dunn's test, Fig 2). There were no significant differences in the emergence timing of

231   secondary axis branches among treatments, likely due to the large variability in responses and

232   the absence of needle growth in the drought+heat treatment ($p > 0.05$). For both axes, standard

233   error around needle emergence timing is higher in heat, drought, and drought+heat treatments

234   relative to ambient, indicating increased variability with climate stress.

235

236   *Needle and shoot growth.*

237   In primary axis branches, piñon needle growth in the ambient and chamber control

238   treatments was greater than that in the drought, heat, and drought+heat treatments ($p < 0.05$, Fig

239   3). For the secondary axis, there were no significant differences in piñon needle growth among

240   ambient, heat and drought trees ($p > 0.05$). However, no piñon needle growth was observed in

241   drought+heat trees, and control chamber trees had higher needle growth than trees in the heat and

242   drought treatments for the secondary axis ($p < 0.01$, Fig 3). Shoot growth of piñon pine over the

243   course of 2013 was higher, and increased earlier, in the ambient and chamber control treatments

244   relative to the heat, drought, and drought+heat treatments for both primary and secondary axes (p

245   < 0.05, Fig 4). For juniper, there was no difference in shoot growth among treatments ($p > 0.05$),

246   and shoot growth was much lower than for piñon pine ($p < 0.001$, Fig 4).

247

248   *Reproductive structures.*

249   Observed differences in the growth of reproductive structures among treatments in both

250   species was minimal. We observed no female cone production for either species, and only one

251　piñon branch in the control chamber treatment had male cones. We observed substantial

252　production of male cones in juniper, both early (cones produced in 2012 which released pollen in

253　early 2013) and later in the year (cones produced in 2013 for pollen release in early 2014, Fig

254　S3). There were no significant treatment effects on juniper male cone production for either year,

255　likely due to the high variability among trees and branches. In the drought+heat treatment, male

256　pollen cones were reduced from 2012 to 2013 on secondary axis branches ($p < 0.05$, Fig S4,

257　Kruskal-Wallis analysis with posthoc Dunn's test).

258

259　*Phenology and Physiology Correlations.*

260　　　　　Shoot and needle growth of piñon were positively correlated with pre-dawn water

261　potential while needle emergence timing was negatively correlated with water potential ($p <$

262　0.05, Fig 5a and c, Table S1). There were no correlations for juniper between shoot growth and

263　water potential ($p > 0.05$). Correlations between shoot growth, needle growth, and needle

264　emergence timing were also found with soluble sugars, starch, and total NSC in bole, needle, and

265　shoot tissues ($p < 0.05$, Fig 5b and d, Table S2). Growth measurements were positively

266　correlated with NSC components with one exception, an inverse relationship for primary axis

267　shoot growth with shoot glucose and fructose ($r = -0.48$, $p < 0.05$). Needle emergence timing

268　was negatively correlated with NSC variables in primary axis branches ($p < 0.05$), but this

269　relationship was not significant for secondary axis branches ($p > 0.05$). Shoot growth, needle

270　growth and needle emergence timing were most consistently correlated with shoot starch content

271　and these relationships generally had the highest correlation coefficients (Table S2, Fig 5c and

272　d). Although the distribution of points in Fig 5c and d suggests a non-linear relationship, non-

273　linear regression did not result in a statistical improvement over a linear trend. For juniper, only

13

274   two correlations with NSC were found, shoot growth in secondary axis branches with shoot

275   glucose and fructose (r = -0.53, p < 0.05) and with shoot total NSC (r = -0.46, p < 0.05; data not

276   show). Monthly and mean growing season pre-dawn water potential were correlated with June

277   NSC variables by tissue in piñon pine (Table S3). Soluble sugars were inversely correlated with

278   water potential, but a greater number of tissue starch and total NSC variables were positively

279   correlated with water potential (p < 0.05, Table S3).

280

281   **Discussion.**

282   Overall, we found that experimental drought and heat reduced growth and delayed

283   phenological development of piñon pine, while juniper growth was unresponsive to treatment.

284   We hypothesized that increased temperature would lead to earlier development and increased

285   shoot and foliar growth. We found no support for this hypothesis in any measured phenological

286   or growth variable. In contrast, the heat treatment (+4.8°C) reduced phenological development

287   in secondary axis branches (Fig 1), delayed needle emergence in primary axis branches (Fig 2),

288   reduced foliar growth (Fig 3), and reduced shoot growth (Fig 4) in piñon pine relative to the

289   ambient treatment. These results contrast starkly with findings from much phenological

290   research, predominately from more mesic ecosystems where increased temperature was

291   associated with earlier development and/or increased growth (Aono and Kazui 2008, Miller-

292   Rushing and Primack 2008, Gordo and Sanz 2009, Primack et al. 2009, Richardson et al. 2013,

293   Keenan et al. 2014). Our results demonstrate that this paradigm may not apply for trees in drier

294   regions.

295   Drought alone delayed phenological development and reduced foliar growth (primary

296   axis), and shoot growth, relative to the ambient treatment, consistent with our hypothesis (Figs 1-

297   4). Moreover, there were no differences in any measured variable between the heat and drought

298   treatments in either branch axis, indicating that these two stresses affected the growth and

299   phenology of piñon pine similarly. Our results were consistent with the phenological and growth

300   responses of several Mediterranean tree species to experimental and natural drought (Borghetti et

301   al. 1998, Ogaya and Peñuelas 2004, Peñuelas et al. 2004, Girard et al. 2011, 2012).

302   Given that the heat and drought treatments both delayed development and reduced

303   growth it was surprising that the drought+heat treatment did not have more of a synergistic effect

304   on piñon pine (Figs 1-4). The effects of heat and drought were most striking for foliar

305   development, where heat delayed emergence by 19 days, drought by 39 days, and drought+heat

306   by 57 days in the primary axis branches (Fig 2), while in secondary axis branches in the

307   drought+heat treatment had no needle growth at all in 2013 (Figs 1 and 2). Although evergreen

308   conifers can still photosynthesize with previous years' foliar growth, these trees went without the

309   higher photosynthetic efficiency of younger foliage for part or all of the growing season (Ethier

310   et al. 2006, Warren 2006).

311   Experiments have been found to under predict advances in leaf-flushing from warming,

312   perhaps due to treatment artifacts (Wolkovich et al 2012). We found no differences in response

313   between our ambient and chamber control treatment making this explanation for our results less

314   likely. Previous research has shown that advances in development from heating can be reduced

315   as temperature increases from ~+1 to ~+3°C (Repo et al. 1996, Morin et al. 2010, Kuster et al.

316   2014), and our temperature treatment was ~+4.8°C. Our result of reduced growth in the heated

317   treatment is consistent with previous research finding that that radial growth of piñon pine and

318   other conifers in the southwest US is negatively correlated with temperature (Adams and Kolb

319   2005, Williams et al. 2013).

320   Our phenology and growth results should be considered in the context of the unusually

321   dry climate of 2011, 2012, and 2013 prior to September (Fig S2; Williams et al. 2014). From

322   January through August 2013 the SUMO experiment site received only 146 mm of precipitation,

323   51% of the 25-year mean of 284 mm for the same period. Prior to this, 2012 annual precipitation

324   was only 48% of the 25-year mean. Thus for the majority of 2013, our heat and drought

325   treatments were imposed on top of an already strong drought. Effects of this background

326   drought on the ambient trees in our experiment may explain why we did not observe any earlier

327   development or increased growth in the heat treatment for piñon pine, relative to the ambient

328   treatment. The lack of any reproductive development in ambient piñon, despite our observations

329   that male pollen cones were common in 2012 in these trees, could also be explained by the dry

330   start to 2013. The low precipitation in 2011-2013 may also explain the low shoot growth of

331   juniper, relative to piñon pine, and also the lack of any treatment effect in juniper shoot growth.

332   Juniper is known for its highly conservative growth in dry years (Howell 1941, Herman 1956).

333   This conservative growth strategy likely enables juniper's survival at hotter and drier sites than

334   piñon pine. Notably, the conditions in the drought+heat treatment during the already dry 2013

335   were not unrealistic, but analogous to those anticipated with future warming in megadrought

336   climate conditions projected for later in the century (Williams et al. 2013, Cook et al. 2015).

337   Consistent with our hypothesis, lower values of water potential appeared to constrain

338   growth and timing of needle emergence in piñon pine (Fig 5, Table S1). Correlation coefficients

339   were typically highest for mean growing season water potential, and values in the months of

340   March, May, June, and August were consistently correlated with growth and phenology,

341   indicating that physiological drought stress over the entire growing season influenced growth and

16

342   development (Fig 5, Table S1). In juniper, low observable variability in growth likely

343   contributed to the lack of correlation with water potential.

344   We hypothesized that non-structural carbohydrates (NSC) would be unrelated to growth

345   and phenology, or that reduced growth and delayed development due to drought stress would be

346   associated with increased NSC, consistent with a reduction in C sink demand preceding a C

347   source limitation (Körner 2003, Fatichi et al. 2014). However, low NSC in June was correlated

348   with reduced growth and delayed needle emergence in piñon pine (Table S2). Correlations were

349   strongest and most consistent across growth and phenology variables for shoot starch (Fig 5),

350   indicating that trees with higher NSC reserves in shoots in the dry early growing season were

351   more likely to show earlier needle emergences and greater growth over the rest of the season.

352   This relationship, while unexpected given previous results from some species (e.g. Körner 2003),

353   is consistent with reduced NSC in piñon pine observed in response to seasonal and prolonged

354   drought stress (Adams et al. 2013, Sevanto et al. 2014, Dickman et al. 2015). The response of

355   piñon is not unique; Mediterranean *Arbutus unedo* with low lignotuber NSC following 14 years

356   of experimental drought had reduced basal area increment growth (Rosas et al. 2013). Despite

357   low variability in juniper growth, we found two significant positive correlations of shoot growth

358   with shoot glucose and fructose, and total NSC, indicating that higher juniper growth was related

359   to increased NSC. Glucose and fructose have been shown to increase in juniper in response to

360   drought and may be important for osmotic adjustment that enables growth under drought

361   conditions in this species (Dickman et al. 2015). The paucity of significant relationships in this

362   species is consistent with our hypothesis that NSC would be primarily unrelated to growth.

363   We urge caution in interpreting positive (inverse) relationships between growth (needle

364   emergence timing) and NSC in these trees as a source limitation. We have correlated growth

365   with NSC from relatively early in the growing season (June 19th, DOY 170). At this time for

366   piñon pine, some shoot growth had been observed in the ambient and control chamber

367   treatments, but needle emergence had not yet occurred in any measured branches. It is possible

368   that NSC increased later in the growing season in trees which had reduced growth. June NSC

369   and its components were correlated with shoot water potential across the growing season (Table

370   S3), complicating the attribution of causation. For all cases where a significant relationship was

371   found for both mean growing season water potential and shoot starch with growth or needle

372   emergence (Fig 5), the adjusted r^2 from a linear regression was always higher for the correlation

373   with water potential than for that with shoot starch concentration. This indicates that direct

374   drought stress on xylem water tension was a stronger constraint for growth and development than

375   NSC.

376   Our results demonstrate that a 4.8C increase in temperature, a change consistent with

377   climate projections for 2100 in many regions with increasing anthropogenic CO_2 emissions

378   (IPCC 2013), can cause phenological delay and reduced growth. Moreover, drought in

379   combination with elevated temperature resulted in either a ~2 month delay in needle emergence

380   or a complete lack of needle growth in piñon pine, which would cause a severe reduction in tree

381   vigor over multiple growing seasons. These strong effects were observed after only ~1 year of

382   treatment, demonstrating that phenological and growth responses to temperature and drought

383   stress can be rapid. Our results indicate that projections based on typical phenological responses

384   of an earlier and longer growing season in response to higher temperatures, and consequent

385   increased ecosystem C sink strength, may not be valid for regions where plants will face

386   increased drought stress with future climate change (Weiss et al. 2012). Trees that experience

387   growth reductions from drought and temperature stress have reduced vigor and are often more

18

388   prone to drought-induced mortality (Manion 1980, Waring 1987, Macalady and Bugmann 2014).

389   Outside of the Mediterranean region, phenological responses of semi-arid tree species to drought

390   and temperature have not been well-studied, and our results highlight the need to evaluate these

391   tree responses in other dry regions. The physiological link between xylem tension and

392   phenological response could provide a mechanistic, process-based approach to account for

393   drought- and temperature-induced developmental delay and reduced productivity in global

394   vegetation models. Correctly representing such phenological feedbacks on the terrestrial C sink

395   with a changing climate is necessary for robust projections of global climate change.

396

397   **Acknowledgments.**

403

404   **References.**

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546

547   **Supporting Information.**

548

549   **Table S1.** Correlations between water potential and shoot growth, needle growth, and emergence

550   timing.

551   **Table S2.** Correlations between NSC and components and shoot growth, needle growth, and

552   emergence timing.

553   **Table S3.** Correlations between water potential and NSC components.

554   **Fig S1.** Design of the Los Alamos Survival-Mortality experiment.

555   **Fig S2.** Daily means in temperature and vapor pressure deficit for the ambient and heated

556   treatments, and total daily precipitation at the SUMO experiment in 2013.

557   **Fig S3.** An illustration of phenological phases in our classification scheme for piñon pine

558   **Fig S4.** Pollen cone production in one-seed juniper by treatment.

27

559   **Figures and Tables**

560

561   **Table 1.** Phenophase descriptions of piñon pine shoot development. This classification scheme

562   was adapted for piñon pine from Girard et al. (2011, 2012). Photographic examples of

563   phenophases in piñon pine are shown in Fig S3.

Phenophase	Description
1	Bud dormant and unchanged in size
2	Bud swelling or growth observed
3	Needle scales open (budbreak)
4	Needle emergence
5	Needle pairs separate

564

565   **Fig 1.** The proportion of piñon pine shoots with needle emergence (phenophase > 4, Table 1) on

566   primary and secondary branches over the 2013 growing season under 5 environmental

567   treatments. Significant differences among treatments (over the growing season) are indicated by

568   letters separately for both primary and secondary axis branches (repeated measures ANOVA, $p <$

569   0.05). Phenophase classification is defined in Table 1 and illustrated in Fig S3. Overlapping

570   values are offset slightly to illustrate all treatments. Error bars are standard errors.

571

572   **Fig 2.** Timing of needle emergence for primary (black) and secondary (gray) axis branches.

573   Significant differences were found for needle emergence timing in the primary, but not

574   secondary, axis branches, and are indicated with letters (Kruskal-Wallis analysis and posthoc

575   Dunn's test). Note that no needle growth was observed in secondary axis branches in the

576   drought+heat treatment during the 2013 growing season. Error bars are standard errors.

577

578   **Fig 3.** Piñon pine needle elongation on primary and secondary axis shoots over the 2013

579   growing season under 5 environmental treatments for shoots that had needle emergence.

580   Significant differences among treatments (over the growing season) are indicated by letters

581   separately for both primary and secondary axis branches (repeated measures ANOVA, $p > 0.05$).

582   Piñon pine trees under ambient conditions had greater needle elongation than trees in the heat,

583   drought, and drought+heat treatments on the primary axis, and the drought treatment on the

584   secondary axis. A line is shown at zero for the lack of needle growth observed on secondary axis

585   shoots in the drought+heat treatment; this was not included in statistical analysis. The occasional

586   appearance of decline in mean needle growth over the growing season is due to the error in the

587   growth measurement. Error bars are standard errors.

588

589   **Fig 4.** Shoot growth on primary and secondary axis branches of piñon pine (*Pinus edulis*) and

590   one-seed juniper (*Juniperus monosperma*) over the 2013 growing season under 5 environmental

591   treatments. Shoot growth increased significantly in both species over the growing season

592   (repeated measures ANOVA, $p < 0.001$). Treatment effects and treatment by time interactions

593   were significant only for piñon pine ($p < 0.001$). Letters indicate significant differences in effects

594   among treatments for piñon pine over the growing season; shoot growth was reduced in the heat,

595   drought, and drought+heat treatments, relative to control treatments ($p < 0.05$). The occasional

596   appearance of decline in mean shoot growth over time is a due to the error in the growth

597   measurement. Error bars are standard errors.

598

599   **Fig 5.** Relationships in piñon pine between mean growing season pre-dawn water potential (Ψ_{pd})

600   and growth (**a**), June twig starch concentration and growth (**b**), mean growing season Ψ_{pd} and

601   timing of needle emergence (**c**), and June twig starch concentration and timing of needle

602   emergence (**d**), for primary (red) and secondary axis (blue) branches. Relationships with growth

603   are shown for both shoots (circle, solid line) and needles (triangle, dashed line) in **a** and **b**.

604   Significant linear regressions between water potential and primary axis shoot ($r^2 = 0.39$) and

605   needle ($r^2 = 0.45$) growth, secondary shoot ($r^2 = 0.51$) and needle ($r^2 = 0.68$) growth, and primary

606   needle emergence timing ($r^2 = 0.49$) are shown in **a** and **c**. Significant linear regressions between

607   June twig starch concentration and primary axis shoot ($r^2 = 0.24$) and needle ($r^2 = 0.29$) growth,

608   secondary shoot ($r^2 = 0.39$) and needle ($r^2 = 0.59$) growth, and primary needle emergence timing

609   ($r^2 = 0.43$) are shown in **b** and **d**. Additional growth and phenology correlations with water

610   potential and non-structural carbohydrate components for bole, needle, root and twig tissues are

611   found in Tables S1 and S2. Analogous relationships for juniper shoot growth were not observed

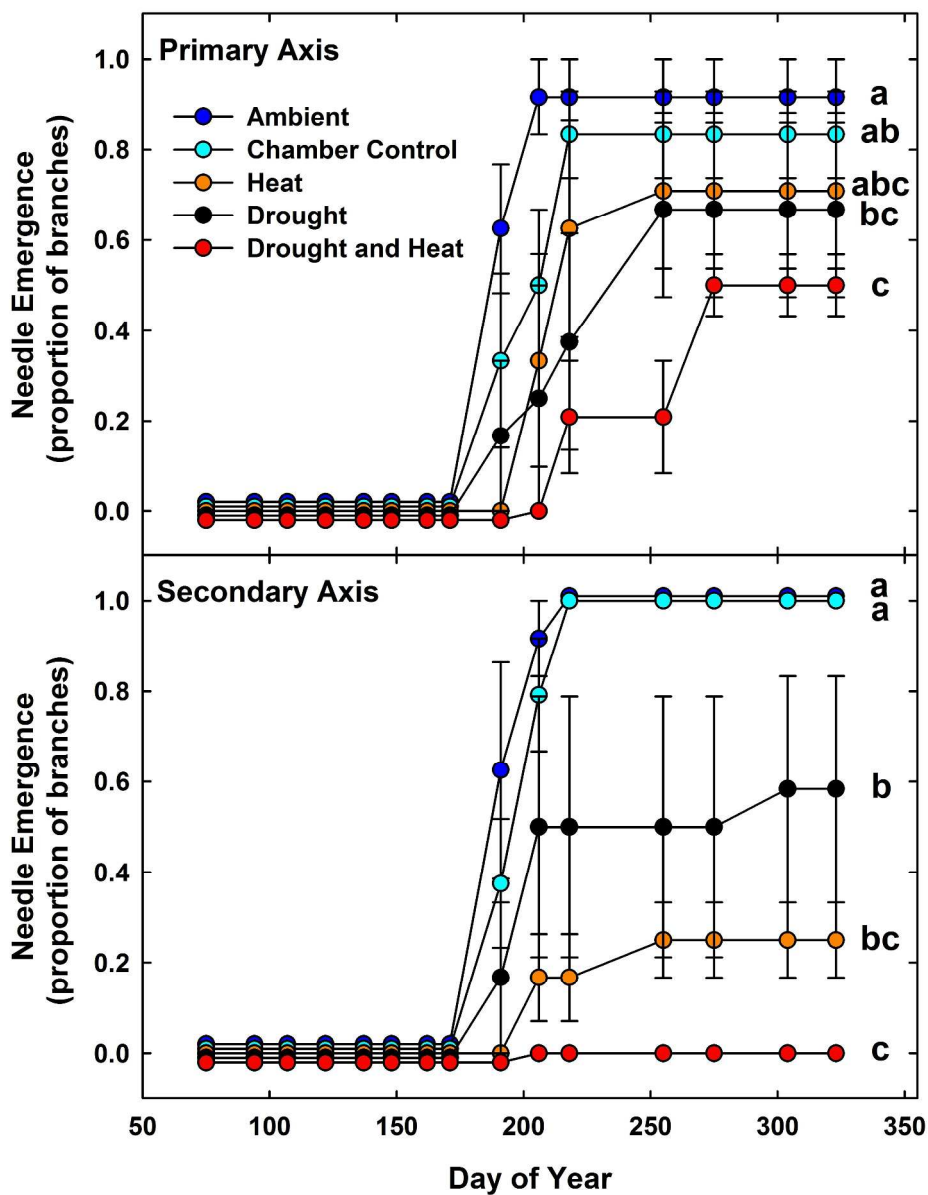612   and are not shown (p > 0.05).

613

Fig 1. The proportion of piñon pine shoots with needle emergence (phenophase > 4, Table 1) on primary and secondary branches over the 2013 growing season under 5 environmental treatments. Significant differences among treatments (over the growing season) are indicated by letters separately for both primary and secondary axis branches (repeated measures ANOVA, $p < 0.05$). Phenophase classification is defined in Table 1 and illustrated in Fig S3. Overlapping values are offset slightly to illustrate all treatments. Error bars are standard errors.
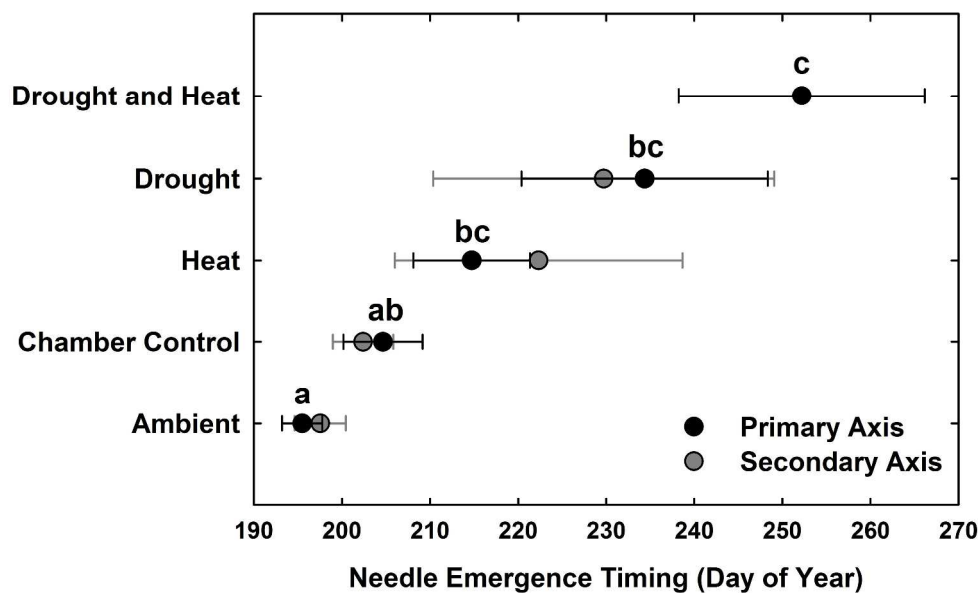611x786mm (150 x 150 DPI)

Fig 2. Timing of needle emergence for primary (black) and secondary (gray) axis branches. Significant differences were found for needle emergence timing in the primary, but not secondary, axis branches, and are indicated with letters (Kruskal-Wallis analysis and posthoc Dunn's test). Note that no needle growth was observed in secondary axis branches in the drought+heat treatment during the 2013 growing season. Error bars are standard errors.
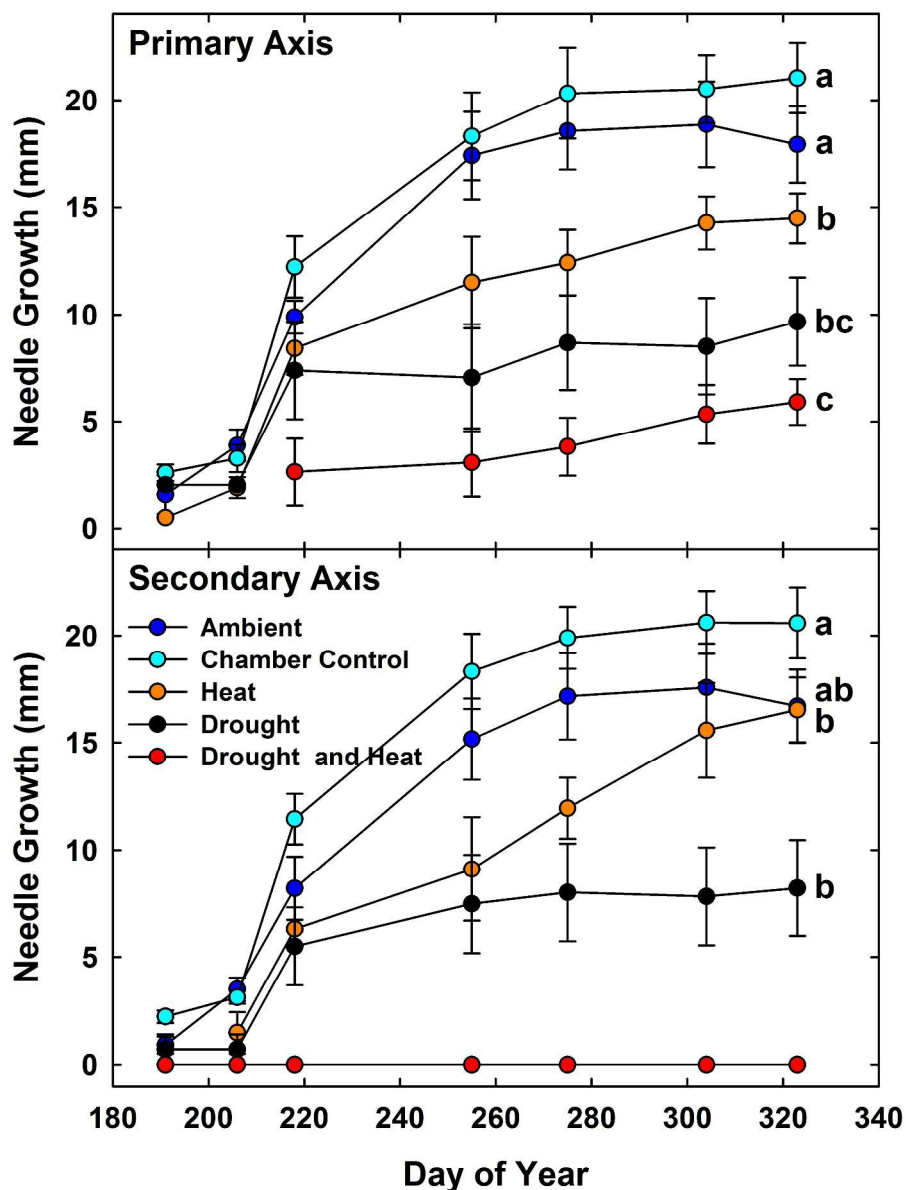721x434mm (150 x 150 DPI)

Fig 3. Piñon pine needle elongation on primary and secondary axis shoots over the 2013 growing season under 5 environmental treatments for shoots that had needle emergence. Significant differences among treatments (over the growing season) are indicated by letters separately for both primary and secondary axis branches (repeated measures ANOVA, p > 0.05). Piñon pine trees under ambient conditions had greater needle elongation than trees in the heat, drought, and drought+heat treatments on the primary axis, and the drought treatment on the secondary axis. A line is shown at zero for the lack of needle growth observed on secondary axis shoots in the drought+heat treatment; this was not included in statistical analysis. The occasional appearance of decline in mean needle growth over the growing season is due to the error in the growth measurement. Error bars are standard errors.
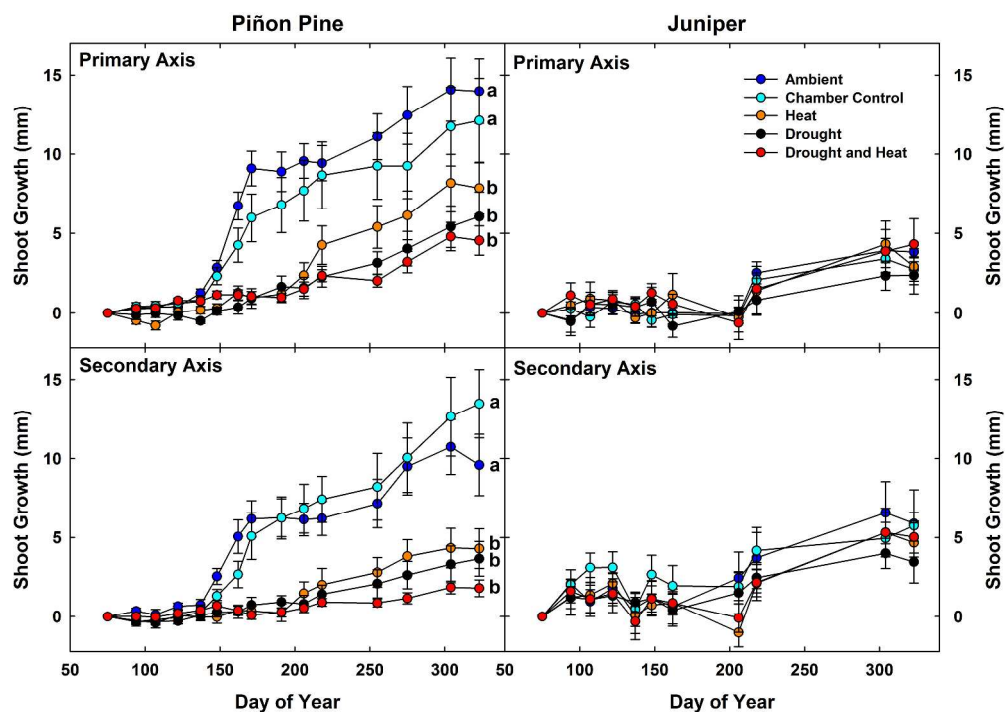602x796mm (150 x 150 DPI)

Fig 4. Shoot growth on primary and secondary axis branches of piñon pine (Pinus edulis) and one-seed juniper (Juniperus monosperma) over the 2013 growing season under 5 environmental treatments. Shoot growth increased significantly in both species over the growing season (repeated measures ANOVA, $p < 0.001$). Treatment effects and treatment by time interactions were significant only for piñon pine ($p < 0.001$). Letters indicate significant differences in effects among treatments for piñon pine over the growing season; shoot growth was reduced in the heat, drought, and drought+heat treatments, relative to control treatments ($p < 0.05$). The occasional appearance of decline in mean shoot growth over time is a due to the error in the growth measurement. Error bars are standard errors.
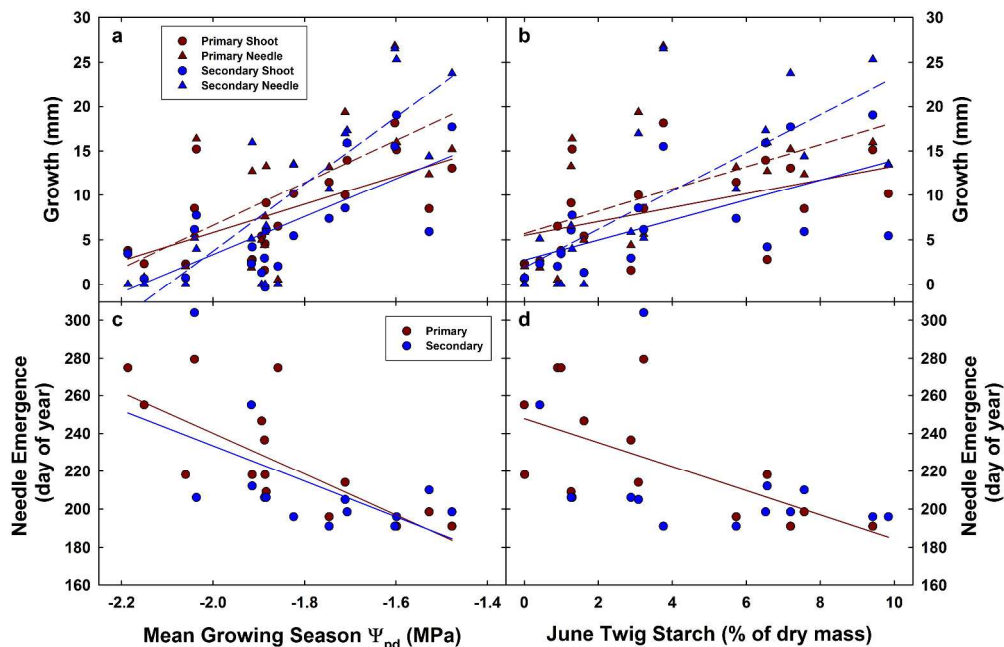1168x826mm (150 x 150 DPI)

Fig 5. Relationships in piñon pine between mean growing season pre-dawn water potential (Ψpd) and growth (a), June twig starch concentration and growth (b), mean growing season Ψpd and timing of needle emergence (c), and June twig starch concentration and timing of needle emergence (d), for primary (red) and secondary axis (blue) branches. Relationships with growth are shown for both shoots (circle, solid line) and needles (triangle, dashed line) in a and b. Significant linear regressions between water potential and primary axis shoot ($r2 = 0.39$) and needle ($r2 = 0.45$) growth, secondary shoot ($r2 = 0.51$) and needle ($r2 = 0.68$) growth, and primary needle emergence timing ($r2 = 0.49$) are shown in a and c. Significant linear regressions between June twig starch concentration and primary axis shoot ($r2 = 0.24$) and needle ($r2 = 0.29$) growth, secondary shoot ($r2 = 0.39$) and needle ($r2 = 0.59$) growth, and primary needle emergence timing ($r2 = 0.43$) are shown in b and d. Additional growth and phenology correlations with water potential and non-structural carbohydrate components for bole, needle, root and twig tissues are found in Tables S1 and S2. Analogous relationships for juniper shoot growth were not observed and are not shown ($p > 0.05$).

1249x816mm (150 x 150 DPI)

1   **Experimental drought and heat can delay phenological development and reduce growth in**

2   **semiarid trees.**

3

4   Henry D. Adams[1], Adam D. Collins[1], Samuel P. Briggs[1], Michel Vennetier[2], L. Turin Dickman[1],

5   Sanna A. Sevanto[1], Núria Garcia-Forner[3,4], Nate G. McDowell[1].

6

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11

12   **Supporting Information.**

13

14

15   **Supporting Figures**

Precipitation
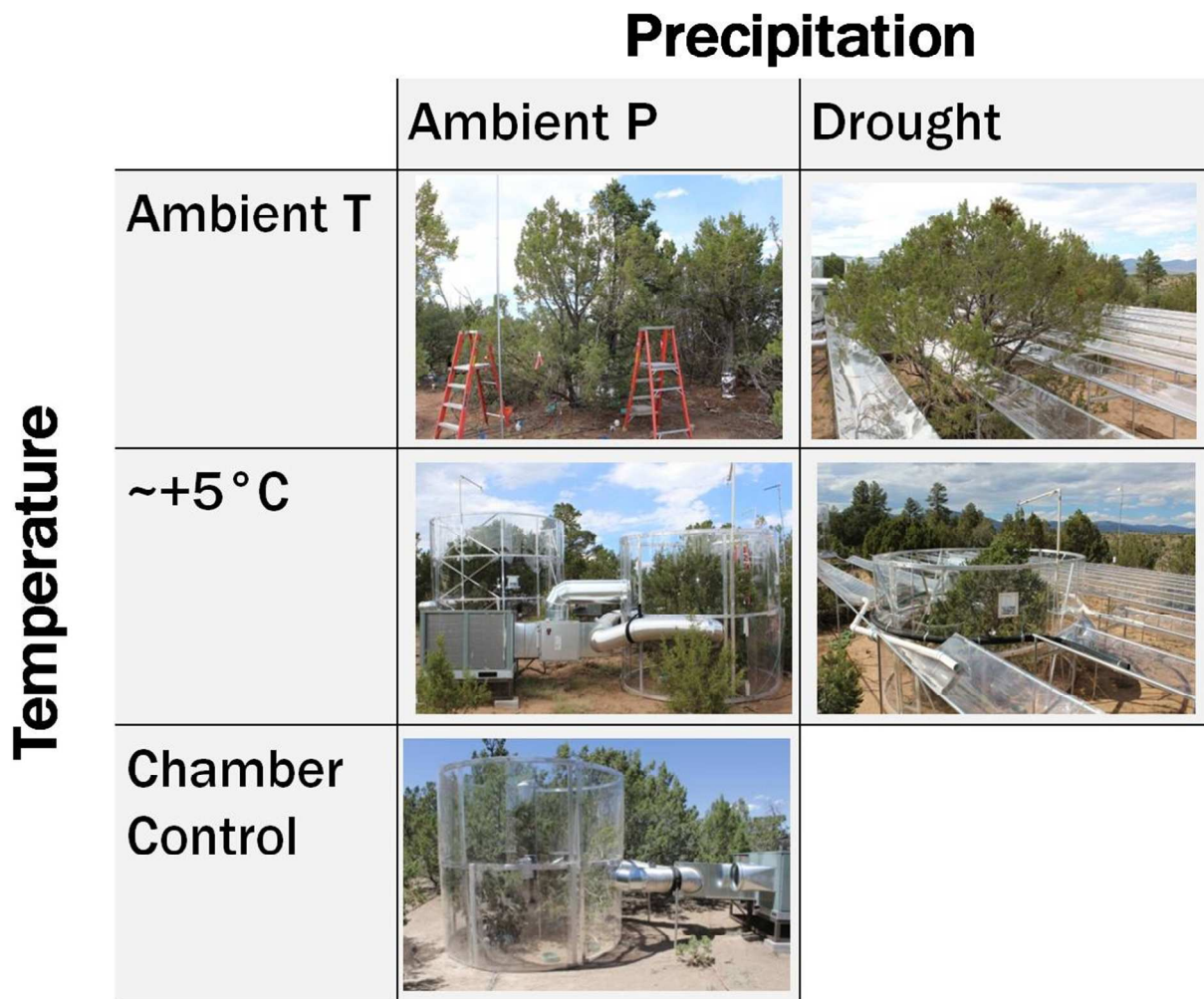
16

17

18   **Fig S1.** Design of the Los Alamos Survival-Mortality experiment (SUMO). Drought was

19   induced with a ~45% throughfall rain-out structure and temperature was modified with

20   transparent plastic open-top chambers regulated by heating and cooling units. Precipitation and

21   temperature factors were combined to provide ambient, drought, heat, and drought+heat

22   treatments. A chamber control treatment was implemented with an additional set of open-top

23   chambers regulated to ambient field air temperature.

24
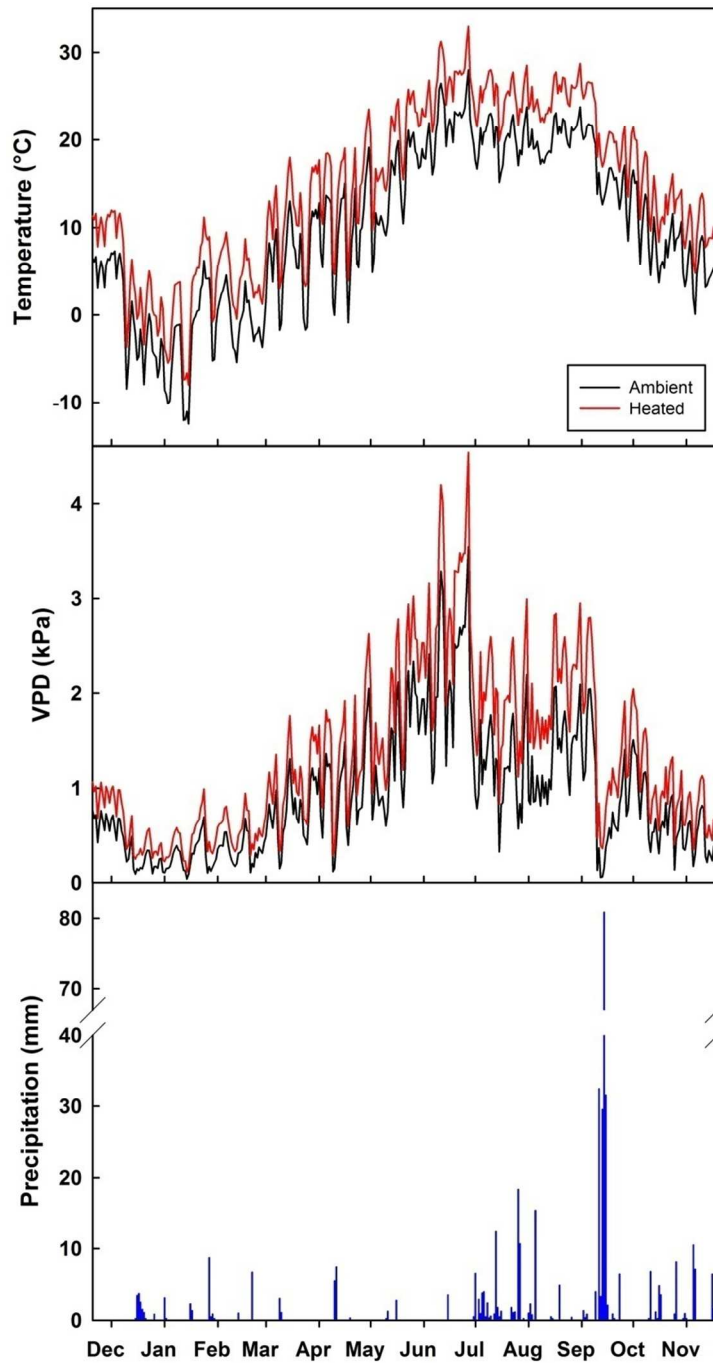
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26

27   **Fig S2.** Mean daily temperature and vapor pressure deficit (VPD) for the ambient and heated

28   treatments (heat and drought+heat), and total daily precipitation at the SUMO experiment from

29   December 2012 to November 2013.
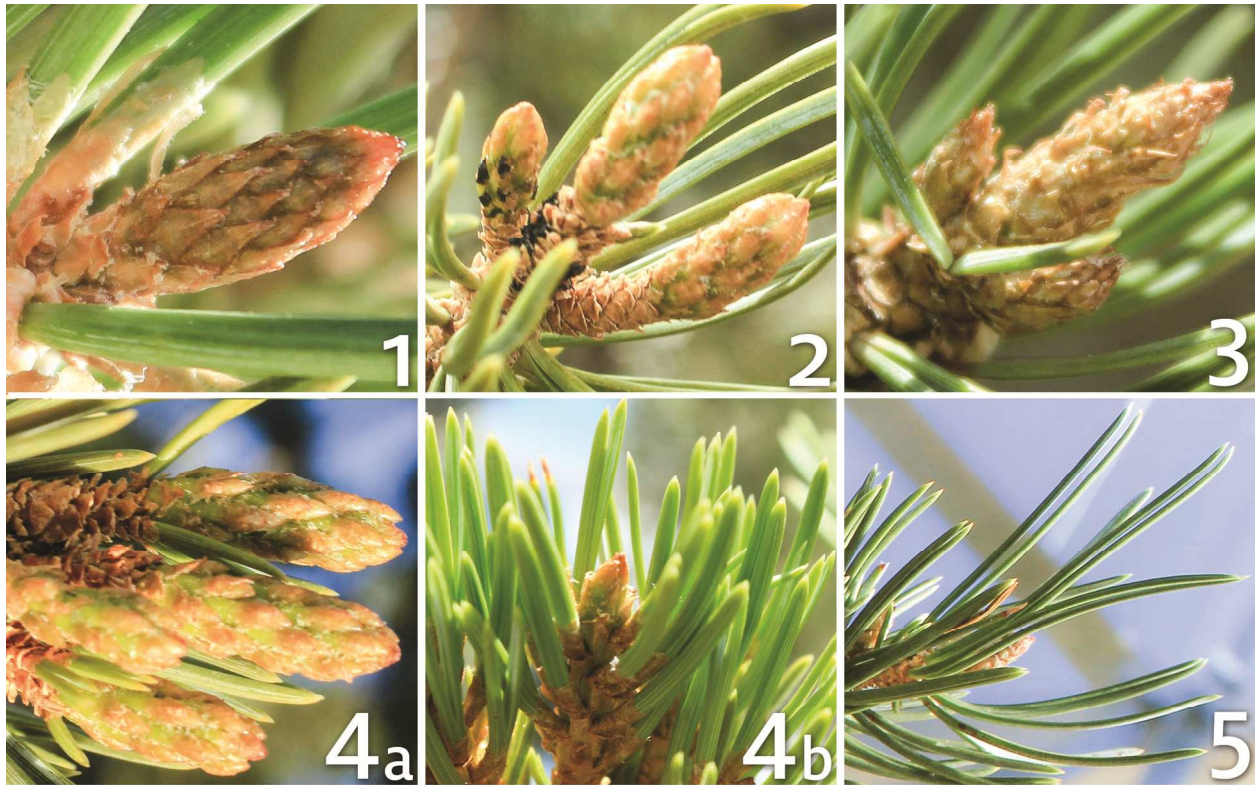
Fig S3. Phenological phases in our classification scheme for piñon pine: 1) bud dormant and unchanged in size, 2) bud swelling or growth observed, 3) needle scales open (budbreak), 4) new needle emergence and growth (both early (4a) and later (4b) examples of this stage are shown), 5) needle pairs separate.

35
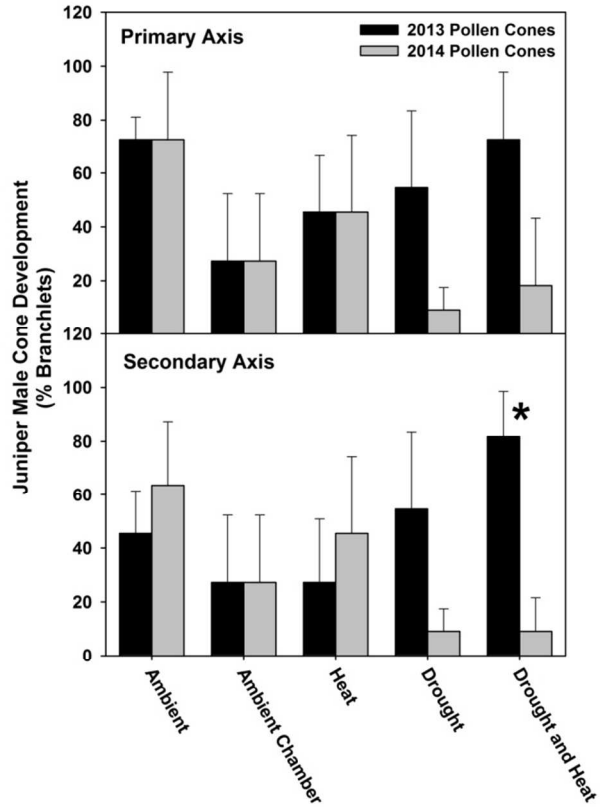
Fig S4. Pollen cone production in one-seed juniper by treatment. Pollen cones observed

releasing pollen in early 2013 and also those developed late in the growing season for 2014

pollen release are shown. Significant differences between 2013 and 2014 cones are noted with

an asterisk (Kruskal-Wallis test, $p < 0.05$). Error bars are standard errors.

Supplemental Tables

Table S1. Correlations are shown between pre-dawn shoot water potential (monthly and mean growing season) and shoot growth, needle growth, and needle emergence timing in primary and secondary axis branches of piñon pine. Only significant correlation coefficients are shown ($p < 0.05$). The relationships between mean growing season water potential and shoot growth, growth, and needle emergence timing are also shown in Fig 5. There were no significant correlations for juniper between shoot growth and water potential.

Axis	Phenology Measurement	March	April	May	June	July	August	Sept.	Oct.	Mean
				Correlation coefficient with water potential						
Primary	Shoot growth	0.59		0.70	0.49		0.46			0.62
	Needle growth	0.66		0.66	0.48		0.59			0.66
	Needle emergence	-0.61		-0.60			-0.65			-0.70
Secondary	Shoot growth	0.73		0.64	0.48			0.50		0.71
	Needle growth	0.79		0.60	0.57		0.60	0.45		0.82
	Needle emergence									-0.55

Table S2. Correlations between June non-structural carbohydrate (NSC) concentrations and shoot growth, needle growth, and needle emergence timing in primary and secondary axis branches of piñon pine. Correlations are shown separately for NSC components of glucose and fructose (Gluc & Fruc), sucrose, starch, and total NSC concentrations. Only significant correlation coefficients are shown ($p < 0.05$). The relationships between shoot starch content and shoot growth, growth, and needle emergence timing are also shown in Fig 5. Significant correlations were found for juniper shoot growth in secondary axis branches with shoot glucose and fructose ($r = -0.53$, $p < 0.05$) and shoot total NSC ($r = -0.46$, $p < 0.05$; data not show).

Tissue	NSC Component	Primary Axis			Secondary Axis		
		Shoot growth	Needle growth	Needle emergence	Shoot growth	Needle growth	Needle emergence
Bole	Gluc & Fruc						
	Sucrose						
	Starch					0.54	
	Total NSC						
Needle	Gluc & Fruc						
	Sucrose						
	Starch			-0.45	0.51	0.55	
	Total NSC				0.47	0.46	
Root	Gluc & Fruc						
	Sucrose						
	Starch						
	Total NSC						
Shoot	Gluc & Fruc	-0.48					
	Sucrose					0.52	
	Starch	0.49	0.55	-0.65	0.62	0.77	
	Total NSC		0.51	-0.57	0.58	0.77	

Table S3. Correlations between pre-dawn shoot water potential (monthly and mean growing season) and June NSC and components in piñon pine. Only significant correlation coefficients are shown ($p < 0.05$).

Tissue	NSC Component	March	April	May	June	July	August	Sept.	Oct.	Mean
Bole	Gluc & Fruc									
	Sucrose	-0.51	-0.68							-0.52
	Starch					0.55				
	Total NSC		-0.58							
Needle	Gluc & Fruc							0.47		
	Sucrose									
	Starch	0.55						0.44		0.55
	Total NSC							0.53		
Root	Gluc & Fruc									
	Sucrose									
	Starch					0.60	0.61			0.59
	Total NSC									0.58
Shoot	Gluc & Fruc			-0.61	-0.53					
	Sucrose									
	Starch	0.50			0.67	0.58	0.49			0.70
	Total NSC	0.49			0.54	0.56	0.45			0.63