

## STUDIES

# Resource allocation trade-offs in a mast-seeding conifer: piñon pine prioritizes reproduction over defence

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## Abstract

The cost of plant reproduction or defence at the expense of other fitness traits is a central component of life history theory. Yet the three central resource allocation pathways of growth, reproduction and defence have rarely been assessed simultaneously nor across individual to landscape scales. This information is critical towards identifying the physiological, environmental and genetic mechanisms underpinning resource allocation. This study assessed trade-offs in resource allocation between tree growth, defence and reproduction across scales among piñon pine (*Pinus edulis*), a widespread mast-seeding conifer of the southwestern USA. Time series (2004–16) of tree growth (radial and primary shoot growth), defence (resin duct production; a key constitutive defence for this species) and cone production among individual trees from populations across a broad environmental gradient were used to investigate these trade-offs in resource allocation across three scales: individual, population and landscape. We found evidence for a defence–reproduction trade-off among individuals whereby total resin duct area in annual xylem rings was lower during years of above-average cone production. Despite variability in cone and resin duct production across trees within a population and across populations, there was no association between these fitness traits at either of those scales. There was no evidence of trade-offs between cone production and growth at any scales measured, whereas resin duct production and growth were positively related at all scales. Our study suggests that a strategic trade-off occurs whereby investment into defence is temporarily curtailed to favour reproduction, despite increased risk of exposure to natural enemies and the ability of piñon pine to simultaneously allocate carbon to growth and defence. Our study provides new insights into physiological expressions of growth, defence and reproduction over time in this long-lived mast seeding conifer and indicates the presence of trade-offs with direct importance for individual fitness and population dynamics under global change.

**Keywords:** Defence; mast seeding; piñon pine; reproduction; resource allocation; trade-offs; tree growth.

## Introduction

A variety of vital functions are important for the persistence of long-lived tree populations on landscapes. Broadly speaking, trees must acquire and allocate resources to the maintenance or increase of biomass, tolerate or resist biotic and abiotic stressors,

and pass genetic material to new generations (Berntson and Wayne 2000; Niinemets 2010; Neale and Kremer 2011). The complex suite of collective physiological mechanisms governing these processes may be reflected by resource allocations to tree

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growth, defence and fecundity. Early theory in plant physiology proposed that the simultaneous allocation of resources to growth traits and defence traits is likely constrained (Loomis 1932; Coley 1988; Herms and Mattson 1992), as individuals should be limited by the availability of carbon resources that can be distributed to each respective process (Stamp 2003). A considerable body of research has focused on evaluating the relationships between growth and defence traits or fitness costs to test this theory across plant taxa, including a diverse assortment of tree species (e.g. Coley et al. 1985; Bazzaz et al. 1987; Gulmon and Mooney 1986). Yet few general conclusions can be made as some systems exhibit negative growth–defence relationships but in others these associations may be neutral or positive (Koricheva 2002; Karasov et al. 2017; Züst and Agrawal 2017).

Models of physiological trade-offs postulate that carbohydrates are preferentially invested in growth with remaining quantities then invested in other functions (Loomis 1932). The trade-off in growth and defence predicted by this model has long been presumed to apply to conifers (Lorio 1986) and is supported by evidence from a number of conifer species (Lombardero et al. 2000; Sampedro et al. 2011; Moreira et al. 2015). Similarly, a trade-off between growth and reproduction across years has been demonstrated in numerous species of trees including various conifers (Eis et al. 1965; Owens 1969; Tappeiner 1969; Gross 1972; Tuomi et al. 1982; Dick et al. 1990; Koenig and Knops 1998; Koenig and Knops 2000; Obeso 2002; Monks and Kelly 2006). While investigations of trade-offs between reproduction and defence are far less common, evidence from deciduous and coniferous species over short (<5 years) timescales corroborate their existence (Herms and Mattson 1992; Tuller et al. 2018) yet highlight the paucity of evidence.

Trade-offs between inter-annual investments in growth and defence have been more extensively investigated relative to reproduction among pines (Ferrenberg et al. 2015), largely due to metrics developed to reconstruct defence allocation throughout a tree lifespan. This is because pines have evolved a complex defensive system that is primarily based on oleoresin (hereafter referred to as ‘resin’) to protect against insect infestations, which is produced and stored in a system of specialized cells known as ‘resin ducts’ that remain visible in annual growth rings (Trapp and Croteau 2001; Franceschi et al. 2005). Across various pine species, resin flow has been found to positively correlate with resin duct number, density and total cross-sectional area (Blanche et al. 1992; Lombardero et al. 2000; Baier et al. 2002; Rodriguez-Garcia et al. 2014; Hood and Sala 2015); and these same measures have all been linked to either deterring bark beetle colonization (Kane and Kolb 2010; Kläy 2011; Gaylord et al. 2013, 2015)—or to increasing the likelihood of survival following bark beetle attack (Ferrenberg et al. 2014; Hood and Sala 2015). Yet it is a challenge to incorporate reproductive investment into comparisons of growth and resin duct production as most authors do not repeatedly measure seed production while simultaneously measuring defence and growth metrics. This is particularly true for masting species, for which reproductive efforts are highly episodic due to high synchrony and high inter-annual variability in seed production. Accordingly, reproduction in masting species must be observed for relatively longer periods to adequately test hypotheses related to potential trade-offs among growth, defence and reproduction.

Yet trade-offs in resource allocation over time are likely more prevalent among these long-lived masting species. The resource-switching hypothesis was developed to explain the high inter-annual variability in reproductive output among masting species and postulates that trees allocate resources

to reproduction 1 year and then away from reproduction the next (Pearse et al. 2016). In support of this hypothesis, negative associations between annual growth and seed production have been reported across a wide array of masting species (Koenig and Knops 1998; Monks and Kelly 2006), and, in conifers, masting has been shown to reduce stored nutrients (Sala et al. 2012), suggesting that these large reproductive events do occur at the expense of other vital functions. Yet few authors have considered the role of allocation to defence in understanding trade-offs among mast-seeding species, likely because of the challenges of simultaneously measuring growth, defence and reproduction over time.

Evolutionary adaptation to various abiotic and biotic pressures may lead to the presence of different growth–defence–reproduction phenotypes within and across populations (Herms and Mattson 1992; Cobb et al. 2002). As a result, not only may trade-offs exist across years within individuals, but also across individuals and populations (Bazzaz et al. 1987). Pine defences are subject to adaptive and directional selection that lead to inherited phenotypic variation in defence traits (Moreira et al. 2012; Westbrook et al. 2015). These traits have been shown to vary across pine populations (Martin et al. 2010; Esteban et al. 2012; Zas et al. 2015) as well as among familial lines within populations (Alfaro et al. 2004; Moreira et al. 2012; Moreira et al. 2015). Seed production among pines has also been shown to strongly vary within and across populations (Linhart et al. 1981; Climent 2008) and is likely linked to differential investment in resource allocation. Indeed, in a common garden study, Climent et al. (2008) found that individuals with high fecundity tended to have reduced growth rates, and that seeds of populations from resource-poor environments tended to have higher fecundity but lower growth rates. This suggests that resource allocation trade-offs to growth, defence and reproduction is likely not only prevalent within individual’s overtime but also across individuals and populations due to adaptation to specific abiotic and biotic pressures.

This study focuses on trade-offs in resource allocation to tree growth, defence and reproduction in two-needle piñon pine (*Pinus edulis*), which is a widely distributed masting conifer across the semi-arid ecosystems of the southwestern USA. Piñon pine populations are frequently subjected to biotic challenges from bark beetle species, especially *Ips confusus* (pinyon ips beetle, Coleoptera: Curculionidae), which can erupt into local outbreaks or regional epidemics when piñon pines are more susceptible to attack by environmental stressors such as drought (Gaylord et al. 2013; Meddens et al. 2015). Indeed, the combination of drought and bark beetles caused recent severe tree mortality across large areas of the US Southwest (Breshears et al. 2005; Meddens et al. 2015). Previous research has found high variability in the size and area of piñon pine resin ducts (Gaylord et al. 2015), and that those trees with a greater amount of resin ducts were less likely to die during recent drought and bark beetle infestations (Gaylord et al. 2015). At the same time, declines in piñon pine cone production have been documented (Redmond et al. 2012), perhaps because these remaining live trees allocate more resources to defences. The combined variability in expenses of terpene-based defences, annual growth and a masting strategy of reproduction, coupled with the ability to reconstruct not only growth but also defence (i.e. resin ducts; Gaylord et al. 2013) and reproduction (i.e. cone production; Redmond et al. 2016) make piñon pine an ideal species for investigating trade-offs among growth, defence and reproduction.

Here, we use a time series (2004–16) of tree growth, resin duct production and cone production taken from individual

piñon pine trees at multiple populations across a broad environmental gradient to investigate inter-annual correlations in resource allocation between these traits. We compare variability in allocation patterns across scales (i.e. within individuals and populations, and between populations) to test the following hypotheses: (1) the expression of growth, defence and reproduction are negatively correlated in piñon pine due to associated resource costs; (2) trade-offs between growth, defence and reproduction will be most pronounced among individuals but will scale similarly across individuals and populations; and (3) climate conditions constrain the relative strength of correlations between growth, defence and reproduction. Our study uses a unique data set of within-individual time series to provide new insights into allocations between physiological expressions of growth, defence and reproduction in a long-lived masting conifer species, and indicate trade-offs do occur between tree defence and reproduction among individuals but not between growth and defence.

## Materials and Methods

### Study area

We surveyed seven sites in southwestern Colorado and one site in New Mexico (Fig. 1) that spanned gradients of elevation (1852–2306 m) and soil available water capacity (6.1–11.4 cm). We used a combination of geographic information systems (Gorelick et al. 2017; ESRI 2011) and soil maps (NRCS 2004) to identify potential sites located within 1 km of roads on US Forest Service and BLM land (Redmond et al. 2018). In the field, we selected study sites from our potential sites that contained a minimum of six adult and reproductively mature piñon trees. Sites that lacked adequate numbers of suitable piñon trees, largely due to high levels of drought-associated die-off (Breshears et al. 2005; Floyd et al. 2009), were subsequently excluded from sampling. Mean annual precipitation from 1980 to 2010 at our sites range from 320 to 460 mm and mean monthly temperatures ranged from 0 to  $-4.2$  °C (December) and from 19.7 to 22.3 °C (July; PRISM Climate Group 2018).

### Field methods

At each site, we established three 50-m transects (perpendicular to the slope) and recorded all trees (>20 cm diameter at root collar) within a 5-m belt upslope of the transect. We then randomly selected six to eight trees to sample for our analyses. Thirteen years (2004–16) of historical cone production and primary shoot growth were reconstructed using annual bud scale scars and the cone scar abscission method (Forcella 1981; Redmond et al. 2016). In brief, on 6–8 branches per tree we counted the number of cones and cone abscission scars on each annual branch segment and measured primary shoot growth, which were dated using annual bud scale scars. The number of cones sampled per year per branch was then multiplied by the total number of cone-bearing branches, which was obtained by visual counts as in Redmond et al. (2016). The cone abscission scar method used here has been highly effective at reconstructing past cone production for piñon pine (Redmond et al. 2016), but is limited in that it is unable to assess whether the cone was fertilized and subsequently aborted or if the cone produced viable seeds. Damaged bark limits the ability to accurately record cone scar data; therefore, we excluded trees with evidence of severe canopy dieback for sampling in our analyses.

### Dendrochronological methods

One intact core was taken from each tree sampled for cone production and primary shoot growth to quantify radial growth and estimate allocation to defence (total resin duct area and mean resin duct size). Cores were 12 mm in diameter and taken at 20–30 cm height. In the laboratory, tree cores were air-dried, mounted and then progressively sanded following standard dendrochronological techniques (Fritts 1976; Stokes and Smiley 1996). Tree cores were then visually cross-dated using piñon pine tree-ring chronologies. For the seven study sites in southwestern Colorado, we used piñon pine tree-ring chronologies that were collected from those exact same study sites (Redmond et al. 2017), whereas for the study site near Albuquerque, NM, we used a nearby published piñon pine tree-ring chronology (Woodhouse et al. 2010). All tree rings between 1980 and 2016 (Colorado sites) and 1950 and 2016 (New Mexico site) were measured to the nearest 0.0001 mm using a sliding stage micrometer (Velmex Inc., Bloomfield, NY, USA). We recorded growth for a longer time period at the New Mexico study site because the chronology used for cross-dating was from a different site location and only went to 2003. Visual cross-dating was confirmed using the program COFECHA and we omitted any tree cores that were weakly correlated ( $r < 0.4$ ) with the other trees from the site. This resulted in a total of 37 trees from eight sites used for analyses. For our metric of radial growth, we used raw tree-ring width rather than basal area increment, because previous analyses have shown raw ring widths of piñon pine at our study sites to not be as strongly influenced by age compared to basal area increment (see Appendix S1 of Redmond et al. 2017). For all analyses, we only used radial growth data from 2003 to 2016 to match our cone production and primary shoot growth data and there was no association between tree size and mean raw ring width during this time period (Pearson's  $r = -0.11$ ;  $P = 0.50$ ).

### Resin duct methods

We scanned all cores at 4800 dpi using Epson Perfection V550 scanner and measured vertical oleoresin duct size using the ellipse tool in ImageJ (version 1.46r) and assigned the calendar year in which each duct formed. Ducts were measured in all years in which we had growth and cone production data (2004–16). We also recorded the length of each tree ring in which we were able to obtain accurate resin duct sizes. These lengths were generally the inner diameter of our increment borer (12 mm) but varied if the rings were oriented at an angle or if there was a scar or crack on part of the core. For our analyses we focused on two resin duct variables: total resin duct area and mean resin duct size (Table 1). Mean resin duct size and total resin duct area divided by radial growth have been associated with piñon mortality during drought (Gaylord et al. 2015). We did not divide total resin duct area by radial growth because we included radial growth as a separate predictor variable in our reproduction and defence models.

### Statistical analyses

#### Trade-offs in reproduction, growth and defence across years.

To assess for trade-offs between reproduction, growth and defence across years among individual trees, we modelled seed cone production and two metrics of defence using linear mixed effect analyses. Our seed cone production model assessed how two metrics of growth (shoot and radial growth) and two metrics of defence (total resin duct area and mean resin duct size) were associated with seed cone production (see Table 1 for a description of all variables). Similar to many other pine species,

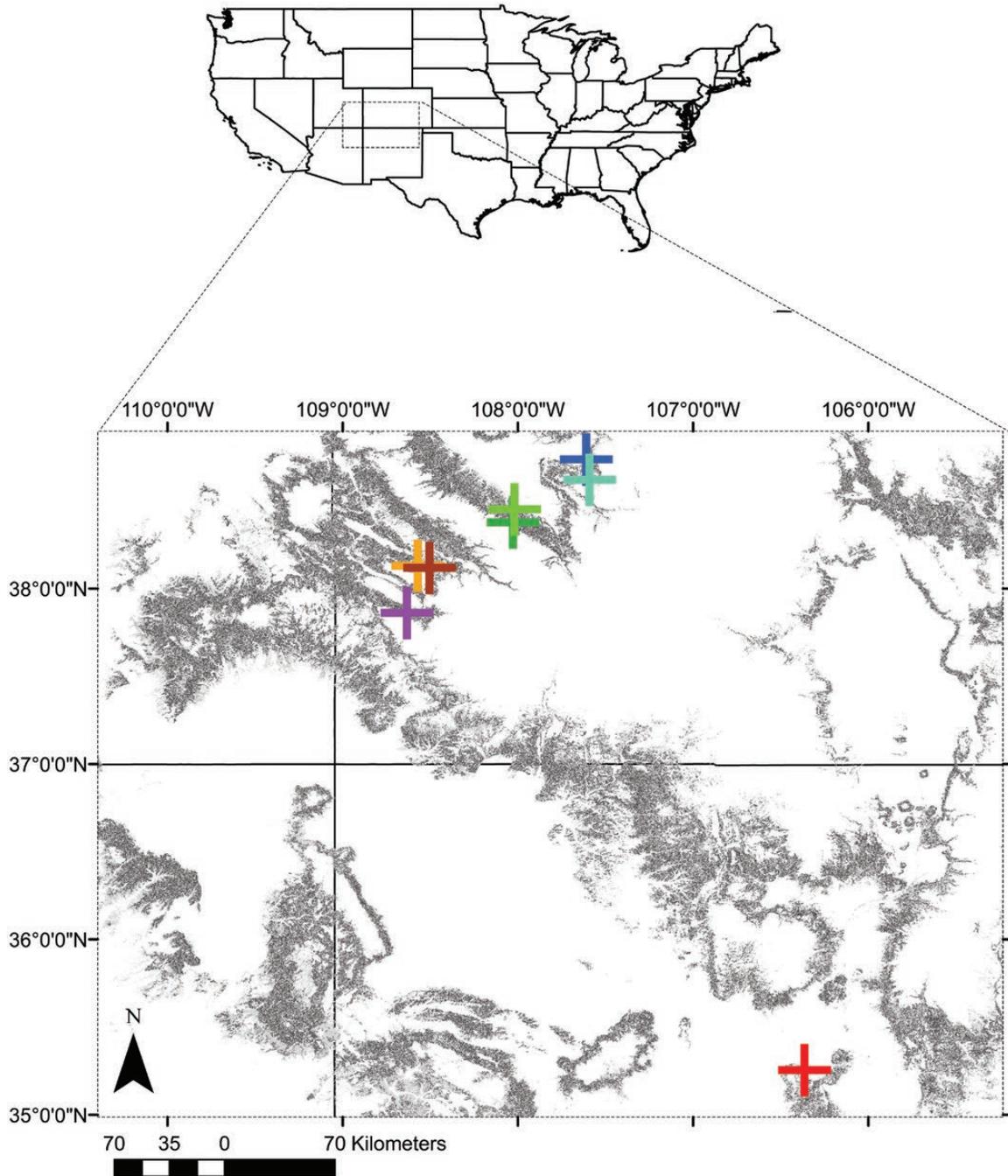


Figure 1. Location of the eight study sites sampled in southwestern Colorado and northern New Mexico to assess trade-offs in piñon growth, defence and reproduction. Shaded grey area shows the distribution of piñon pine and coloured crosses denote each study site. The three pairs of sites in southwestern Colorado (coloured with shades of blue, green and orange) differ in elevation, with high elevation (>2150 m) sites (light blue, dark green and orange crosses) paired with a low elevation (<2000 m) site (dark blue, light green and brown crosses).

piñon pine seed cones take 2 years to mature (Ronco 1990). We focused our analyses on the year of cone maturation as that is the year when the majority of the cone biomass, including the nutritious seeds, is developed. As a mast-seeding species, piñon pine cone production has high inter-annual variability, such that some years trees produce very high numbers of cones while many years have very little to no cones. As a result, Gaussian, Poisson and even negative binomial with zero inflation distributions poorly fit the cone production data. Because of this, we modelled seed cone production as a binary variable,

with years of above-average seed cone production for that tree characterized as 'high' and given a value of 1 and years of below-average seed cone production characterized as 'low' and given a value of 0. Cone production was then modelled with a binomial distribution.

In addition to modelling cone production, we also modelled our two metrics of defence: total resin duct area and mean resin duct size. Specifically, we modelled how growth (shoot and radial growth) and cone production ('high' vs. 'low') during the year of maturation was associated with total resin duct area and,

**Table 1.** Description of the metrics used for reproduction, growth and defence for all analyses.

Category	Description
Reproduction	
Cone production	Mean number of seed cones produced per tree, estimated using cone abscission scars (Redmond et al. 2016). For annual analyses, cone production was calculated as ‘high’ or ‘low’ based on whether the tree produced an above-average number of cones that year (e.g. ‘high’) or below-average (e.g. ‘low’) and was subsequently modelled using a binomial distribution.
Growth	
Shoot growth	Mean primary shoot growth (mm per year) for each year based on 6–8 branches per tree.
Radial growth	Mean radial (i.e. secondary) growth for each year, measured as raw ring width (mm per year) rather than basal area increment.
Defence	
Total resin duct area	Total area of vertical oleoresin ducts per annual growth ring (mm <sup>2</sup> per year). This metric was standardized by the length of the annual ring sampled, which varied slightly across tree cores, and thus total resin duct area is: resin duct area (mm) per year per sample length (mm).
Mean resin duct size	Mean area of each individual vertical oleoresin duct in each annual growth ring (mm <sup>2</sup> ).

in a separate model, mean resin duct size. For the variables in which we detected a negative association indicating a trade-off (cone production and total resin duct area; see Results), we then assessed whether climate conditions influenced the strength of any negative associations (e.g. Hypothesis 3). We used an integrative climatic variable, the forest drought severity index (FDSI; Williams et al. 2013), that has been strongly associated with past growth rates as well as drought and beetle-induced tree mortality events of southwest US forest trees, including piñon pine. Forest drought severity index was calculated as a combination of early summer vapour pressure deficit and winter–spring precipitation of the current year and late summer vapour pressure deficit of the year prior (see Williams et al. 2013 for details) and was standardized so that the FDSI value of each site had a mean of 0 and a standard deviation of 1. We then modelled how total resin duct size (response variable) was associated with our two growth metrics, cone production, FDSI and the interaction between FDSI and cone production.

All analyses were performed in R (R Core Team 2016). For all cone production and defence models, the intercepts for site and for tree (nested within site) were included as random effects to account for the nested structure of our data. Variance inflation factors of all models were checked using the `vif` function in the R package ‘car’ (Fox and Weisberg 2011) and were always <3 (Zuur et al. 2009), including the models discussed below. Predictor variables were always z-scored prior to analyses and thus standardized regression coefficients are reported. Cone production was modelled using the `glmer` function in R package ‘lme4’ (Bates et al. 2015) and the defence metrics were modelled using the `lme` function in the R package ‘nlme’ (Pinheiro et al. 2017). Data calculations and merging of data sets were done using the R package ‘data.table’ (Dowle et al. 2015) and partial residual plots were made using the function `visreg` in the R package ‘visreg’ (Breheny and Burchett 2015).

#### Trade-offs in reproduction, growth and defence across trees.

We assessed trade-offs among reproduction, growth and defence across trees using a similar linear mixed effect modelling approach as our analyses above. Specifically, we performed three separate analyses to model seed cone production and our two defence metrics. The predictor variables in each model included the two growth metrics, the two defence metrics (cone production model only) and

cone production (defence models only). Basal area of each tree was also included as a predictor variable in each model to account for potential differences in tree size influencing cone production or our defence metrics. For these analyses, we quantified cone production, growth and defence metrics for each tree by averaging across all years. Cone production was calculated as the mean number of cones produced per year and thus modelled as a continuous variable rather than a binary variable. All cone production and defence models were modelled with a Gaussian distribution and an intercept for site was included as a random effect.

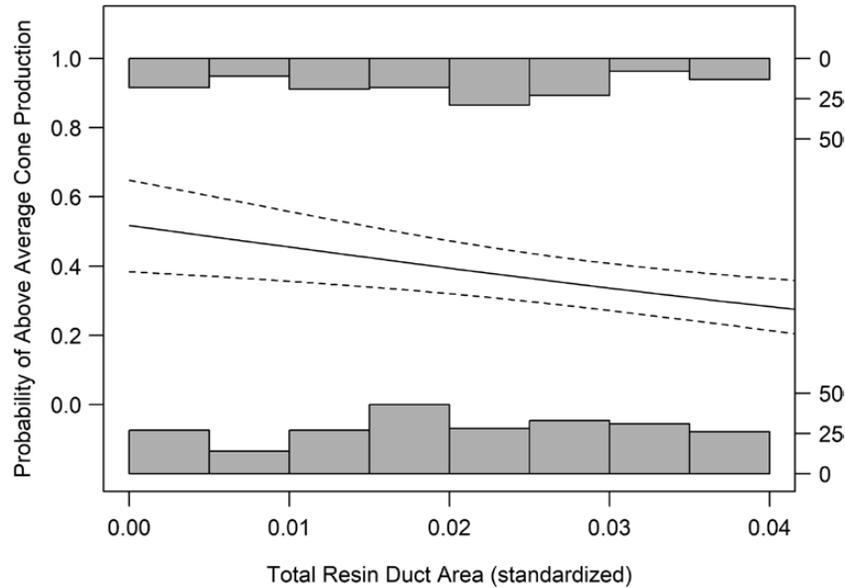
#### Trade-offs in reproduction, growth and defence across sites.

We performed correlation analyses to assess for trade-offs between reproduction, growth and defence across sites. Cone production, growth and defence metrics for each site were quantified by averaging the data across all trees within a site. As a result, our sample size for this analysis was low ( $n = 8$ ) and subsequently we limited our analyses to Pearson’s correlation analyses of cone production and the two growth metrics, cone production and the two defence metrics, and between the defence metrics and the growth metrics (eight correlations total).

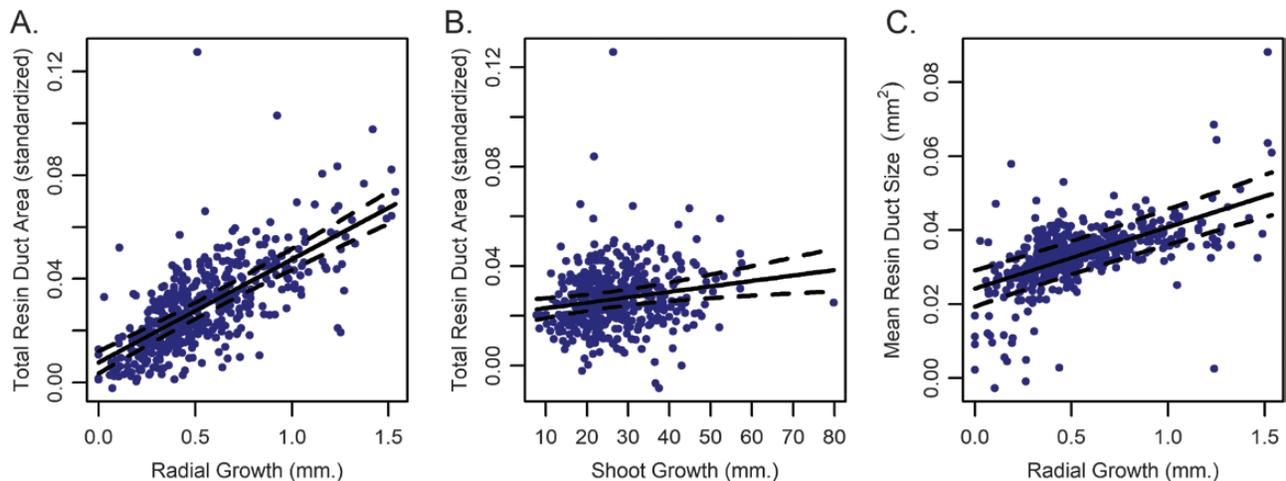
## Results

### Trade-offs in reproduction, growth and defence across years

Cone production was negatively associated with total resin duct area, such that in years of above-average cone production trees generally had a lower amount of resin duct area ( $\beta_{\text{std}} = -0.46$ ,  $P = 0.003$ ; Fig. 2). There was no association between cone production and either growth metrics ( $P > 0.12$ ; see Supporting Information—Figure S1) or the other defence metric, mean resin duct size ( $P = 0.29$ ), across years. In addition to being strongly negatively associated with cone production ( $\beta_{\text{std}} = -0.004$ ,  $P < 0.001$ ), total resin duct area was strongly and positively associated with radial growth ( $\beta_{\text{std}} = 0.011$ ,  $P < 0.0001$ ; Fig. 3A) and also positively associated with primary shoot growth ( $\beta_{\text{std}} = 0.001$ ,  $P = 0.004$ ; Fig. 3B). Mean resin size was also strongly and positively associated with radial growth ( $\beta_{\text{std}} = 0.002$ ,  $P < 0.001$ ; Fig. 3C), but not with primary shoot growth or cone production ( $P > 0.10$ ).



**Figure 2.** The predicted relationship (dashed lines show 95 % CI) between total resin duct area ( $\text{mm}^2$ , standardized by length sampled) of a given year and the probability of having an above-average cone production year. Histograms show how the frequency (secondary y-axis) of years with above-average cone production (top histogram) and below-average cone production (bottom histogram) varies by total resin duct area. The highest histogram bin includes all data with a resin duct area greater than 0.035.



**Figure 3.** Partial residual plots showing the predicted relationship (dashed lines show 95 % CI) between total resin duct area and radial growth (A) and shoot growth (B) as well as between annual mean resin duct size and radial growth (C). Each data point is 1 year of data nested within each tree and nested within each site and thus shows relationships at the annual level.

We found no evidence that climate conditions associated with forest drought stress (e.g. FDSI, calculated as a combination of winter–spring precipitation and summer vapour pressure deficit during the year of cone production and the year prior) influenced the relationship between total resin duct area and cone production ( $P > 0.10$ ).

#### Trade-offs in reproduction, growth and defence across trees

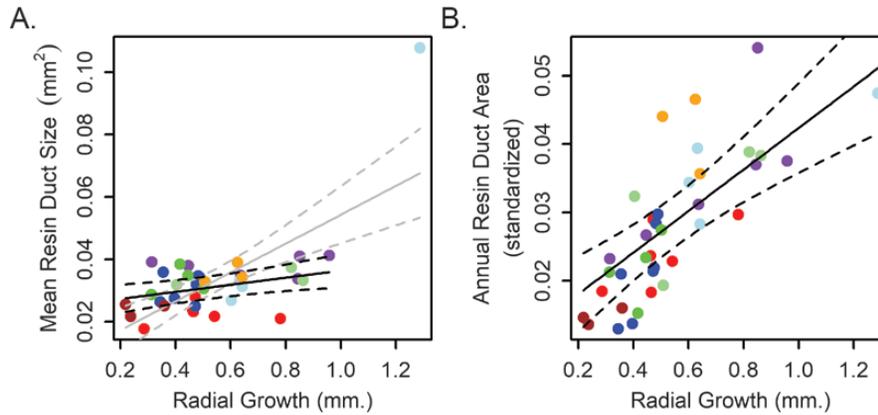
There was no association between tree cone production and tree growth or defence metrics ( $P > 0.20$ ). However, both defence metrics (total resin duct area and mean resin duct size) were associated with radial growth ( $\beta_{\text{std}} = 0.007$  and  $0.002$ ,  $P < 0.0001$  and  $0.02$  for total resin duct area and mean resin duct size, respectively; Fig. 4), indicating that trees that allocated more resources to radial growth also allocated more resources to defence.

#### Trade-offs in reproduction, growth and defence across sites

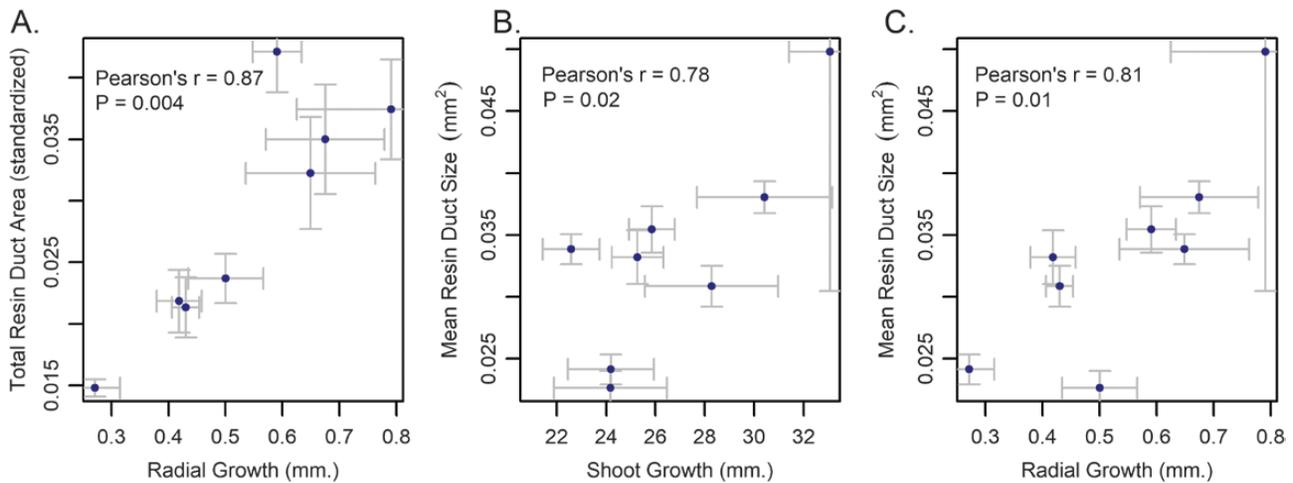
We found no evidence for trade-offs between tree growth, reproduction and defence across our eight sites. However, there were positive correlations between tree radial growth and our two defence metrics, total resin duct area (Pearson's  $r = 0.87$ ,  $P = 0.005$ ; Fig. 5) and mean resin duct size (Pearson's  $r = 0.82$ ,  $P = 0.01$ ; Fig. 5). There was also a positive correlation between mean resin duct size and primary shoot growth (Pearson's  $r = 0.78$ ,  $P = 0.02$ ; Fig. 5). These results suggest that sites with greater growth rates on average were generally more defended.

#### Discussion

Due to their long lifespans, trees are subject to a dynamic array of attacks from natural enemies as well as changing



**Figure 4.** The predicted relationship (dashed lines show 95 % CI) between radial growth and mean resin duct size ( $\text{mm}^2$ ) (A) and annual resin duct area ( $\text{mm}^2$  per year, standardized by length sampled) (B) across trees. Each data point is a tree and data points of the same colour are trees all within the same site, with the colours matching the sites in Fig. 1. There was one outlier in panel A (see top right of figure) and the grey lines show the modelled relationship with the outlier included in the analyses, whereas the black lines in panel A show the modelled relationship with the outlier excluded.



**Figure 5.** Relationship between mean site tree defence metrics (total resin duct area and mean resin duct size) and mean site growth metrics (radial growth and primary shoot growth). Each data point is a site, calculated as the mean value among all trees sampled within that site, and light grey brackets show the standard error.

environmental conditions that alter resource availability. How trees balance the production of growth, defence and reproduction by allocating among these costly functions is thus central to our understanding of forest ecology. Our study focused on a widespread mast-seeding conifer, piñon pine, using long-term measures of tree growth alongside cone and resin duct production—traits that are conserved on the surface of limbs or in annual growth—to measure potential trade-offs between these functions across individual, population and landscape scales.

We did not detect a growth–defence trade-off for piñon pine, but did detect a defence–reproduction trade-off whereby total resin duct area in annual xylem rings, a constitutive defence, was lower during years of above-average cone production. This result suggests that a strategic trade-off occurs whereby investment into defence is temporarily curtailed to favour reproduction, even though piñon pine appear to be able to simultaneously grow and defend against natural enemies. Seed production has direct importance for fitness while primary and secondary tree growth occur for nearly the entirety of an individual's lifespan and are linked to overall performance and survival (Moustakas and Evans 2015)—factors that could underlie our finding that

these functions do not exhibit trade-offs in piñon pine. At the same time, resin duct defences have been demonstrated to have clear importance for pine survival in the face of bark beetle attacks (Kane and Kolb 2010; Kläy 2011; Ferrenberg et al. 2014; Hood and Sala 2015). Nevertheless, our results indicate that seed production is favoured over defences, despite a risk of exposure to natural enemies. This apparently risky trade-off is consistent with the resource-switching hypothesis (Pearse et al. 2016) and could stem from piñon pine being adapted to semi-arid environments where they must tolerate relatively frequent periods of drought and resource limitation that raise mortality risk regardless of defence allocation. Alternatively, this trade-off may be necessary in order to develop large enough seed crops to satiate predators or increased pollination efficiency (i.e. economies of scale hypothesis; Pearse et al. 2016). The resource scarcity in these semi-arid ecosystems may make it particularly important to allocate resources to pulsed, large reproductive efforts during favourable climatic or resource conditions, regardless of exposure to natural enemies.

The negative association between defence investment and cone production suggests that the resources needed to produce these are both limited and linked, unlike xylem growth. Sala et al.

(2012) provided direct evidence of nutrient (N and P) depletion following a mast event in whitebark pine (*P. albicaulis*), and there is strong evidence that nutrient limitations (specifically P) constrain resin production in pines (Cobb *et al.* 1997; Sampedro *et al.* 2011; Moreira *et al.* 2015). We thus hypothesize that the greater demand for resources in these processes likely leads to switching of these relatively more 'expensive' resources, resulting in lower allocation to resin duct production during mast years.

Contrary to growth–defence trade-offs predicted by theory, we found a positive relationship among growth and resin duct defences within piñon pine. Our results, however, join those from a majority of previous studies that have examined resin duct characteristics of pines and revealed positive growth–defence relationships whereby more growth led to an increase in resin duct production, size or total area within the xylem (Kane and Kolb 2010; Ferrenberg *et al.* 2014; Hood and Sala 2015; Westbrook *et al.* 2015; Klutsch and Erbilgin 2018). The association between total resin duct area and radial xylem growth in our study and others may be partly tautological as there is greater space for resin ducts, although notably we also found a similar positive relationship between primary shoot growth and total resin duct area. This positive relationship of growth and defence indicates that factors which promote more growth, such as greater nutrient or water availability, also lead to more resin duct defence production. This finding is also supported by a meta-analysis which found that terpenoid-based defences tend to have a positive relationship to growth and only exhibit negative relationships when resources are highly abundant (Koricheva 2002)—a result predicted by the now-defunct carbon–nutrient balance hypothesis (Hamilton *et al.* 2001).

The presence of different growth–defence–reproduction phenotypes in piñon pine could promote the development of ecotypes in response to abiotic and biotic pressures over time. Pine defences are subject to adaptive and directional selection, as both resin duct characteristics and overall production of resin are genetically controlled and at least a moderate amount of the observed phenotypic variation has been shown to be heritable in congeners of *P. edulis* (Moreira *et al.* 2012; Westbrook *et al.* 2015). In previous studies, resin duct traits have been shown to vary across pine populations (Martin *et al.* 2010; Esteban *et al.* 2012; Zas *et al.* 2015) as well as among familial lines within populations (Alfaro *et al.* 2004; Moreira *et al.* 2012; Moreira *et al.* 2015). Additionally, evidence from Norway and Sitka spruce also indicates that resin duct characteristics can significantly vary among genotypes from the same family group (Hannrup *et al.* 2004; Rosner and Hannrup 2004; King *et al.* 2011). Despite this earlier work, we did not find trade-offs in resource allocation among trees or among populations, only across years. This suggests that either selection pressures are similar across sites, or that piñon pines at the spatial scale of our study are not differentiated and instead are members of the same population or familial line.

This study provides strong support for trade-offs between defence and reproduction among individuals yet failed to detect trade-offs across individuals or between growth and defence or reproduction. The lack of these other trade-offs may be partially due to the limitations of our study design. Most importantly, this study was observational and as a result we were unable to control differences in resource availability across trees or populations. The positive associations between tree growth and defence may thus be due to microsites with greater resource availability, allowing a given tree to allocate more resources to both growth

and defence. Another key limitation is the cone abscission scar method used to reconstruct historic cone production. Whereas this method is highly effective at reconstructing past cone production (Redmond *et al.* 2016), we do not know if these cones produced viable seeds or whether the quantity of seeds produced varied among cones. As a result, there may have been years that trees stopped allocating resources to seed production, leading to noise in our model and thus reducing our ability to detect trade-offs at the ultimate level of embryos. In addition, we were unable to sample trees that had highly damaged branches and thus no available markers of cone production, which may have been exceptionally poor or high producers of resin ducts or cone production. Our metrics of defence allocation were also limited to the amount of defence structures produced and did not include variation in monoterpene production, composition and volatile emissions from resin that all contribute to a tree's ability to defend against insect infestations (Keeling and Bohlmann 2006). Lastly, we sampled a relatively small ( $n = 8$ ) number of sites that did not span the full range of piñon pine, which could have reduced our ability to detect trade-offs across populations. These limitations underscore the importance of continued research on trade-offs in resource allocations given the challenges of observational studies, especially those reconstructing past investment in growth, reproduction and defence.

## Conclusions

The allocation of resources to plant reproduction or defence at the expense of other fitness traits has been a central component of plant life history theory. Assessing potential allocation trade-offs among different plant functions is challenging for long-lived plants given the potential for changes in allocation over time. This challenge is even greater for long-lived, masting species, such as piñon pine, as an increasing number of years of data are required in order to capture masting events. Despite this challenge, masting species are likely the ideal model for studies of allocation trade-offs given the large, pulsed investment of resources required for reproduction. Our study focused on a widespread mast-seeding conifer using long-term measures of tree growth alongside cone and resin duct production—traits that are conserved on the surface of limbs or in annual growth—to measure potential trade-offs between these functions across individual, population and landscape scales. We found evidence for trade-offs among reproduction and defence within individuals, such that trees allocated less resources to defences during mast years. However, we found no evidence of a growth–reproduction trade-off across all scales, and growth and defence were positively associated at all scales in our study. We hypothesize that a greater demand for carbohydrates and nutrients in reproduction necessitates a lower allocation to resin duct and terpene production during mast years, while continued allocation to growth would support continued resource allocation and transport. A key next step for understanding these trade-offs is to evaluate the physiological mechanisms underpinning changing resource allocation between reproductive and defensive pathways within individuals. Because masting in piñon pine and various other tree species is cued by the climatic conditions in preceding years (e.g. Mooney *et al.* 2011; Redmond *et al.* 2012; Parmenter *et al.* 2018), determining the temporal and resource threshold at which carbon is shunted away from defences

towards reproduction carries importance for forecasting large-scale, spatio-temporal increases in not only tree recruitment but also in susceptibility to natural enemies and associated mortality.

## Supporting Information

The following additional information is available in the online version of this article—

**Figure S1.** Relationship between cone production and radial growth (left panel) and shoot growth (right panel).

**Metadata.** Metadata for each data file are provided.

**R code.** The R code used for the analyses is provided.

All data included in this study are available under the following file names: ‘ba\_growth.csv’, ‘cone\_data.csv’, ‘fdsi\_data.csv’, ‘resin\_data.csv’, ‘shootgrowth\_data.csv’, ‘tree\_data.csv’ and the metadata provided in the supporting information describe each data set.

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## Conflict of Interest

None declared.

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