**A robust method to determine historical annual cone production among slow-growing
conifers**

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Abstract

Forest and woodland ecosystems may be strongly affected by climate change influences on tree population processes such as seed production and seedling recruitment. Yet climate effects on seed production are generally poorly understood, particularly for trees that exhibit masting behavior (i.e. high synchronicity and high inter-annual variability in seed production). This is largely due to the limited amount of long-term datasets on seed production, which are necessary to characterize the highly variable reproductive outputs of masting species. The cone abscission scar method provides a promising approach to accurately determine historical (past 10-20 years) annual cone production, but the method has not been rigorously validated. Here we use a long-term dataset of cone abundance on individually monitored pinyon pine (*Pinus edulis*) trees to validate the cone abscission scar methodology. Tree cone production estimated using abscission scars was positively associated with observed mature cone and conelet abundances from 8 -13 years previously (Spearman's $\rho = 0.52$ and 0.66 , respectively), the time period of our observed historical cone production data. Further, we show that between 4-5 branches per tree and 4-6 trees per site need to be sampled to minimize the variance in cone abundance estimates. Thus, only approximately 3-4 hours are needed to obtain an estimate of historical annual cone production in a stand. Overall, we show that the cone abscission scar method provides a robust and time efficient approach to accurately determine historical annual cone production for *Pinus edulis* and likely other slow-growing conifer trees.

Keywords: tree reproduction, seed production, cone abscission scar, cone scar, *Pinus*, conifer

1. Introduction

Forest ecosystem response to changing climate and land-use practices will depend upon species-specific effects on fundamental tree population processes, including reproduction, growth, and mortality. Whereas we are able to obtain long-term data on tree mortality and growth through dendrochronology studies, satellite imagery, and long-term monitoring plots, we have limited long-term data on tree reproductive potential due to the difficulty in obtaining historical seed production data (but see Crone et al., 2011; Krebs et al., 2012; Mutke et al., 2005a; Pérez-Ramos et al., 2010; Redmond et al., 2012). Of particular interest are the potential climate change responses of the many tree species that exhibit ‘masting’ behavior, or high synchronicity and high inter-annual variability in seed production. The necessary long-term data are generally lacking to evaluate the endogenous and exogenous drivers of tree reproduction among masting species.

The historical reproductive output of certain coniferous species can be estimated using the visible abscission scars that remain when female cones are dropped from cone-bearing branches. Cone abscission scars allow temporal variations in seed cone production to be observed by counting scars (as well as any remaining seed cones) at each terminal bud scale scar on a subset (generally 5-10) of cone-bearing branches. Because seed cones of many pine species take multiple years to mature, this methodology estimates potential reproductive output as the total number of conelets (immature cones) that were subsequently aborted, in addition to mature cones. Thus, the cone abscission scar method provides a promising approach to estimate seed cone production over the past 10-20 years among several pine species, including *Pinus albicaulis*, *P. edulis*, *P. halepensis*, *P. pinea*, *P. pinaster*, *P. pumilo*, and *P. silvestris* (Crone et al., 2011; Forcella, 1981a, 1981b; Girard et al., 2011; Kajimoto et al., 1998; Mutke et al., 2005b;

Thabeet et al., 2009; Weaver and Forcella, 1985). With increased drought and projected impacts of climate change on cone production, the ability to reconstruct past cone production will become increasingly important for both basic and applied research.

Yet despite the breadth of studies using this methodology (Crone et al., 2011; Forcella, 1981b; Girard et al., 2011; Kajimoto et al., 1998; Mutke et al., 2005b; Redmond et al., 2012; Thabeet et al., 2009; Vennetier et al., 2013; Weaver and Forcella, 1985), there has been limited validation, likely due to the necessity of obtaining long-term data on individually monitored trees. Forcella (1981a) found that cone abscission scars of the recent year were strongly correlated with the total number of observed new cones on the tree and ground surface in pinyon pine (*Pinus edulis*), yet it remains unclear how effective this methodology is at quantifying cone production further back in time. Morgan and Bunting (1992) also conducted a validation study using historical cone production data on whitebark pine (*Pinus albicaulis*), yet this study did not have individually monitored trees and was thus only able to assess whether the cone abscission scar method could distinguish between qualitatively different levels of cone production (e.g. high vs. low cone production years). Given the considerable effort required to accurately date cone scars on a given branch, it is also important to quantify how many branches are necessary to obtain a robust estimate of cone production in a tree, as well as how many trees need to be sampled to accurately estimate cone production at the stand level.

Here we use a long-term dataset of cone abundance on individually monitored pinyon pine trees to validate the cone abscission scar methodology. Our specific objectives were to: 1. Evaluate whether the cone abscission scar methodology is effective at measuring conelet and/or mature cone abundance 8 to 13 years previously; 2. Determine the sample size necessary to

obtain a robust estimate of cone production within an individual tree and among trees within a stand.

2. Materials and methods

Nineteen individually tagged pinyon pine trees, previously sampled between 2003 and 2008 to determine conelet and cone production, were revisited in 2015 for our validation study. These trees were located near Sunset Crater National Monument (5 trees) and Red Mountain (14 trees) in northern Arizona, USA (see Cobb et al., 2002 for site location and tree selection details). On average, sampled trees were 21.9 cm in basal trunk diameter (range: 15.5 - 29 cm), 9.8 m² in canopy area (range: 4.2 - 19.6 m²), and 3.7 m in height (range: 1.9 – 5.3 m).

2. 1. Pinyon Pine Seed Cone Production and Cone Abscission Scar Methodology

Similar to many pine species, pinyon pine seed cones require multiple growing seasons to mature (Little, 1938; Mirov, 1967). At cone initiation in August or September, microscopic buds develop and not until early that following summer, when pollination occurs, do the microscopic buds develop into visible seed conelets (or 1st year cones), which then overwinter. Mature seed cones form the following fall, 26 months after cone initiation (Little, 1938; Mirov, 1967). Similar to other pine species (Crone et al., 2011; Kajimoto et al., 1998; Thabeet et al., 2009; Weaver and Forcella, 1985), pinyon pine seed cones and conelets leave visible abscission scars on tree branches (Fig. 1). These abscission scars allow temporal variations in seed cone production to be observed by counting cone scars (as well as any remaining cones or conelets) at each terminal

bud scale scar on cone-bearing branches (see Fig. 1 for a description; Forcella, 1981b). Cone-bearing branches of pinyon pine are noticeably more erect and sturdy than purely vegetative branches and are generally in the top two thirds to top third of the tree canopy. Following the methodology in Forcella (1981b), for each branch sampled, all cones, conelets, and cone abscission scars were counted on the dominant branch stem as well as all recent (<13 years old) lateral offshoots. To ensure accurate dating of annual growth increments along tree branches, offshoots without any cone scars were also dated to confirm cone abscission dates by cross-dating within each branch system. Finally, the total number of cone-bearing branches on each tree was counted to obtain an estimate of total cone production for each year by multiplying the mean scar number per branch for a given year by the total number of branches.

2.2. Field Sampling

Between late July and early September of each year from 2003 to 2008, all individually tagged trees were visited and all conelets (i.e. juvenile 1st year cones) and mature cones (i.e. mature 2nd year cones) were counted by two independent observers, and the observer counts were then averaged. These two observers were present in the field together, but each observer counted the total number of conelets and mature cones on the tree without prior knowledge of the other observer's estimates. In October of 2015, we revisited each tree and used the cone abscission scar method to quantify cone production during that same time period. To do this, we counted the number of conelets, mature seed cones, and seed cone abscission scars at each annual node from 2003-2008 on 6-10 cone-bearing branches on each tree following the methodology outlined above (see Fig. 1 for details). We had difficulty determining cone scars

past 2004 (year of maturity, i.e. conelets of 2003), likely due to a drought event that occurred in 2002 and resulted in extensive pinyon pine mortality in the area (Clifford et al., 2011; Floyd et al., 2009; Mueller et al., 2005). We were thus able to compare the cone abscission scar abundance to mature seed cone abundance from 2004-2008, whereas we were able to compare the cone abscission scar abundance to seed conelet abundance from 2003-2008 (years of maturity: 2004-2009).

2.3. Statistical Analyses

To determine whether the cone abscission scar method accurately measures conelet and/or mature cone abundance, we performed Spearman's rank correlation analyses to evaluate the relationship between estimated cone abundance (calculated for each tree and each year) and observed conelet abundance (analysis 1) and mature cone abundance (analysis 2). We performed these two separate analyses to assess whether our estimated cone abundance is a better estimate of conelet abundance, which includes conelets that were subsequently aborted in addition to conelets that developed into mature cones, or a better estimate of mature cone abundance. We also assessed the accuracy of the cone abscission scar method at distinguishing between years of high and low cone production across our study area. To do this, we calculated the mean estimated cone abundance and mean observed cone and conelet abundance for each year (averaged across all trees in our study area) and then performed Pearson's correlation analyses. We similarly assessed whether the cone abscission scar method accurately detects high and low cone-producing trees by calculating the mean estimated cone abundance and mean observed

cone and conelet abundance for each tree (averaged across all years) and then performing Pearson's correlation analyses.

To evaluate the appropriate branch sample size needed to determine cone production for each tree, we assessed how the variance and mean of estimated cone abundance changed with an increasing sample size (from 1 branch to 8 branches). For this analysis, we used the cone abscission scar data only from the year with the highest cone production (2007) and only with trees that had at least 9 branches sampled and had at least one cone scar in 2007 on one of the branches, which resulted in 8 trees retained for this analysis. For each sample size (1 - 8 branches) and each tree, we then randomly selected branches to match our sample size and calculated the mean cone production, repeating this permutation for every combination of branches. Following, we calculated the variance of the mean cone production for each tree and each sample size. We then graphically analyzed the relationship between sample size and variance to identify the inflection point where the variance was no longer reduced with increasing sample size. We also graphically analyzed the relationship between the sample size and the mean to identify the point where the mean value of cone production became stable.

We used a similar approach to evaluate the appropriate tree sample size needed to determine cone production for a site. We similarly used the cone abscission scar data only from the year with the highest cone production (2007), but for this analysis we only used trees from one site in Red Mountain that had the most trees ($n = 12$). For each sample size available for permutation (1 - 11 trees), we then randomly selected trees to match our sample size, calculated the mean cone production, and repeated this for every combination of trees. Following, we calculated the variance of the mean cone production for each sample size. Similar to above, we

then graphically analyzed the relationship between sample size and variance to identify the inflection point where the variance was no longer greatly reduced with increasing sample size.

3. Results

We found a strong relationship between cone abundance estimated using the cone abscission scar methodology and observed conelets (Spearman's $\rho = 0.66$; Fig. 2) and mature cones (Spearman's $\rho = 0.52$; Fig. 2). The errors in our estimates of cone production were relatively small given the high variability in cone production among trees and years (ranging from 0 to 192 cones per tree): our estimates of cone abundance were within 35 cones of observed conelet and cone abundances 90% of the time (Fig. 2) and our estimates were within 10 cones of observed conelet and cone abundances over 62% of the time (Fig. 2). These errors suggest that the cone abscission scar method is effective when comparing between years/individuals with high, moderate, and low amounts of cone production, but is less effective when just comparing among years/individuals with low (<35 cones per tree) amounts of cone production. Results also suggest that during years of high conelet abundance (>100 conelets per tree), cone scar estimates underestimate the number of conelets (Fig. 2), suggesting that not all small conelets leave detectable cone scars for 10+ years. However, the relationship between cone scar abundance and mature cones closely matches the one-to-one line (Fig. 2), suggesting that this method measures cone abundance accurately and with minimal bias.

The cone abscission scar method was highly effective at distinguishing between years of high and low cone production across our study area (Pearson's $r > 0.97$; Fig. 3). This method was also effective at distinguishing between high and low cone producing trees: mean estimates

of cone abundance among each tree (averaged across all years) were strongly correlated with observed conelet and cone abundance (Pearson's $r = 0.81$ and 0.82 , respectively).

Variance in mean cone abundance rapidly declined with increasing sample size until the sample size reached 4-5 branches in a tree and 4-6 trees in a site (Fig. 4). Similarly, the median value of cone production stabilizes once 5 branches are sampled within a tree and 5-6 trees are sampled within a site (Fig. 4). This result suggests that these sample sizes are sufficient to obtain a robust estimate of stand-level cone production. We estimate that approximately 3-4 hours are needed to sample 6 trees in a site and 5 branches per tree, and thereby obtain a robust estimate of historical annual cone production in a stand.

4. Discussion

Long-term, historical data on tree seed production are needed to predict how tree population dynamics will be affected by changing climate and land-use practices, which may dramatically alter forest and woodland ecosystems (Clark et al., 2011). Yet to date we have a limited understanding of how tree reproduction may be affected by these predicted changes in climate and current land-use practices, likely due to the difficulty in obtaining long-term records of tree reproductive outputs. Such long-term data are particularly necessary for understanding reproduction dynamics of 'masting' species, such as many species of pine and oak. Here, we show that the cone abscission scar method provides a robust and time-efficient approach to accurately determine historical (past 13 years) annual cone production among conifer species.

Annual cone production estimated using the cone abscission scar method was strongly and positively associated with the observed numbers of conelets and mature cones. The total

number of estimated cone scars tightly matched the total number of observed mature cones, such that the relationship was similar to the one-to-one line (Fig. 2). Unlike the relationship with mature cones, the total number of estimated cone scars appear to underestimate the total number of observed conelets during periods of high cone production (Fig. 2). This underestimation of conelets using the cone scar method suggests that all small conelets may not reliably leave detectable abscission scars for 10+ years. Regardless, the overall approach is effective at measuring historical annual cone production with minimal sampling effort.

Relatively small sample sizes (4-5 branches from 4-6 trees) were needed to minimize sampling variance and thereby obtain a robust estimate of cone production in a stand. These results agree with previous research (Forcella, 1981a) and also support previously used sample sizes in other studies across multiple species (Crone et al., 2011; Thabeet et al., 2009; Weaver and Forcella, 1985).

Even though we were only able to validate the cone abscission scar method for pinyon pine, the approach used here has already been applied to a wide range of species in the Pinaceae family, including *Abies alba*, *Pinus albicaulis*, *P. halepensis*, *P. nigra*, *P. pinea*, *P. pinaster*, *P. pumilo*, *P. sylvestris* (Crone et al., 2011; Girard et al., 2011; Kajimoto et al., 1998; Mutke et al., 2005b; Thabeet et al., 2009; Vennetier et al., 2013; Weaver and Forcella, 1985). We hypothesize that this approach is less effective at detecting cone scars farther back in time among faster-growing conifer species, although validation data would be needed. There are several factors that limit the effectiveness of the cone abscission scar approach. These include those that reduce the detection accuracy of cone scars, such as branch injuries, formation of thick bark, and rapid radial growth of branches, as well as those that reduce the ability to date annual growth increments using terminal bud scale scars, such as shoot abortions due to moths or other insects,

high levels of polycyclism (i.e. multiple growth flushes within a growing season), missing annual growth increments, and branch dieback.

Data collected in a spatially-explicit manner using the cone abscission scar method can be used to assess how spatiotemporal variability in climate affects tree reproduction potential across the landscape. This information can be used to develop spatially-explicit long-range pine nut forecasts and to assess how seed production may change under a changing climate. Furthermore, land managers can use this approach to determine which trees have high reproductive outputs in an area. In the case of pinyon pine, such information can be used to target highly reproductive trees for removal where the management goal is to slow the rate of pine expansion into adjacent nonforested ecosystems (Jacobs et al., 2008). Alternatively, if the management goal is to retain several large seed producing trees for regeneration, wildlife, and/or pine nut harvesters, then land managers can use these data to identify those trees to remain on the landscape following fuel-reduction treatments. Overall, this method provides a robust and time efficient approach to accurately determine historical annual cone production, data that are critically important to determine the endogenous and exogenous drivers of seed production.

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Figure Captions:

Figure 1. (A.) Image of a ponderosa pine (*Pinus ponderosa*) branch with the year provided for each annual segment and the year of the cone and conelets given. Images of pinyon pine (*Pinus edulis*) cone scars that would form a 2014 mature cone (**B**) and a 2009 mature cone (**C**). Dates given for all images assume the branch was collected in October or November of 2015. We provide the image of a ponderosa pine branch because the bud scars are more detectable at a

distance due to the lower density of needles immediately surrounding the bud scar. These photos also illustrate the utility of this approach across very different conifer species.

Figure 2. Relationship between cone scar abundance and observed conelets (1st year cones, *left*) and mature cones (2nd year cones, *right*) across all trees (19 total) and years (2004-2009, based on the year of maturity). Observed conelet abundances, which were observed the year prior to mature cone production (i.e. 1st year cones), include conelets that were subsequently aborted in addition to conelets that developed into mature cones. Observed cone abundances include only mature cones that were observed at the time of cone maturity. The line illustrates where cone scar abundance is equivalent to observed conelet (*left*) and mature cone (*right*) abundance.

Figure 3. Estimated cones using the cone abscission scar method (red circles), observed conelets (blue squares), and observed cones (green triangles) from 2004 to 2009 (based on the year of maturity), averaged across all trees in the study area. These mean estimates of cone abundance were strongly correlated with observed conelet and cone abundance (Pearson's $r = 0.98$ and 0.97 , respectively). Error bars are ± 1 SE.

Figure 4. Changes in the variance (*top*) and mean (*bottom*) of estimated cone production using abscission scars with increasing sample sizes of branches within a tree (*left*) and trees within a stand (*right*). Boxplots are not included for the variance of trees for each sample size because we only had data from one stand to calculate the variance.

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Highlights

- We assessed whether cone abscission scars reflect historical annual cone production
- We compared cone scar estimates with observed historical (8-13 yrs prior) cone data
- Cone scars were strongly correlated with observed cone abundances
- Minimal sampling effort was required: 4-5 branches per tree and 4-6 trees per site
- Cone scars are an accurate and time-efficient way to reconstruct historic cone production