PREDICTING AND QUANTIFYING POLLEN PRODUCTION IN *JUNIPERUS ASHEI* FORESTS

Landon D. Bunderson
Department of Biology, The University of Tulsa, Tulsa, OK 74104, landon-bunderson@utulsa.edu

Peter Van de Water
California State University, Fresno, 2576 E. San Ramon Ave., Mail Stop ST-24, Fresno, CA 93740

Harrington Wells and **Estelle Levetin**
Department of Biology, The University of Tulsa, Tulsa, OK 74104

ABSTRACT

Juniperus ashei pollen has been reported as a major airborne allergen in regions of Texas and Oklahoma. Pollen production across these populations was examined in order to support a pollen forecast system. Four locations in Texas and 2 locations in Oklahoma were chosen as study sites. Trees in each location were measured, cone production was evaluated based on a rating system, and percent tree cover was determined. Cone production was estimated by counting cones from 1/8th sections of 10 representative trees. Additionally, vials of cones collected at each location were used to determine the number of pollen grains per cone. The 10 representative trees were used to test three models describing the relationship of pollen production to tree size: height, surface area, and volume. Using the pollen count data, tree measurements, the rating system, and the three models, three estimates of total pollen grains per hectare were produced. The highest producing area using the most conservative model predicted a total pollen production of 3.3×10^{13} pollen grains per hectare. Although there is great variability between the locations making it difficult to determine which factors are most important, differences in the amount of pollen production in each location could be partially predicted by percent juniper cover. *Phytologia 94(3): 417-438 (December 1, 2012).*

KEY WORDS: *Juniperus ashei*, pollen, allergy, pollination ecology

Airborne allergens are a major contributor to allergic disease. As such, airborne pollen concentrations have been monitored for decades in many parts of the world (Gregory, 1973; Lacey & Venette, 1995). Much effort has gone into predicting the season start, daily concentration, and peak date of various allergenic pollen types (Garcia-Mozo et al., 2002; Adams-Groom et al., 2002; Schappi et al., 1998; Norris-Hill, 1995; Raynor and Hayes, 1970; Garcia-Mozo et al., 2009). Although many studies have focused on local release and deposition, daily concentrations can be affected by both local and long-distance pollen producers (Skjøth et al., 2007; Van de Water and Levetin, 2001). Wind trajectories have been used to track the long-distance transport of pollen and mold spores (Gregory, 1973; Aylor et al., 1982; Van de Water and Levetin, 2001; Skjøth et al., 2007; Skjøth et al., 2008). These deterministic models predict trajectory, but are not predictive of how much pollen is transported. In order to produce a more precise pollen forecast, the disciplines of aerobiology, meteorology, plant phenology, and plant ecology must be combined (Levetin and Van de Water, 2003). More complicated prediction systems which use estimates of source contribution have been created to track dust, pollen, smoke, and other bioaerosols (Sofiev et al., 2006; Nickovic et al., 2001; Jain et al., 2007). Although little information exists on pollen source contribution, there are some noteworthy studies.

Anemophilous plants often produce large amounts of pollen and there have been attempts to quantify production per anther in several angiosperms (Subba Reddi and Reddi, 1986) and per cone in gymnosperms (Hidalgo et al., 1999). One study estimated source contribution of Poaceae pollen production in NW Morocco. This study identified various representative zones and estimated pollen production in each area. Number of pollen grains per anther, anthers per flower, flowers per spikelet, and number of spikelets per inflorescence were estimated. Also, the number of inflorescences per square meter was estimated using a quadrat system (Aboulaich et al., 2009). In a similar approach, potential pollen production of three *Cupressus* species was approximated by estimating cones per tree and pollen grains per cone (Hidalgo et al., 1999). Another approach to predicting pollen source is the use of plant distribution maps. Often, smaller surveys are used to determine prevalence of species of interest and then combined with less detailed distribution maps to create an extrapolated relative pollen contribution map (Pauling et al., 2012; Sofiev et al., 2006). Once relative pollen concentrations have been determined with this method, modelers often use trial and error to determine input values. There has been no attempt to estimate pollen production within the genus *Juniperus*.

Juniperus ashei is among the most important aeroallergens in the Cupressaceae (Weber and Nelson, 1985). The airborne pollen *J. ashei* produces is well documented to affect inhabitants of cities and towns adjacent to juniper woodlands and, because juniper pollen can be transported over long distances, it also is well known to affect people in cities far from the pollen source. Instances of long distance transport of *J. ashei* pollen from the Edwards Plateau, Texas into Tulsa, OK and transport from the Arbuckle Mountains in south central Oklahoma into Tulsa, OK have been well documented (Levetin and Buck, 1986; Rogers and Levetin, 1998; Levetin, 1998). In fact, even though the nearest upwind source of *J. ashei* pollen is over 200 km away, it has been detected in Tulsa, Oklahoma for over 30 years (Levetin and Buck, 1986; Van de Water et al., 2003). *J. ashei* releases pollen from December to February (Pettyjohn and Levetin, 1997; Levetin and Buck, 1986). Since 1998, Levetin and Van de Water have used HY-SPLIT trajectory modeling to predict long-distance dispersal of *J. ashei* pollen (http://pollen.utulsa.edu/pollen.html).

Juniperus ashei inhabits large areas across Oklahoma and Texas. It is distributed throughout central Texas, New Mexico, northern Mexico, the Arbuckle Mountains of south central Oklahoma, and the Ozark Mountains of northern Arkansas and southwestern Missouri (Adams, 2008) *J. ashei* is dioecious, grows to 15 m in height, and inhabits limestone glades and bluffs at elevations of 150-600 m. Dry, eroded, nutrient-poor sites favor *J. ashei* and it often becomes the predominant species in such sites (Diamond et al., 1995). Other sites, including grasslands, have been subject to encroachment by *J. ashei* and as a result, lands are often actively managed to prevent such encroachment (Noel and Fowler, 2007). *J. ashei* encroachment is apparently due in large part to fire suppression (Noel and Fowler, 2007; Allred et al., 2012). One study from a location with shallow, limestone soils in Uvalde County, TX found that 90% of the 1,000 trees per ha were *J. ashei* (Hicks and Dugas, 1998).

The current study was undertaken to estimate *J. ashei* pollen production per tree as well as across the landscape in order to provide information for a pollen forecasting model. Hidalgo et al. (1999) used surface area of trees as a measure of pollen production. On the other hand, fecundity (at least in animals) is quite often related to volume of the individual (Wenner et al., 1991). In this study, both of these models of male fecundity are tested, as well as a simple linear relation between tree height and pollen production, using *J. ashei*. In addition, tree stand characteristics are tested as predictors for high rates of pollen production.

MATERIALS AND METHODS

Study Sites

The information from this study will be combined with a pollen air sampling study to support the previously mentioned pollen forecast model. The air sampling locations were positioned to cover the geographic range of *J. ashei*. Survey locations for this study were established near pollen sampling stations and were intended to be representative of the air sampling locations. Additional considerations were based on accessibility and recommendations of local representatives of the United States Departments of Agriculture and Interior as well as colleagues from local universities. Two locations in the Arbuckle Mountains of Oklahoma and four locations in the Edwards Plateau region of Texas were chosen as sampling sites. The sites were at Camp Classen (Classen) and Crossbar Ranch (Crossbar) in the Arbuckle Mountains near Davis, Oklahoma, San Marcos (San Marcos), TX, Llano River State Park near Junction, TX (Junction), the Texas Agrilife Research and Extension Center near Sonora, TX (Sonora), and the Balcones Canyonlands National Wildlife Refuge near Cedar Park, TX (Balcones). Most of the data were collected in December 2009 and January 2010. Some of the cone data for representative tree cone counts and pollen grains per cone counts were collected in December of 2010 and January 2011 which included an additional site for cone collection at the Cedar Ridge Preserve near Dallas, TX.

Representative quadrats measuring 100×100 m were chosen at each location. Within each quadrat six 10×10 m sub-quadrats were randomly selected. Quantitative measures of size were recorded for all trees within a sub-quadrat. Tree height, trunk diameter and canopy diameter were measured. The majority of trees exhibited radial symmetry, so the canopy diameter was estimated by taking measurements in 2 directions perpendicular to one another (Hicks and Dugas, 1998). Since juniper trees are difficult to age (Panshin and Dezeeuw, 1964; Van Aukin, 1993), diameters were used as a measure of size class rather than age class. Many of the trees measured were too small to achieve valuable measurements at the standard breast height. Instead, trunk diameter was measured at a height of approximately 15 cm. Also, many *J. ashei* trees have multiple trunks. Because of this, diameters of multiple trunk trees were converted to basal area and summed (Rodgers et al., 1996).

Finally, the gender of each tree was noted and for male trees a subjective measure of pollen cone production was given: high cone producing (HCP), low cone producing (LCP) or no cone producing (0CP). The 0CP category consisted of trees that had very few male cones per tree which allowed for gender determination but did not contribute much to pollen load. This subjective measure was made because of the striking difference in number of cones on trees of the same height. The subjective measure was tested to determine whether the population was indeed made of two classes of male trees (HCP, LCP) which could improve prediction of total pollen production from the juniper population. Since parts of many trees were dead or dying, estimates of percent live vegetation per tree were made by visual inspection.

Percent canopy coverage of the quadrat area was determined using a line-intercept method (Floyd and Anderson, 1987). Three 100 m transects radiating outward from a central point were selected randomly as measures for canopy cover.

Quantifying Pollen Cone Production

Five trees were chosen from each of the HCP and LCP categories from the combined tree pool of all of the locations. Preliminary observations revealed that cone production varied more from top to bottom than it did left to right. This phenomenon has been observed in female cones of *Picea glauca* (Turkington et al., 1998).

Due to this type of cone distribution, representative trees were divided longitudinally into eighths. A $1/8^{th}$ section was harvested and the number of cones counted for that portion of the tree. All $1/8^{th}$ sections were collected from trees with no dead vegetation. Due to this requirement, it was difficult to find representative trees from each location.

Some large trees were so laden with cones that it was necessary to do a sub-count on the representative trees. In this case, approximately 1/3 of the cones in the 1/8th section were counted. The remaining cones were estimated by obtaining the weight of the green vegetation associated with 500 cones. This was repeated at least 10 times. The remainder of the cones and green vegetation were removed from the branches and weighed and the number of cones was extrapolated.

Pollen Grains Per Cone

Ten mature but unopened cones per tree (Hidalgo et al., 1999) from at least 5 *J. ashei* trees in each location were placed in sterile tubes. Vials were returned to the lab and refrigerated until processed. Each ten-cone sample was thoroughly crushed in a vial and suspended in 10 ml of a 50:50 glycerol and water solution. Approximately 10 μl of the solution was placed on each side of the hemocytometer (Pettyjohn and Levetin, 1997). For each 10-cone tube, this process was repeated six times. The number of pollen grains was estimated using standard hemocytometer dilution conversions. Not all cone vials were processed due to fungal growth in 28 of 40 vials.

Statistical Analysis

Three models predicting cone production per tree (cone production being proportional to height, surface area, or volume) were tested through linear regression on log transformed data (i.e. $\log(y) = \log(b) + a \log(x)$). A slope of 3 suggests a volume relationship, a slope of 2 suggests a surface area relationship, and a slope of 1 suggests a one-dimensional relationship (i.e., height). An ANOVA was used to test for significance of the regression for each slope (SAS JMP 2010).

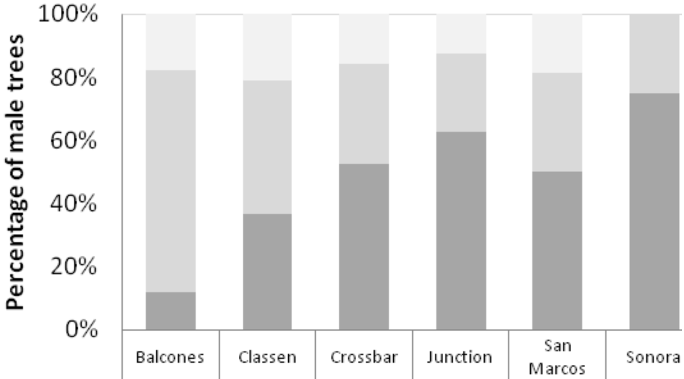
Combining the cone count data and each model of cone production with the quadrat sampling data, total cone production was estimated. Adjustments were made to the cone production model by multiplying by the percent live vegetation per tree.

A chi-square test was used to determine whether the distribution of the HCP, LCP, and 0CP trees varied in their ratios across locations. Tree stand characteristics were compared using one-way ANOVAs and mean comparisons were made using a Tukey Kramer HSD test. Male to female ratio was analyzed using chi-squared distribution tests (test for 50:50, and test for different frequencies among locations). A regression analysis was performed to determine whether individual stand characteristics were good predictors of pollen production. The number of trees in each cone production rating (HCP, LCP, 0CP) for every sub-quadrat (36) was plotted against the mean or number value for each stand characteristic (36) for the regression analysis. All statistical analyses were performed using SAS JMP 2010.

RESULTS AND DISCUSSION

Stand Characteristics

Analysis of stand characteristics was based on trees that were ≥2 m tall. This threshold was determined by finding the shortest cone producing tree (2 m). The estimated number of HCP trees per hectare was greatest at Crossbar and Junction with 166.7 trees per hectare (Figure 1). The Balcones had the least HCP trees with 33 trees/ha but the highest number of LCP trees with 200 trees/ha (Figure 1). A chi square test to test the ratio of HCP, LCP, and 0CP trees between locations was not significant ($p>0.05$). Location and individual HCP, LCP, and 0CP groups were compared using a one-way ANOVA. Although the number of HCP trees per hectare varied widely between locations (Figure 1), differences were not significant. The number of LCP trees was significantly different ($F_{5,30} = 3.1$, $p<0.05$) and means comparisons reveal that the number of LCP at Balcones was significantly greater than at Sonora (Figure 1). No differences were found between locations for the number of 0CP trees.



	Balcones	Classen	Crossbar	Junction	San Marcos	Sonora
Male 0CP	50	67	50	33	50	0
Male LCP	200	133	100	67	83	17
Male HCP	33	117	167	167	133	50

Figure 1. Number and percentage of male HCP, LCP, and 0CP tree groups per hectare in each location. Bars represent percentage of trees in each group. Values in table are the number of trees per hectare in each group.

Juniperus ashei tree density was highest in San Marcos with 1500 mature trees per hectare and lowest in Sonora with 150 mature trees per hectare (Table 1). The remaining locations were between 683 and 1100 trees per hectare (Table 1). The one-way ANOVA indicated the differences were significant ($F_{5,30} = 3.8$, $p<0.01$) for tree density and means comparison show that the only significant difference was the density between Balcones and Sonora (Table 1). There was a positive correlation between tree density and 0CP, LCP, and HCP, but the relationship was not significant (Table 2).

In all 6 locations, the genus *Juniperus* was the dominant arboreal vegetation. *Juniperus ashei* and *J. pinchotii* were both present in Sonora with *J. pinchotii* making up a slightly higher percentage of the canopy cover. *J. ashei* was 2.7% of the cover and *J. pinchotii* was 3.3%. Allred et al. measured tree cover in Sonora and found that percent cover ranged from below 10% to around 30% (2012).

Differences between percent juniper canopy cover and percent total canopy cover were less than 6% for all locations except San Marcos (Table 1). San Marcos was the only location where a large percentage of the tree canopy cover consisted of species other than juniper. Of the total 67.3% cover, 35.2% was juniper while the remainder consisted mostly of *Quercus* species (Table 1). Total canopy cover was significantly different across locations ($F_{5,12} = 5.44$, $P < 0.01$). Means comparisons show that total cover was significantly higher in San Marcos and Balcones than in Sonora (Table 1). Crossbar and Junction were 46.8% and 44.1%. Classen and Sonora were 32.7% and 6%, respectively (Table 1). For juniper canopy cover, locations were significantly different ($F_{5,12} = 5.0$, $p<0.05$). Means comparisons reveals that juniper canopy cover at Balcones was significantly higher than Sonora (Table 1).

Table 1. *Juniperus ashei* stand characteristics by location: mean (standard deviation) and letter grouping based on Tukey-Kramer means comparisons*.

Location	Tree Height (m)	Canopy Diameter (m)	*J. ashei* Cover(%)	Total Cover (%)	Live Vegetation (%)	Mature Density†	Trunk Basal Area (cm)
Balcones	5.8(1.7)A	3.6(1.6)A	65.3(14.8)A	69.1(9.8)A	26.3(22.3)C	900(119)AB	309(473)A
Classen	3.0(0.7)B	2.4(0.8)B	29.0(21.5)AB	32.7(26.1)AB	65.1(21.8)A	733(69)AB	133(87)B
Crossbar	4.0(1.1)B	3.7(1.5)A	41.0(18.9)AB	46.8(12.1)AB	40.2(19.8)BC	1100(112)AB	283(274)A
Junction	3.5(0.9)B	2.5(1.2)B	40.0(20.1)AB	44.1(18.9)AB	73.3(11.5)A	683(52)AB	111(106)B
San Marcos	4.1(1.7)B	2.3(1.6)B	35.2(1.6)A	67.3(22.8)A	42.2(15.6)BC	1500(137)AB	85(154)B
Sonora	3.5(0.8)B	3.6(1.4)AB	2.7(4.0)B	6.0(4.5)B	58.3(15.4)AB	150(18)B	187(178)AB

*In each column values follow by the same letter are not significantly different.
†Mature tree density per hectare including male, female, and gender unidentifiable trees. Mature tree based on the shortest cone producing tree (>2 m).

Percent juniper cover was significantly correlated with number of LCP trees ($r = 0.90$, $p < 0.05$) (Table 2). Juniper canopy cover was the only stand characteristic that was significantly correlated with the number of LCP trees.

Mean tree height in Classen was 3 m, which was much shorter than the mean 5.8 m tree height in Balcones (Table 1). Mean tree height for all locations except Balcones was between 3 and 4.1 m (Table 1). A one-way ANOVA showed that mean tree height was significantly different ($F_{5,298}$ = 24.8, p<0.0001). Means comparisons show that trees in the Balcones were significantly taller than all other locations (Table 1). Classen trees were significantly shorter than those in the Balcones, Crossbar, and San Marcos. Canopy height was positively correlated with number of LCP trees, but the relationship was not significant. The number of 0CP and the number of HCP trees was not strongly correlated with tree height (Table 2).

Table 2. Correlation coefficients of stand characteristics and HCP, LCP, 0CP. Number of male trees in a given category (HCP, LCP, 0CP) of each sub-quadrat were plotted with a given stand characteristic of each sub-quadrat.

Male Cone Production Rating	Tree Density*	Tree Height*	Canopy diameter	Total %Cover	Juniper %Cover	%Live Vegetation	Trunk Basal Area
LCP	0.39	0.70	0.15	0.68	0.90*	-0.59	0.14
HCP	0.44	-0.49	-0.60	0.14	0.05	0.40	0.05
0CP	0.68	0.16	-0.42	0.64	0.64	-0.25	0.15

*p<0.05

Trees at Classen, Junction, and San Marcos had the smallest mean canopy diameters (2.4, 2.5, and 2.3 m respectively) and those at Crossbar had the largest mean canopy diameter (3.7 m). Mean canopy diameter of trees at Balcones and Sonora was 3.6 m (Table 1). The one-way ANOVA for canopy diameter across locations showed significant differences ($F_{5,298}$ = 7.7, p<0.0001). Means comparisons showed tree diameters at Balcones and Crossbar were greater than Classen, Junction, and San Marcos. Canopy diameter was negatively correlated with 0CP and HCP trees with r values of -0.42 and -0.60 respectively but the relationship was not significant for either (Table 2).

Tree trunk basal area was highest in Balcones and Crossbar with 309 cm^2 and 283 cm^2, respectively. San Marcos had the smallest basal area at 85 cm^2. Although there were several large trees in San

Marcos, a large number of trees in the area had small trunk diameters and were near the minimum height of 2 m which is the reason for the low mean basal area. Basal areas at Junction, Classen, and Sonora were 111, 133, and 187 cm^2 respectively (Table 1). A one-way ANOVA showed that basal area differences were significant ($F_{5, 287} = 7.8$, $p<0.0001$). Means comparison showed trees at Balcones and Crossbar had a larger mean trunk size than Classen, Junction, and San Marcos. Sonora was not significantly lower or higher than any other location (Table 1). Trunk basal area was not correlated with 0CP, LCP, or HCP (Table 2).

Mean percent live tree vegetation varied from 26% in Balcones to 73% in Junction. San Marcos and Crossbar were similar with 42% and 40% respectively (Table 1). Live vegetation at Classen was 65% and at Sonora it was 58% (Table 1). A one-way ANOVA found that differences in percent live vegetation were significant ($F_{5, 241} = 38.7$, $p<0.0001$) and means comparison revealed that Junction and Classen percent live vegetation ratings were significantly higher than at San Marcos, Crossbar, and Balcones; and Sonora was significantly higher than Balcones (Table 1). Negative non-significant correlations exist between percent live vegetation and LCP and 0CP. While HCP trees were positively correlated with live vegetation, the correlation was not significant (Table 2).

Male trees were more abundant than female in Balcones, Classen, Junction, and San Macros. Across the six locations, percent male (m/(m+f)) varied from 45% in Crossbar to 71% in Balcones. In Sonora, there was an equal amount of male trees to female trees. Classen, Junction, and San Marcos had male percentages of 58, 62, and 63 respectively. A chi-squared analysis showed that the locations were not significantly different from each other ($p > 0.05$) nor were they significantly different from a 50:50 ratio ($p > 0.05$).

Tree stand characteristic results from this study are generally comparable with other studies. For example, mean tree heights from this study were in the same range as other studies. Two locations in Guadalupe River State Park, TX (30 km north of San Antonio) had mean tree heights of 6.2 m and 7.9 m. Another location in the same study in Bosque County, TX (west of Waco) had a mean tree height of

7.2 m (Mclemore et al., 2004). Hicks and Dugas found that trees in a location in Uvalde County, TX averaged 2.7 m in height (1998). Mean tree height in this study ranged from 3.0 m to 5.8 m (Table 1). Hicks and Dugas also found that there were approximately 1,000 trees per ha and 90% of those trees were *J. ashei* (1998). In other words, the juniper density was 900 trees/ha. Balcones tree density was also 900 trees/ha and the highest density area in this study was San Marcos which reached 1500 *J. ashei* trees/ha (Table 1).

Pollen Grains Per Cone

The average number of pollen grains per cone was significantly different across the population per the one-way ANOVA ($F_{11, 60}$ = 10.3, p<0.0001). Means comparison shows that Sonora was higher than San Marcos and Junction (Table 3).

Table 3. *Juniperus ashei* pollen grains per cone by location.

Location	Pollen grains/cone
San Marcos	$3.74 \times 10^5 \pm 7.04 \times 10^4$
Junction	$3.63 \times 10^5 \pm 6.32 \times 10^4$
Sonora	$4.72 \times 10^5 \pm 4.23 \times 10^4$
Dallas	$4.02 \times 10^5 \pm 5.91 \times 10^4$

The mean across all sites was 402,000 pollen grains per cone and the standard deviation across all sites was 74,794. Hidalgo et al., found that the mean pollen per cone of *Cupressus sempervirens* was 365,722 and the standard deviation was 40,058 (1999). The mean pollen grain per cone value will be used in the pollen production estimate.

Pollen Cone Production Model

Observation at each location confirmed preliminary findings that cone production varied more from top to bottom than left to right. Male cones were counted from ten trees. The largest HCP tree countedwas 4.8 m tall with a mean canopy diameter of 5.0 m and estimated to have produced 1.38 million cones. By comparison, the tallest LCP tree was 5.2 m tall with a mean canopy diameter of 5.7 m and estimated to have produced 140,000 cones (Figure 2). Hidalgo et al. estimated male cone production in three *Cupressus* species and found that mean cone production ranged from 176,233 cones per tree in the lowest cone producing species and 2,974,651 cones per tree in the highest producing species (1999). When cone count was plotted against tree height for the HCP and LCP cone producers, the slopes fell onto separate and distinct lines. Further, using the test statistic, Student's t, the slopes were found to be significantly different (p<0.001) thus justifying separate regression analyses for the two groups (Figure 2).

The HCP regression was significant ($F_{1,3}$ = 44.1, P<0.01) as was the LCP regression ($F_{1,3}$ = 15.6, P<0.05).

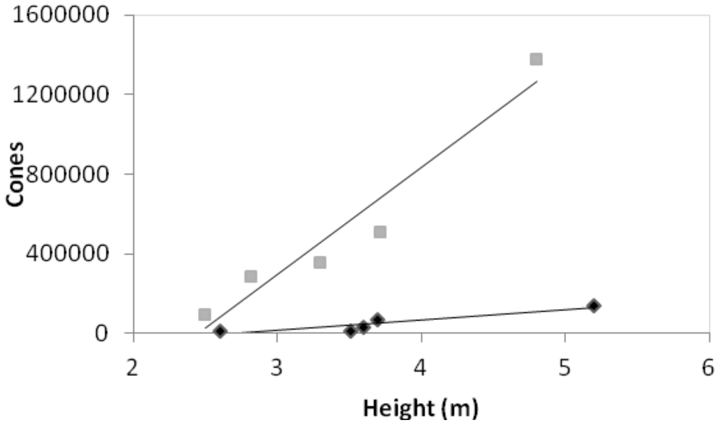


Figure 2. Height of representative trees and cone production for low cone producing (LCP) trees and high cone producing (HCP) trees. Estimates of cones were based on counts of 1/8 tree × 8. HCP = gray squares, LCP = dark diamonds.

The three models (cone production proportional to height, surface area, and volume) were tested through linear regression using log transformed data: log(cones) = a log(height) + log(b) (Table 4). Slope for the log transformed LCP data was approximately 3.39 (95% CI, -0.77, 7.54) and for HCP it was 3.73 (95% CI, 1.93, 5.53). Externally studentized residuals were calculated to test for outliers and none were identified. A slope of 3 suggests a volume relationship, a slope of 2 suggests a surface area relationship, and a slope of 1 suggests a one-dimensional relationship (i.e., height). The 95% confidence interval for the log transformed HCP slope includes 2 and 3 while the LCP interval includes 1, 2, and 3. The estimate of total cone production, therefore, used the 3 models and location stand characteristics.

Potential pollen production was estimated for each location using the three models (Table 4). Using the values and equations from Table 4, and tree heights (x) from the six locations, the totals were multiplied by percent live vegetation estimates and the mean number of pollen grains per cone (402,000) and extrapolated and values are expressed in pollen grains per hectare. Total estimated pollen production varied widely (Figure 3). The volume model predicts San Marcos as the highest pollen producing location while the surface area and height models predict Junction as the highest (Figure 3). Tree heights and number of HCP trees per location were the driving factors. The HCP trees often produce an order of magnitude greater number of cones (pollen). There were 167 HCP trees/ha in Junction and Crossbar

Table 4. Height, surface area, volume, and full-log models of pollen cone production including equation and constants (a,b).

		LCP		HCP	
Model	Equation	a	b	a	B
Height	$Y = ax + b$	51,819	-135,132	538,770*	-1,320,926*
Surface Area	$Y = bx^2$	2	2750.86	2	33011.64
Volume	$Y = bx^3$	3	757.38	3	9890.16
Full-log	$Y = bx^a$	3.39	459.20	3.73	4102.04

*Full-log test of height, surface area, and volume models led to rejection of simple height relationship in HCP trees.

and only 133 HCP trees/ha in San Marcos (Figure 1). The HCP trees in San Marcos were taller (data not shown) on average than the Junction trees and Junction HCP trees were taller than Crossbar HCP trees which is the reason the volume model predicted more pollen grains in San Marcos. Rankings of the total production for Crossbar, Classen, Sonora, and Balcones were the same for all three models (Figure 3).

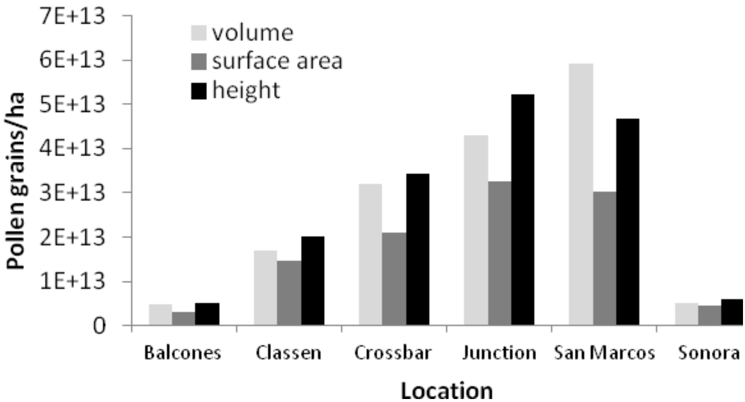


Figure 3. Estimated pollen production per hectare using volume, surface area, and height models.

A representative HCP and LCP tree was not collected in every location due to the requirement that the representative trees have a live vegetation rating of approximately 100%. Another shortcoming is that the tallest HCP tree with cone counts was 4.8 m which means that HCP trees taller than 4.8 m were predicted to follow the same cone to height relationship established by our slope without an actual count to support it (Figure 2). Many of the HCP trees in the San Marcos location were taller than 5 m and it is possible that the relationship between cone production and tree size was different for taller trees. It is interesting that Sonora and Balcones were very similar in their estimated production with vastly different stand characteristics (Table 1, Figure 3). The trees in Sonora were much shorter and smaller than the Balcones trees, but the number of HCP trees per ha was higher (Figure 1, Table 1). Although these data provide a pollen production range, the slopes of the HCP and LCP trees created by the 10 trees counted do not have multiple trees counted for the same height in each cone production class. This means that the model does not test the variability in the relationship between trees of the same height. Rather, it provides a range of possible pollen production based on a single count for each height. More representative tree cone counts would be necessary to determine which model is the most accurate. It is also possible that the model varies by location or that groups of trees fit one model and other groups fit another.

This study compared plots at six woodland locations. It is important to point out that forest descriptions by location as well as pollen cone production estimates by location reflect immediate localities and not greater geographic areas. For example, Classen and Crossbar are approximately 5 kilometers apart yet have very different stand characteristics (Table 1). This is due in part to an age mosaic created by wild and prescribed fires as well as other human removal practices. Some stand characteristics are likely a function of precipitation, stand age, climate zone and soil types. For instance, the average precipitation from the west end of the Edwards Plateau to the east end of the Plateau ranges from 600 mm/yr to 900 mm/yr respectively which likely contributes to the tree density difference between locations (Owens et al., 2006).

Light was one major factor affecting pollen cone production and could also contribute to the number of pollen grains per cone. Incident light is increased when forest edges are created which promotes plant growth (Murcia, 1995). The effect of light availability was tested on seed production of balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) in western Quebec. While mean annual seed production is typically proportional to basal area, light conditions affected production. Sub-canopy trees were found to produce half as many seeds as canopy trees. Height at which seed cones are produced differed between sub-canopy and canopy white spruce trees with heights of 14 m and 3 m respectively (Greene et al., 2002). In the locations sampled for this study, more male cones were typically produced where there was full sun as on the upper portion of the trees or trees in the open and this is documented in other junipers (Raatikainen and Tanksa, 1993). The edge effect was especially apparent along roadways, but this study was not designed to test the edge effect. Density and number of HCP trees was not negatively correlated as one would expect, and this is probably in part due to edge effects along clearings. In other words, a high tree density area could be an area where trees were evenly spread or in clumps. It is interesting to note that in the least dense area (Sonora) 75% of all male trees were HCP trees (Figure 1).

The only significant relationship between stand characteristic and HCP, LCP, or 0CP trees was LCP and juniper percent cover (Table 2). If more sunlight results in more cones, then low cone producing (LCP) trees would be expected in areas with less light (i.e. tall trees, dense forest, high forest cover). Balcones produced the highest number of LCP trees and had the highest percent juniper cover (Table 1, Figure 1). Other trees associated with juniper forests in Oklahoma and Texas were various species of *Quercus* some of which may have less dense canopies than junipers. This could result in more light available in a stand with high percent canopy cover that is made up of *Quercus* as opposed to a high percent cover juniper monostand. For example, percent juniper cover in San Marcos was relatively low (35%) but overall cover was high (67%) and number of HCP trees was relatively high which may be due to better access to light (Table 1, Figure 1). Compare this to the Balcones location where there were very few HCP trees and juniper made up 65% out of the 69% total cover (Table 1).

Clearly, the canopy characteristics are many and varied across the *J. ashei* distribution and these six locations may not represent all possibilities. It should be noted that the large amount of variability within each location can have a significant effect on the various models presented. More data is necessary in order to determine the best model and to ensure that the range in each variable is representative. Another avenue of testing the models may be comparing aerobiological data with predicted pollen production. Although analysis of aerobiological data is ongoing, preliminary results indicated that the mean pollen concentrations were not well correlated with estimated pollen load from the models in this study (Levetin et al., 2011).

CONCLUSION

This study showed that *Juniperus ashei* trees have the potential of producing enormous amounts of pollen with up to 1.3 million pollen cones per tree and approximately 402,000 pollen grains per cone. While the large amount of pollen in juniper woodlands is difficult to quantify with a high degree of accuracy, loads per hectare can be estimated through statistical models based on tree and landscape characteristics. Typically, very dense areas are not producing as many pollen cones. The data from this study also indicate that only a fraction of the trees on the landscape are producing most of the pollen. More field work is needed to distinguish between the pollen production models and to quantify the effect of forest edge on pollen production.

ACKNOWLEDGEMENTS

This work is funded through NASA's Applied Sciences, Public Health program proposal called: "Decision support through Earth Science Research Results" element of the NASA ROSES 2008 omnibus solicitation. The authors would also like to acknowledge the help of Jason Robinson and Michael Callegari with the field work.

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