Seed cone volume, a new methodology for a metric to differentiate *Hesperocyparis* (western cypress, Cupressaceae) taxa

Jim A. Bartel
San Diego Botanic Garden, P. O. Box 230005, Encinitas, CA 92023
jbartel@sdbgarden.org

and

Robert P. Adams
Baylor-Utah Lab, Baylor University, 201 N 5500 W, Hurricane, UT, 84737

ABSTRACT

Treating the irregularly surfaced seed cone of Monterey cypress (*Hesperocyparis macrocarpa*) as an ellipsoid, cone volume (cm^3) for 25 sampled cones was calculated by using cone length and diameter measurements in the mathematical volume formula for a spheroid. A graduated cylinder was used to directly measure volume (ml) of the same 25 cones to compare the two volume methodologies. The two methods were always within 0.9 cm^3 (ml) of each other for each cone sampled and were not statistically different. The easily measured seed cone length and width of the calculated methodology may make for precise cone volume comparison among populations and taxa. Published on-line www.phytologia.org *Phytologia 100(1): 111-116 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: *Hesperocyparis*, *H. abramsiana*, *H. macrocarpa, Cupressus,* western cypress, New World cypress, seed cone, seed cone volume, seed cone density, taxonomy, Cupressaceae.

Seed cone (ovulate cone or megastrobilus) length is a measurable character or metric that has been used in taxonomic keys to distinguish among pine family (Pinaceae) genera (*Picea*, *Pseudotsuga*, and *Tsuga*) and various California pines (*Pinus*) (Baldwin et al. 2012). This metric was similarly used in the cypress family (Cupressaceae), along with leaf morphology, to distinguish between the coast redwood (*Sequoia*) and giant sequoia (*Sequoiadendron*) (Bartel 2012). Additionally seed cone length and other cone characteristics (e.g., shape, color, woody versus fleshy) were used in part to distinguish among other cypress family genera (*Callitropsis*, *Chamaecyparis*, *Juniperus*, *Hesperocyparis*) and between western or New World cypress (*Hesperocyparis*) species in California (Bartel 2012). In one case, ranges in seed cone length and diameter were used in tandem to depict obvious differences in overall cone size to differentiate between two varieties of *H. abramsiana* (Bartel 2012).

Maturing the second year, the woody seed cones of western cypress (*Hesperocyparis*) are serotinous and, thus, generally remain closed and attached beyond maturity (> 2 years) (Bartel 2012). Cones range from 10-50 mm in length and are generally spheric to widely cylindric in shape (Bartel 2012). Pairs of decussate cone scales are peltately attached around the cone axis, which is often longer than the cone width or diameter. Because the range of seed cone length and width measurements typically overlap between taxa and populations, comparing cone volumes may make seed cone size differences more readily apparent than by length and width measurements alone. This paper presents a new methodology for a metric to calculate seed cone volume and discusses its possible use.

MATERIALS AND METHODS

Twenty-five (25) mature seed cones were collected from 10 naturalized Monterey cypress (*Hesperocyparis macrocarpa*) trees growing within the highway divider of Carlsbad Boulevard near its intersection with Avenida Encinas in Carlsbad, CA. A variety of sizes and shapes, from spheric to widely cylindric, were included in the 25 sampled cones (Fig. 1). The seed cones were measured using a DoWorld Tools 6-inch electronic digital caliper at a resolution of 0.01 mm. Cone length was measured from stem to cone bottom, while two cone diameter or width readings were made equidistant along the cone axis at 90° from each other. The seed volume in cubic centimeters (cm^3) was calculated in Microsoft Excel using the volume formula for an ellipsoid (Burnside 2017) [$V = (4/3)\cdot\pi\cdot a\cdot b\cdot c$] where a = cone length radius, b = first cone diameter radius, and c = second cone diameter radius (Fig 2).

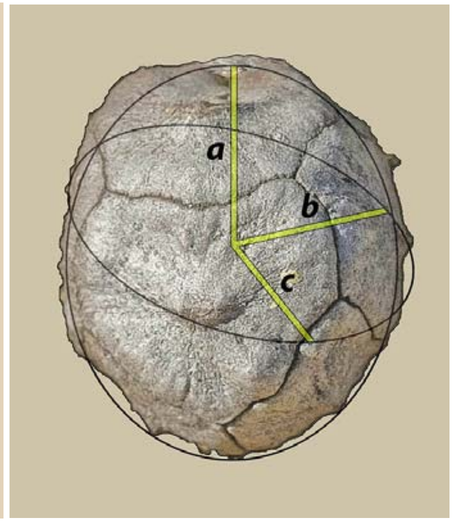

Figure 1. The 25 sampled seed cones identified by cone number. Figure 2. Seed cone depicting the three measured radii within a cone used to calculate volume. All angles between each radius pair are 90°.

After the seed cones were measured with the caliper, the cones were placed one at a time into a 250 ml graduated cylinder with a measured volume of water. The measured volume in milliliters (ml) for each sampled cone was the difference to the nearest 0.5 ml between the cylinder readings with and without the seed cone.

RESULTS AND DISCUSSION

The cylinder measured and caliper calculated seed cone volumes and the differences between these methods are given for each of the 25 sampled seed cones in Table 1. While the two methods produced identical volume numbers only once, the two methods always were within 0.9 cm^3 (ml) of each other. In Excel, the two methods were analyzed using a t-Test of paired two sample for means, which is presented in Table 2. This statistical test is used to determine whether the null hypothesis (H_0) should be rejected that the difference between the means of the two methods is zero ($\mu_d = 0$). At a 95% confidence level ($\alpha = 0.05$), the t-Statistic number is smaller than the t Critical two-tail number. Consequently, the null hypothesis is not rejected and, therefore, the means from the two volume measuring methods are not statistically different.

In determining which method to choose to measure the volume of an irregularly shaped object, Bruce and Schumacher (1950) noted that measuring the displaced volume was the most accurate method. However in practice the cylinder methodology used in this study for seed cones had issues because it requires two readings (one with and one without the cone) that are approximated to the nearest 0.5 ml, which impacts accuracy. In addition, cones occasionally floated in the cylinder water making readings

Table 1. Cylinder measured and caliper calculated seed cone volumes and the differences between these methods for each of the 25 sampled seed cones

Cone number	Cylinder measured volume (ml)	Caliper calculated volume (cm^3)	Difference of calculated volume to measured volume
1	12.0	11.8	-0.2
2	10.5	10.6	0.1
3	14.0	14.0	0.0
4	7.5	8.0	0.5
5	9.5	9.2	-0.3
6	16.0	15.5	-0.5
7	6.0	6.2	0.2
8	7.0	6.8	-0.2
9	15.5	14.9	-0.6
10	6.0	6.5	0.5
11	11.5	11.9	0.4
12	9.5	9.0	-0.5
13	8.0	7.6	-0.4
14	16.5	16.7	0.2
15	12.5	11.6	-0.9
16	7.0	6.3	-0.7
17	12.0	11.8	-0.2
18	9.5	10.3	0.8
19	9.5	9.7	0.2
20	7.5	6.6	-0.9
21	4.5	4.2	-0.3
22	5.0	5.3	0.3
23	4.5	4.3	-0.2
24	10.5	10.3	-0.2
25	11.0	10.3	-0.7

Table 2. Results of t-Test of paired two sample for means for the cylinder measured and caliper calculated seed cone volumes

	Cylinder measured volume (ml)	Caliper calculated volume (cm3)
Mean	9.72	9.58
Variance	12.231	11.911
Observations	25	25
Pearson Correlation Coefficient	0.991	
Hypothesized Mean Difference	0	
Degrees of Freedom	24	
t Statistic	1.579	
P(T<=t) two-tail	0.127	
t Critical two-tail	2.064	

difficult. Moreover, because the cylinder methodology requires that the cone be immersed in water, seed cones likely would require drying so as to not affect their further use in the study. Lastly, caliper measurement of seed cones is already occurring, along with other observations like number of cone scale pairs per cone. As a result, using the caliper method to precisely obtain seed cone volume would only come at the cost of further analyzing previously collected data in a spreadsheet.

Adams and Bartel (2009) used combined Inter-Simple Sequence Repeats (ISSRs) and terpenoid data to support the varietal recognition of the Butano Ridge population of *Hesperocyparis abramsiana* from the other four populations. To distinguish *H. abramsiana* var. *butanoensis*, the authors used mean seed cone length and diameter in a table (Adams and Bartel 2009). In developing a taxonomic key, Bartel (2012) primarily used ranges of seed cone length and diameter in tandem to separate the varieties of *H. abramsiana*. Using the original caliper data of seed cone length and diameter from Adams and Bartel (2009) to calculate cone volumes and inserting those values into an updated table from the same article, the differences in mean cone volume between the two varieties of *Hesperocyparis abramsiana* in Table 3 are more obvious. McMillan (1952) used a photograph of a “random selection” of seed cones from the then three known populations to demonstrate the “striking difference” in cone size. Recently in a self-admitted “too small cone sample size,” Malone et al. (2012) provided additional seed cone images and a confusing table of the five populations of *H. abramsiana* with 3 “indices” involving unexplained computations of seed cone length and/or width. Whereas using the mean cone volumes in addition to various cone length and width measurements in Table 3, the seed cones from Butano Ridge proved to be demonstrably larger ranging from nearly twice to more than four times the size of the other populations of *H. abramsiana*.

Table 3. Revised grove-by-grove comparison of *Hesperocyparis abramsiana* seed cones by adding mean cone volume data to Table 1 from Adams and Bartel (2009) and by adding cone length and width range data from Bartel (2012).

	Butano Ridge	**Eagle Rock**	**Bracken Brae**	**Bonny Doon**	**Majors Creek**
Mean of seed cone length (mm)	27.0	22.1	16.7	22.0	19.9
Range of seed cone length (mm)	22–32(35)	(14)16–25			
Mean of seed cone width (mm)	25.5	20.0	16.1	18.8	18.3
Range of seed cone diameter (mm)	22–31	14–22			
Mean number of scale pairs per cone	4.7	4.2	3.6	4.3	4.4
Mean of seed cone volume (cm^3)	9.2	4.1	1.9	4.2	4.8

While seed cone length and width are frequently used to identify differences in cypress (Wolf 1948, Farjon 2005, Bartel 2012), an attempt to quantify seed cone size or volume appears to be quite rare. Goggans and Posey (1968) measured “cone size” of several *Hesperocyparis* (*Cupressus*) taxa by weighing in grams 100 dried, open, and seed-free cones within each sampled population. The authors made general correlations by comparing the cone size of various populations in Arizona, California, New Mexico, and northern Mexico, including the Eagle Rock population of *H. abramsiana*. Ignoring that weighing cones after drying the cones and extracting the seeds, a 1 to 2 month process (Johnson and Karrfelt 2008), makes cone weight of no practicable use in the field, cone mass and cone volume are not synonymous given the varying densities of cones (see discussion below). Pandit and Ram (2002), working with *Cupressus torulosa* at two sites in the Himalayas of India, evidently measured seed cone weight of 100 seed cones per collection site prior to or during cone opening for this non-seritinous species. Though the authors reported that "cone size was the mean diameter of two axes (at right angle) of the cone measured" with a digital caliper, they also correctly noted that "cone density was the value of mass divided by the volume of the cone." Despite the confusing methodology section, the 2002 article focus was on the correlation of cone and seed moisture with seed germination and not taxonomy. Though not to calculate cone volume, Westman and Whittaker (1975) treated *H. pygmaea* cones as spheres to calculate cone surface area in a study of pygmy forest biomass in Mendocino County, California. Finally in a study of insect pests of *C. sempervirens* seed cones in Tunisia, Ben Jamaa and Roques (1999) used an unexplained formula to measure the volume of seed cones where l = cone length and w = cone width ($V = \pi \cdot l \cdot (3w^2 + l^2)/24$). Because a quick test using their formula for our *H. macrocarpa* seed cones resulted in larger cone volumes for 18 of the 25 cones and a statistically different mean volume from our calculated mean volume, we did not pursue the formula further.

Though compact digital scales make the accurate weighing of individual seed cones relatively easy in the field, cone weights likely will not correlate well with cone size because of the effects of cone maturation or aging on density. Kafton (1976) reported that while "full-size" seed cones of *Hesperocyparis macrocarpa* mature from greenish-brown (6 to 11 months in age) to brown (> 11 months in age) to grey in color (> 15 months), the cones continue to become "less soft and moist" as the cones age and desiccate. Older grey seed cones of *H. macrocarpa* often will naturally open up and release their seeds (Fig. 3). Despite collecting seed cones up to 32 years in age with viable seeds, Kuhlman (1986) in studying the Bracken Brae population of *Hesperocyparis abramsiana* reported that a slow release of seeds from trees appears to be a result of ongoing vascular constriction in the cone peduncle resulting in a loss of fluids allowing the cone scales to open. In a quick test to compare the relative densities of young versus older mature seed cones, the calculated volumes and measured mass (digital scale with 0.1 gram resolution) were taken for 15 light brown (young) and 15 dull brown to grey (older) seed cones. Using the density formula $D = m/v$, where density (D) is equal to mass (m) divided by volume (v), the mean mass per unit volume for young cones was 1.12 and for older cones 1.00. In light of the fact that the density of water is 1, not surprisingly 13 of the 15 young cones sank in water, while 12 of the 15 older cones floated in the water. Where species like *H. abramsiana* retain their closed cones for decades, this density difference likely will be greater for the oldest cones.

Figure 3. Naturally opening, older grey seed cones and dispersing seeds of *Hesperocyparis macrocarpa*.

CONCLUSIONS

Because seed cone volume is not an easily obtain metric in the field, seed cone length and diameter still will be required for taxonomic keys. Nonetheless, calculating seed cone volume appears to be a precise method to obtain an additional promising metric to easily recognize substantial differences in cone size. In the example of *Hesperocyparis abramsiana* var. *butanoensis* where using the mean cone volumes in addition to various cone length and width measurements in Table 3, the seed cones from Butano Ridge proved to be demonstrably larger using seed cone volume with cones ranging from nearly twice to more than four times the size of the other populations of *H. abramsiana*.

Though 3 radii were used in this article to calculate seed cone volume, typical cone measurements made in the field involve measuring 3 diameters that would then need to be halved. An alternative and simplified seed cone volume formula using diameters in lieu of radii would be $V = \pi \cdot a \cdot b \cdot c/6$ where a = cone length diameter, b = first cone width diameter reading made equidistant along the cone axis, and c = second cone width diameter reading at 90° from b.

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