# CORRELATION OF MESCALINE CONCENTRATIONS IN *LOPHOPHORA WILLIAMSII* (CACTACEAE) WITH RIB NUMBERS AND DIAMETER OF CROWN (U.S.A.)


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## ABSTRACT

*Lophophora williamsii*, peyote, is a small cactus growing to approximately 10 cm in diameter with a flat to dome-shaped spineless crown with fissures or ribs that develop following the Fibonacci series and whose numbers indicate relative degree of maturing. In this study crown tissue of 30 wild-collected specimens was analyzed to determine whether there was a correlation between the concentration of the primary alkaloid mescaline in crown tissue with the average diameter of the crown. We also compared mescaline concentration in three groups of ten individuals: 5 ribs (juvenile stage), 8 ribs (intermediate), and 13 ribs (elder/mature stage), since these Fibonacci numbers are the most stable and long-lasting on *L. williamsii*. This was designed to test the hypothesis that there is a positive correlation between mescaline concentration and both diameter and rib number. Nine greenhouse-grown specimens were likewise analyzed to serve as a control group for the study. All 39 tissue samples were subjected to an alkaloid extraction procedure followed by an acid-base washing procedure. Mescaline was identified using liquid-chromatography and mass spectroscopy and then quantified using reverse-phase high-pressure liquid chromatography. The Pearson's Chi-squared test showed no statistical correlation between increasing mescaline concentration and increasing rib numbers for field-collected samples and greenhouse-raised control samples. Field-collected samples: *P*-value of 0.392; greenhouse control samples: *P*-value of 0.313. Similarly, field and greenhouse samples showed no statistical correlation between mescaline concentration and average diameter of the crown. Field-collected samples: *P*-value of 0.251; greenhouse control samples: *P*-value of 0.229. This study contributes to the understanding of this vulnerable species and to approaches to its overall conservation and the preservation of Native American culture.

## RESUMEN

*Lophophora williamsii* es un pequeño cactus que crece hasta aproximadamente 10 cm de diámetro, con una corona sin espinas entre plana y domo. En las especies de cactus con fisuras en las costillas, el número de costillas puede ayudar a determinar una etapa relativa aproximada de desarrollo; costillas se desarrollan siguiendo la serie Fibonacci, con el número de costillas aumentando a medida que un individuo madura. En este estudio se analizó el tejido de la corona de 30 especímenes silvestres recogidos para determinar si había una correlación entre la concentración de mescalina en el tejido de la corona y el diámetro promedio de la corona. También comparamos la concentración de mescalina en tres grupos de diez individuos por grupo: 5 costillas (etapa juvenil), 8 costillas (etapa mayor) y 13 costillas (etapa madura), ya que estos números de Fibonacci son observables en *L. williamsii* durante períodos más largos de tiempo. Nueve especímenes cultivados en invernadero también fueron analizados para servir como una variable de control para el estudio. Las 39 muestras de tejido fueron sometidas a un procedimiento de extracción de alcaloides seguido de un procedimiento de lavado en base ácida. La mescalina se identificó mediante espectroscopia de masas y luego se cuantificó mediante cromatografía líquida de alta presión de fase inversa. La prueba estadística de Pearson Chi-cuadrado no mostró correlación estadística entre el aumento de la concentración de mescalina y el aumento del número de costillas tanto para muestras recogidas en el campo como para muestras controladas en invernadero (muestras recogidas en el campo: valor P de 0.392; muestras de control en invernadero: valor P de 0.313). Del mismo modo, las muestras de campo e invernadero no mostraron correlación estadística entre la concentración de mescalina y el diámetro medio de la corona (muestras recogidas en el campo: valor P de 0.251; muestras de control en invernadero: valor P de 0.229). Este estudio se suma aún más a la comprensión de esta especie vulnerable y su conservación general por su ecosistema único y la preservación de la cultura Nativa Americana.


## INTRODUCTION

Plants, the primary source of energy for the earth's ecosystems, are faced with numerous biotic stresses and adverse environmental conditions; they are under constant threat of predation by pathogens—fungi, bacteria, nematodes, and viruses—as well as relentless interactions with herbivores (Gimenex et al. 2018). Over time, many species of plants developed unique characteristics to reduce herbivory through constitutive and inducible defenses (Alijbory & Chen 2018).


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Constitutive defenses include barriers such as cell walls, waxy epidermal cuticles, and bark (Freeman & Beattie 2008). While inducible defenses are classified as direct, including traits that increase production of toxic chemicals, pathogen-degrading enzymes, and autophagy, and as indirect defenses, that entice a mutualistic relationship involving intervention from higher predators (Freeman & Beattie 2008; Alijbory & Chen 2018). Most of these defenses come as a fundamental trade-off between plant growth and their available resources. One prominent defense theory is the resource availability hypothesis (RAH) which suggests that plants in high-resource environments select for the investment of replacing biomass over the relatively high cost of defense production; while in stressful environments with low resources, the majority of plants evolve slow growth rates for the allocation of high levels of defenses (Hahn et al. 2019; Tuller et al. 2018).

Prior to the late 1800s, secondary metabolites such as alkaloids had been largely overlooked, as they consisted of a diverse assortment of organic compounds with unknown physiological purpose (Buchanan et al. 2000). Unlike primary metabolites (e.g., phytosterols, acyl lipids, nucleotides, and amino acids), which have obvious metabolic roles in plants, secondary metabolites seemed to be more of an ill use of nitrogen (Buchanan et al. 2000; Crozier et al. 2006). However, with the isolation of the first alkaloid, morphine—extracted from *Papaver somniferum* by Friedrich Wilhelm Sertürner in 1803—alkaloids and other secondary metabolites (including flavonoids, phenols, and terpenoids) began to be understood as important adaptive products of evolution that served to protect plants against herbivory and/or microbial infection, to attract pollinators, and as allelopathic agents (Cipollini & Levey 1997; Buchanan et al. 2000; Schaefer 2015).

Currently the Dictionary of Natural Products (DNP) lists 21,512 alkaloids that have been isolated since the discovery of morphine (updates from dnp.chemnetbase.com, accessed 9/18). While the definition of alkaloids has varied over the years, their role as chemical defenses is strongly supported by their wide range of physiological effects on animals, as well as the efficacy of some as antibiotics (Crozier et al. 2006). In chemical terms, alkaloids are diverse naturally occurring compounds that contain at least one nitrogen atom and a phenyl ring (Hesse 2002; Walsh 2017). Previously thought to be produced only by plants, recent studies have shown that alkaloid-bearing species have been found in nearly all classes of organisms, including frogs, ants, butterflies, bacteria, sponges, fungi, spiders, beetles, and mammals (Buchanan et al. 2000; Hesse 2002). Alkaloids may contain amines, may be heterocyclic, and are categorized by the metabolic pathway by which each is synthesized (Ibarra-Laclette et al. 2015; Walsh 2017). A popular broad scheme divides them into three groups; a protoalkaloid (e.g., mescaline) contains a nitrogen atom derived from an amino acid in the structure, but not in the benzene ring. A true alkaloid (e.g., nicotine) has a nitrogen atom derived from an amino acid appearing in the heterocyclic ring. A pseudoalkaloid (e.g., ephedrine) is not derived from an amino acid (Amirkia & Heinrich 2014).

The alkaloids of the peyote cactus, *Lophophora williamsii* (Lem. ex Salm-Dyck) J.M. Coult. (Cactaceae) have brought it fame or notoriety in different cultures. Peyote is a small cactus (up to approximately 10 cm in diameter) with a flat to dome-shaped spineless crown with tufts of hairs growing from the areoles (Correll & Johnston 1970; Terry et al. 2012). Its geographic distribution includes the Chihuahuan Desert through much of central and northern Mexico and into the Trans-Pecos region of western Texas, as well as a much smaller region in several Texas border counties in the ecological region known as the Tamaulipan Thornscub of South Texas. Over 90 percent of the *L. williamsii* range in Texas is on private land (Anderson 1995; TPWD 2016). Populations are most prevalent on south-facing slopes of limestone or partly-limestone hills with altitude ranges from 50 to 1200 meters above sea level (Correll & Johnston 1970; Anderson 1996; Terry 2013). Peyote is psychoactive due in large part to the presence of the alkaloid mescaline, approximately 90% of which occurs in the chlorophyllaceous layer (the chlorenchyma layer) of the cactus in the aerial portion (crown) of the stem (Klein et al. 2015). Mescaline, or 3,4,5-trimethoxyphenethylamine (Fig. 1) is produced from the primary metabolite tyrosine (Fig. 1)—a common metabolite in both plants and mammals (Kulma & Szopa 2006; Lundström 1971; Rosengarten & Friedhoff. 1976)—mescaline has been acknowledged as a norepinephrine mimic that produces strong dose-dependent sensory distortions when ingested (Huxley 1952; Rosenberg and Friedhoff 1974). In addition to mescaline, *L. williamsii* is reported to contain some 50 other alkaloids, including

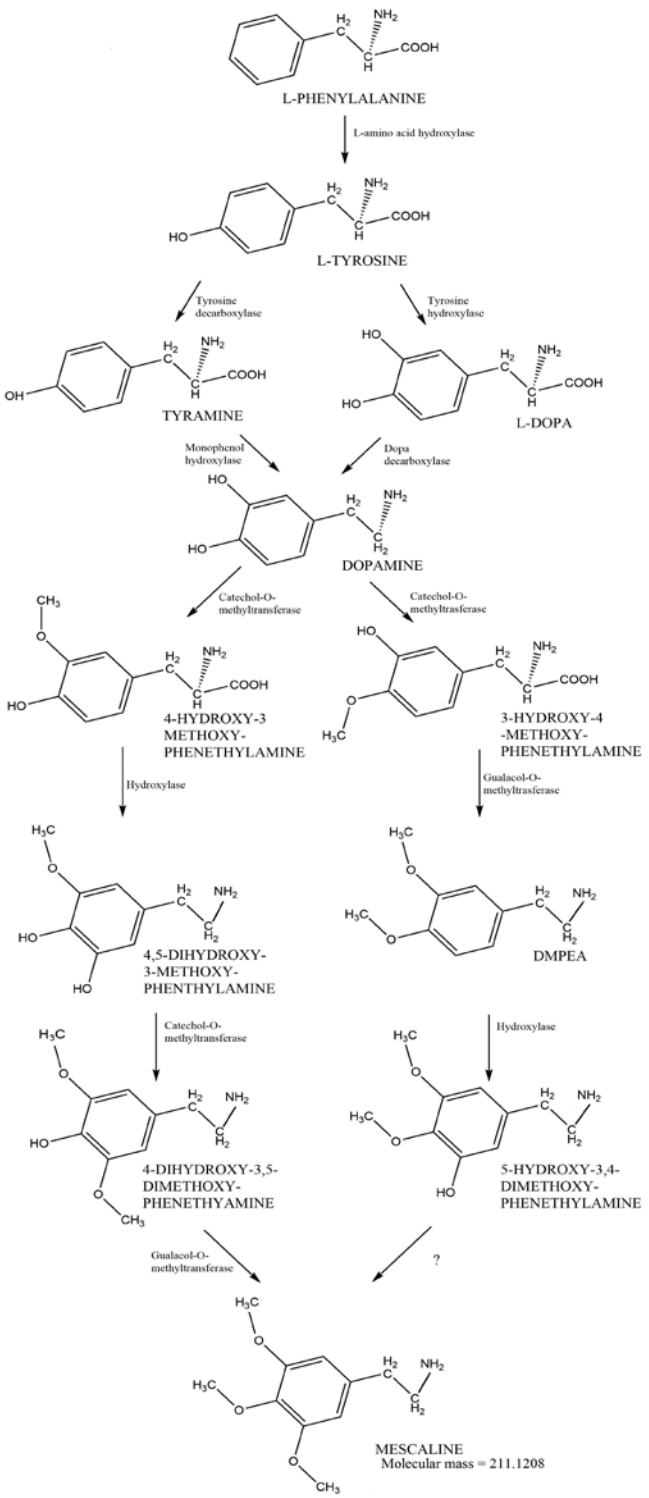


Fig. 1. Metabolic pathway of mescaline (Hesse 2002; Rosenberg & Stohs 1974).

pellotine, anhalonidine, tyramine, and hordenine (Lundström 1972; Anderson 1996). These amines not only have a deterrent taste, but may also produce aversive physiological effects (such as nausea and vomiting in naïve humans) which appear to be efficient in preventing herbivory in most mammalian species. However, certain terrestrial snails (such as those in the genus *Rabdotus*) and humans are known not to be daunted by the unpleasant taste of this cactus, and choose to consume it willingly (Terry et al. 2011).

Mescaline is categorized by the U.S. Drug Enforcement Agency (DEA) as a Schedule I controlled substance, which is defined as a substance with high potential for abuse and no currently accepted medical use (Drug Enforcement Administration, Department of Justice 2017). Despite the legal and gustatory stigmas associated with peyote and its alkaloids, the Native American Church (NAC) constitutes a conspicuous example of the positive relationship people currently hold with this cactus. The common and widespread legally-sanctioned ceremonial use of peyote by the NAC is shared by an unknown but large number of Native Americans throughout the U.S. and Canada who currently practice religious rites in which peyote is consumed for its spiritual (psychotropic) effects (Anderson 1995). Due to widespread, intensive and persistent harvesting and lack of conservation efforts, sales data on *Lophophora williamsii* during the last two decades have shown a sharp decline in the annual number of harvested buttons (the cut tops of the plants, consisting of aerial chlorophyllaceous stem tissue) of this species (cactusconcservation.org, accessed 10/19). The species is listed as Vulnerable in the IUCN Red List (IUCN 2013). Increased conservation efforts in recent years have provided education for authorized harvesters on how to properly harvest peyote in a manner that would maximize the likelihood of regrowth from the areoles on the subterranean stem which remains in the ground after harvesters have removed the crown (Terry & Mauseth 2006; Terry et al. 2011).

In other plant families such as the Fabaceae, Convolvulaceae, and Solanaceae, newer shoots and leaves have a higher alkaloid concentration than older plant material, possibly in order to protect vulnerable and valuable organs (McKey 1974); in contrast, oak trees (*Quercus* spp.) are known to have a higher concentration of tannins in more mature plant material, and instead produce several new shoots at one time during a period of decline in predators (ibid.). In another study, with *Trichocereus* spp. (San Pedro cactus), which, like peyote, produces mescaline, the concentration of mescaline-derived alkaloids stored in the plant tissues increases gradually over a long period of time (Carod-Artal & Vázquez-Cabrera 2006).

In those cactus species that exhibit a range of observable rib numbers (e.g., *Lophophora williamsii*), the total number of ribs present on an individual can be a rough indicator of its degree of maturity (Gibson & Nobel 1986; Terry, pers. comm.). The exact age of a wild cactus of unknown history is impossible to determine with contemporary dating methods, due to the fact that the growth rates of the plant vary markedly with environmental conditions, which cause temporary contraction during drought and expansion/growth during periods of wet weather (Gibson & Nobel 1989). It is reasonable to suggest that older plants will generally be larger and have more ribs than younger plants if measured at the same time and in the same environment in which they grew (Terry, pers. comm.). Furthermore, *L. williamsii* plants transition relatively quickly from the 5-ribbed juvenile stage to the next stable rib number (8), and eventually from the 8-ribbed stage to the stable (and final) stage of 13 ribs (5, 8 and 13 being consecutive numbers in the Fibonacci series). It is possible to observe these cacti with other-than-Fibonacci numbers of ribs, such as 6, 7, and 9; however, these stages are generally not stable and will soon transition to one of the more stable rib numbers: 5, 8, or 13 (Gibson & Nobel 1986; Terry, pers. comm.). In some populations, the 10-rib configuration appears to be more stable than in other populations (Terry, pers. obs. 2018).

According to Teodoso Herrera, the spiritual leader of the Native American Church of the Rio Grande, there is a prevalent belief among NAC members that older/larger peyote buttons constitute "stronger medicine" than smaller or younger buttons. This notion is compatible with the analytical findings of Kalam et al. (2013), who found that a decrease in button size was accompanied by a decrease in mescaline concentration. The delicate balance between storage and production of anti-herbivory alkaloids varies greatly to suit the needs of each plant species. The present study will examine the accuracy of the widespread belief that the average mescaline concentration found in 5-ribbed plants (Fig. 2) is significantly lower than the average concentration found in

Fig. 2. *Lophophora williamsii*, two 5-ribbed juvenile specimens (wild population, Presidio Co., Texas).

8-ribbed plants (Fig. 3), and similarly, the average concentration of mescaline found in the 8-ribbed plants will be lower than the average concentration in 13-ribbed plants (Fig. 4)

## MATERIALS AND METHODS

Samples of plant tissue were collected from a wild population in Presidio County, TX the exact location will not be disclosed in order to protect the cactus from poaching. Thirty peyote buttons with healthy characteristics (10 with 5 ribs, 10 with 8 ribs, and 10 with 13 ribs) selected, and tissue samples from the exposed crowns, which averaged about ⅓ of the crown volume, were excised from each specimen and immediately weighed using a TR-100 digital pocket scale to calculate fresh weight to the nearest 0.01 gram. Collected samples ranged from 1.16 to 10.64 grams. Inconsistency among weights in field-collected samples was in part to protect smaller plants from excessive excision that could have proved to be detrimental to their survival in a very dry year, as well as substantial differences in overall tissue density of samples at the time of excision. An average diameter of each cactus sampled was calculated (maximum diameter plus minimum diameter divided by two) to determine the approximate volume of each crown; (Tables 1, 2, and 3).

Nine specimens of *Lophophora williamsii* (3 with 5-ribs, 3 with 8-ribs, and 3 with 13-ribs), previously collected from Brewster, Presidio, and Starr counties and then concurrently grown at the Sul Ross State University greenhouse in Alpine, TX, were analyzed as a control group for this study. The nine greenhouse specimens were excised for crown tissue samples and measured for average diameter, volume, and dry weight as described above (Table 4). This section of the study served to decrease the amount of untestable variables associated with environmental conditions (e.g , nurse plants, drought/wet seasons, soil type, predation, and sun exposure) that might be expected to increase/decrease mescaline concentrations.

All 39 tissue samples were then thinly sliced and placed on a mesh screen with circulating air above and below the screen, and allowed to air-dry at room temperature for two weeks. Individual samples were then ground to a fine powder using a Proctor-Silex coffee grinder, weighed using an Acculab V-200 (Acculab, Satorius Group©) electronic balance to calculate dry weight to the nearest 0.01 gram, and then subjected to alkaloid extraction by a method similar to the procedure of Klein et al. (2015).

All 39 collected samples of ground dried tissue and 25 mL of HPLC-grade methanol were placed in 39 separate 100 mL beakers. All beakers were then covered with paraffin film to avoid evaporation and swirled daily for one week. The methanol slurry of cactus tissue was then filtered through No. 5 grade filter paper, and the filtered methanol extract was left to dry for one week.

The dry methanol extract of each sample was dissolved first into 25 mL of water acidified to pH 3, using dropwise addition of 1 M HCl; secondly, 25 mL of methylene chloride was used to dissolve the remaining extract, both were then placed into a separatory funnel (pH was determined using a Fisher Scientific Accument AE150 benchtop pH meter [Thermo Fisher Scientific Inc]). At this low pH, the polar protonated alkaloids have a higher affinity for the acidified aqueous solution than for the less polar and less electronegative methylene chloride phase. Once emulsions had resolved, the methylene chloride layer was drained from the separatory funnel and discarded. This defatting process was repeated twice to ensure a thorough extraction of nonpolar substances. The remaining acidic aqueous solution was drained into a 150-mL beaker and then alkalinized to pH 12 by adding 1.0 M NaOH dropwise in order to deprotonate the phenolic alkaloids (such as mescaline and tyramine). The alkalinized water and 25 mL of methylene chloride were then placed into a clean separatory funnel and mixed thoroughly. Once the emulsions had resolved, the methylene chloride layer was drained into a 250-mL round-bottomed flask and placed in a Buchi Rotavapor® R-200 (Buchi Labortechnik AG©) rotary evaporator at 50°C until the solution had completely dried (Robert LeBlanc, pers. comm.). Two mL of methanol was then added to the round-bottomed flask to dissolve the alkaloids left on the inside of the flask and then strained through a 0.2-micron MILLEX nylon syringe-driven filter unit (MilliporeSigma™) to remove small particulates. The sample alkaloid extracts thus obtained were then placed into labeled centrifuge tubes and stored in lab freezer at -20°C (alkaloid extraction method as per Klein et al. 2015).

All 39 sample extracts were then analyzed using reverse-phase high-pressure liquid chromatography

Fig. 3. *Lophophora williamsii*, an 8-ribbed mature specimen (Sul Ross State University greenhouse specimens).

(HPLC) to determine the mescaline concentration of each of the original tissue samples; this was performed using an Agilent 1260 Infinity HPLC (Agilent Technology, Inc.) instrument with a Phenomenex Gemini 5-micron C18 column (250 mm × 4.6 mm). The eluent suggested for this particular column, which also provided clean peak values, was disodium phosphate: methanol: acetonitrile, 35:55:10 (Na2HPO4: MeOH: MeCN), with a 0.10 mL/sec flow rate. The detector wavelength was set at the known UV-absorbance maximum of 205 nm for mescaline (Klein et al. 2015). A certified mescaline standard (1 mg/mL) was purchased from Fisher Scientific to provide retention-time verification and instrument calibration. The mescaline standard was analyzed repeatedly throughout sample analysis to derive an average retention time, peak height (milli-Absorbancy units generated by HPLC) and AUC (area under the curve measured in mAu × seconds, generated by HPLC). Each of the 39 samples was run three times in order to generate average peak heights and AUC that were then interpolated with the average peak heights/AUC of the mescaline standard.

The total mescaline concentration of the crown (as percentage of dry tissue weight) was calculated as follows: the mescaline concentration (µg/µL) for each sample was obtained from the average peak height values as well as the average AUC values (acquired from HPLC), that were then divided by the total injection volume. This value was then multiplied by the total sample volume; resulting in the amount of mescaline extracted from

Fig. 4. *Lophophora williamsii*, a 13-ribbed specimen that had been growing through this rock crevasse that has distorted its spherical shape: note the production of betalains indicated by the reddish/purple color of the crown of the plant (wild population, Presidio Co., Texas).

Table 1. Crown and sample data for five-ribbed peyote specimens from which tissue samples were collected March 7, 2018 in Presidio County, Texas.

| Specimen | Max/Min Diameter of Crown (cm) | Average Diameter of Crown (cm) | Average Volume of Crown ($cm^3$) | Fresh Weight of Excised Sample (g) | Dry Weight of Excised Sample (g) |
|---|---|---|---|---|---|
| 1 | 5.5cm / 4.8cm | 5.15cm | 35.86$cm^3$ | 5.14g | 1.00g |
| 2 | 5.0cm / 2.9cm | 3.95cm | 16.18$cm^3$ | 5.52g | 0.70g |
| 3 | 4.6cm / 3.1cm | 3.85cm | 14.98$cm^3$ | 4.11g | 0.60g |
| 4 | 6.0cm / 4.9cm | 5.45cm | 42.49$cm^3$ | 6.07g | 1.13g |
| 5 | 5.4cm / 4.3cm | 4.85cm | 29.95$cm^3$ | 4.48g | 0.85g |
| 6 | 6.4cm / 6.3cm | 6.35cm | 67.21$cm^3$ | 9.26g | 1.12g |
| 7 | 6.8cm / 6.2cm | 6.5cm | 72.09$cm^3$ | 7.42g | 0.86g |
| 8 | 6.5cm / 4.8cm | 5.65cm | 47.35$cm^3$ | 5.9g | 0.78g |
| 9 | 3.8cm / 3.7cm | 3.75cm | 13.84$cm^3$ | 3.90g | 0.80g |
| 10 | 5.4cm / 2.9cm | 4.15cm | 18.76$cm^3$ | 3.55g | 0.72g |

Table 2. Crown and sample data for eight-ribbed peyote specimens from which tissue samples were collected March 7, 2018 in Presidio County, Texas.

| Specimen | Max/Min Diameter of crown (cm) | Average Diameter of crown (cm) | Average Volume of crown ($cm^3$) | Fresh Weight of excised sample (g) | Dry Weight of excised sample (g) |
|---|---|---|---|---|---|
| 1 | 3cm / 2.5cm | 2.75cm | 5.46$cm^3$ | 2.94g | 0.50g |
| 2 | 4.1cm / 4.0cm | 4.05cm | 17.44$cm^3$ | 3.55g | 0.76g |
| 3 | 3.1cm / 2.3cm | 2.7cm | 5.17$cm^3$ | 2.34g | 0.63g |
| 4 | 2.7cm / 2.7cm | 2.7cm | 5.17$cm^3$ | 3.16g | 0.60g |
| 5 | 2.2cm / 2.1cm | 2.15cm | 2.61$cm^3$ | 1.16g | 0.18g |
| 6 | 3.6cm / 3.4cm | 3.5cm | 11.25$cm^3$ | 4.39g | 0.74g |
| 7 | 2.9cm / 2.5cm | 2.7cm | 5.17$cm^3$ | 1.63g | 0.31g |
| 8 | 3.2cm / 2.4cm | 2.8cm | 5.76$cm^3$ | 1.53g | 0.32g |
| 9 | 2.9cm / 2.5cm | 2.7cm | 5.17$cm^3$ | 1.97g | 0.41g |
| 10 | 3.4cm / 3.3cm | 3.35cm | 9.87$cm^3$ | 3.01g | 0.55g |

Table 3. Crown and sample data for thirteen-ribbed peyote specimens from which tissue samples were collected March 7, 2018 in Presidio County, Texas.

| Specimen | Max/Min Diameter of Crown (cm) | Average Diameter of Crown (cm) | Average Volume of Crown ($cm^3$) | Fresh Weight of Excised Sample (g) | Dry Weight of Excised Sample (g) |
|---|---|---|---|---|---|
| 1 | 6.8cm / 6.7cm | 6.75cm | 80.73$cm^3$ | 10.57g | 1.16g |
| 2 | 7.0cm / 5.4cm | 6.2cm | 62.56$cm^3$ | 10.10g | 1.69g |
| 3 | 6.7cm / 6.2cm | 6.45cm | 70.44$cm^3$ | 8.35g | 1.87g |
| 4 | 5.8cm / 5.7cm | 5.75cm | 49.90$cm^3$ | 6.27g | 1.03g |
| 5 | 5.4cm / 5.0cm | 5.2cm | 36.91$cm^3$ | 5.57g | 0.70g |
| 6 | 7.1cm / 5.6cm | 6.35cm | 67.21$cm^3$ | 8.14g | 1.05g |
| 7 | 6.5cm / 6.0cm | 6.25cm | 64.09$cm^3$ | 6.02g | 0.78g |
| 8 | 6.6cm / 5.1cm | 5.85cm | 52.55$cm^3$ | 7.03g | 1.36g |
| 9 | 8.1cm / 7.3cm | 7.7cm | 119.84$cm^3$ | 10.64g | 1.32g |
| 10 | 7.3cm / 7.2cm | 7.25cm | 100.03$cm^3$ | 8.0g | 0.96g |

an excised portion of the peyote plant collected in the field. Lastly, in order to express the mescaline concentration as a percentage of the dry weight, the total sample volume was divided by the dry weight of the excised sample and multiplied by 100%, (Klein et al. 2015; Trout 2014).

In order to confirm the identification of mescaline, each sample was analyzed using an AccuTOF JMS-T100LC (JOEL Ltd) mass spectrometer (LC-MS) at the University of Texas at El Paso supervised by Dr. Sohan de Silva. Helium was used as an eluent/carrier for each sample while each peak was analyzed and recorded twice. All 30 samples had an identical relative intensity that was within the range of 0.0001 m/z to the protonated mono-isotopic mass of mescaline 212.12867 m/z, which was calculated using the chemical formula of mescaline

Table 4. Crown, sample and rib and county data for control group: greenhouse peyote specimens with tissue samples and collected counties, tissue samples.

| Specimen | Rib Numbers Present/ Collection County | Average Diameter of Crown (cm) | Average Volume of Crown ($cm^3$) | Dry Weight of Excised Sample (g) |
|---|---|---|---|---|
| 1 | 5 (Presidio Co.) | 6.75cm | 80.51$cm^3$ | 0.53g |
| 2 | 5 (Presidio Co.) | 6.2cm | 62.39$cm^3$ | 0.36g |
| 3 | 5 (Presidio Co.) | 6.45cm | 70.25$cm^3$ | 0.72g |
| 4 | 8 (Presidio Co.) | 5.75cm | 49.77$cm^3$ | 0.51g |
| 5 | 8 (Presidio Co.) | 5.2cm | 36.81$cm^3$ | 0.32g |
| 6 | 8 (Brewster Co.) | 6.35cm | 67.03$cm^3$ | 0.45g |
| 7 | 13 (Presidio Co.) | 6.25cm | 63.92$cm^3$ | 0.93g |
| 8 | 13 (Brewster Co.) | 5.85cm | 52.41$cm^3$ | 0.31g |
| 9 | 13 (Starr Co.) | 7.7cm | 119.52$cm^3$ | 0.52g |

and its molecular mass (211.1208 g/mol) to create a simulation spectrograph to compare tested samples. All thirty tested samples had a range of less than 1% of the mono-isotopic mass of mescaline.

The method employed for statistical analysis involved developing a Pearson's Chi-squared test in SPSS (IBM SPSS® Statistics) software, which determines any statistically significant differences existing between non-normally distributed means of the dependent variable. In this study the variables were the three independent groups (5, 8, and 13 rib numbers), the average diameter of the crown, measured during field collection, and the dependent variable, concentration of mescaline. Before the test was performed, a Grubbs outlier test was used to eliminate extreme outlying data that would obscure the *P*-value while considering sample size. Data were also logarithmically transformed, and a Shapiro-Wilk test was performed to determine normality of the data set. Excel (Microsoft Office®, Microsoft corporation) software was used to create regression line plots to calculate two calibration curves allowing for calculations of sample concentrations, as well as developing two box plots in order to conceptualize raw data.

## RESULTS

Thirty *Lophophora williamsii* crowns collected from a single population in Presidio County and nine greenhouse-grown crowns were analyzed for their individual concentrations of mescaline. The concentrations were then evaluated to determine whether there was any correlation between mescaline concentrations and rib numbers. Each crown was subjected to an alkaloid extraction, detailed above, and compared against a mescaline standard to follow retention times during analysis. The mescaline standard was also used to create two standard calibration curves (Figs. 5 & 6) to test statistical variability within the samples, using the height of curve and the area-under-the-curve (AUC) data obtained from the averages of HPLC chromatograms. The calibration curves provided the formulas y = 4576.2x + 130.7 for height of curve and y = 19185x + 1493.2 for AUC, where the variable 'y' is the peak height (mAu) or AUC (mAu × seconds) and 'x' is the concentration of mescaline (in µg/µL) injected into the HPLC instrument. Injected concentrations were then divided by the total injection volume (0.002 µg/µL) and multiplied by the total vial volume (2 ml). Total vial concentration volumes were then multiplied by the original weight of the dried crown to calculate an overall percentage of mescaline per microgram of tissue (Tables 5, 6, 7, and 8).

From the height of curve data, samples 5.4, 8.5, 8.7, 8.9, 8.10, 13.9 were omitted from the data set after being labeled as outliers by the Grubbs outlier test for either having too minute of a concentration of mescaline or being drastically higher than other plants with the same rib number. After discarding outlying samples and logarithmically transforming the data set, the Pearson's Chi-squared analysis showed there is no statistically significant difference between the 5, 8, and 13 rib numbers sampled and their respective mescaline concentrations in this population ($P = 0.392$, df =24; likelihood ratio $P = 0.253$). The following (Fig. 7) contains a box plot showing the average, as well as high and low, concentrations calculated by height of curve data and by group rib number in order to display the non-normality of the data (Shapiro-Wilk: 5-ribs *P*-value = 0.089; 8-ribs *P*-value = 0.751; 13-ribs *P*-value = 0.115). The Pearson's Chi-squared analysis also determined no significant

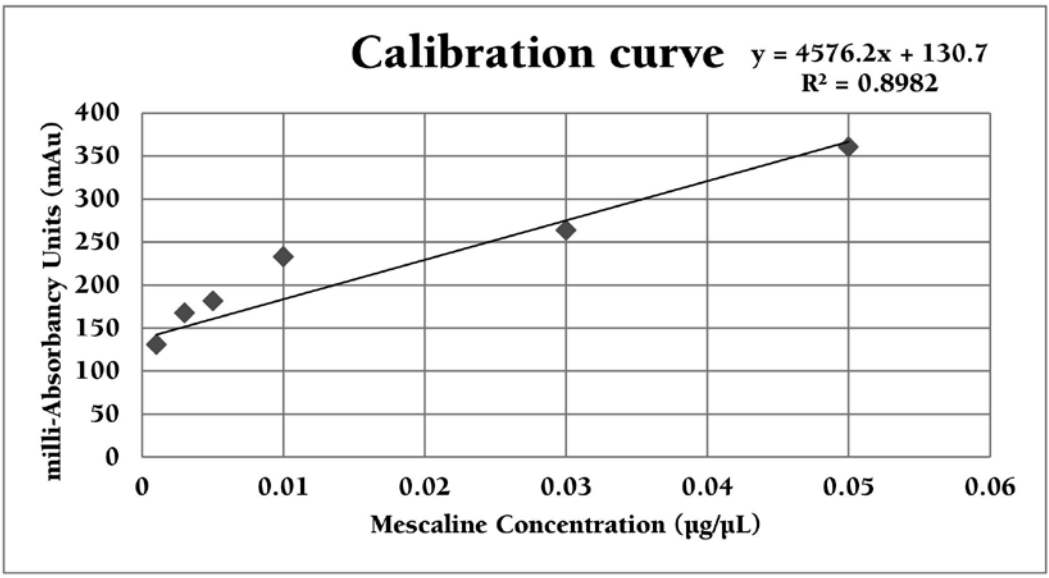


Fig. 5. Mescaline calibration curve based on peak heights of HPLC data obtained using a purchased mescaline standard sample.

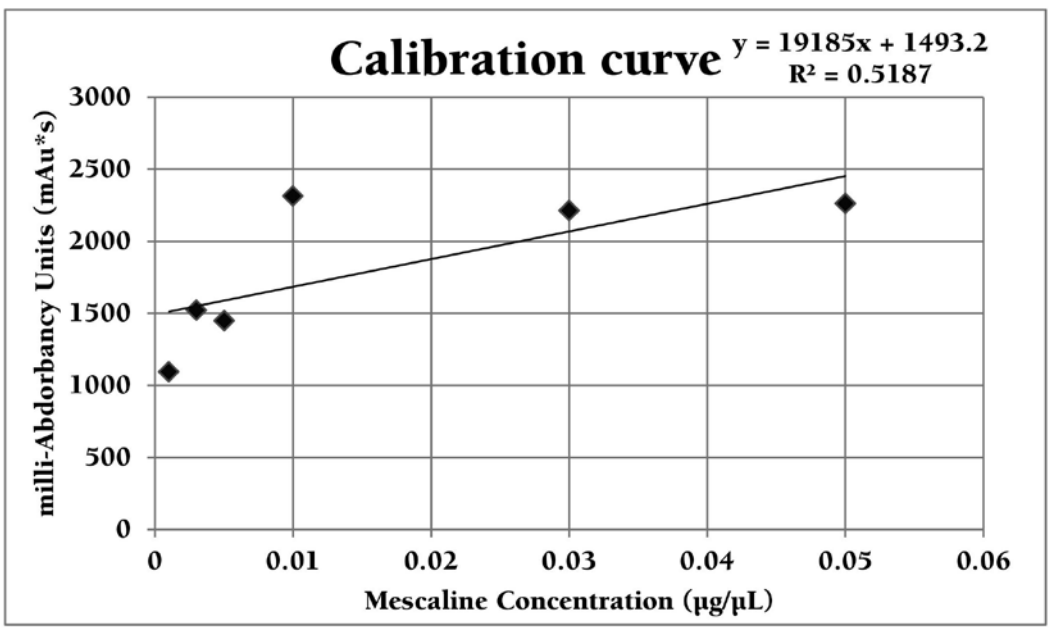


Fig. 6. Mescaline calibration curve based on peak heights of AUC (area under curve) data obtained using a purchased mescaline standard sample.

correlation between the average diameter of plants with each rib number (5, 8, and 13) collected and average mescaline concentration, displaying a *P*-value of 0.251 and a likelihood ratio of *P* = 1.000 (Fig. 8).

For the height of curve data 5-ribbed samples ranged from 0.0398 to 0.1884 µg/µL (average of 0.0865 µg/µL), 8-ribbed samples ranged from 0.0597 to 0.2141 µg/µL (average of 0.2114 µg/µL), and 13-ribbed samples

Table 5. Dry weight in µg and mescaline concentration in µg/µL calculated from peak height values for all 10 samples of wild-collected peyote with 5 ribs, 8 ribs, and 13 ribs.

| Specimen | Dry Weight of Excised 5-ribbed Sample (µg) | Mescaline Concentration in dry weight; Peak Height (µg/µL) | Dry Weight of Excised 8-ribbed Sample (µg) | Mescaline Concentration in dry weight; Peak Height (µg/µL) | Dry Weight of Excised 13-ribbed Sample (µg) | Mescaline Concentration in dry weight; Peak Height (µg/µL) |
|---|---|---|---|---|---|---|
| 1 | 500 | 0.0473 | 1000 | 0.0771 | 1160 | 0.1198 |
| 2 | 760 | 0.1884 | 700 | 0.0597 | 1690 | 0.1482 |
| 3 | 630 | 0.0449 | 600 | 0.0806 | 1870 | 0.3558 |
| 4 | 600 | 0.4209 | 1130 | 0.1560 | 1030 | 0.5418 |
| 5 | 180 | 0.1536 | 850 | 0.3687 | 700 | 0.1907 |
| 6 | 740 | 0.0398 | 1120 | 0.1255 | 1050 | 0.1198 |
| 7 | 310 | 0.1298 | 860 | 0.6095 | 780 | 0.1084 |
| 8 | 320 | 0.0421 | 780 | 0.2141 | 1360 | 0.4913 |
| 9 | 410 | 0.0555 | 800 | 16.6318 | 1320 | 3.6095 |
| 10 | 550 | 0.0770 | 720 | 3.2271 | 960 | 0.3851 |

Table 6. Dry weight in µg and mescaline concentration in µg/µL calculated from AUC values for all 10 samples of wild-collected peyote with 5 ribs, 8 ribs, and 13 ribs.

| Specimen | Dry Weight of Excised 5-ribbed Sample (µg) | Mescaline Concentration in dry weight; AUC (µg/µL) | Dry Weight of Excised 8-ribbed Sample (µg) | Mescaline Concentration in dry weight; AUC (µg/µL) | Dry Weight of Excised 13-ribbed Sample (µg) | Mescaline Concentration in dry weight; AUC (µg/µL) |
|---|---|---|---|---|---|---|
| 1 | 500 | 0.0066 | 1000 | 0.0111 | 1160 | 0.0165 |
| 2 | 760 | 0.0224 | 700 | 0.0083 | 1690 | 0.0187 |
| 3 | 630 | 0.0064 | 600 | 0.0108 | 1870 | 0.0439 |
| 4 | 600 | 0.0338 | 1130 | 0.0208 | 1030 | 0.0482 |
| 5 | 180 | 0.0110 | 850 | 0.0359 | 700 | 0.0213 |
| 6 | 740 | 0.0088 | 1120 | 0.0171 | 1050 | 0.0165 |
| 7 | 310 | 0.0129 | 860 | 0.0468 | 780 | 0.0140 |
| 8 | 320 | 0.0056 | 780 | 0.0243 | 1360 | 0.0515 |
| 9 | 410 | 0.0074 | 800 | 0.0777 | 1320 | 0.0144 |
| 10 | 550 | 0.0101 | 720 | 0.0660 | 960 | 0.0388 |

Table 7. Dry weight in µg and mescaline concentration in µg/µL calculated from Peak Height values for all nine greenhouse control group samples with 5 ribs, 8 ribs, and 13 ribs.

| Specimen | Dry Weight of Excised 5-ribbed Sample (µg) | Mescaline Concentration in dry weight; Height (µg/µL) | Dry Weight of Excised 8-ribbed Sample (µg) | Mescaline Concentration in dry weight; Height (µg/µL) | Dry Weight of Excised 13-ribbed Sample (µg) | Mescaline Concentration in dry weight; Height (µg/µL) |
|---|---|---|---|---|---|---|
| 1 | 530 | 0.1563 | 510 | 0.2766 | 930 | 0.0112 |
| 2 | 360 | 0.2493 | 320 | 0.2474 | 310 | 0.0217 |
| 3 | 720 | 0.2775 | 450 | 0.0 | 520 | 0.0811 |

ranged from 0.1084 to 0.5418 µg/µL (average of 0.2734 µg/µL) of mescaline per dry weight. The average standard deviation between 5- and 8-ribbed samples was 0.0883 µg/µL; 13-ribbed samples had an average standard deviation of 0.0883 µg/µL for the 8-ribbed samples and 0.1321 µg/µL for the 5-ribbed samples.

From the AUC data, samples 5.2, 5.4, 8.7, 8.9, 8.10 were likewise omitted from the data set for minute or drastically higher mescaline concentrations by the Grubbs outlier test. Once discarded and logarithmically transformed, the Pearson's Chi-squared analysis showed there is no statistically significant difference among

Table 8. Dry weight in µg and mescaline concentration in µg/µL calculated from AUC values for all nine greenhouse control group samples with 5 ribs, 8 ribs, and 13 ribs.

| Specimen | Dry Weight of Excised 5-ribbed Sample (µg) | Mescaline Concentration in dry weight; AUC (µg/µL) | Dry Weight of Excised 8-ribbed Sample (µg) | Mescaline Concentration in dry weight; AUC (µg/µL) | Dry Weight of Excised 13-ribbed Sample (µg) | Mescaline Concentration in dry weight; AUC (µg/µL) |
|---|---|---|---|---|---|---|
| 1 | 530 | 0.0380 | 510 | 0.0717 | 930 | 0.0258 |
| 2 | 360 | 0.0645 | 320 | 0.0634 | 310 | 0.0155 |
| 3 | 720 | 0.0600 | 450 | 0.0 | 520 | 0.1086 |

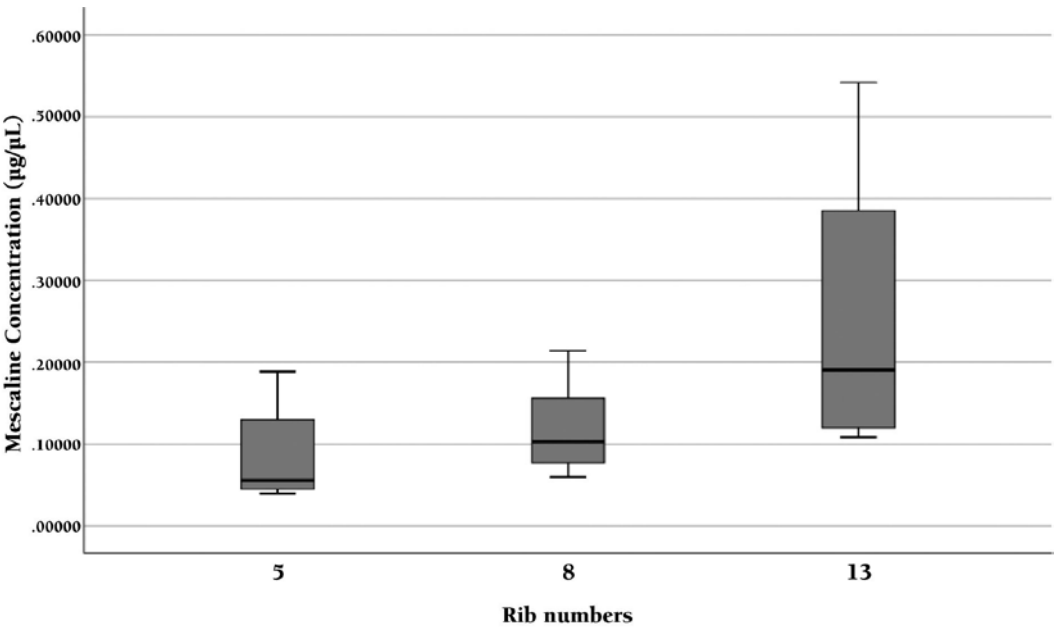


Fig. 7. Mescaline concentration of dry weight samples (µg/µL) for all 5-ribbed, 8-ribbed, and 13-ribbed *Lophophora williamsii* collected (prepared without outlying samples 5.4, 8.5, 8.7, 8.9, 8.10, and 13.9; logarithmically transformed data set).

the 5, 8, and 13 rib ($P = 0.394$, df =25; likelihood ratio $P = 0.253$). A histogram box plot (Fig. 9) of the concentrations of mescaline was calculated by AUC data and their corresponding rib numbers. This data set was also shown to display the non-normality of the data (Shapiro-Wilk: 5-ribs $P$-value = 0.796; 8-ribs $P$-value = 0.819; 13-ribs $P$-value = 0.044). The Pearson's Chi-squared analysis also determined no significant correlation between the average diameter of each rib number (5, 8, and 13) collected and average mescaline concentration, displaying a $P$-value of 0.250 and a likelihood ratio of $P = 1.000$ (Fig. 10).

For the AUC data 5-ribbed samples ranged from 0.0066 to 0.0129 µg/µL (average of 0.0086 µg/µL), 8-ribbed samples ranged from 0.0083 to 0.0359 µg/µL (average of 0.0186 µg/µL), and 13-ribbed samples ranged from 0.0140 to 0.0515 µg/µL (average of 0.0284 µg/µL) of mescaline per dry weight. The average standard deviation between 5- and 8-ribbed samples was 0.0068 µg/µL; 13-ribbed samples had an average standard deviation of 0.0071 µg/µL for 8-ribbed samples and 0.0139 µg/µL for the 5-ribbed samples. In the control group samples, 8.3 was omitted from statistical analysis due to its unmeasurably low concentration of mescaline. This group of variables had similarly been logarithmically transformed then tested for correlation using Pearson's Chi-squared analysis, resulting in no statistically significant difference among the 5, 8, and 13 ribs (Peak Height: $P = 0.313$, df = 14; likelihood ratio $P = 0.240$) (AUC: $P = 0.313$, df = 14; likelihood ratio $P = 0.240$).

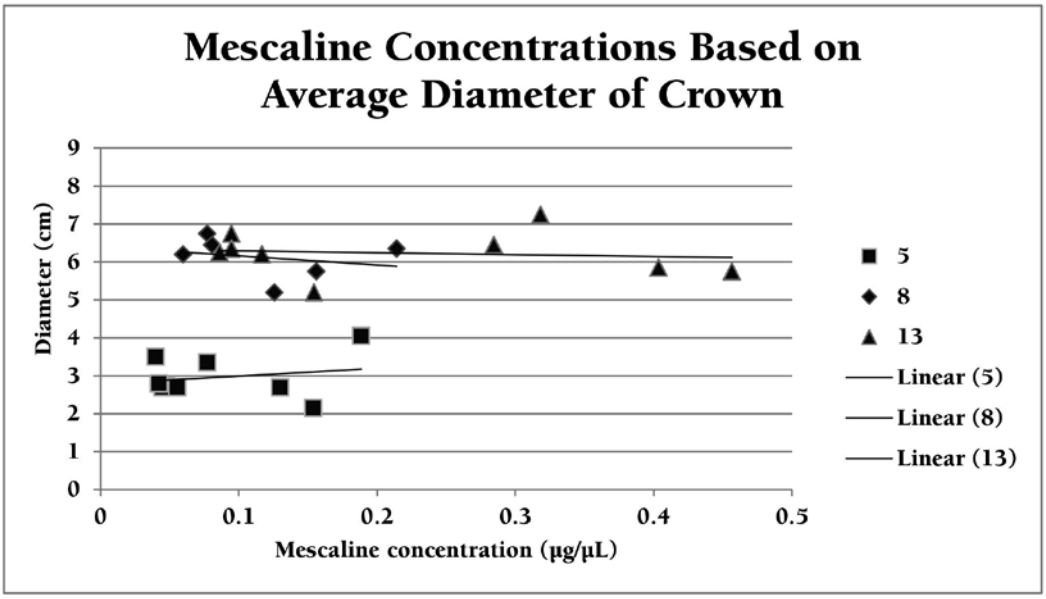


Fig. 8. Regression of mescaline concentrations based on peak height data in relation to average diameter and rib number (5, 8, and 13), Pearson's Chi-square *P*-value of 0.251.

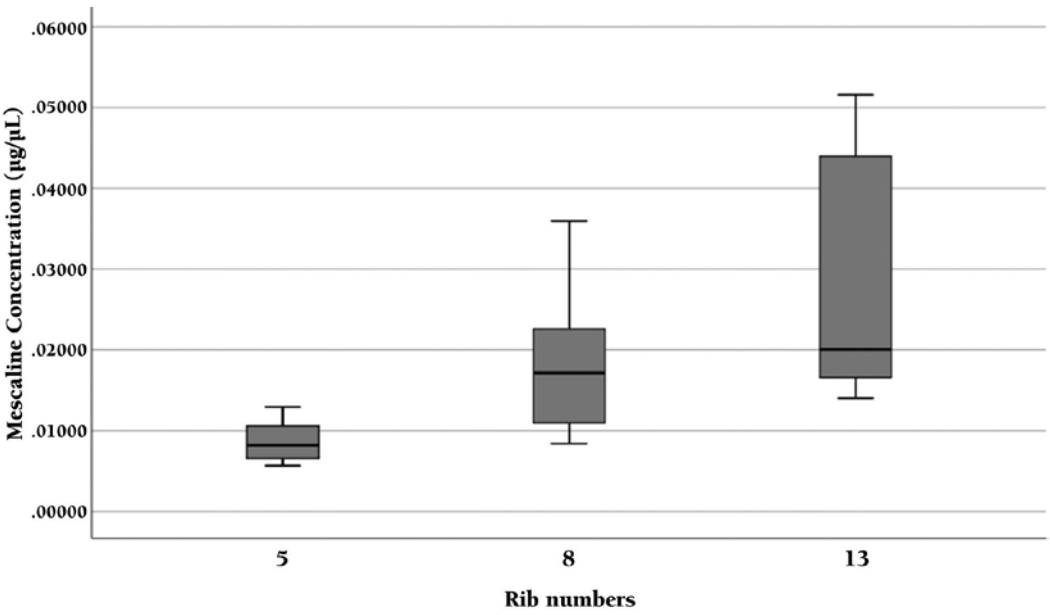


Fig. 9. Mescaline concentrations of dry weight samples (µg/µL) using Area Under the Curve (AUC) for all 5-ribbed, 8-ribbed, and 13-ribbed *Lophophora williamsii* collected (prepared without outlying samples 5.2, 5.4, 8.7, 8.9 and 8.10; and logarithmically transformed data set).

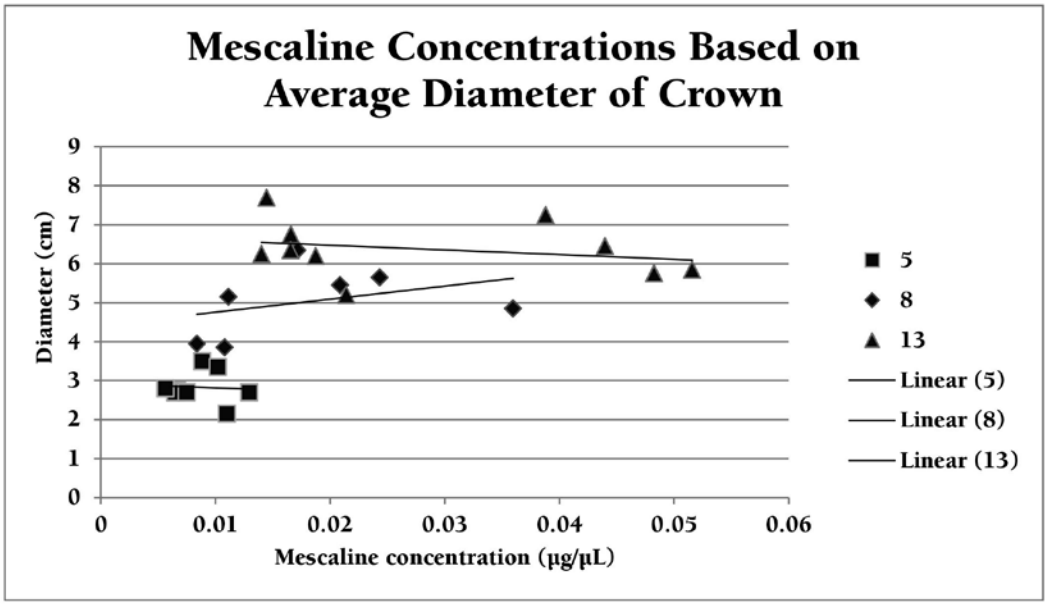


**Fig. 10. Regression of mescaline concentrations based on Area Under Curve (AUC) data in relation to average diameter and rib number (5, 8, and 13), Pearson's Chi-square *P*-value of 0.250**

## DISCUSSION

It is widely accepted that many secondary metabolites, such as alkaloids, cyanogenic glycosides, glucosinolates, terpenes, saponins, tannins, anthraquinones, and polyacetylenes, represent evolving traits that have diversified over time to defend against viruses, bacteria, fungi, competing plants, and herbivores (Buchanan et al. 2000; Bennett & Wallsgrove 1994). In many cases, these secondary metabolites have been found to go through a process called Evolutionary Molecular Modeling, just as several alkaloids form a quaternary nitrogen configuration creating a structural antagonist/agonist present in most vertebrates (Wink 2003). However, the relationship between the organism and the secondary metabolite it produces is complex and dynamic. This has been observed though varied concentrations among different tissues and organs, differences in storage percentages at different developmental stages, between populations and individuals, or observable increases in concentrations as an active state for defense against predation (Wink 2003). Alkaloids are present in approximately 20% of all angiosperms (Buchanan et al. 2000) and are unevenly distributed between plant families, with the exception of Papaveraceae, in which each genus that has been studied produces at least one alkaloid (Bennett & Wallsgrove 1994).

In this study of peyote's primary alkaloid (mescaline), numerous variables must be observed to obtain a complete picture of how mescaline is stored over time. As described above, alkaloid concentrations can drastically change due to varying circumstances; for instance, a study done by Hulsey et al. (2011) showed a statistically significant difference in the concentration of mescaline between two geographically and ecologically distinct regions in Texas, in which a population in the Trans-Pecos region of the Chihuahuan Desert had a higher concentration than a population in the South Texas Tamaulipan Thornscrub. In another study performed by Klein et al. (2015), *Lophophora williamsii* was found to have the highest concentration of produced mescaline in the crown portion of the cactus, and then dropped ten-fold in concentration in the subterranean stem, and lastly dropped another ten-fold entering root structures. It is conjectured to be due to the fact that in most instances the aerial crown is the only portion of the plant exposed to predation. Furthermore, the cactus will primarily store mescaline in the chlorophyllous subcutaneous layer of crown tissue (apical stem). Degrees of

variation in mescaline concentration may also be affected by environmental stress factors that can change drastically from one hillslope to the next (Terry pers. comm. 2018).

The preliminary data obtained in this study show that there is no positive correlation, in either field-collected or greenhouse control samples, between average mescaline concentration and rib number for samples of 5, 8, and 13 ribs. Similarly, there is no correlation between the average diameters of the crown and the corresponding mescaline concentrations. Within all three groups of wild-collected peyote samples, mescaline concentration varied drastically, and in some cases, surpassed or fell below the concentration of mescaline found in plants with the next higher rib number. In order to correct for statistical anomalies, concentration of mescaline was calculated using height of curve and area under curve (AUC) from the HPLC chromatograms. After logarithmically transforming the concentrations, the statistical test Pearson's Chi-square analysis had rejected the current hypothesis for a positive correlation between mescaline concentration and rib numbers 5, 8 and 13. Height of curve concentrations had a *P*-value of 0.392 and AUC yielded a *P*-value of 0.394. In order to correct for extreme data ranges, the Gibbs's outlier test was performed, which resulted in discarding data from samples 5.4, 8.5, 8.7, 8.9, 8.10, and 13.9 for concentrations calculated from height of curve and 5.2, 5.4, 8.7, 8.9, and 8.10 for concentrations calculated by AUC. Samples 5.2, 5.4, and 13.9 had a higher than average concentration that did not fit in with remaining samples despite not having the highest dry weight overall. It is theorized that these three field specimens were in locations that provided a slightly more stressful environment, i.e., direct sunlight without nurse plants/non-organic coverage, that would have reduced the amount of water intake, forcing the plants to concentrate the available alkaloids as a defense mechanism to protect against herbivory. For the remaining 8-ribbed samples, *Lophophora williamsii* has been observed to spend significantly more time as an 8-ribbed plant before transitioning to the following (13-rib) stage; signifying that in theory any samples collected with eight ribs could have just transitioned to that stage or are at the stage of transitioning to the following rib number without any physical indication (Terry pers. comm.). This allows for the high variability in mescaline concentration observed in among 8-ribbed samples collected. Samples 8.9 and 8.10 most likely represent younger individuals since their mescaline concentrations were so minuscule that they were unmeasurable with the current calibration curve established. Samples 8.5 and 8.7 show specimens that were at a later stage in the 8-ribbed transition, showing their overabundance of mescaline that does not correlate with the rest of the samples.

These results can be interpreted to suggest that rib numbers/age is a small underlying factor that determines the concentration of mescaline when viewed without any other environmental variables, including presence of nurse plants and the diversity of such nurse plants. For example, many nurse plants documented could provide for allopathic agents that could prove beneficial to overall survival/stress factors, much as a species in the family Fabaceae would be responsible for higher nitrogen concentrations in surrounding soils due to its mutualistic relationship with nitrogen-fixing bacteria..

Values for mescaline concentration in 8-ribbed samples varied from so minute as to be un-measurable with the calibration curve established, to having the highest value of all the plants collected (Fig. 5); the present data suggest that during this stage environmental or regulatory factors may cause variation in mescaline storage. Most five-ribbed specimens collected had a substantial surface area showing betalain pigmentation, indicating physiological distress that could change mescaline concentrations in the living plants. However, betalain levels were not measured and so could not be compared in this study. Nevertheless, by identifying and selecting for outlying individuals with high mescaline concentration could be used for intentional greenhouse propagation having implications for conservation for ceremonial use.

In the greenhouse, sample 8.3 had been noteworthy due to its immeasurable concentration of mescaline detected through the HPLC, along with an unusually high concentration of another unknown polar alkaloid that is frequently visible as a minuet peak when analyzing *Lophophora williamsii* crowns. This sample will undergo subsequent testing to determine the identity of the unknown alkaloid and its high concentration. Any differences found in this sample can offer some insight into how some of these alkaloid concentrations can vary due to any unique variables not explored in this study (e.g., soil composition and effects of grafting).

Previous evidence suggests that secondary metabolites such as catecholamines (e.g , dopamine) perform many regulatory functions such as involvement with oxidative status, pairing with phytohormones in the regulation of plant growth, as well as regulation of sugar metabolism in many plant species that metabolize them (Kulma & Szopa 2007; Rosengarten & Friedhoff 1976). This suggests that high concentrations of secondary metabolites can revert to a primary metabolite such as tyrosine during periods of growth and expansion (ibid.). With the variables tested in this study, namely rib number and crown diameter alongside wild and greenhouse peyote plants, 13-ribbed specimens would still be the most likely candidate for harvesting to be used in ceremony by members of the Native American Church (NAC). Not only do they have a dense storage of mescaline per dry weight (unlike 8-ribbed samples that showed greatly varied concentrations within the group), they also, contain a well-developed subterranean stem structure that could provide for healthy offshoots in the future, contributing to the survival of this unique species and its alkaloids. This study contributes a brief understanding of morphology relative to chemistry in peyote with hopeful implications towards conservation of the species and preservation of Native American culture.

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