INTRODUCTION

*Lophophora williamsii* (Lem. ex Salm-Dyck) J.M. Coul., commonly known as peyote, is a low, flat-to-domed spineless cactus with a chlorophyllous crown and substantial non-chlorophyllous stem and taproot (Fig. 1). It is found in the wild occurring most frequently on limestone hills and in other calcareous soils from 50 to 1850 m above sea level (Anderson 1996).

Peyote has been harvested and used by humans for at least 6,000 years (Terry et al. 2006) and historically has been used by native peoples as a medicine (Schultes 1938; 1940) and for religious purposes (Stewart 1987). Federal U.S. legislation (Controlled Substances Act, U.S. Congress 1970) prohibited its use as a drug and categorized the plant itself as a Schedule 1 controlled substance; its principle psychoactive alkaloid, mescaline (3,4,5-trimethoxy-β-phenethylamine), was also listed as Schedule 1. The sacramental use of the plant in religious ceremonies by members of the Native American Church (NAC) continued, largely protected by the U.S. Constitution, the American Indian Religious Freedom Act Amendments of 1994 (U.S. Congress 1994), and

Abstract: We evaluated the pharmacological consequences of tissues other than crown being included with harvested peyote. Mean mescaline concentrations were determined for crown, non-chlorophyllous stem, and root, using mature individuals from the same population in South Texas. Samples of each tissue—crown, non-chlorophyllous stem, and root—were taken from each of 13 individual plants. Samples were dried, triturated, defatted, and extracted with methylene chloride, using an acid-base aqueous wash to recover the alkaloids. The concentration of mescaline in each sample was determined by HPLC. The average mescaline concentration in non-chlorophyllous stem was an order of magnitude lower than that in crown, whereas the mescaline concentration in root was two orders of magnitude lower than that in crown. These results show that non-chlorophyllous stem is a poor source of mescaline, and root is an extremely poor source. These results have important implications for conservation, suggesting that non-traditional harvesting of peyote for religious or medicinal use involving the cutting of non-chlorophyllous tissue are contributing to the death of plants and the subsequent failure to regenerate new crowns. Therefore, this practice should be reevaluated by peyote harvesters and users.

Keywords: peyote harvesting, peyote conservation, crown tissue, non-chlorophyllous stem tissue, root tissue
a provision in the Code of Federal Regulations, 21 C.F.R. § 1307.31 (Drug Enforcement Administration 2013). Total membership of the NAC has been reported at 250,000 members for several decades (e.g. Anderson 1995), although there is no evidence that a census has ever been conducted to determine the number of NAC members. It should be noted that to speak of “the NAC” is actually misleading, as the NAC is not a single entity, but rather a highly heterogeneous collection of individual churches and multi-church “chapters” that span the continental U.S. and Canada. Churches that identify themselves as NAC vary geographically, culturally, linguistically, socio-economically, in the content and format of their religious ceremonies, and even in their legal status (because of the disparate state laws that determine the legality or illegality of the religious use of peyote). The only thing that all NAC groups have in common is their ceremonial use of peyote. All peyote plants so used are harvested from wild populations.

In the United States, populations of *Lophophora williamsii* that are large enough to support commercial harvesting occur only in the Tamaulipan Thornscrub ecoregion of South Texas (Terry and Mauseth 2006). Over the past four decades, a marked decline in numbers and average size of the plants has been observed in South Texas, as well as a decline in density and extent of the populations (Anderson 1995; Terry et al. 2011, 2012; Kalam et al. 2013). Licensed peyote distributors and their employees (sometimes known as “peyoteros”) have harvested and distributed about 1.4 to 2.3 million peyote tops (“buttons”) per year for the last quarter of a century in South Texas (Texas Department of Public Safety, unpublished data).

*Lophophora williamsii* can be sustainably harvested by transversely cutting off the crown of the plant at its base, usually at or near ground level. Properly harvested peyote plants usually produce regrowth of new crowns in a few months, by axillary branching from areolar buds found on the non-chlorophyllous stem. The only parts of the plant which are capable of producing new branches are the crown (i.e. the aerial, chlorophyllous stem) and the (usually, but not always, subterranean) non-chlorophyllous stem (Fig. 2), which produces new growth as axillary branches, particularly in response to removal of the apical mer-
Texas most often occurs on sloping soil surfaces, and its non-chlorophyllous stem exposed above ground. Figure 3.

In previous publications, the non-chlorophyllous stem has been referred to as the "subterranean" stem, but we consider it appropriate to change the terminology to reflect the fact that a portion of that part of the stem may sometimes be exposed above ground in the natural habitat of the plant. Peyote in South Texas most often occurs on sloping soil surfaces, and while the crown usually protrudes just above ground level, it is not uncommon for some plants to experience anything from complete burial to having up to several centimeters of non-chlorophyllous stem exposed by the erosion and gravel redistribution that comes with torrential rains (Fig. 3).

Mescaline, the predominant psychoactive compound in *L. williamsii*, has long been recognized as a sympathomimetic agent, producing vivid changes in sensory perception when ingested (Huxley 1954; Kumla and Szopa 2007; Simpson and Ogorzaly, 2013). Mescaline concentrations in *L. williamsii* have typically been found to range from a little less than 2% to around 4% of the dry weight of each crown (Bruhn and Holmstedt 1974; Hulsey et al. 2011).

The objective of the present research was to determine whether there was a significant statistical difference in the mescaline concentrations of isolated crown, non-chlorophyllous stem, and root tissues of *Lophophora williamsii*. This bears directly on differences between traditional harvesting technique—where only the crown is harvested, leaving the non-chlorophyllous stem and root intact—and the technique more recently adopted by some groups (including some commercial distributors), whereby a considerable amount of the non-chlorophyllous stem is harvested along with the crown (Frioli 2003; Vilchez 2014; Terry, personal observation). Rigorous demonstration of substantial differences in mescaline concentrations among the three different parts of the plant could encourage conservation of the plant by identifying precisely where the great majority of the psychoactive “medicine” (i.e. mescaline) is found in peyote and, more importantly, where it is not found. Our hope is that the harvesters of peyote—both indigenous NAC members and non-indigenous licensed peyote distributors—will weigh any short-term convenience of harvesting parts of the plant with low mescaline concentrations against the long-term desirability of maximizing clonal regrowth from harvested plants by removing only the crown of the plant, which has been found to contain the higher levels of mescaline desired by NAC members for ceremonial purposes.

### MATERIALS AND METHODS

#### Plant selection and alkaloid extraction.

Plants used in this study were collected in December 2004 from a population of *Lophophora williamsii* in Starr County, Texas, in the Tamaulipan Thornscrub ecoregion, by M. Terry, who holds the appropriate licensing from the Drug Enforcement Administration (DEA) necessary to perform this research. Thirteen plants were selected from this South Texas population in order for alkaloid content to be representative of the area of commercial peyote harvesting. In order to protect the plants at this collection site from poaching, the exact collection location will not be disclosed. The plants were potted and placed in a protected greenhouse for future study.

In preparation for alkaloid extraction, 13 whole uprooted *Lophophora williamsii* specimens were rinsed with tap water, and each individual was cut transversely into three separate segments: crown, non-chlorophyllous stem, and root (Fig. 1). These three segments of each individual plant were then thinly sliced and dried on a 1 mm screen, at room temperature, for one week. Each tissue sample of dried plant material from each of the 13 specimens was separately ground into a fine powder with a
The procedure for extraction of alkaloids from the pulverized tissue samples was similar to that of Ogunbodede (2010) and Hulsey et al. (2011): an initial methanol extract was paper-filtered and evaporated to dryness, followed by redissolving the residue in dichloromethane, which underwent washing with acidic (pH 3) and basic (pH 12) aqueous solutions; then finally the dried dichloromethane extract was redissolved in methanol, which was filtered through a 0.2 micron filter and stored at −20°C until it was analyzed by HPLC (Snyder and Kirkland 1974; Ogunbodede 2010; Hulsey et al. 2011). During this part of the procedure, three samples were accidently spilled so that, of the 13 plants, complete sets of data exist for only 10 plants.

Analytical instrumentation and methodology. An Agilent 1260 Infinity HPLC instrument with a Phenomenex Gemini 5-micron C18 column (250 mm × 4.6 mm) was used to determine the concentration of mescaline in each sample. The solvent used for data collection consisted of 70% HPLC-grade methanol and 30% HPLC-grade water, with a 1.2 mL/minute flow rate. The detector wavelength was set at the known UV-absorbance maximum of 205 nm for mescaline (Helmlin and Brenneisen 1992). A 1-to-5,000 (w/v) ratio of mescaline standard to methanol (3.2 mg mescaline per 16 mL methanol) was used to determine the standard curve of mescaline in milli-absorbance units (mAU). Four different injection volumes (8.0 µL, 9.0 µL, 10.0 µL, and 11.0 µL) of a 1-to-5,000 ratio of mescaline standard to methanol were each run on the HPLC instrument three times, and the average of the 3 values at each injection volume yielded one of the four points used to generate a standard curve of the HPLC mescaline peaks in milli-absorbance units (mAU) (Lindsay, 1987; Snyder and Kirkland, 1974). Data were analyzed using this standard curve, which was developed by plotting the height of the mescaline HPLC peaks (in mAU) as a function of micrograms of mescaline in each injection volume (as in Hulsey et al. 2011; Kalam et al. 2013).

Based on the high concentrations of mescaline found in crown tissue in preliminary data, the original crown extracts were diluted appropriately (1:9 to 1:27), in order for the mescaline peaks to fall in the linear interval of the standard curve. Based on the low mescaline levels determined in preliminary data for non-chlorophyllous stem and root tissues, measured amounts of standard mescaline were added to each of the non-chlorophyllous stem and root extracts in order to raise the mescaline peak heights so that they would fall within the linear interval of the standard curve of mescaline. After calculating the total micrograms of mescaline in each spiked sample from the HPLC peak height of the spiked sample, the weight of the mescaline that had been added to the original sample was subtracted from the total amount of mescaline measured in the spiked sample.

The identity of mescaline was confirmed by GC-MS (as in Rösner et al. 2007; Ogunbodede, 2010; Hulsey et al., 2011).

Statistical analysis. The method employed for statistical analysis was the procedure PROC MIXED in SAS software (SAS Institute, Cary, NC). Plant ID was treated as a random effect and plant part as a fixed effect. Residuals met the assumption of normality. The response variable was the logarithm (base 10) of mescaline concentration.

RESULTS

Extracts of crown, non-chlorophyllous stem, and root tissues of Lophophora williamsii in one population from Starr County, Texas, were analyzed and were all found to contain a detectable amount of mescaline (Table 1). Each tissue was analyzed following the protocol described above and uniformly

### Table 1. Mescaline content of dry tissues of Lophophora williamsii (% of dry tissue weight). In those fields where “No data” appears, the loss of data was caused by spillage of unmeasurable quantities of extract in the course of laboratory procedures.

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>crown</th>
<th>non-chlorophyllous stem</th>
<th>root</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No Data</td>
<td>0.183</td>
<td>0.0773</td>
</tr>
<tr>
<td>2</td>
<td>2.63</td>
<td>0.186</td>
<td>0.0773</td>
</tr>
<tr>
<td>3</td>
<td>2.18</td>
<td>0.183</td>
<td>No Data</td>
</tr>
<tr>
<td>4</td>
<td>3.33</td>
<td>0.118</td>
<td>0.0520</td>
</tr>
<tr>
<td>5</td>
<td>No Data</td>
<td>0.166</td>
<td>0.0333</td>
</tr>
<tr>
<td>6</td>
<td>No Data</td>
<td>0.156</td>
<td>0.0520</td>
</tr>
<tr>
<td>7</td>
<td>1.90</td>
<td>0.166</td>
<td>0.0497</td>
</tr>
<tr>
<td>8</td>
<td>1.87</td>
<td>0.179</td>
<td>0.0400</td>
</tr>
<tr>
<td>9</td>
<td>2.20</td>
<td>0.163</td>
<td>0.0400</td>
</tr>
<tr>
<td>10</td>
<td>5.50</td>
<td>0.125</td>
<td>0.0400</td>
</tr>
<tr>
<td>11</td>
<td>1.86</td>
<td>0.376</td>
<td>0.0253</td>
</tr>
<tr>
<td>12</td>
<td>1.82</td>
<td>0.163</td>
<td>0.0147</td>
</tr>
<tr>
<td>13</td>
<td>2.29</td>
<td>0.313</td>
<td>0.0218</td>
</tr>
</tbody>
</table>
yielded a mescaline chromatogram peak with a retention time of approximately 1.7 minutes. The concentration of mescaline in *Lophophora williamsii* varied among the three tissues examined in this study, and varied among individuals for each tissue. The relative differences in mescaline content among the three plant parts were consistent for all individuals.

The highest mescaline concentrations were found in the crowns of these plants, where the concentration range was 1.82–5.50% mescaline. Non-chlorophyllous stem samples contained concentrations an order of magnitude lower: 0.118–0.376% mescaline on a dry-weight basis. Root samples contained the lowest concentrations: 0.0147–0.0773% mescaline—two orders of magnitude less than the mescaline concentrations in crown tissue.

Log concentrations of mescaline in the three plant parts (i.e. location in crown, non-chlorophyllous stem, or root) were significantly different ($F_{2,20} = 300.47$, $P < 0.0001$) (Fig. 4). Each pairwise contrast between plant parts was also significant ($F_{1,20}$ between 94 and 595, $P < 0.0001$ in each comparison). The means of the log-transformed concentrations were +0.3804 (crown), −0.7427 (non-chlorophyllous stem), and −1.4048 (root). Back-transformed, these means become 2.40%, 0.18%, and 0.04%. (Note that, as expected, they are close to but not identical to the raw means of 2.56%, 0.19%, and 0.04%.)

**DISCUSSION**

An understanding of the differences in mescaline concentration between the peyote historically harvested in the Chihuahuan Desert and the peyote currently being commercially harvested in the United States portion of the Tamaulipan Thornscrub is crucial for managing the conservation of the species. The populations in those two geographic regions are considered to be dissimilar in their alkaloid content (Anderson 1996; Weniger 1984). It is presently unclear how much of that difference is due to genetic and environmental differences and how much may be attributable to chronic overharvesting in the South Texas populations (see Kalam et al. 2013). In any case, much of the research pertaining to the Chihuahuan Desert populations may not apply to the South Texas populations.

The recent work by Hulsey et al. (2011) showing a quantitatively limited but statistically significant geographic mescaline concentration gradient in *Lophophora williamsii* populations across Texas raises some important questions. Leading those is the need for understanding what roles the known genetic differences, climatic differences, and overharvesting in South Texas may play. Todd (1969) compared Mexican *L. williamsii* in the northern Chihuahuan Desert (Coahuila) to *L. williamsii* in the southern Chihuahuan Desert (San Luis Potosí). In his semi-quantitative thin-layer chromatographic analysis, Todd (1969) reported the northern plants to have significantly higher levels of mescaline in both crown and root than did the southern population. Todd’s “root” sample from the San Luis Potosí population, 8.6 lbs. of dried “roots,” showed only trace amounts of mescaline. (By “root”, Todd appears to have meant the combined non-chlorophyllous stem and true root combined, inasmuch as, by comparison with the total weight of the “roots,” [8.6 lbs.], his
crown sample was only 3.5 lbs. by dry weight.) Trout (1999) mentions that the mescaline content in South Texas populations seems to be similar to that of the San Luis Potosí population in Todd’s (1969) research, in terms of both crown and root. That observation is compatible with the results of the present study of a South Texas population, where we found 0.118–0.376% mescaline on a dry-weight basis in non-chlorophyllous stem, and 0.0147–0.0773% mescaline on a dry-weight basis in root.

In Todd’s (1969) study, the Coahuila population showed a higher concentration of mescaline in its “root” than did the plants in the present study. However, the San Luis Potosí population examined in Todd’s study showed only trace amounts of mescaline in the “root,” which is comparable to the present results.

Significant statistical differences among the mescaline concentrations exist for the crown, the non-chlorophyllous stem, and the root of the *Lophophora williamsii* specimens examined in the current study, indicating that plants in commercially harvested areas in the United States have substantially less mescaline in the non-chlorophyllous stem than in the crown and much less still in root tissue. If there were a widespread understanding that mescaline concentrations diminish by a factor of 10 in the non-chlorophyllous stem when compared with the crown and by a factor of 100 in the root compared to the crown, such knowledge could result in changes in harvesting behavior. A reduction in the amount of non-chlorophyllous stem that is actually harvested would help to lower the mortality rates of the harvested plants. Since mescaline is produced in the chlorophyll-containing parenchyma (chlorenchyma) cells in the crown tissue (Janot and Bernier 1933), it stands to reason that most of the mescaline is, in fact, in the crown tissue of the cacti. The non-chlorophyllous stem tissue was crown tissue at one time in its development, which may explain why there is mescaline present at greater levels in the non-chlorophyllous stem than in the root tissue. No studies on mescaline content in comparable tissues of other cacti have been published (Terry, personal observation), although it stands to reason that similar alkaloidal concentration gradients would exist in the tissues of cacti with similar morphology (e.g. *Ariocarpus fissuratus*).

The present findings indicate that plants in South Texas have extremely low levels of mescaline in the non-chlorophyllous stem and root and, therefore, effectively no psychotropic activity in those organs. This implies that only the green crowns of the plant should be harvested.

The implications of the current findings with respect to therapeutic uses of peyote are less clear, largely because those uses are so diverse and culture-specific, and the mechanisms of action and potential efficacy of other major peyote alkaloids and nonalkaloidal components are only now beginning to be studied (Terry, personal observation).

![Figure 5. Example of “pomada de peyote” as sold on the internet.](image)

Commercial harvesting of wild populations of *Lophophora williamsii* in the United States is at risk. Dwindling populations in the “Peyote Gardens” of South Texas have been reported for decades (Morgan 1976; Anderson 1995; Powell and Weedin 2004; Morales 2007). Harvesting too frequently, harvesting juvenile plants, cutting too deeply into the non-chlorophyllous stem during harvest, and uprooting whole plants for possible therapeutic value of the root have led to the rapid decline of this species in the U.S and Mexico (IUCN 2013). The present study provides clear evidence that only the crowns of the plant contain adequate levels of the primary psychoactive compound in peyote (viz. mescaline) that are desired for religious use by the NAC. The fact that any regrowth after harvesting can originate only from areoles located on the non-chlorophyllous stem emphasizes the importance of this section of the plant body in enabling regeneration of new growth following harvesting. A broader awareness of these findings and subsequent changes in harvesting techniques could potentially slow the rate of destruction of the South Texas peyote populations by decreasing the mortality associated with the adverse harvesting practices of “deep cutting.”

The problem is multiplied as people are inadvertently taught destructive harvesting practices (Fig. 2), including practices that were acceptable several decades ago. Common examples would include the use of a digging stick to pry plants out of the ground, most often along with a significant portion of the non-chlorophyllous stem, and the deliberate harvesting of roots for consumption in the form of a tea.
Those destructive practices are clearly not sustainable in wild populations, although sustainable root harvesting would become quite feasible in cultivation. The acceptability of root harvests in the course of procuring wild plants for sacramental use needs to be questioned in the face of shrinking populations of peyote and increased consumption for both ceremonial and therapeutic purposes. The issue of deep cutting is complicated by the fact that some NAC members are requesting that distributors provide them with deep-cut peyote, and even some of the distributors themselves are inclined to accept deep-cut buttons from their employees because the buttons remain fresher for a longer period of time when some of the non-chlorophyllous stem is attached to the crown (K. Feeney, personal communication).

Another possible threat to *Lophophora williamsii* is industrial-scale manufacturing of healthcare products for therapeutic use, which appears to be on the rise in Mexico, exemplified by products like "pomada de peyote" (peyote salve or ointment). Such products can be found advertised on the Internet and sold openly in the markets of Mexican cities for the treatment of arthritic and muscular pain through topical application (Fig. 5). A major unanswered question is, what is the approximate amount of peyote that is being harvested annually from wild populations to supply the demand for the pomadas? Chemical analysis to determine how many of these products actually contain peyote—and how much they may contain—is currently in progress (Terry et al., in preparation).

**CONCLUSIONS**

The present results suggest that consumers of peyote may need to make a choice between the potentially sustainable harvesting of peyote crowns for religious use and the clearly unsustainable harvesting of the "root" of wild plants. Whether the roots and non-chlorophyllous stems are used with virtually no incremental effect in religious ceremony (due to their very low mescaline content), or whether they are used as a therapeutic herbal remedy to treat conditions for which more efficacious treatments may be available, every peyote plant harvested for consumption of its root is removed absolutely and irreversibly from its population. This realization, clearly not a comforting one, is becoming less avoidable year by year, as the availability of *Lophophora williamsii* for any use continues to decrease.

Changes in the procedure for harvesting peyote have occurred in recent years (Frioli 2003; Vilchez 2014; Terry and Trout, personal observations). The most usual traditional method employed in Texas was to harvest by cutting off the aerial, photosynthetic crown of the plant at ground level (or at the base of the crown), leaving the non-chlorophyllous stem and the root intact in the ground (Terry and Mauseth 2006). The traditional procedure directly stimulates regrowth in the form of new crowns. Removal of the original crown concomitantly removed the apical meristem of the plant, thereby eliminating the source of secretion of auxin, which suppresses axillary branching in intact unharvested plants. Removal of the crown and its aerial areoles without damaging the non-chlorophyllous stem, leaves intact the areoles on the non-chlorophyllous stem. These areoles contain dormant axillary buds (meristematic tissue), which in an unharvested plant are inhibited from developing into axillary branches by the suppressive effect of auxin. But with removal of the crown and its apical meristem, which is the primary locus of auxin secretion, some of the dormant axillary buds in the areoles of the non-chlorophyllous stem become de-repressed and develop into new axillary branches, each of which develops its own crown.
and its own adventitious tap root. The results of the present study support the wisdom of the traditional practice of limiting harvesting to the crown of the plant.

There has always been cultural variation in peyote harvesting practices, ranging from sustainable to destructive, but most groups have traditionally followed approaches which permit subsequent re-harvesting on their return visits to the harvested population. This was certainly true of the prehistoric inhabitants of the region around Cuatrociénegas, Coahuila. The CM-79 burial cave is the only known archaeological site where identifiable peyote buttons have been found (see Terry et al. 2006). These approximately 1,000-year-old buttons showed classic harvesting of the crowns only, with no non-chlorophyllous stem or root attached (Fig. 6). Even in instances of traditional Huichol harvesting using a digging stick, efforts are typically made to return the root to the earth. In recent years, there has been a visible rise in the prevalence of poor harvesting practices that remove part or all of the “root” (the term “root” colloquially refers to the combined non-chlorophyllous stem and true root) (Frioli 2003; Vilchez 2014). This is probably in response to reductions in population size and in the average size of individual plants, resulting in requests being made of the licensed distributors to include more non-chlorophyllous stem tissue with the crown to provide a longer shelf life for the fresh crown before it eventually dries (K. Feeney, personal communication). There has also been a reported increase in root tea consumption. It may be noteworthy that requests for and use of peyote to make root tea appear to be limited to those tribes that have known peyote only as a trade item and for a relatively short time (about a century or less).

The traditional method of harvesting peyote promotes the development of ramets to replace the parent plant whose crown was harvested. However, to an increasing extent the traditional method of harvesting is not being followed—as when plants are being harvested entire, with roots incidentally attached to the stem, or when entire plants are dug up with the specific intention of consuming the roots in the form of root tea. In all instances where much (or all) of the non-chlorophyllous stem is removed along with the crown, many (or all) of the areolar axillary buds are also removed along with the non-chlorophyllous stem, and that reduces (or eliminates) the capacity of the harvested plant to produce ramets with new crowns. Consequently, nontraditional harvesting that damages or removes the non-chlorophyllous stem, reduces or eliminates regrowth, thus increasing the mortality rates associated with harvesting (Terry et al. 2011, 2012). In short, the non-traditional practice of “low cutting” that removes non-chlorophyllous stem and its vital areoles is non-sustainable. The comparative analytical determination of mescaline concentration in crown, non-chlorophyllous stem and root in the present study shows that such unsustainable harvesting practices confer no compensatory benefit in terms of alkaloid concentrations, leading to the unavoidable conclusion that such practices are simply destructive.

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