INTRODUCTION

Approximately 20% of world cycad species are found in Mexico, 80% of which are endemic (Yovides, 2000). At least nine species are present in the state of San Luis Potosí: *Dioon edule*, *Zamia fischeri*, *Z. loddigesii*, *Ceratozamia hildae*, *C. kuesteriana*, *C. latifolia*, *C. mexicana*, *C. microstrobila*, and *C. zaragozai* (Yáñez-Espinosa, 2006) [Ed’s note: *Zamia vazquezii* is also found in San Luis Potosi]. *Dioon edule* Lindl. is the most widely distributed, particularly in the Huasteca zone located in the Sierra Madre Oriental. Habitat deterioration of *D. edule* is evident, since many natural forests have been converted into either pastureland for cattle or sugarcane fields. Cattle breeding has been devastating for many *D. edule* populations, because the species is considered extremely toxic to animals, and exterminating these plants is considered an effective option to prevent harm to livestock.

According to the farmers, cattle consuming young leaves or cones is fatal; the effects start with diarrhea, progress to paralysis of the hind legs, and finally result in death. This could be attributed to toxic secondary metabolites produced in cycads that apparently serve as protection against predators. Although it has been suggested that these compounds are responsible for the control of development and maintenance of herbivores associated with cycads, two of these compounds, cycasin and macrozamin, can cause severe intoxication in addition to carcinogenic and neurotoxic effects in mammals (Schneider et al., 2002). One study has reported low values of cycasin (0.1% fresh weight) and macrozamin (0.6% fresh weight) in *Dioon edule* seeds and concluded that the seeds are highly toxic for rodents.

One portion of the Huasteca region is inhabited by the indigenous Xi’iuy people, whose major activity is agriculture, based on cultivation of maize, beans, and sugar cane. Hunting does not represent a major source of food, but gathering products, like maguey (*Agave* spp.) and chamal (*Dioon edule*) derivatives are consumed. The harvest of chamal has played a major role in the Xi’iuy diet from time immemorial, since this is a substitute when maize and bean yields are low. It is common for the Xi’iuy to consume tamales, gorditas, and tortillas made from chamal, the dough made from boiled and ground seeds. Some recipes alert the maker that improperly prepared chamal foodstuffs may cause vomiting, gastrointestinal dis-

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ease, or “fatal consequences” in people when consumed (Bassler, 2000).

Besides the common use of chamal as a food source, it also has medicinal, ornamental, and other uses. Our research approach to study chamal was multidisciplinary, and the proposal included ecological, ethnobotanical, and toxicological investigations. We were aware that this would be a long-term project, but our experiences by the end of the first year include benefits like the promotion of conservation of existing populations of *Dioon edule*, a species that is poorly known in the state of San Luis Potosí; compilation of traditional knowledge of the Xi’iuy people related to this species; and exploration of possible toxic effects of the seeds of this species as a food source. Thanks to the Cycad Society grant in 2007, we were able to carry on the research, with complementary funds and infrastructure provided by the Autonomous University of San Luis Potosí, and collaboration of three B.Sc. students from three different disciplines—agronomy, geography, and chemistry—that worked together to develop the research project.

The study area comprises the municipalities of Tamasopo, Rayón, and Santa Catarina in the Huasteca region. These were selected to carry on the research based on previous knowledge of the utilization of chamal by the Xi’iuy people. We stated three main questions, as follows:

1) What is the situation of the natural populations and habitat of *Dioon edule*?
2) What and how many ethnobotanical categories can be recognized for *Dioon edule* plants?
3) How can toxins present in the seeds of *Dioon edule* affect the Xi’iuy people that eat them?

The following sections represent the results of three separate investigations that were conducted to address these three questions. They were written independently, and each has its own title and authors.

**Population Structure of Chamal**

G. Rubio Méndez, R. Mora Cardona & L. Yáñez-Espinosa

Preliminary exploration of an altitude gradient (400, 1000 and 1200 m above sea level [masl]) in San Luis Potosí indicated an abundance of *Dioon edule* plants, as well as a high frequency of use of chamal for food, medicine, and ornamental and trade objects. Planned field routes were traversed in the company of male key informants that were selected on the basis of their knowledge, leadership, and moral influence on their communities, as well as their willingness to cooperate with us.

Six selected sites were located with GPS, and the habitat in each was characterized by defining vegetation type, floristic composition, soil characteristics, and threats, such as deforestation, fire, cattle incendence, and others. *Dioon edule* population structure and spatial distribution was characterized in two sampling transects of 100 m length by 2 m width. Plants intercepted in each transect were counted and their heights and diameters measured; also assessed were the number of leaves per plant and the life stage. *Dioon edule* life stages were classified as: 1) seedlings - individuals with no visible stem, one to few leaves not arranged in a crown; 2) juveniles - non-reproductive individuals with no visible stem or barely visible, and leaves arranged in one or more well-developed crowns; and 3) adults - reproductive individuals with a visible erect stem and one or more crowns. Adult individuals were classified as male or female from observation of the mature or immature cones, or their vestiges, on the stem.

Analyses of the soil sampled from the six sites indicated that *Dioon edule* has a preference for clay soils consisting of over 50% clay particles, 17% organic matter, and a slightly basic pH (average pH = 7.2). This soil, because of its density, retains moisture well and tends to be more nutrient-rich than other soil types. The average calcium content of 82.43 meq 100g⁻¹ is very high, and the soil is slow-draining and compacts easily (Barnes et al., 1998).

We found two populations of *Dioon edule* with conditions of well conserved habitat of high quality. There were two populations with intermediate quality habitat and intermediate plant density, and two populations with low quality habitat, mainly caused by cattle and land conversion to agriculture (Fig. 1). In the six sites, most of the *Dioon edule* plants showed signs of herbivory by *Eumaeus childrenae* (Lepidoptera: Lycanidae) caterpillars, that have been previously reported for the Huasteca region (Contreras-Medina et al., 2003).

Species richness in the high-quality habitats—distributed in the ecotone of submontane shrubs and *Quercus* forest—was high and included *Flourensia laurifolia* DC. (Asteraceae) and *Quercus polymorpha* SCHILD. & CHAM. (Fagaceae). In the lower quality habitats—distributed in the ecotone of submontane shrub and dry tropical forest—were *Bursera simaruba* (L.) SARG. (Burseraceae) and *Pseudobombax ellipticum* (KIRKHO) DUGAND (Bombaceae).

All populations showed a low frequency of female adults (Table 1), but it is interesting that populations inhabiting conserved habitats were located closer to Xi’iuy indigenous communities, and less conserved habitats were farther away from Xi’iuy communities. Ironically, female cone harvest pressure is higher in the closer populations. The sex ratio in the medium and highly conserved habitats averaged 2:1 (M:F). Populations from more distant habitats have cattle present, and all plants in the population are threatened in order to avoid toxic damage. Seedlings and juveniles showed a low frequency in the lower quality habitats, and the sex ratio was of 1:4:1.

We also evaluated cone and seed development, seed production, and sporophyll morphology in 13 mature female cones harvested from two sites authorized by the Mexican government (SEMARNAT No. 00507/08) (Fig. 2). Average weight of female cones was 1.4 kg, and the average number of sporophylls was 220, with an average of 180 well-developed seeds.

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**Fig. 2.** (A) Female cone of *Dioon edule* being weighed and (B) dissected; (C) sporophylls with attached seeds removed for characterization.

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These seeds were sowed last July 2008 in greenhouse conditions to evaluate germination, but this process has not finished.

**Alimentary Uses of Chamal**

E. Tristán Martínez & J. Fortanelli Martínez

After exploratory trips into the Xi’iuy region, five localities with abundant *Dioon edule* populations were selected for this portion of the study. Seventeen individuals were interviewed, mostly women that agreed to share their knowledge about the alimentary uses of chamal. The questionnaire included questions pertaining to ethnobotanical categories, type of use, harvest process, and food processing. To include more detailed information about the processing of the ‘tamales de chamal’, as they are locally known, we worked with three families who allowed us to monitor and record—through digital photos and video—the entire process. Data taken included volumetric and chronometric measurements, organoleptic attributes, and Spanish and Xi’iuy names for each activity and kitchen utensil. Data processing was merely descriptive and qualitative because of directed non-random sampling.

The botanical categories related to *Dioon edule* and its uses were as follows: 1) ornamental-ceremonial - the leaves or the entire plant are used to decorate altars; 2) ornamental with trade purpose - the entire plant is removed from the root and sold to dealers who transport the plant to the city; and 3) alimentary. Different parts of the seed are used to process the most frequent made ‘tortillas’, ‘atoles’, ‘gorditas de horno’ and ‘tamales’. Although nearly all people self-consume the tamales, there are also some that sell them.

It is necessary to mention that the harvest of this plant for alimentary purposes is apparently independent of ethnic identity, and is, instead, more related to economic level. In other words, frequent consumers of chamal are typically indigenous people and poor mestizos. However, chamal also represents a threat for peasants mainly dedicated to livestock, because plants “invade” grasslands and grazing lands in forests. The highly toxic new leaves are palatable for cattle, and shorty afterwards the animals lose motor function.

Regarding the ornamental use, in one community plants are gathered and sold to buyers from San Luis Potosí. They pay around US$40.00 for four sacks with four to six plants in each. Here, *Dioon edule* is not only a complement to the inhabitants’ diet but also a source of income. Considering that *D. edule* is an endemic threatened species (PROY-NOM-059-ECOL-2001) (Yáñez-Espinosa, 2006), it is critical to regulate its use with the aim of conserving populations without detriment of everyone’s benefit.

Food processing of chamal seeds must be done correctly to avoid symptoms like vomiting, diarrhea, nausea, and motor function loss in people—termed ‘enchamalar’. There are variations in the processing procedures, ingredients, amounts, etc., and this apparently is related to regional variants and economical purposes of the final product. The most frequent alimentary product is the ‘tamales de chamal’, which is described below.

**Seed harvest.** Female cones are removed from the plant during April and May, when the chamal is ‘sazón’ (physiologically mature) and then opened with a knife or stricken with a rock.

**Gametophyte removal.** Complete seeds are dried, to store, or consumed immediately after removing the sclerotesta. The gametophyte is sectioned in three to four portions, with or without embryo.

**Gametophyte cooking.** The gametophytes are boiled in bleach prepared with a mix of water, ash, and lime for 5 to 10 minutes, and then left to stand for 40 minutes to 2 hours. Favorite ashes come mainly from Quercus laeta LIEBM., Q. polymorpha SCHLTDL. & CHAM., and Cordia boisieri A.DC.

**Grinding.** After some testing to assure seeds are cooked and have absorbed the exact quantity of bleach, they are ground in a mill and a ‘metate’ (flat stone used for grinding corn) to produce a dough. The tamales are small dough balls of 4-7 cm, plain or filled with egg or chickpea stew, and occasionally wrapped with leaves of *Arundo donax* L. Finally, the tamales are boiled in water for some minutes until they float, and then they are ready to eat (Fig. 3). There are some women from one community dedicated to cooking the tamales and selling them nearby in packages of five, and occasionally the tamales are sent to another country.

**Chamal (Dioon edule)** is a plant strongly related to agriculture-gatherer Xi’iuy culture because it has allowed them to subsist in marginalized conditions by functioning as a substitute for maize in times of food shortage. Nevertheless, the knowledge of the proper cooking procedure is endangered because new generations are not interested to learn.

**Toxicity of Chamal**

Covarrubias Camarillo & E. Garcia Chávez

The aim of this component of the study was to evaluate the toxicity of the seeds of *Dioon edule* LINDL. (seed, gametophyte, and sarcotesta) and the possible damage to the kinematics (locomotion) of male Wistar rats by repeated exposure of different treatments (0.5 g / kg / day) for 15 days. Rats received humane care, and the experiment adhered to the Institution’s guidelines and the Mexican Official Norm (NOM-062-ZOO-1999) regarding technical specifications for production, care, and use of laboratory animals.

The animals were assigned to five groups: one control group and four groups of *D. edule* - seed, gametophyte, sar-
cotesta, and 2-Amino-3-(methylamino)-propionic acid (BMAA, which is a chemical found in cycads and thought to have neurodegenerative effects on mammals). Control rats received deionized water daily for 15 days by gavage; BMAA rats received the dose by IV acutely; and rats in the other three groups received a water extract of seed, gametophyte, and sarcotesta flour by gavage for 15 days. The experimental dose was based upon previous reports in rats (González, 1990) which showed that steady-state brain concentrations of 10-30 μg/g could possibly cause alterations in behavioral and locomotor activity (Fig. 4).

The results showed a significant increase in body weight gain in the group of rats treated with sarcotesta 6 days after exposure, which remained at 9 and 15 days (p<0.002). The group of rats exposed to BMAA only showed an increase after 9 days of exposure (Fig. 5). However these changes were not associated with the consumption of food, because an increase in the consumption of food was not observed in the same periods of time (Table 2). The group of animals exposed to BMAA significantly reduced their consumption of food after 12 days of exposure, but this decrease had no effect on the body weight of the animals (Table 2).

In relation to the effect of treatments on the kinematics of the animals, the results showed alterations in the locomotion of the rats exposed to seeds, characterized by a significant decrease in the support phase and a significant increase in the length and duration of stride compared with the control group (p<0.001, Table 3). These results were translated into an increase in the velocity of the stride. The group of animals exposed to gametophyte showed a significant decrease in the length and velocity of stride, compared with the control group. This behavior was also observed in the group treated with BMAA (Table 3). These results were associated with a decrease in the velocity of the step, and with a slow and eventful displacement. The results of the phases of flexion, extension, and balance showed no significant differences (data not presented).

Consumption of seeds of Cycas microsperma by Chamorro people on Guam has been associated with neurodegenerative disease (amyotrophic lateral sclerosis) as a result of the content of BMAA in the seeds (Banack & Cox, 2003). Studies in animals exposed to BMAA showed alterations characterized by inability to extend legs, dragging gait, hyper-excitability state, ataxia, and difficulty in walking (Karamyan & Speth, 2008).

**Table 1.** Dioon edule population density in the Huasteca region, San Luis Potosí, México.

<table>
<thead>
<tr>
<th>Habitat quality</th>
<th>Seeding (no. ha⁻¹)</th>
<th>Juvenile (no. ha⁻¹)</th>
<th>male (no. ha⁻¹)</th>
<th>female (no. ha⁻¹)</th>
<th>Total (no. ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1413</td>
<td>963</td>
<td>238</td>
<td>100</td>
<td>2713</td>
</tr>
<tr>
<td>Medium</td>
<td>550</td>
<td>438</td>
<td>175</td>
<td>88</td>
<td>1250</td>
</tr>
<tr>
<td>Low</td>
<td>363</td>
<td>263</td>
<td>175</td>
<td>125</td>
<td>925</td>
</tr>
</tbody>
</table>

**Table 2.** Daily food consumption of Wistar rats (g).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.0 ± 5.5</td>
<td>21.3 ± 6.5</td>
<td>25.7 ± 3.7</td>
<td>25.7 ± 3.4</td>
<td>27.5 ± 3.8</td>
<td>19.9 ± 7.0</td>
</tr>
<tr>
<td>Seeds</td>
<td>17.0 ± 3.7</td>
<td>23.0 ± 2.1</td>
<td>20.7 ± 2.7</td>
<td>23.9 ± 1.5</td>
<td>26.8 ± 2.0</td>
<td>23.1 ± 2.4</td>
</tr>
<tr>
<td>Gametophyte</td>
<td>24.8 ± 3.8*</td>
<td>25.2 ± 4.7</td>
<td>24.2 ± 5.2</td>
<td>23.8 ± 5.2</td>
<td>23.0 ± 6.5</td>
<td>13.0 ± 2.9</td>
</tr>
<tr>
<td>Sarcotesta</td>
<td>30.0 ± 0.0*</td>
<td>30.0 ± 0.0*</td>
<td>30.0 ± 0.0*</td>
<td>29.6 ± 0.9</td>
<td>27.4 ± 2.1</td>
<td>13.3 ± 3.0</td>
</tr>
<tr>
<td>BMAA</td>
<td>18.9 ± 2.8</td>
<td>26.2 ± 2.3</td>
<td>22.9 ± 2.9</td>
<td>25.8 ± 2.9</td>
<td>7.2 ± 2.9*</td>
<td>3.4 ± 2.3*</td>
</tr>
</tbody>
</table>

BMAA = beta-methylamino alanine; values represent mean ± SD; *p < 0.001 vs control; °p < 0.001 vs BMAA; °p < 0.001 vs sarcotesta; °p < 0.001 vs gametophyte; n=6

**Table 3.** Analysis of kinematics (locomotion) of Wistar rats fed various parts of chamal seeds.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Seeds</th>
<th>Gametophyte</th>
<th>Sarcotesta</th>
<th>BMAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase height (s)</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1*</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Length of stride (cm)</td>
<td>9.0 ± 1.3</td>
<td>10.3 ± 1.2*</td>
<td>7.2 ± 1.3*</td>
<td>8.2 ± 1.3</td>
<td>6.5 ± 0.9*</td>
</tr>
<tr>
<td>Duration of stride (s)</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.1*</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1*</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Velocity of stride (cm/s)</td>
<td>13.9 ± 3.7</td>
<td>21.7 ± 6.7*</td>
<td>10.3 ± 3.7*</td>
<td>13.3 ± 3.6</td>
<td>9.3 ± 3.1*</td>
</tr>
</tbody>
</table>

*p < 0.001 vs control; °p < 0.001 vs BMAA; °p < 0.001 vs sarcotesta; °p < 0.001 vs gametophyte; n=6