Pine seed predation by mice: an experimental assessment of preference

R. Flores–Peredo & B. S. Bolívar Cimé

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Abstract

Pine seed predation by mice: an experimental assessment of preference.— Seed traits are considered an essential factor influencing rodents' foraging preferences. We evaluated the mouse's preferences for seeds of four pine species, Pinus patula, P. pseudostrobus, P. teocote and P. montezumae, that differ in length, width, nutritional content, and concentrated tannins. In 'cafeteria experiments' in the laboratory, we tested six of the nine mice species commonly found in the temperate forest of Southern Mexico. Longer and wider seeds were those of P. teocote and P. montezumae. P. teocote seeds had the highest protein content, P. patula were highest in lipids, and P. montezumae seeds were highest in carbohydrates. In concentrated tannins, gallic acid content was highest in P. patula seeds and tannic acid content was highest in P. teocote seeds. Mice preferred small pine seeds with a high lipid and gallic acid content, a low tannic acid content, and an intermediate protein and carbohydrate content. The foraging behavior of rodents, their energy optimization, and the likely effects on seed fate and plant composition would thus be mediated by combinations of seed traits rather than by single seed traits such as size or tannin contents.

Key words: Bromatological analysis, Concentrated tannins, Energy optimization, Temperate forest, Seed size, Small rodents

Resumen

Depredación de semillas de pino por roedores: una evaluación experimental de las preferencias.— Se considera que las características de las semillas son un factor esencial que influye en las preferencias de forrajeo de los roedores. Evaluamos en laboratorio, mediante experimentos tipo cafetería, las preferencias alimentarias de seis de las nueve especies de roedores que se observan con frecuencia en el bosque templado del sur de México por las semillas de cuatro especies de pinos: Pinus patula, P. pseudostrobus, P. teocote y P. montezumae, que difieren entre sí en el largo, el ancho, el contenido nutricional y la concentración de taninos. Las semillas más largas y anchas fueron las de P. teocote y P. montezumae. Las de P. teocote tuvieron el mayor contenido de proteína, las de P. patula fueron las más ricas en lípidos y las de P. montezumae fueron las más ricas en carbohidratos. En cuanto a la concentración de taninos, el mayor contenido de ácido gálico se encontró en las semillas de P. patula y el de ácido tánico, en las de P. teocote. Los roedores prefirieron semillas pequeñas con un alto contenido de lípidos y ácido gálico, un bajo contenido de ácido tánico y un contenido intermedio de proteína y carbohidratos. En consecuencia, el comportamiento de forrajeo de los roedores, su optimización energética y los posibles efectos en el destino de las semillas y la composición de la vegetación estarían determinados por una combinación de características de las semillas más que por una única característica como el tamaño o el contenido de taninos.

Palabras clave: Análisis bromatológico, Concentración de taninos, Optimización energética, Bosque templado, Tamaño de las semillas, Pequeños roedores

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Rafael Flores–Peredo & Beatriz del Socorro Bolívar Cimé, Inst. de Investigaciones Forestales (INIFOR), Parque Ecológico El Haya, Antigua Carretera Xalapa–Coatepec, Univ. Veracruzana, Xalapa, Veracruz, México.

Corresponding author: Rafael Flores–Peredo. E-mail: pereodof@gmail.com
Introduction

Seed production is a fundamental process in forest ecosystem dynamics as seeds are the template of forest regeneration. Seeds are the most nutritious part of plants because of the concentration of carbohydrates, fats and proteins necessary for early seedling development. For this reason, seeds are important food items for small vertebrates, such as rodents and birds, and invertebrates such as insects (Janzen, 1971). Among rodents, squirrels and mice are responsible for the loss of most tree seeds in northern temperate and boreal forests because they are efficient predators and seed dispersers (Yi et al., 2015). In pine forest, they remove and consume 24 to 99% of seeds (Steele et al., 2005; Vander Wall, 2010; Flores–Peredo et al., 2011, Wang et al., 2012), while birds and arthropods usually play a smaller role (Hulme, 2002). As a result, rodents regulate seedling establishment, influencing the dynamics and distribution of plant communities and also the evolution of their reproductive strategies (Hulme & Kollmann, 2005; Wang et al., 2014).

Seed traits such as size, nutrient content (proteins, lipids, carbohydrates) and secondary compounds (alkaloids and tannins) are considered one of the most essential factors influencing foraging preferences of granivores (e.g., Janzen, 1971; Díaz, 1996), which in turn may regulate seed fate and plant structure (Hoshizaki & Miguchi, 2005; Wang & Chen, 2009). Regarding seed size, it has been documented that large seeds are consumed more because of their nutritional value (Xiao et al., 2004; Wang & Chen, 2012). However, as larger seeds take longer to consume than smaller seeds, rodents can be at greater risk from predators during consumption. Large seeds are thus more likely to be removed and cached than eaten in situ by scatter–hoarding rodents (Boman & Casper, 1995; Jansen et al., 2004; Hulme & Kollmann, 2005; Sivy et al., 2011). Rodents are also known to select seeds of high nutritional value by smell, avoiding in situ those with secondary toxic compounds such as tannins and terpenes (Wang & Chen, 2009; Zong et al., 2010; Rubino et al., 2012). Tannins (naturally astringent compounds) and terpenes (mainly hydrocarbons found in volatile oils and resins) reduce the digestibility of seeds because of their high affinity for proteins (Wang & Chen, 2012). This can lead to body weight loss and even death (Vander Wall, 2001; Shimada & Saitoh, 2003). Nevertheless, rodents may choose seeds with tannins in periods of low food availability (Xiao et al., 2008; Vander Wall, 2010), attenuating their effects by consuming seeds that are high in protein and fat content (Wang & Chen, 2012; see also Díaz, 1996). Identifying which seeds rodents prefer in forest ecosystems may help to understand their influence on plant community dynamics (Hulme, 2002), their foraging strategies, energy optimization (Kasparian & Millar, 2004; Hoshizaki & Miguchi, 2005) and the way in which these resources are used by granivore species in their habitats (Millar et al., 1985).

In temperate forests in central Veracruz, Mexico, the feeding preferences of rodents seems to involve several factors that favor the dominance of Pinus teocote, together with the regenerative strategies of the species, herbivory and light intensity, because seeds of this species and those of P. montezumae are removed little (Flores–Peredo et al., 2011). It has been reported that wood, needles and bark from P. teocote contain two of the principal concentrated tannins, tannic and gallic acids (Rosales–Castro & González–Laredo, 2003; Rosales–Castro et al., 2009; Sáenz–Esqueda et al., 2010), and those from P. montezumae have high levels of tannic acid and turpentine (Rodríguez–Franco, 1997; Bernabé–Santiago et al., 2013), but tannic and gallic acid levels in seeds are unknown. Reported seed sizes for these species are 4.0 ± 0.42 (SE) mm length and 2.4 ± 0.21 mm width for P. teocote (Ramirez–Garcia, 2000), and 8.0 ± 0.27 mm length and 4.0 ± 0.32 mm width for P. montezumae (Perry, 2009). Both species are dispersed by wind, rodents and insects. Studies with rodents using artificial seeds (Wang & Chen, 2008, 2012), acorns (Smallwood et al., 2001; Shimada & Saitoh, 2003; Steele et al., 2005; Takahashi & Shimada, 2008), pine seeds (Chen & Chen, 2011; Zong et al., 2010) and seeds from other plant species (Downs et al., 2003) would suggest rodents have clear preferences for seeds from among these pine species in relation to size and nutrient contents.

Materials and methods

Study area

The study was conducted in the central region of the state of Veracruz, in the San Juan del Monte Ecological Reserve in Mexico (19º 39’ 00”, 19º 35’ 00” N, 97º 05’ 00”, 97º 07’ 30” W, between 2,327 and 3,100 m a.s.l.), which has an area of 609 ha. The vegetation mosaic is comprised of three types of vegetation: 1) pine forest, with Pinus teocote as the dominant species (ca. 69% of the forest cover in the
area, followed by *Pinus patula*, *P. pseudostrobus* and *P. montezumae* (GEV & CEMA, 2002). These trees are 15 to 25 m high, and 6 to 8 m apart; 2) oak–alder forest consisting mainly of *Quercus crassifolia* Humb et Bonpl and *Alnus jorullensis* HBK that are 6 to 12 m high; and 3) subalpine grassland, consisting mainly of patches of *Brachypodium mexicanum* Roem et. Schult and *Muhlenbergia macourea* Muhl, fairly close together (1 m apart) or, sometimes packed together with tillers that are up to 1 m high, in association with occasional shrubs such as *Baccharis conferta* Kunth that are 1 to 3 m high (GEV & CEMA, 2002).

Seed collection, measurement and site selection

For each species of pine, ten trees were randomly selected during the seeding season (November–January) and 800 mature cones (200 per species) were collected. The cones were dried in the sun and the seeds were removed. The dispersal wing was removed before laboratory experiments. To assess intra– and inter–specific variation in seed size, three samples of 50 seeds were selected from a total of 350 g per pine species. Each seed of each sample was measured (length and width) with a digital caliper to the nearest 0.01 mm (Rodriguez–Laguna et al., 2012).

Nutrient analysis

The bromatological analyses were made on 300 g of seeds from each pine species, using three subsamples of 100 g according to standardized methods (AOAC, 2005). In order to determine moisture, ash, protein, lipid, fiber and carbohydrate content, the seeds were pulverized in a mortar. The Soxhlet method (AOAC, 2005) was used to determine crude fat content. For moisture content, samples were over–dried at 70°C for 72 h. Ash was determined by incineration and crude fiber was determined by acid and alkaline digestion of the samples. Protein analysis was automated, using micro Kjeldahl equipment (Brand: Buchi Labortechnik, Type: B–339) and the conversion method for total protein nitrogen (N × 6.25) was used as recommended by FAO/OMS (1973). Total carbohydrate was obtained from the additive difference in the percentages of moisture, ash, protein and lipids.

We determined two of the most common concentrated tannins, gallic acid and tannic acid (Alasalvar & Shahidi, 2009; Rosales–Castro et al., 2009; Sàenz–Esqueda et al., 2010). Gallic acid was determined using 400 g of seeds per species in four subsamples of 100 g, and 300 g of seeds per species in three subsamples of 100 g were used for tannic acid determination. To extract gallic acid, about 150 mg of the extract was dissolved in 10 mL of methanol, and 2 mL of this solution was filled up with 0.3% HCl to 5 mL. A 100 µL aliquot of the resulting solution was added to 2 mL of 2% Na₂CO₃, and after 2 min 100 µL of Folin–Ciocalteau reagent (Merck, Darmstadt, Germany) (diluted with methanol 1:1) was added. After a further 30 minutes, the absorbance was measured at 750 nm using a spectrophotometer. The concentration was calculated using gallic acid as standard, and the results were expressed as milligrams gallic acid equivalents (GAE) per gram extract (Matthaus, 2002). To extract tannic acid, we took into account amendments to the ISO–1988 International standard for the determination of tannins number 9648.5 (ISO Norm 9648, 1988).

Rodent capture

Between February 2006 and January 2007, rodents were captured within the three vegetation types (pine forest, oak–alder forest and subalpine grassland) with two replicate sampling plots (1.7 hectares approximately, 130 m × 130 m), separated by 3 km on average. We sampled each replicate twice a month over 12 months, with a 1–week break between visits. The sampling effort was 144 trap–nights per vegetation type, and 7,200 trap–nights overall. In each replicate, we established four random linear transects (two of 130 m and two of 120 m) with a width of 5 m, where we set 13 and 12 Sherman traps, respectively (1 trap/10 m, 50 traps in total) baited with oats, vanilla and peanut butter. Traps remained active for 12 h (6.00 p.m.–6.00 a.m.). When it was necessary to identify the individuals captured, we euthanized by asphyxia an adult male and female of each recognizable rodent species following the American Society of Mammalogists guidelines and procedures on animal ethical care and use (Sikes et al., 2011) and authorized by the Mexican federal authority (SEMARNAT). Taxonomical identification of species was based on cranial morphology according to Wilson & Reeder (2005). Feeding preference experiments were done with the animals and species that were recorded throughout the year until we obtained data from 20 individuals for each mouse species.

Feeding preferences

The feeding preference experiments were performed using a wood box (60 x 30 x 30 cm) divided into two areas (isolation and feeding). The feeding area was covered with black plastic to facilitate cleaning and reduce animal stress (Moberg & Mench, 2000). For each test, we used a plastic Petri dish with 20 seeds (five of each species of pine) to be eaten *ad libitum*. For each run of the experiment, one mouse from each captured species, which had fasted during the previous 6 h, was placed in the isolation area for about 10 minutes. The connecting door was then opened and the mouse was allowed to enter the feeding area. We evaluated the choice of seeds by pine species, identifying in the feeding area in each trial (five times for each mouse) (Heroldova et al., 2008) the first seed chosen by olfaction among 20 seeds of four pine species. After identifying the seed chosen, we allowed the mouse to eat freely for 5 minutes before it was removed from the box at the end of the experiment. At the end of each trial, we added together the number of times a certain seed was chosen for each pine species and counted the number of seeds consumed. This was repeated five times with the same mouse every 40 min. After the experiment, the mouse was released at the place where it was captured.
Intra–specific variation in the length and width of the seeds for each pine species was evaluated using Bartlett’s test, considering three groups of 50 seeds as replicates after evaluation of data normality with Shapiro–Wilks tests (Crawley, 2007). If variances were homogeneous for each pine species, we then used an analysis of variance to assess inter–specific differences in seed size. For significant differences, we used a multiple–comparison Tukey test to contrast seed size among pairwise pine species (Crawley, 2007).

To assess differences in the protein, lipid and carbohydrate contents of seeds among the four pine species, we applied a one–way ANOVA. Because these data departed from normality (according to Shapiro–Wilks tests), we performed square–root transformations (Crawley, 2007). For significant differences, we used multiple–comparison Tukey tests. To assess differences in the gallic and tannic acid content in seeds among the four pine species, we applied a Kruskal–Wallis nonparametric test (Crawley, 2007). We used the values obtained in percentage for each component as response variable and the pine species as explanatory variable.

Differences in pine seed species chosen for the first time in the experiment were evaluated using a general linear mixed model (GLMM; Wang et al., 2012), since our data involved repeated measurements from the same individual (five repetitions) and both random (each mouse tested) and fixed (pine seed species) effects (Crawley, 2007). The pine seed species was the single categorical explanatory variable in the model, which had four levels corresponding to each pine species tested. For this analysis, the response variable was binary: pine seed species chosen or not chosen, thus the family was binominal and the link function used was logit (Crawley, 2007). The same analysis was performed to evaluate differences in the number of seeds consumed among pine species; in this case, we used a Poisson distribution (response variables were counts) and log as link function in the model. When the tests were significant, differences between levels of the explanatory variable were evaluated using the multcomp package version 1.3–5 (Bretz et al., 2010). These analyses were performed separately for each rodent species with more than 12 individuals tested using the R software version 2.13.2 (R Development Core Team, 2008).

### Results

For each pine species, seed length and width values were normal. We found no intra–specific variation in seed length and width for the pine species evaluated (table 1). However, length of seeds showed inter–specific significant differences ($F_{3,8} = 271.48$, $P < 0.001$). Longer seeds were those of *Pinus teocote* (6.26 ± 0.04 mm) and *P. montezumae* (5.69 ± 0.04 mm) compared with *P. patula* (5.13 ± 0.04 mm) and *P. pseudostrobus* (4.56 ± 0.04 mm) (fig. 1A). Similarly, the width of the seeds differed among pine species ($F_{3,8} = 1639.00$, $P < 0.001$). Wider seeds were those of *Pinus montezumae* (4.23 ± 0.02 mm) and *P. teocote* (4.04 ± 0.02 mm) compared with *P. patula* (2.62 ± 0.02 mm) and *P. pseudostrobus* (2.55 ± 0.02 mm) (fig. 1B).

Protein ($F_{3,8} = 13291$, $P = < 0.001$), lipids ($F_{3,8} = 25.95$, $P = < 0.001$) and carbohydrate content ($F_{3,8} = 11745$, $P = < 0.001$) of seeds were significantly different among the four pine species (fig. 2). Generally, the protein content of the seeds was relatively low and *Pinus teocote* seeds contained significantly more protein (13.29%). Lipid content was slightly higher than protein content and the seeds of *P. patula* had the highest lipid content (38.48%). Carbohydrate content was the predominant nutrient type in the seeds, and *P. montezumae* had the highest carbohydrate content (75.23%). Seed fiber content did not differ among pine species ($F_{3,8} = 0.44$, $P = 0.729$); *P. patula* 8.60%, *P. pseudostrobus* 8.13%, *P. teocote* 8.53% and *P. montezumae* 11.56%.

### Table 1. Values obtained using the Shapiro Wilk test for length and width values of four pine seed species: normality according to Shapiro–Wilks and homoscedasticity according to Bartlett. The n value corresponds to three groups of 50 seeds for each species of pine.

| Species          | n   | Length (mm) Normality | Homoscedasticity | Width (mm) Normality | Homoscedasticity |
|------------------|-----|-----------------------|-------------------|----------------------|-------------------|
| *Pinus patula*   | 150 | $P > 0.065$           | $F_2 = 1.26$, $P = 0.533$ | $P > 0.100$           | $F_2 = 0.10$, $P = 0.953$ |
| *Pinus pseudostrobus* | 150 | $P > 0.100$           | $F_2 = 4.30$, $P = 0.117$ | $P > 0.100$           | $F_2 = 1.52$, $P = 0.468$ |
| *Pinus montezumae* | 150 | $P > 0.100$           | $F_2 = 2.07$, $P = 0.355$ | $P > 0.100$           | $F_2 = 0.16$, $P = 0.924$ |
| *Pinus teocote*  | 150 | $P > 0.100$           | $F_2 = 4.37$, $P = 0.112$ | $P > 0.100$           | $F_2 = 4.37$, $P = 0.112$ |
Fig. 1. Differences between length (A) and width (B) of seeds of four pine species from the central area of Veracruz State, Mexico: Pp. *Pinus patula*; Pps. *Pinus pseudostrobus*; Pm. *Pinus montezumae*; Pt. *Pinus teocote*. Letters a, b, c and d indicate significant differences at $P < 0.05$; mean ± SE.

Fig. 1. Diferencias entre el largo (A) y el ancho (B) de las semillas de cuatro especies de pino de una zona central del estado de Veracruz, México. Las letras a, b, c y d indican diferencias significativas con $P < 0.05$; media ± EE. (Para las abreviaturas de las especies, véase arriba.)

Fig. 2. Protein, lipid and carbohydrate content of the seeds from four temperate forest pine species from the central area of Veracruz State, Mexico: Pp. *Pinus patula*; Pps. *Pinus pseudostrobus*; Pt. *Pinus teocote*; Pm. *Pinus montezumae*. In each nutritional block, protein, lipids, and carbohydrates. Letters a, b, c, and d indicate significant differences, $P < 0.05$; mean ± SE.

Fig. 2. Contenido de proteínas, lípidos y carbohidratos en las semillas de cuatro especies de pino de un bosque templado del centro del estado de Veracruz, México. En cada grupo nutricional, proteínas, lípidos y carbohidratos. Las letras a, b, c y d indican diferencias significativas con $P < 0.05$; media ± EE. (Para las abreviaturas de las especies, véase arriba.)
Total content of gallic acid among seed species was significantly different ($H = 14.15, df = 3, P = 0.0027$), with seeds of *P. patula* showing the highest percentages (0.364 ± 1.2) (fig. 3A). Among species of seeds, the total amount of tannic acid was also significantly different ($H = 10.42, df = 3, P = 0.0153$), with seeds of *P. teocote* showing the highest levels (0.795 ± 0.5) (fig. 3B). We caught a total of 248 animals belonging to nine species of mice from five genera and one family (Muridae). Five species were granivores, and four were omnivores; all are potential seed predators. We used 124 of these mice for the preference experiments, but only six species reached more than 12 individuals captured and were included in statistical analysis (table 2). The majority of rodents (five species) chose *P. patula* and *P. pseudostrobus* seeds more often than *P. montezumae* (table 3, fig. 4); and all these species consumed more *P. patula* and *P. pseudostrobus* seeds (table 3, fig. 5).

**Discussion**

We identified interspecific variation among the length and width of pine seed species. *P. teocote* and *P. montezumae* seeds were longer and wider than the other species studied. The nutritional contents of seeds also differed significantly among pine species. *P. teocote* seeds had the highest content of protein and tannic acid, *P. patula* seeds were highest in lipids and carbohydrates, and *P. montezumae* seeds had the lowest percentages of gallic and tannic acid. The total content of gallic acid among seed species was significantly different ($H = 14.15, df = 3, P = 0.0027$), with seeds of *P. patula* showing the highest percentages (0.364 ± 1.2) (fig. 3A). Among species of seeds, the total amount of tannic acid was also significantly different ($H = 10.42, df = 3, P = 0.0153$), with seeds of *P. teocote* showing the highest levels (0.795 ± 0.5) (fig. 3B). We caught a total of 248 animals belonging to nine species of mice from five genera and one family (Muridae). Five species were granivores, and four were omnivores; all are potential seed predators. We used 124 of these mice for the preference experiments, but only six species reached more than 12 individuals captured and were included in statistical analysis (table 2). The majority of rodents (five species) chose *P. patula* and *P. pseudostrobus* seeds more often than *P. montezumae* (table 3, fig. 4); and all these species consumed more *P. patula* and *P. pseudostrobus* seeds (table 3, fig. 5).

**Table 2.** Nine mouse species recorded in a temperate forest in central Veracruz, Mexico. The total number of individuals (N), the number of individuals per species used in the experiments (NE) and food guild (FG: O. Omnivore; G. Granivore) are given.

| Mouse species           | N  | NE | FG |
|-------------------------|----|----|----|
| Peromyscus melanotis    | 77 | 20 | O  |
| Peromyscus maniculatus | 54 | 20 | O  |
| Reithrodontomys mexicanus | 41 | 20 | G  |
| Reithrodontomys fulvescens | 32 | 20 | G  |
| Reithrodontomys megalotis | 15 | 15 | G  |
| Reithrodontomys sumichrasti | 12 | 12 | G  |
| Mus musculus            | 11 | 11 | O  |
| Microtus mexicanus      | 3  | 3  | G  |
| Neotomodon alstoni      | 3  | 3  | O  |
| Total                   | 248| 124|    |
Table 3. Summary of the general linear mixed model test evaluating feeding preferences of six rodent species: \( Z \); \( Z \)-score; \( P \); \( P \)-value; \( Pm \). Pinus montezumae; \( Pp \). Pinus patula; \( Pps \). Pinus pseudostrobus; \( Pt \). Pinus teocote. Bold numbers indicate \( Z \) values with significant differences at \( P < 0.05 \).

| Chosen seed | P. maniculatus | P. melanotis | R. mexicanus | R. fulvescens | R. megalotis | R. sumichrasti |
|-------------|----------------|--------------|--------------|---------------|--------------|---------------|
| Z;P         |                |              |              |               |              |               |
| Pp vs. Pm   | 2.920; 0.018   | 1.494; 0.438 | 5.288; <0.001 | 5.641; <0.001 | 4.657; <0.001 | 4.027; <0.001 |
| Pps vs. Pm  | 2.646; 0.039   | 3.331; 0.005 | 4.015; <0.001 | 3.238; 0.006  | 3.106; 0.009  | 3.571; 0.002  |
| Pt vs. Pm   | –1.638; 0.353  | –1.357; 0.523 | 2.927; 0.017  | 3.705; <0.001 | 0.769; 0.866  | 2.134; 0.136  |
| Pps vs. Pp  | –0.295; 0.991  | 1.925; 0.215  | –1.902; 0.220 | –3.651; 0.001 | –2.001; 0.184 | –0.750; 0.872 |
| Pp vs. Pt   | –4.243; <0.001 | –2.759; 0.029 | –3.281; 0.006 | –3.050; 0.012 | –4.229; <0.001 | –2.729; 0.029 |
| Pps vs. Pt  | –4.008; <0.001 | –4.415; <0.001 | –1.454; 0.457 | 0.662; 0.908  | –2.502; 0.058 | –2.036; 0.167 |

Number of seeds consumed

| Number of seeds consumed | Pp vs. Pm | Pp vs. Pm | Pp vs. Pm | Pp vs. Pm | Pp vs. Pm | Pp vs. Pm |
|--------------------------|----------|----------|----------|----------|----------|----------|
| Z;P                      |          |          |          |          |          |          |
| Pp vs. Pm                | 10.190; <0.001 | 7.883; <0.001 | 11.850; <0.001 | 10.431; <0.001 | 9.237; <0.001 | 9.042; <0.001 |
| Pps vs. Pm               | 9.644; <0.001 | 10.342; <0.001 | 9.874; <0.001 | 8.752; <0.001 | 7.790; <0.001 | 7.786; <0.001 |
| Pt vs. Pm                | 0.626; 0.921 | –1.490; 0.4323 | 2.527; 0.054  | 1.282; 0.570  | 0.134; 0.999  | 0.610; 0.927  |
| Pps vs. Pp               | –0.723; 0.884 | 3.102; 0.010  | –2.438; 0.068  | –1.947; 0.205  | –1.711; 0.312  | –1.547; 0.402  |
| Pp vs. Pt                | –9.789; <0.001 | –8.932; <0.001 | –9.927; <0.001 | –9.386; <0.001 | –9.135; <0.001 | –8.603; <0.001 |
| Pps vs. Pt               | –9.223; <0.001 | –11.164; <0.001 | –7.762; <0.001 | –7.639; <0.001 | –7.679; <0.001 | –7.307; <0.001 |

majority of rodents (five species) chose \( P. patula \) and \( P. pseudostrobus \) seeds and all rodents consumed more small seeds from \( P. patula \) that were rich in lipids and gallic acid and from \( P. pseudostrobus \) with an intermediate content of protein, carbohydrates and gallic acid.

The pine seeds studied show variation in characteristics such as length and width (Perry, 2009), as related to the particular biology of each species and environmental conditions (Eguíluz, 1982). Several studies have reported that seed size is a decisive factor for scatter–hoarding rodents in the choice between seed predation and dispersal (Wang & Chen, 2009; Wang et al., 2013) because seed size is frequently correlated with handling time and energy payback (Breuer, 2001), which in turn had a bearing on the efficiency of foraging (Waite & Ydenberg, 1994) and predation risk (Lima et al., 1985; Sivy et al., 2011).

The general consensus is that larger seeds are more likely to be removed rather than eaten \textit{in situ} (Jansen et al., 2004); in our case, this gives a certain advantage to species such as \( P. montezumae \) and \( P. teocote \) for their dispersal and establishment, and is associated with other factors such as the particular regeneration strategies of each species. \( P. montezumae \), for example, grows slowly and has a cespitose habit during its early years, although its root development is substantial (Vera et al., 1988). This confers an advantage to \( P. teocote \), whose longer seeds are related to higher seedling growth rates and production of more vigorous seedlings (Ramírez–García, 2000), and adaptability to impoverished soils, where they are capable of becoming established and emerging as the dominant species (Perry, 2009).

Many tree species that depend on scatter–hoarding animals for seed dispersal also produce massive crops of large seeds at irregular intervals; in pines, this process is known as seedbed years (Perry, 2009). Mast seeding and large seed size in these species have been explained as adaptations to increase animal dispersal and reduce predation (Jansen et al., 2004). Hereby, the comparison between seeds eaten \textit{in situ} and removed by rodents is important because they have different consequences for the reproductive success and composition of plant species, \textit{i.e.}, seeds removed indicate some probabilities of success of seed dispersal and seedling establishment, while seeds eaten \textit{in situ} mean total seed predation (Stiles, 2000; Morán–López et al. 2015; Wang & Yang, 2015). Seed size, however, is not the only determining factor in seed removal and dispersal (Wang & Chen, 2012). Other factors such as the nutritional quality and defensive secondary compounds particularly tannic acids are also involved in the plant–animal interactions (Vander Wall, 1990; Yi et al., 2015).

Several studies have documented that large seeds of \( Quercus \), \( Lithocarpus \), \( Cyclobalanopsis \), \( Castanopsis \)
and Carapa genus in a subtropical forest of Southwest China are more often selected by rodents possibly because of their high nutritional quality (Jansen et al., 2004; Xiao et al., 2004, 2006), as larger food size often maximizes energy payback for seed consumers (Brewer, 2001). Similarly, some studies have suggested that rodents prefer to cache seeds with high tannin levels and consume seeds with low tannin levels (Smallwood et al., 2001; Shimada & Saitoh, 2003). In other studies, rodents have been shown to eat low–tannin acorns in situ and hoard high–tannin acorns (Barthelmess, 2001; Smallwood et al., 2001; Xiao et al., 2008), whereas other studies have found different, even opposite results (Xiao et al., 2006; Wang & Chen, 2009, 2011). Rodents not only feed on readily available resources, but also select foods high in certain chemical components and low in others, thus regulating their needs and energy expenditure (Bozinovic et al., 1997). Understanding seed preference in scatter–hoarding rodents is thus complicated because selection involves a complex decision–making process (Wang & Chen, 2012). Fat and protein are key nutrients that determine the nutritional quality of a food item in mammalian diets, and hence play an important role in rodent foraging processes (Xiao et al., 2006; Takahashi & Shimada, 2008); on the other hand, concentrated tannins also affect rodent foraging behavior and in turn, seed fate (Vander Wall, 2010).

Our results contrast with these assumptions, however, if we consider the individual effect of size, nutritional quality and tannin presence. Seeds of Pinus patula, considered small, had a high fat content and concentrated tannins such as gallic acid and Pinus teocote seeds (big seeds) had a high protein content but also the highest amounts of tannic acid. Fat is an important energy source that directly affects survival and reproduction in animals. Rodents appear to prefer seeds with high quantities of fat (Xiao et al., 2006) because these provide an energy resource in winter (Steele, 2008). Rodents from the

Fig. 4. Proportion of seeds chosen from four pine species by six species of rodents, captured over a one–year period in the temperate forest in the central area of Veracruz State, Mexico: Pp. Pinus patula; Pps. Pinus pseudostrobus; Pt. Pinus teocote; Pm. Pinus montezumae. Letters a, b, c, and d indicate significant differences at \( P < 0.05 \); mean ± SE.

Fig. 4. Proporción de semillas elegidas de cuatro especies de pino por seis especies de roedores capturados en el periodo de un año en el bosque templado del centro del estado de Veracruz, México. Las letras a, b, c y d indican diferencias significativas con \( P < 0,05 \); media ± SE. (Para las abreviaturas de las especies, véase arriba.)
genus *Peromyscus*, for example, have an affinity for seeds with a high lipid content, such as those from *Pinus cembroides* (piñon or pinyon pine), rather than those from *P. discolor* (Martínez–Delgado et al., 1996). In our study, the majority of rodents chose and consumed small seeds of *P. patula* and *P. pseudostrobus*, high in fat and gallic acid content, low in tannic acid content, and an intermediate level of protein and carbohydrate content. These results indicated that rodents commonly performed energy balances based on their digestive capacity. Similarly, desert rodents prefer diets with specific combinations of proteins, lipids and carbohydrates, which also prevent metabolic water loss (Chad et al., 2001).

According to Wang & Chen (2012), rodents choose and remove seeds with a high fat and protein content because these elements can mitigate the negative effects of gallic acid. In contrast, *P. teocote* seeds, that had the highest amount of proteins and tannic acid, were consumed less. In laboratory experiments with rats, gallic acid has been considered an excellent antioxidant with protective effects against toxic elements (Vijaya–Padma et al., 2011). Furthermore, it is a polyphenol involved in the metabolism of carbohydrate assimilation, preventing its antioxidant properties from being converted into fat (Hanhineva et al., 2010), which may explain the consumption of *P. pseudostrobus* seeds with an intermediate carbohydrate content. It is possible that rodents consume seeds with high amounts of this compound because it allows them to regulate their metabolism and energy optimization because of their beneficial anti–inflammatory, anti–allergenic and cardiovascular properties (Martin & Appel, 2009).

Regarding protein content, in a cafeteria experiment, when offered natural and artificial food, *Peromyscus leucopus* selected those foods with 15% protein over...
those that had 5%, 25% and 35% (intermediate content), demonstrating that selection is not only based on nutritional needs, but also on metabolic capacity and digestibility (Chad et al., 2001). Similarly, a study of the food preferences of the rodent Ctenomys gapperi using natural and commercial foods revealed similar results, a preference for food with 14% protein over those with 20 and 30% (Kasparian & Millar, 2004). Protein levels are also important for the growth and reproduction of rodents (Cameron & Eschelman, 1996), which they select depending on their physical requirements (Bensaid et al., 2002). This is due to the fact that in rodents there are critical levels for assimilation of protein that relate to normal growth and maintenance of healthy animals (Shenk et al., 1970).

Our results clearly support the comments of Vander Wall (2010) and Wang & Chen (2012) showing that set traits of certain pine seeds, such as size, nutritional content and chemical defenses, are indicators of seed quality, affect rodent foraging decisions in a temperate forest, and are involved with the plant species composition of the site. Seed traits influence rodent foraging preferences because all seed traits are combined, and it is difficult to distinguish individual trait effects on rodents foraging behavior or the interactions among them. A large number of plants, such as pines (Perry, 2009; Zong et al., 2010; Nopp-Mayr et al., 2011; Lobo, 2013; Yi et al., 2015), show differences in seed traits (Diaz, 1996; Wang & Chen, 2008, 2012), and are a key element for understanding the foraging behavior of rodents and also their physiological condition and energy optimization (Blate et al., 1998; Wang & Chen, 2009; Sivy et al., 2011). However, other aspects, such as regenerative strategies of species, are also involved (Hulme & Kollmann, 2005). Detailed studies of these processes and foraging strategies among rodents are essential to understand the dynamics involved in the establishment and persistence of plant communities.

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