

SEED BANK FORMATION AND REMOVAL OF *PINUS HARTWEGII* (PINACEAE) SEEDS ALONG AN ALTITUDINAL GRADIENT IN THE COFRE DE PEROTE NATIONAL PARK, VERACRUZ, MEXICO  
FORMACIÓN DE BANCOS DE SEMILLAS Y REMOCIÓN DE SEMILLAS DE *PINUS HARTWEGII* (PINACEAE) A LO LARGO DE UN GRADIENTE ALTITUDINAL EN EL PARQUE NACIONAL COFRE DE PEROTE, VERACRUZ, MÉXICO

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#### Abstract

**Background:** Seed banks, an important element in the natural regeneration of plant communities, are regulated mostly by granivores along an altitudinal gradient.

**Question/Hypothesis:** *Pinus hartwegii* forms transient and probably persistent seed banks along an altitudinal gradient. Seed removal will vary according to the removing agents, the altitude and the season.

**Species under study/Data description/Mathematical model:** The formation of seed banks and the removal of *Pinus hartwegii* (Pinaceae) seeds along an altitudinal gradient were evaluated in different seasons using a linear model with two factorial variance analyses.

**Site and years of study:** The study was conducted at the Cofre de Perote National Park, Veracruz, Mexico, over an altitudinal gradient (at 3,400, 3,600, 3,800 and 4,000 m a.s.l.) in July-September 2015 (rainy season), and January-March 2016 (dry season).

**Methods:** In order to understand seed bank formation and identify the removal agents along an altitudinal gradient, two experiments were conducted with seeds from 10 trees at each altitudinal tier.

**Results:** The results of the first experiment, after one year, demonstrated the formation of transient and probably persistent seed banks along an altitudinal gradient ( $P < 0.0001$ ). The results of the second experiment showed significant variations in seed removal according to the removal agents and the seasons ( $P < 0.0001$ ).

**Conclusions:** *Pinus hartwegii* forms transient and probably persistent seed banks along an altitudinal gradient. Seed removal varies according to altitude and season.

**Keywords:** Conifer, granivory, pine, seasonality, temperate forest.

#### Resumen

**Antecedentes:** Los bancos de semillas son importantes en la regeneración natural de comunidades vegetales y son regulados principalmente por granívoros a lo largo de un gradiente altitudinal.

**Pregunta/Hipótesis:** *Pinus hartwegii* forma bancos de semillas transitorios y probablemente persistentes a lo largo del gradiente altitudinal. La remoción de semillas será diferente entre agentes removedores, altitudes y estaciones.

**Especies estudiadas/Descripción de datos/Modelo matemático:** La formación de bancos y remoción de semillas de *Pinus hartwegii* (Pinaceae) por estación a lo largo de un gradiente altitudinal fueron evaluados usando dos análisis de varianza factorial a través del proceso modelo lineal generalizado.

**Sitio de estudio y año de estudio:** El estudio fue conducido en el Parque Nacional Cofre de Perote en un gradiente altitudinal (a los 3,400, 3,600, 3,800 y 4,000 m s.n.m.), durante julio-septiembre 2015 (lluvias) y enero-marzo 2016 (secas).

**Métodos:** Para conocer la formación de bancos de semillas e identificar los agentes removedores a lo largo de un gradiente altitudinal, dos experimentos fueron conducidos con semillas de 10 árboles por altitud.

**Resultados:** Los resultados del primer experimento mostraron después de un año, la formación de bancos de semillas transitorios y probablemente persistentes a lo largo del gradiente altitudinal ( $P < 0.0001$ ). Los resultados del segundo experimento mostraron diferencias significativas en la remoción de semillas entre agentes removedores y estaciones ( $P < 0.0001$ ).

**Conclusiones:** *Pinus hartwegii* forma bancos de semillas transitorios y probablemente persistentes a lo largo de un gradiente altitudinal. La remoción de semillas varía de acuerdo a la altitud y estación.

**Palabras clave:** Bosque templado, conífera, estacionalidad, granivoría, pino.

Seed banks are defined as the mature viable seed stocks present on the soil's surface, or buried in the soil, duff or litter (Simpson *et al.* 1989). Germination is the most important process in the initial composition of plant communities (Thompson & Fenner 2005), essential to their establishment and permanence through time (McGee & Feller 1993, Bueno & Baruch 2011, Pensado-Fernández *et al.* 2014). Seed banks can be classified into transient and persistent, according to the time the seeds remain viable ( $< 1$  year, or  $\geq 1$  year, respectively) (De Souza-Maia *et al.* 2006). Pine-dominated plant communities are known to fail to form seed banks given that, once dispersed, their seeds remain viable for less than a year, due to biotic and abiotic factors (Carrillo-Anzures *et al.* 2009). However, the presence of persistent seed banks has been reported for *Pinus pinaster* Aiton and *Pinus halepensis* Mill. (Trabaud *et al.* 1997, Ferrandis *et al.* 2000). *Pinus patula* Schltld. & Cham. seeds may remain stored up to 10 years at room temperature (Aparicio-Rentería *et al.* 2014), which indicates that knowledge about the ability of pine species to form bank seeds is yet to be obtained.

The altitudinal gradient is of great importance in the formation of seed banks, as it influences the production of female strobili, as well as the quantity and availability of seeds (Sáenz-Romero *et al.* 2006, Zelikova *et al.* 2008). Tree populations growing at the extreme altitudinal limits (lower and upper) of their natural distribution range produce seeds in smaller quantity than their counterparts at medium altitudes (Quiroga & Premoli 2013). At the lower altitudinal limit, a smaller quantity of seeds is associated to the periodic exposition of trees to extreme climatic events such as droughts and high temperatures (Mátyás *et al.* 2010), whereas at the upper limit it is associated to frost (low temperatures) (Sáenz-Romero *et al.* 2006). Environmental resources and conditions at intermediate altitudinal areas may favor a higher concurrence of predators and greater seed displacement (Sánchez-Cordero 2001). López-Toledo *et al.* (2017) found that one of the intermediate altitude populations of *Pinus pseudostrobus* Lindl. presented a larger number of seeds with embryo than those at a lower altitude.

Granivore guilds play an important role in the persistence of seed banks. Their activity as predators and seed removers has a spatial effect, the magnitude of which varies depending on the group to which they belong (*i.e.*, birds, insects or small mammals) (Hulme 1998). In order to evaluate seed removal by different groups of granivores, several studies have resorted to exclusion treatments, consisting in applying a mesh of different opening sizes according to the size of the granivores in question (Campos *et al.* 2007, Álvarez-Aquino *et al.* 2014), or a repellent, in the case of insects (Matamoros-Juárez & Gaitán-Martínez 2017, Bordones *et al.* 2018). In temperate forests, birds, insects and rodents are efficient removers of pine seeds with different foraging strategies (Hulme & Kollmann 2005, Flores-Peredo *et al.* 2011, Vander-Wall & Beck 2012). In the majority of forest ecosystems, rodents are the main seed removers (Hulme & Benkman 2002), and in pine forests they may remove up to 99 % of the seeds that have fallen to the ground (Vander-Wall 2008), which indicates the important role they play

in regeneration dynamics (Vander-Wall 2008, Lobo *et al.* 2009). However, other granivores, such as birds and insects, also play an important role, as has been reported for *Pinus patula*, *P. pseudostrobus*, *P. teocote* Cham. & Schltld. and *P. montezumae* Lamb. in the central part of the state of Veracruz, Mexico (Flores-Peredo *et al.* 2011, Flores-Peredo & Bolívar-Cimé 2016).

Although differences in seed removal rates are usually associated to seasonal changes (rainy versus dry seasons), the altitudinal gradient also influences the production, availability and quality of seeds for food (Fleury *et al.* 2014, Wang *et al.* 2014, López-Toledo *et al.* 2017). Seed production impacts the reproductive periods of granivore animals (Ofori *et al.* 2015). For example, the size of rodent communities changes seasonally due to changes in weather conditions, which determine both ecosystem productivity and the phenology of seed production (Wang *et al.* 2009, Cortes-Flores *et al.* 2011). This is found among pine communities with mast seeding, the number of years varying from species to species (Perry 2009). In *Pinus hartwegii* Lindl., for example, seeding years occur every six or seven years (Musálem-Santiago & Solís-Pérez 2000). *Pinus hartwegii* develops at the altitudinal limits of mountainous areas in Mexico and Central America (up to 4,300 m a.s.l.), and in Veracruz it is present in the Cofre de Perote and Pico de Orizaba national parks (Narave & Taylor 1997). Due to its biological characteristics and geographic location at the altitudinal limit of montane forests, *P. hartwegii* is at risk of losing its habitat as a result of global warming (Farjon *et al.* 1997). Moreover, it is not known whether it forms transient or persistent seed banks, and whether its granivore seed removal dynamics is associated to an altitudinal gradient. In this study we pose the following questions: 1) Do *Pinus hartwegii* seeds remain viable and have the possibility to form transient or persistent seed banks? 2) If that is the case, does its capacity to do so vary along an altitudinal gradient? 3) Does seed removal vary seasonally along an altitudinal gradient and according to the group of granivores (birds, rodents and insects)? The hypotheses are: a) At a high altitude, *Pinus hartwegii* will show potential to produce transient and probably persistent seed banks, as low temperatures help maintain seed metabolism low; and b) Seed removal will vary with altitude, season, and group of granivores, probably being greater at medium altitudes, where food availability for granivores is higher.

## Materials and methods

**Study area.** This study was undertaken at the Cofre de Perote or Nahcampa petl National Park, located in the southern portion of the Sierra Madre Oriental, at its confluence with the eastern end of the Transversal Neovolcanic Axis, in the central-western region of the state of Veracruz, Mexico. The four altitudinal tiers selected were: Site 1 (altitude: 3,400 m; latitude: 19° 31' 13.33'' N; longitude: 97° 09' 49.65'' W); Site 2 (altitude: 3,600 m; latitude: 19° 30' 52.15'' N; longitude: 97° 09' 51.31'' W); Site 3 (altitude: 3,800 m; latitude: 19° 30' 19.42'' N; longitude: 97° 09' 31.37'' W), and Site 4 (altitude: 4,000 m; latitude: 19° 29' 44.47'' N;

longitude: 97° 09' 09.27" W). Climates are semi cold subhumid with long cool summers [Cb'(w2)], and cold subhumid ETH (García 2004). Mean annual rainfall is 1,500-1,800 mm, and the predominant soil is ochric andosol. Plant communities are represented by pure *P. hartwegii* forests in high altitudes, while at low altitudes associations of *P. hartwegii*, *P. montezumae*, and *Abies religiosa* (Kunth) Schltdl. & Cham. are found. The undergrowth is composed of species such as *Vaccinium geminiflorum* Kunth, *Baccharis conferta* Kunth and *Juniperus monticola* Martínez, and the herb stratum includes the following species: *Alchemilla procumbens* Rose, *Agrostis toluensis* Kunth, *Calamagrostis schiedeana* Steud., *Calamagrostis rigens* Fr., *Cirsium jorullense* Spreng., *Draba jorullensis* Kunth, *Echeveria secunda* Booth ex Lindl., *Eryngium proteiflorum* Delaroché, *Erysimum macradenium* J. Gay, *Festuca toluensis* Kunth, *Gnaphalium liebmannii* Sch. Bip. ex Klatt., *Lupinus montanus* Kunth., *Muhlenbergia macroura* Hitch., *Ottoa oenanthoides* Kunth, *Oxylobus arbutifolius* A. Gray, *Penstemon gentianoides* Poir., *Sedum obcordatum* R.T. Clausen, and *Senecio roseus* Sch. Bip. (Narave 1985, Vázquez-Ramírez 2014). As for animal communities, there are 14 species of amphibians, 25 species of reptiles, 89 species of birds, and 51 species of mammals (Morales-Mávil *et al.* 2007).

**Seed bank.** In order to obtain seeds, mature cones were collected from 10 healthy trees at each altitudinal tier. The persistence of seeds on the ground was evaluated at each tier, an experiment was conducted consisting of three treatments with 25 seeds and three repetitions (*i.e.*, 900 seeds in total). Treatments were: a) seeds placed on the soil's surface, b) seeds planted 5 cm deep into the soil, and c) seeds planted 10 cm deep. Before being planted, seeds were wrapped in a 2 mm-opening plastic insect mesh measuring 10 × 10 cm, so as to secure them and prevent mixing with other seeds of the same species. A year later, seeds were retrieved, and their viability was evaluated by the tetrazolium chloride test at 1 %, to determine the type of seed bank (transient or probably persistent) (Corral-Aguirre & Sánchez-Velásquez 2006). Seeds considered viable were those in which at least 75 % of their embryos took on a red-violet dye, and the rest were considered unviable (Kolotelo *et al.* 2001, Flores-Peredo *et al.* 2011).

**Seed removal.** During July-September 2015 (rainy season) and January-March 2016 (dry season), four random granivore exclusion treatments with five repetitions each were conducted at each altitudinal tier (Arias-Le Claire 2001, Côté *et al.* 2003, Flores-Peredo *et al.* 2011). Treatments availed of meshes with different opening sizes to separate birds, rodents, and insects, and evaluate their respective contributions to seed removal (Hulme 1998, Hulme & Kollmann 2005, Álvarez-Aquino *et al.* 2014, Magalhaes *et al.* 2018). The experimental unit was a plastic Petri box with 20 seeds of *P. hartwegii* (n = 20). The treatments were: 1) exclusion of rodents and birds (access to insects only) by means of a cage measuring 16 × 16 × 9 cm, formed with a 0.5 cm-opening mesh; 2) exclusion of birds and insects (access to rodents

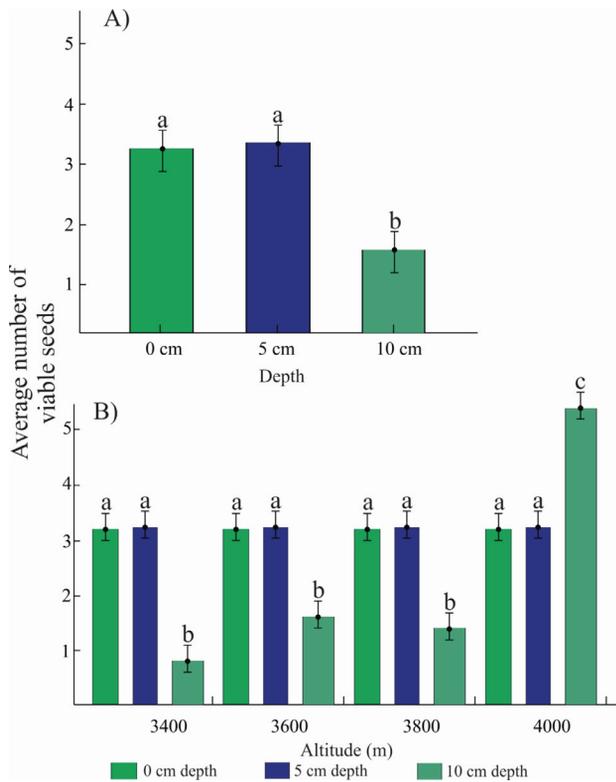
only) with a cage measuring 16 × 16 × 9 cm, formed with a 2 cm opening mesh, around which a garlic-based repellent was sprayed to keep insects out, its effectiveness previously tested with ants and consigned in the literature (Karunamoorthi & Hailu 2014, Matamoros-Juárez & Gaitán-Martínez 2017, Bordonos *et al.* 2018); 3) exclusion of rodents and insects (access to birds only) placing the seeds at the upper side of the cage, far from rodents, and using garlic-based repellent to avoid insects, and 4) control with free access (no exclusions). The cages were fixed to the ground by means of four 10 cm steel nails.

**Statistical analysis.** In order to understand how seed banks are formed, a variance analysis with the general linear model (GLM) of SAS (SAS 2015) was conducted along the altitudinal gradient, considering a nested pattern in which the depth of seed planting (0, 5 and 10 cm) was nested in each tier (3,400, 3,600, 3,800 and 4,000 m a.s.l.) and the response variable was the quantity of viable seeds (natural logarithm [ln]). Mean comparisons were conducted using the Bonferroni adjustments (Zar 1999). Seed removal (by rodents, birds, and insects), altitude (3,400, 3,600, 3,800 and 4,000 m a.s.l.), and seasons (rainy, dry) were evaluated by means of a GLM procedure with Poisson distribution (McCullagh & Nelder 1989, Crawley 2007, Agresti 2015), using as dependent variable the number of seeds removed (count data) because did not assume normality and as independent variables the exclusion, altitude, and season treatments. This is because the Poisson distribution is applied to discrete phenomena of nature, that is, phenomena that imply counts during a defined period of time or in a certain area (Guerriero 2012). For the evaluation of differences between treatments we used the Multcomp package, Version 1.3-5 (Bretz *et al.* 2011) in R language (R Development Core Team 2016).

## Results

A year after the seeds were planted at different depths (at the surface, and 5 and 10 cm deep into the soil), viable seeds were found, which proved the formation of transient and probably persistent seed banks during that period. The number of viable *P. hartwegii* seeds varied significantly with depth ( $P < 0.0001$ ) and altitude ( $P = 0.0110$ ). In average, approximately three viable seeds were retrieved at the surface and at a depth of 5 cm, and approximately one viable seed was retrieved at a depth of 10 cm (Figure 1A). The average number of seeds found at the surface (0 cm) and at a depth of 5 cm was similar in all the altitudinal tiers (approximately three seeds); the smallest number (approximately one seed) was found at a depth of 10 cm at the altitudinal tiers 3,400, 3,600 and 3,800 m a.s.l., and the largest average number of viable seeds (approximately six seeds) was found at a depth of 10 cm at the altitudinal tier of 4,000 m a.s.l. (Figure 1B).

The season-altitude interaction had a significant effect on the removal of *P. hartwegii* seeds ( $F = 13.61$ ,  $df = 3$ ,  $P < 0.001$ ). The greatest removal of seeds was registered at 4,000 m a.s.l. during both the dry season ( $11.79 \pm 0.75$ ) and the rainy season ( $8.40 \pm 0.75$ ). Conversely, at 3,800 m a.s.l.,



**Figure 1.** A. Average number of viable *P. hartwegii* seeds retrieved at different depths along an altitudinal gradient. B. Average number of viable *P. hartwegii* seeds retrieved at different depths along an altitudinal gradient at the Cofre de Perote National Park, Veracruz, Mexico. Dissimilar letters indicate significant differences ( $P < 0.05$ ).

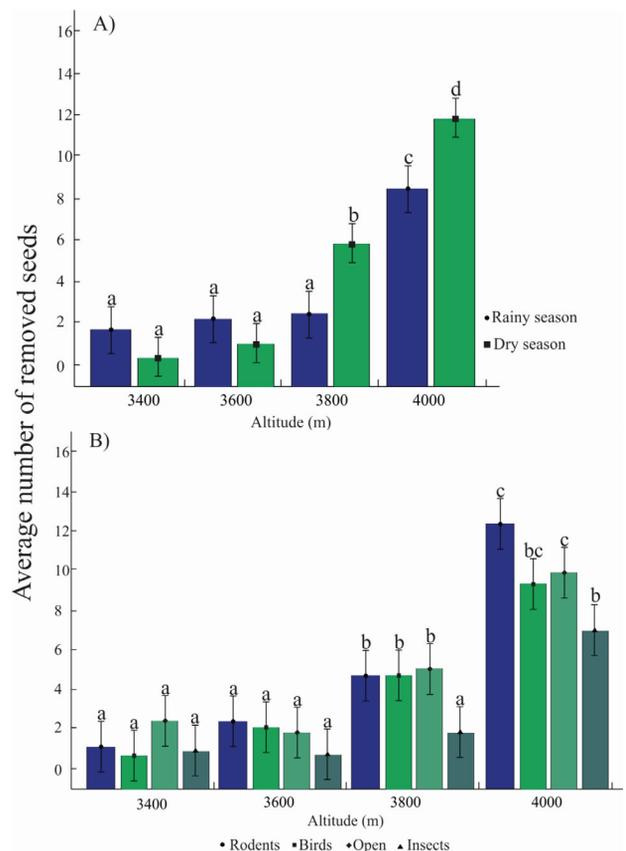
the removal of seeds was greater during the dry season ( $5.76 \pm 0.75$ ) (Figure 2A). Altitude and the exclusion treatments also had a significant effect on the removal of *P. hartwegii* seeds ( $F = 2.24$ ,  $df = 9$ ,  $P = 0.017$ ). At 3,800 m a.s.l., more seeds were removed by rodents, birds, and at the open treatment ( $4.91 \pm 1.05$ ,  $5.11 \pm 1.05$ ,  $5.54 \pm 1.05$ ), whereas at 4,000 m a.s.l., rodents were the main seed removers ( $13.12 \pm 1.05$ ) (Figure 2B).

## Discussion

The percentage of viable *P. hartwegii* seeds found after a year of lying at the soil's surface or planted into it indicates the ability of the species to form transient and probably persistent seed banks. Along with *Pinus pinaster* Aiton, *Pinus halepensis* Mill. (Ferrandis *et al.* 2000, Trabaud *et al.* 1997) and other pine species from eastern Canada (Thomas & Wein 1985), Washington, USA (Pratt *et al.* 1984), and Idaho, USA (Kramer & Johnson 1987), *P. hartwegii* belongs to the list of pines with potential to form transient and probably persistent seed banks. The fact that the number of viable seeds found in the soil increased with depth suggests that the micro-environmental stability found at the soil's deeper layers favor seed

development, as seeds are exposed to less stress than those lying on the soil's surface (Cavieres & Arroyo 2001, Fenner & Thompson 2005). This has also been reported for *Pinus ponderosa* (Pratt *et al.* 1984) and other conifers (Archibold 1989). In the case of *P. hartwegii*, its response has to do also with its life history (Campanhã-Bechara *et al.* 2013). It has been suggested that in high montane environments, low temperatures can be associated to low embryonic metabolic rates, which may favor seed longevity (Murdoch & Ellis 2000) and, therefore, the formation of transient and probably persistent seed banks (Cavieres & Arroyo 2001). This may help explain the larger number of viable seeds found at the deepest level of the highest altitudinal tier, where frost and low temperatures are frequent.

The interactions between season and altitude had a significant effect on seed removal. Seed removal was greater at 4,000 m a.s.l., during both seasons, as opposed to other studies where higher seed removal was greater at medium altitudes (*e.g.*, Sánchez-Cordero 2001). During the dry season (February-March), a temperature rise occurs, which favors cone aperture and seed dispersal. This results in a higher



**Figure 2.** A. Average number of removed *P. hartwegii* seeds in two evaluation seasons along an altitudinal gradient. B. Average number of *P. hartwegii* seeds removed through an exclusion treatment along an altitudinal gradient at the Cofre de Perote National Park, Veracruz, Mexico. Dissimilar letters indicate significant differences ( $P < 0.05$ ).

availability of pine seeds on the ground (Perry 2009), which generates a movement of granivores towards the areas with higher availability of resources, a process called denso-dependency (Hulme & Kollmann 2005, Flores-Peredo *et al.* 2011).

On the other hand, the greatest seed removal at 4,000 m a.s.l. during the rainy season (particularly that carried out by several species of rodents) suggests a synchronicity between food availability, reproductive activity, and the presence of juveniles in large numbers (Ceballos & Oliva 2005). In pine forests of the state of Veracruz, Mexico, for example, a greater richness of species of granivore rodents during the rainy season has been documented, although they were more abundant during the dry season (Flores-Peredo & Vázquez-Domínguez 2016). This suggests that seasonal variations in the availability of food influence the movements of granivores among plant communities (Hulme & Benkman 2002, Rautenbach *et al.* 2014, Ofori *et al.* 2015) and altitudinal gradients (Shuai *et al.* 2017).

The exclusion treatments carried out showed that rodents and birds were the most important seed removal agents at 4,000 m a.s.l. Coinciding with this study is a report from the San Juan del Monte Ecological Reserve in Veracruz, Mexico, where the natural vegetation is pine-oak forest, and where rodents and birds were reported as the main seed removers for four pine species, the former acting at night and the latter during the day (Flores-Peredo *et al.* 2011). Although the lesser removal of seeds found in altitudinal tiers between 3,500 and 3,600 m confirms the meaningful effect of altitude, it may also be due to the lower density of *P. hartwegii* in a more diverse forest (Murrieta-Hernández *et al.* 2014). In a situation in which seeds from diverse plants are abundant, *P. hartwegii* seeds may not be the preferred food resource to granivores. Conversely, the greater removal registered at 4,000 m a.s.l., where *P. hartwegii* is the only tree species, suggests that granivores feed on its seeds because little is available of other resources.

In order to understand the dynamics of the establishment and permanence of endangered plant species, and of the animal groups associated to their regeneration, it is essential to evaluate the presence of transient and probably persistent seed banks, and the effect of the different seed-removing granivores. The results suggest that even though the percentage of viable seeds after 1 year was low (from 12 to 24 %) *Pinus hartwegii* has the potential to form a seed bank (transient and probably persistent). The most important seed removal agents were rodents and birds and removal varies according to altitude and season.

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### Literature cited

- Agresti A. 2015. *Foundations of Linear and Generalized Linear Models*. New Jersey: John & Wiley Sons, Inc, Hoboken, ISBN: 978-1-118-73003-4
- Álvarez-Aquino C, Barradas-Sánchez L, Ponce-González O, Williams-Linera G. 2014. Soil seed bank, seed removal, and germination in a seasonally dry tropical forest in Veracruz, Mexico. *Botanical Sciences* **92**: 111-121. DOI: <https://doi.org/10.17129/botsci.42>
- Aparicio-Rentería A, Juárez-Cerrillo SF, Sánchez-Velásquez LR. 2014. Propagación por enraizamiento de estacas y conservación de árboles plus extintos de *Pinus patula* procedentes del norte de Veracruz, México. *Madera y Bosques* **20**: 85-96. DOI: <https://doi.org/10.21829/myb.2014.201178>
- Archibold OW. 1989. Seed banks and vegetation processes in coniferous forests. In: Leck MA, Parker VT, Simpson RL, eds. *Ecology of Soil Seed Banks*, pp. 107-122. San Diego, USA: Academic Press. ISBN: 978-0-12-440405-2
- Arias-Le Claire H. 2001. Remoción y germinación de semillas de *Dipteryx panamensis* y *Carapa guianensis* en bosques fragmentados de Sarapiquí, Costa Rica. *Revista Forestal Centroamericana* **34**: 42-46.
- Bordones A, De Gracia N, Díaz D, Rodríguez R, Chen A. 2018. Comparación de la efectividad en la protección de cultivos de tomates con insecticidas orgánicos a base de: ajo (*Allium sativum*) y Nim (*Azadirachta indica*). *Revista de Iniciación Científica* **4**: 39-42. DOI: <https://doi.org/10.33412/rev-ric.v4.0.1817>
- Bretz F, Hothorn T, Westfall PH. 2011. *Multiple Comparisons Using R*. FL, USA: CRC Press, Boca Raton: ISBN: 9781584885740
- Bueno A, Baruch Z. 2011. Soil seed bank and the effect of needle litter layer on seedling emergence in a tropical pine plantation. *Revista de Biología Tropical* **59**: 1071-1079. DOI: <https://doi.org/10.15517/rbt.v0i0.3379>
- Campos CM, Giannoni SM, Taraborelli P, Borghi CE. 2007. Removal of mesquite seeds by small rodents in the Monte desert, Argentina. *Journal of Arid Environments* **69**: 228-236. DOI: <https://doi.org/10.1016/j.jaridenv.2006.10.002>
- Campanhã-Bechara F, Reis A, Bourscheid K, Vieira NK, Trentin BE. 2013. Reproductive biology and early establishment of *Pinus elliottii* var. *elliottii* in Brazilian sandy coastal plain vegetation: implications for biological invasion. *Scientia Agricola* **70**: 88-92. DOI: <https://doi.org/10.1590/S0103-90162013000200005>
- Carrillo-Anzures F, Vera-Castillo G, Magaña-Torres OS, Guldin JM, Guries RP. 2009. Seeds stored in the forest floor in a natural stand of *Pinus montezumae* Lamb. *Revista Ciencia Forestal en México* **34**: 41-60.
- Cavieres LA, Arroyo MT. 2001. Persistent soil seed banks in *Phacelia secunda* (Hydrophyllaceae): experimental detection of variation along an altitudinal gradient in the Andes of central Chile (33 S). *Journal of Ecology* **89**: 31-39. DOI: <https://doi.org/10.1046/j.1365-2745.2001.00514.x>
- Ceballos G, Oliva G. 2005. *Los mamíferos silvestres de México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Ciudad de México: Fondo de Cultura Económica,

- Colección: Sección de Obras de Ciencia y Tecnología. ISBN 970-9000-30-6
- Corral-Aguirre J, Sánchez-Velásquez LR. 2006. Seed ecology and germination treatments in *Magnolia dealbata*: an endangered species. *Flora* **201**: 227-232.  
DOI: <https://doi.org/10.1016/j.flora.2005.07.004>
- Cortes-Flores J, Cornejo-Tenorio G, Ibarra-Manríquez G. 2011. Fenología reproductiva de las especies arbóreas de un bosque neotropical. *Interciencia* **36**: 608-613.
- Côté M, Ferron J, Gagnon R. 2003. Impact of seed and seedling predation by small rodents on early regeneration establishment of black spruce. *Canadian Journal of Forest Research* **33**: 2362-2371. DOI: <https://doi.org/10.1139/x03-167>
- Crawley MJ. 2007. *The R Book*. Chichester, UK: John Wiley & Sons, Ltd, ISBN-13: 978-0-470-51024-7.
- De Souza-Maia M, Maia FC, Pérez MA. 2006. Bancos de semillas en el suelo. *Agriscientia* **23**: 33-44.  
DOI: <https://doi.org/10.31047/1668.298x.v23.n1.2689>
- Farjon A, Pérez de la Rosa JA, Styles BT. 1997. *A field guide to the pines of Mexico and Central America*. Oxford: Kew Publishing, Royal Botanical Garden. ISBN-13: 978-1900347372
- Fenner M, Thompson K. 2005. *The Ecology of Seeds*. Cambridge, UK: Cambridge University Press. ISBN: 9780511614101; DOI: <https://doi.org/10.1017/CBO9780511614101>
- Ferrandis P, De las Heras J, Martínez-Sánchez JJ, Herranz JM. 2000. Influence of a low-intensity fire on a *Pinus halepensis* Mill. forest seed bank and its consequences on the early stages of plant succession. *Israel Journal of Plant* **49**: 105-114.
- Fleury M, Rodrigues RR, Do Couto HTZ, Galletti M. 2014. Seasonal variation in the Fate of Seeds under Contrasting Logging Regimes. *PLoS ONE* **9**: e90060.  
DOI: <https://doi.org/10.1371/journal.pone.0090060>
- Flores-Peredo R, Sánchez-Velásquez LR, Galindo-González J, Morales Mávil JE. 2011. Post-dispersed pine seed removal and its effect on seedling establishment in a Mexican Temperate Forest. *Plant Ecology* **212**: 1037-1046.  
DOI: <https://doi.org/10.1007/s11258-010-9884-9>
- Flores-Peredo R, Bolivar-Cimé BS. 2016. Pine seed predation by mice: an experimental assessment of preference. *Animal Biodiversity and Conservation* **39**: 173-184.
- Flores-Peredo R, Vazquez-Domínguez G. 2016. Influence of vegetation type and season on rodent assemblage in a Mexican temperate forest mosaic. *Therya* **3**: 357-369.
- García E. 2004. *Modificaciones al Sistema de Clasificación Climática de Köppen*. México, DF: Instituto de Geografía-Universidad Nacional Autónoma de México. ISBN 9703210104.
- Guerriero V. 2012. Power law distribution: method of multi-scale inferential statistics. *Journal of Modern Mathematics Frontier* **1**: 21-28.
- Hulme PE. 1998. Post-dispersal seed predation and seed bank persistence. *Seed Science Research* **8**: 513-519.  
DOI: <https://doi.org/10.1017/S0960258500004487>
- Hulme PE, Benkman CW. 2002. Granivory. In: Herrera C, Pellmyr O, eds. *Plant Animal Interactions: An Evolutionary Approach*, pp. 132-154, Oxford: Blackwell. ISBN: 978-0-632-05267-7
- Hulme PE, Kollmann J. 2005. Seed Predator Guilds, Spatial Variation in Post-dispersal Seed Predation and Potential Effects on Plant Demography: a Temperate Perspective. In: Forget PM, Lambert JE, Hulme PE, Vander Wall SB, eds. *Seed Fate: Predation, Dispersal and Seedling Establishment*, pp. 9-30, Wallingford: CAB International. ISBN-13: 978-0851998060
- Karunamoorthi K, Hailu T. 2014. Insect repellent plants traditional usage practices in the Ethiopian malaria epidemic-prone setting: An ethnobotanical survey. *Journal of Ethnobiology and Ethnomedicine* **10**: 22-32.  
DOI: <https://doi.org/10.1186/1746-4269-10-22>
- Kolotelo D, Van Steenis E, Peterson M, Bennett R, Trotter D, Dennis J. 2001. *Seed Handling Guidebook*, Canada: British Columbia Ministry of Forests. ISBN 0772646589, 9780772646583
- Kramer NB, Johnson FD. 1987. Mature forest seed banks of three habitat types in central Idaho. *Canadian Journal of Botany* **65**: 1961-1966. DOI: <https://doi.org/10.1139/b87-269>
- Lobo N, Duong M, Millar JS. 2009. Conifer-seed preferences of small mammals. *Canadian Journal Zoology* **87**: 773-780.  
DOI: <https://doi.org/10.1139/Z09-070>
- López-Toledo L, Heredia-Hernández M, Castellanos-Acuña D, Blanco-García A, Saénz-Romero C. 2017. Reproductive investment of *Pinus pseudostrobus* along an altitudinal gradient in Western Mexico: implications of climate change. *New Forests* **48**: 867-881.  
DOI: <https://doi.org/10.1007%2Fs11056-017-9602-8>
- Magalhaes VB, Espirito-Santo NB, Salles LFP, Soares H, Oliveira PS. 2018. Secondary seed dispersal by ants in Neotropical cerrado savanna: species-specific effects on seeds and seedlings of *Siparuna guianensis* (Siparunaceae). *Ecological Entomology* **43**: 665-674.  
DOI: <https://doi.org/10.1111/een.12640>
- Matamoros-Juárez DA, Gaitán-Martínez DA. 2017. *Evaluación de cuatro alternativas de producción en huertos urbanos sobre el crecimiento, rendimiento y fluctuación poblacional de insectos plagas en el cultivo de la chiltoma (Capsicum annum L.) CV. Nathalie Managua*. 2016. Engineering Thesis. Universidad Nacional Agraria.
- Mátyás C, Berki I, Czúcz B, Gálos B, Móczis N, Rasztovits E. 2010. Future of beech in Southeast Europe from the perspective of evolutionary ecology. *Acta Silvatica* **6**: 91-110.
- McCullagh P, Nelder JA. 1989. *Generalized Linear Models*. London: Chapman & Hall Press.
- McGee A, Feller MC. 1993. Seed banks of forested and disturbed soils in southwestern British Columbia. *Canadian Journal of Botany* **71**: 1574-1583. DOI: <https://doi.org/10.1139/b93-192>
- Morales-Mávil JE, Suárez-Domínguez EA, Mestizo-Rivera LR, Villa-Cañedo JT, Enríquez-Roa J, Corona-López C, Bello-Gutiérrez J, González-Christen A. 2007. Riqueza, diversidad y distribución de anfibios, reptiles y mamíferos en las Áreas Naturales Protegidas del estado de Veracruz. Segundo Informe Técnico. Xalapa, Veracruz: Universidad Veracruzana/CONACYT.
- Murdoch AJ, Ellis RH. 2000. Dormancy, viability and longevity. In: Fenner M. ed. *Seeds: The Ecology of Generation in Plant Communities*, pp. 183-214, Wallingford, UK: CABI Publishing. ISBN: 9780511614101; DOI: <https://doi.org/10.1093/aob/mcf038>

- Murrieta-Hernández DM, Pineda-López M del R, Noa-Carranza JC, Mata-Rosas M, Zulueta-Rodríguez R, Flores-Estévez N. 2014. The structure of *Pinus hartwegii* at the Cofre de Perote, Veracruz, México. *Open Journal of Forestry* **4**: 291-301. DOI: <https://doi.org/10.4236/ojf.2014.44035>
- Musálem-Santiago MA, Solís-Pérez MA. 2000. *Monografía de Pinus hartwegii*. Estado de México: SAGAR.INIFAP. ISBN: 968-800-482-0
- Narave H. 1985. La Vegetación del Cofre de Perote, Veracruz, México. *Biótica* **10**: 35-64.
- Narave H, Taylor K. 1997. Pinaceae. In: *Flora de Veracruz*. Fascículo 98. Xalapa, Veracruz, México: Instituto de Ecología y Universidad de California. ISBN: 968-7863-02-1
- Ofori BY, Attuquayefio DK, Owusu EH, Musah RKY, Quartey JK, Ntiama-Baidu Y. 2015. Seasonal changes in small mammal assemblage in Kogyae Strict Nature Reserve, Ghana. *International Journal of Biodiversity and Conservation* **7**: 238-244. DOI: <https://doi.org/10.5897/IJBC2015.0835>
- Pensado-Fernández JA, Sánchez-Velásquez LR, Pineda-López MR, Díaz-Fleischer F. 2014. Plantaciones forestales vs. regeneración natural in situ: el caso de los pinos y la rehabilitación en el Parque Nacional Cofre de Perote. *Botanical Sciences* **92**: 617-622. DOI: <https://doi.org/10.17129/botsci.109>
- Perry JP. 2009. *The Pines of Mexico and Central America*. Timber Press, Inc, Portland, Oregon.
- Pratt DW, Black RA, Zamora BA. 1984. Buried viable seed in a ponderosa pine community. *Canadian Journal of Botany* **62**: 44-52. DOI: <https://doi.org/10.1139/b84-008>
- Quiroga MP, Premoli AC. 2013. El rol de las poblaciones marginales en la conservación del acervo genético de la única conífera del sur de Yungas en Argentina y Bolivia, *Podocarpus parlatorei* (Podocarpaceae). *Ecología en Bolivia* **48**: 4-16.
- Rautenbach A, Dickerson T, Schoeman MC. 2014. Diversity of rodent and shrew assemblages in different vegetation types of the savannah biome in South Africa: no evidence for nested subsets or competition. *African Journal of Ecology* **52**: 30-40. DOI: <https://doi.org/10.1111/aje.12081>
- R Development Core Team. 2016. R: A language and environment for Statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org> (consulted May 1, 2016)
- SAS. 2015. SAS/IML® 14.1 User's Guide. Cary, NC: SAS Institute Inc.
- Sáenz-Romero C, Guzmán-Reyna RR, Rehfeldt GE. 2006. Altitudinal genetic variation among *Pinus oocarpa* populations in Michoacán, Mexico. *Forest Ecology and Management* **229**: 340-350. DOI: <https://doi.org/10.1016/j.foreco.2006.04.014>
- Sánchez-Cordero V. 2001. Elevational gradients of diversity for rodents and bats in Oaxaca, Mexico. *Global Ecology and Biogeography* **10**: 63-76. DOI: <https://doi.org/10.1046/j.1466-822x.2001.00235.x>
- Shuai LY, Ren ChL, Yan WB, Song YL, Zeng ZG. 2017. Different elevational patterns of rodent species richness between the southern and northern slopes of a mountain. *Scientific Reports* **7**: 1-12. DOI: <https://doi.org/10.1038/s41598-017-09274-2>
- Simpson RL, Leck MA, Parker VT. 1989. Seed banks: general concepts and methodological issues. In: Leck MA, Parker VT, Simpson RL, eds. *Ecology of Soil Seed Banks*, pp. 3-8. New York, USA, Academic Press. ISBN: 978-0-12-440405-2; <https://doi.org/10.1016/B978-0-12-440405-2.X5001-5>
- Thomas PA, Wein RW. 1985. Delayed emergency of four conifer species on postfire seedbeds in eastern Canada. *Canadian Journal of Forest Research* **15**: 727-729. DOI: <https://doi.org/10.1139/x85-119>
- Thompson K, Fenner M. 2005. *The Ecology of Seeds*. Cambridge: University Press. ISBN: 9780511614101; DOI: <https://doi.org/10.1017/CBO9780511614101>
- Trabaud L, Martínez-Sánchez JJ, Ferrandis P, González-Ochoa AI, Herranz JM. 1997. Végétation épigée et banque de semence du sol: leur contribution à la stabilité cyclique des pinédes mixtes de *Pinus halepensis* et *P. pinaster*. *Canadian Journal of Botany* **75**: 1012-1021. DOI: <https://doi.org/10.1139/b97-112>
- Vander-Wall SB. 2008. On the relative contributions of wind vs. animals to seed dispersal of four Sierra Nevada Pines. *Ecology* **89**: 1837-1849. DOI: <https://doi.org/10.1890/07-0409.1>
- Vander-Wall SB, Beck MJ. 2012. A comparison of frugivory and scatter-hoarding seed-dispersal syndromes. *The Botanical Review* **78**: 10-31. DOI: <https://doi.org/10.1007/s12229-011-9093-9>
- Vázquez-Ramírez J. 2014. *Fenología reproductiva de las comunidades vegetales del Parque Nacional Cofre de Perote, Veracruz, México*. MsC. Thesis. Universidad Veracruzana.
- Wang GM, Wolff JO, Vessey SH, Slade NA, Whitam JW, Merritt JF, Hunter Jr ML, Elias SP. 2009. Comparative population dynamics of *Peromyscus leucopus* in North America: influences of climate, food and density dependence. *Population Ecology* **51**: 133-142. DOI: <https://doi.org/10.1007/s10144-008-0094-4>
- Wang Y, Wang J, Lai L, Jiang L, Zhuang P, Zhang L, Zheng Y, Baskin JM, Baskin CC. 2014. Geographic variation in seed traits within and among forty-two species of Rhododendron (Ericaceae) on the Tibetan plateau: relationships with altitude, habitat, plant height, and phylogeny. *Ecology and Evolution* **4**: 1913-1923. DOI: <https://doi.org/10.1002/ece3.1067>
- Zar JH. 1999. *Biostatistical analysis*. New Jersey: Prentice-Hall. ISBN: 0-13481S42-X
- Zelikova TJ, Dunn RR, Sanders NJ. 2008. Variation in seed dispersal along an elevational gradient in Great Smoky Mountains National Park. *Acta Oecologica* **34**: 155-162. DOI: <https://doi.org/10.1016/j.actao.2008.05.002>

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