UNIVERSIDAD AUTONOMA DE AGUASCALIENTES

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TESIS

DISPERSIÓN POR ENDOZOOCORIA Y DIPLOENDOZOOCORIA DE ESPECIES VEGETALES EN BOSQUES DEL ANP SIERRA FRÍA, MÉXICO.

PRESENTA

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PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS BIOLÓGICAS

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Endozoochorous dispersal of forest seeds by carnivorous mammals in Sierra Fría, Aguascalientes, Mexico

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Abstract

Some carnivorous mammals ingest fruit and disperse seeds of forest plant species capable of colonizing disturbed areas in ecosystems. The objective of the present study was to evaluate the dissemination of *Arctostaphylos pungens* and *Juniperus deppeana* seeds by the gray fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), and other carnivores in the Protected Natural Area Sierra Fría, in Aguascalientes, Mexico. Scat collection was undertaken via transects using the direct search method, while the seasonal phenology of *A. pungens* and *J. deppeana* was evaluated by recording flower and fruit abundance on both the plant and the surrounding forest floor ground. Seed viability was assessed by optical densitometry via X-ray and a germination test. It was found that the gray fox, coyote, ringtail (*Bassariscus astutus*), and bobcat (*Lynx rufus*) disseminated seeds of *A. pungens* (212 ± 48.9 seeds/scat) and *J. deppeana* (23.6 ± 4.9 seeds/scat), since a large proportion of the collected scat of these species contained seeds (28/30 = 93.33%, 12/43 = 27.9%, 6/12 = 50% and 7/25 = 28% respectively). The gray fox, coyote, ringtail, and bobcat presented an average of seed dispersion of both plant species of 185.4 ± 228.7, 4.0 ± 20.0, 12.1 ± 30.4, and 0.8 ± 1.5 per scat; the seed proportions in the gray fox, coyote, ringtail, and bobcat were 89.6/10.4%, 82.3/17.7%, 90.4/9.6%, and 38.1/61.9% for *A. pungens* and *J. deppeana*, respectively. The phenology indicated a finding related to the greater abundance of ripe fruit in autumn and winter ($p < .01$). This coincided with the greater abundance of seeds found in scats during these seasons. Endozoochory and diploendozoochory enhanced the viability and germination of the seeds ($p > .05$), except in those of *A. pungens* dispersed by coyote. These results suggest that carnivores, particularly the gray fox, the coyote, and the bobcat, play an important role in forest seed dissemination, and thus forest regeneration, by making both a quantitative and qualitative contribution to the dispersal of the two pioneer species under study.

KEYWORDS

Canis latrans, endozoochory, scats, seed dispersal, *Urocyon cinereoargenteus*

ORIGINAL RESEARCH

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Seed dispersal by carnivores in temperate and tropical dry forests

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Abstract

The seed dispersal mechanisms and regeneration of various forest ecosystems can benefit from the actions of carnivores via endozoochory. This study was aimed to evaluate the role of carnivores in endozoochory and diploendozoochory, as well as their effect on seed viability, scarification, and germination in two forest ecosystems: temperate and tropical dry forest. We collected carnivore scat in the Protected Natural Area of Sierra Fría in Aguascalientes, Mexico, for 2 years to determine the abundance and richness of seeds dispersed by each carnivore species, through scat analysis. We assessed seed viability through optical densitometry using X-rays, analyzed seed scarification by measuring seed coat thickness using a scanning electron microscope, and evaluated seed germination in an experiment as the percentage of seeds germinated per carnivore disperser, plant species, and forest type. In the temperate forest, four plant species (but mainly *Arctostaphylos pungens*) were dispersed by four mammal species. The gray fox dispersed the highest average number of seeds per scat (66.8 seeds). Bobcat dispersed seeds through diploendozoochory, which was inferred from rabbit (*Sylvilagus floridanus*) hair detected in their scats. The tropical dry forest presented higher abundance of seeds and richness of dispersed plant species (four species) than in the temperate forest, and the coati dispersed the highest number of seeds (8,639 seeds). Endozoochory and diploendozoochory did not affect viability in thick-testa seeds (1,480 μm) in temperate forest and thin-testa seeds (281 μm) in tropical dry forest. Endozoochory improved the selective germination of seeds. Nine plant species were dispersed by endozoochory, but only one species (*Juniperus* sp.) by diploendozoochory. These results suggest that carnivores can perform an important ecological function by dispersing a great abundance of seeds, scarifying these seeds causing the formation of holes and cracks in the testas without affecting viability, and promoting the selective germination of seeds.

KEYWORDS

carnivores, scanning electron microscopy, seed dispersion, temperate forest, tropical dry forest

AGRADECIMIENTOS

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ACRÓNIMOS Y ABREVIATURAS

ANP	Área Natural Protegida		**P**	Valor de significancia
ANP-SF	Área Natural Protegida Sierra Fría		**r**	Índice de correlación
BT	Bosque templado		**s**	Segundo
BTS	Bosque tropical seco		**SD**	Desviación Estándar (Standard Deviation)
cm	Centímetro		**Sp.**	Especie
dds	Días después de la siembra		**UTM**	Universal Transversal Mercator
et al.	Y colaboradores		**x**	Multiplicación
g	Gramos		**%**	Porcentaje
ha	Hectárea		**'**	Minuto
h	Horas		**"**	Segundo
HUAA	Herbario de la Universidad Autónoma de Aguascalientes		**°C**	Grados Celsius
km	Kilómetro		**>**	Mayor
km^2	Kilómetro cuadrado		**≤**	Menor o igual
			α	Coeficiente de significancia
Kv	Kilovatio		$\square\bar{x}$	Media aritmética /Promedio
L	Litro		**µm**	Micrómetros
m	Metro			
MEB	Microscopía Electrónica de Barrido			
ml	Mililitro			
mm	Milímetro			
N	Norte/ Número de muestra			
O	Oeste			

RESUMEN GENERAL

Los mecanismos de dispersión de semillas forestales y de regeneración de diversos ecosistemas boscosos alterados se complementan con la participación de los carnívoros a través de la endozoocoria y diploendozoocoria, es así, que una de las fases más críticas de estos sistemas de dispersión por carnívoros, es el paso de las semillas a través del sistema digestivo del animal. De acuerdo con lo anterior, los objetivos del estudio fueron: 1) Definir las especies de mamíferos del orden carnívora que realizan la dispersión de semillas por endozoocoria y las que lo realizan por diploendozoocoria en los bosques templados (BT) y bosques tropicales secos (BTS) de la Sierra Fría, 2) Examinar la endozoocoria y diploendozoocoria de los mamíferos en el paisaje a través de los SIGs y 3) Comparar la eficiencia de la endozoocoria y la diploendozoocoria a través de la viabilidad, proceso de germinación y análisis del grosor (desgaste) de las testas por MEB. Encontramos que la zorra gris, coyote, cacomixtle, el coatí y gato montés son los dispersores en el BT y BTS, este último con mayor abundancia y riqueza de especies vegetales dispersas. En ambos bosques, la endozoocoria y la diploendozoocoria no afectaron la viabilidad y germinación de todas las especies de semillas. Estos resultados sugieren que los carnívoros dispersores se adaptan a la abundancia y riqueza de semillas en los bosques donde habitan, para lo cual desarrollan importantes funciones ecológicas al dispersar, escarificar y promover la germinación selectiva de semillas de testa gruesa en BT y semillas de testa delgada en BTS.

Palabras clave: Bosques, carnívoro, endozoocoria, diploendozoocoria

ABSTRACT

The mechanisms of forest seed dispersal and regeneration of various altered forest ecosystems are complemented by the participation of carnivores through endozoochory and diploendozoochory, thus, one of the most critical phases of these dispersal systems by carnivores is the passage of the seeds through the digestive system of the animal. In accordance with the above, the objectives of the study were: 1) Define the species of mammals of the carnivorous order that carry out seed dispersal by endozoochory and those that carry it out by diploendozoochory in temperate forests (TF) and dry tropical forests (DTF) of the Sierra Fría, 2) Examine endozoochory and diploendozoochory of mammals in the landscape through GIS and 3) Compare the efficiency of endozoochory and diploendozoochory through viability, germination process and thickness analysis (wear) of the testas by SEM. We found that the gray fox, coyote, ringtail, coati and bobcat are the dispersers in the temperate forest and dry tropical forest under study, the latter with a greater abundance and richness of dispersed plant species. In both forests, endozoochory and diploendozoochory did not affect the viability or germination of all seed species. These results suggest that dispersing carnivores adapt to the abundance and richness of seeds in forests where they inhabit, for which they develop important ecological functions by dispersing, scarifying and promoting the selective germination of thick-testa seeds in temperate forests and thin-testa seeds in dry tropical forests.

Keywords: carnivorous, diploendozoochory, endozoochory, forests

CAPÍTULO I

LA IMPORTACIA DE LA FAUNA EN LA DISPERSIÓN DE SEMILLAS: LA ENDOZOORIA Y DIPLOENDOZOOCORIA

INTRODUCCIÓN

Los bosques templados

Los bosques templados son importantes biomas que brindan servicios ecosistémicos en todo el mundo, sin embargo, también se presentan diferentes tipos de disturbios que alteran su estructura, composición y funcionamiento (Pickett y White, 1985). Las actividades humanas y los procesos naturales son los principales disturbios que han cambiado significativamente las características de estos ecosistemas en la tierra (Foley *et al.*, 2005).

Debido a lo anterior, las perturbaciones/alteraciones naturales y las provocadas por el ser humano modifican la estructura de los bosques de una manera única, ocasionando cambios en el tamaño, la composición y distribución de las reservas de carbono forestal y transferencia de carbono a la atmósfera (Lorenz y Lal, 2010). Los factores antrópicos más importantes son: los incendios forestales y el cambio de uso del suelo; Escribano-Ávila *et al.* (2015) hacen referencia a las implicaciones que tiene el cambio de uso de suelo en las comunidades vegetales y animales, ya que esta actividad es uno de los motores de cambio climático más importantes a escala global (Sala *et al.*, 2000) y los efectos negativos como consecuencias de la intensificación en los usos del suelo como la pérdida de biodiversidad, fragmentación y destrucción de hábitat (World Resources, 2005).

Dentro del cambio del uso de suelo están las actividades para fines agrícolas, siendo las más comunes y que generalmente resultan en la alteración o la eliminación de la estructura del bosque, matando o eliminando todos los árboles (Franklin *et al*., 2007), esto se ve reflejado en la reducción de la cobertura terrestre del dosel de los árboles (Mascorro *et al*., 2014).

Por otro lado, el bosque templado mexicano presenta un patrón de distribución muy fragmentada, ocasionada por las altas tasas de deforestación, prácticas agrícolas mal realizadas y la ganadería de animales domésticos (Luna *et al*., 2000). No solo en México, sino en todos los ecosistemas del planeta, la fragmentación junto con la reducción en superficie y la perturbación han aumentado recientemente a un ritmo alarmante, así como la ocupación humana; la deforestación ha producido la erosión y disminución de la fertilidad del suelo durante miles de años (Mascorro *et al*., 2014).

Particularmente, el Área Natural Protegida Sierra Fría, localizada al Noroeste del Estado de Aguascalientes está constituida por diferentes tipos de ecosistemas, entre ellos por los bosques templados (Sosa-Ramírez *et al*., 2015). De acuerdo a los estudios de Minnich *et al*. (1994), estos han tenido disturbios recurrentes en los últimos años provocados tanto por factores naturales como antropogénicos, que van desde la introducción de fauna exótica (Sosa-Ramírez *et al*., 2015) hasta la aparición esporádica de plagas y enfermedades (Sánchez-Martínez *et al*., 2012; Díaz-Núñez *et al*., 2014) Todos estos disturbios han ocasionado, como respuesta, un proceso de recuperación y cambios en la estructura de la vegetación, observándose una dinámica en la cual las especies del género *Arctostaphylos* y *Juniperus* son las pioneras, sobre todo en las mesas y los sitios de fácil acceso (Díaz-Núñez *et al*., 2016).

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Los bosques tropicales secos

Los bosques o selvas secas tropicales y subtropicales están en regiones generalmente entre 20 y 10º de latitud y una de sus principales características es que siempre presentan una larga temporada de sequía. Por lo anterior, pueden perder entre el 50 y 100% del follaje en esta época, además tienen otras características propias de este ecosistema como presentar coberturas de dosel mayor al 30%, predominancia de especies de hoja ancha que ocupan más del 75% del dosel. En ellas se concentra una gran variedad de flora y fauna que pueden presentar extraordinarias adaptaciones a las presiones climáticas (Olson *et al.*, 2000).

De un total de 130, 740, 587 km^2, que corresponden a la superficie terrestre del planeta, el 35% está compuesta por las selvas y los bosques. Particularmente, las selvas secas y subcaducifolias cubren una superficie total de 3, 178 00 km^2, es decir, el 2.4% de la superficie terrestre y el 7% por bosques (WCMC, 2000). El 8.8% de la superficie de América Latina y el Caribe corresponde a este bioma (PNUMA-CEPAL, 2001). Además, encontramos que las nominaciones más comunes, además de selvas secas o bajas caducifolias, incluyen las de bosque tropical seco (Gentry, 1982) y bosque tropical seco estacional o seasonally dry tropical forest (Bullock *et al.*, 1995).

Por otra parte, las selvas tropicales secas tienen una amplia distribución en México, ya que se extienden desde el paralelo 29° de latitud Norte (Búrquez *et al.*, 1999) hasta la frontera con Guatemala, en el sur del país. Principalmente, en el Estado de Aguascalientes, la distribución de vegetación más grande de bosque tropical seco se encuentra en el municipio de Calvillo que está amenazado principalmente por el avance de la frontera agrícola (IEFyS, 2012).

Mamíferos en los bosques o selvas secas tropicales

Los mamíferos de las selvas secas del Pacífico de México y Centroamérica según Baker (1967) y Ceballos y Rodríguez (1993) están bien caracterizados. En México más del 30% de los mamíferos del país se encuentran en este ecosistema, lo que hace que sea un importante centro de concentración (Ceballos y García, 1995) al albergar también, poco más del 30% de las especies endémicas del país (Ceballos y Martínez, 2010). Sin embargo, 47 especies de las selvas secas se encuentran dentro de alguna categoría de riesgo como consecuencia de la fragmentación y pérdida del hábitat (IUCN, 2008; Semarnat, 2002)

Particularmente, la marcada estacionalidad que presentan las selvas secas causa una extraordinaria adaptación de la fauna a través de las estaciones, principalmente en la época de secas, donde los recursos son limitados. De esta manera, una respuesta adaptativa a esta condición es la migración estacional de algunas especies hacia otros lugares para encontrar una mayor disponibilidad de recursos y alimento (Ceballos, 1995; Galindo, *et al*., 2004). Por lo tanto, la estacionalidad de las selvas secas, con una marcada época de lluvias y secas, es uno de los factores ambientales más relevantes para los mamíferos, ya que el tipo de vegetación que esté presente en la estación influye en su diversidad y abundancia (Ceballos, 1995; Janzen, 1983).

Es así, que muchas especies de mamíferos utilizan este ecosistema durante las lluvias que causa la presencia de follaje, flores, frutos y semillas, permitiendo que las poblaciones de mamíferos puedan mantenerse, y al mismo tiempo propiciar la polinización y dispersión de las especies vegetales (Janzen, 1982a, b). Dentro de los diversos sistemas de dispersión de semillas de plantas en estos ecosistemas, una mayor diversidad de mamíferos propicia esta diseminación. La ventaja de la mamalocoria (dispersión de semillas por mamíferos) es, el transporte efectivo de semillas y su diseminación en áreas extensas. Por ello, la dinámica de las

comunidades vegetales depende en gran parte de los patrones de dispersión de las semillas (Hubbell, 1979).

La endozoocoria

Los ecosistemas forestales son valiosos, no sólo por los servicios de aprovisionamiento que proporcionan y la conservación de la biodiversidad (Díaz *et al.*, 2009), sino también por su papel crucial para mitigar el cambio climático (Turner, 2010). Estos se ven afectados por diferentes tipos de disturbios: los ocasionados naturalmente o por causa del ser humano (Peters *et al.*, 2013) que han cambiado significativamente las características de estos ecosistemas en la Tierra (Foley *et al.*, 2005). Los cambios producidos por las actividades humanas amenazan la sostenibilidad de estos ecosistemas y afectan su capacidad para proporcionar bienes y servicios diversos, tales como la regulación del clima (Laurance y Williamson, 2002), la biodiversidad (Jenkins, 2003), la producción de agua y el secuestro de carbono.

Por otro lado, los paisajes forestales modificados por el ser humano, están en peligro de perder su capacidad de recuperación y por lo tanto obstaculizan su potencial para autorecuperarse después del disturbio (Chazdon, 2003) y por esta razón, la plantación de árboles se ha adoptado en muchas regiones del mundo como uno de los principales métodos para la restauración forestal (Rodrigues *et al.*, 2011). Sin embargo, los ecosistemas tienen su propio mecanismo de autorrecuperación a través de los sistemas de dispersión de semillas y los rebrotes de las especies vegetales alteradas.

Los sistemas de dispersión son una parte esencial en la distribución natural de las especies vegetales, en la movilización y el intercambio de material genético dentro y fuera de las poblaciones (Rocas, 1982). Por lo tanto, la dispersión es un

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proceso activo y dinámico de transporte que tiende a ubicar la unidad de dispersión en sitios seguros desde el punto de vista físico y competitivo (De Noir, 2002).

Existen factores abióticos y bióticos asociados a los sistemas de dispersión en bosques (Correa *et al.*, 2015). En los factores bióticos encontramos a los animales dispersores, cuya dispersión a través de estos es denominada Zoocória. El gremio de dispersores animales es clave en la configuración de las comunidades vegetales, ampliación de las poblaciones, fundando otras nuevas y creando bancos de semillas en el suelo. Es de importancia para la configuración del paisaje este tipo de dispersión que son considerados uno de los primeros arquitectos paisajistas porque determinan la diversidad, abundancia y distribución espacial de los bancos de semillas disponibles para establecer comunidades de plantas (Herrera, 1985).

Por otra parte, la dispersión de semillas en numerosas especies de plantas se alcanza con el proceso de endozoocoria en el que las plantas producen los frutos carnosos nutritivos que son consumidos por los animales y que, más adelante, defecan las semillas (Cypher y Cypher, 1999). El vertebrado frugívoro representa el comienzo de la dispersión primaria para los propágulos de muchas plantas (Montiel, 2000). De esta manera, los animales que dispersan semillas y afectan la capacidad de la germinación son considerados legítimos dispersores de semillas (Silverstein, 2005) y se reconoce que los carnívoros consumen grandes cantidades de frutos, retienen las semillas en el tracto digestivo por largos periodos de tiempo y recorren áreas extensas, llegando así a ser un factor importante en el transporte y movimiento de las semillas (Cypher y Cypher, 1999).

Tradicionalmente, los mamíferos terrestres no han sido considerados como dispersantes importantes en comparación con otros grupos como las aves o los murciélagos (Herrera, 1989; Willson, 1993). Sin embargo, recientes estudios muestran que en el contexto ecológico de campos abandonados, así como en otras áreas degradadas, son los mamíferos terrestres frugívoros los principales

responsables en la dispersión de las semillas (Escribano-Avila *et al*., 2012; Escribano-Avila *et al*., 2014) y regeneración de la vegetación.

Así mismo, los mamíferos tienen grandes áreas de distribución y periodos de tiempo en la retención de semillas en el intestino, lo que les permite ser clave para la dispersión a larga distancia (Jordano et al., 2007), sin embargo, el paso del fruto a través del tracto gastrointestinal puede tener un efecto benéfico, perjudicial o neutro en las semillas (Murray *et al*., 1994).

Por lo tanto, el patrón de dispersión realizado por este grupo simula una práctica de restauración activa basada en la plantación de árboles pioneros o grupos (islotes de bosques) que actúan como un detonante para la actividad de un conjunto complejo de dispersores en las tierras deforestadas (Lamb *et al*., 2005; Benayas *et al*., 2008). Lo anterior termina por ser una ventaja excepcional que los carnívoros producen y lo hacen de forma constante en el paisaje (Escribano-Avila *et al*., 2013).

La diploendozoocoria

Las estimaciones del número de plantas dispersadas por los animales varían ampliamente, pero se estima que el mecanismo principal de dispersión es el endozoocoria en hasta el 94% de las plantas leñosas, según la región (Jordano, 2000; Buitrón-Jurado y Ramírez, 2014). A pesar de las adaptaciones de las plantas, existe evidencia creciente de que el destino final de las semillas no se determina necesariamente por los animales que los eliminan o dispersan de la planta madre. En lugar de ello, múltiples vectores de dispersión pueden estar implicados en la toma de semillas a su destino final o destrucción (Ozinga *et al*., 2004; Vander Wall y Longland, 2004).

Actualmente, ha aumentado el interés en la diplocoria (dispersión en dos fases, también conocida como "dispersión secundaria" o "dispersión indirecta") que implica una segunda fase de dispersión por hormigas, escarabajos peloteros o

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roedores dispersos que transportan físicamente las semillas a una nueva ubicación (Vander Wall y Longland 2004), pero se ha prestado relativamente poca atención a la "diploendozoocoria", es decir, la dispersión de semillas que implica la ingestión de la semilla por dos o más especies diferentes de animales en secuencia, generalmente implicados una presa y su depredador (Hämäläinen, 2017).

La diploendozooria fue investigada y documentada por primera ocasión por Darwin en (1859) y desde ese año las observaciones para este proceso de dispersión han sido reportados con poca frecuencia. Aunque este tipo de dispersión se ha abordado recientemente de forma más rigurosa utilizando experimentos (Nogales, 1999; Nogales *et al.*, 2007; Padilla y Nogales, 2009; Padilla *et al.*, 2012), sigue siendo desconocida la significación ecológica más amplia de este fenómeno, ya que hasta ahora se han hecho pocos intentos para establecer la importancia de este mecanismo. Presumiblemente, los efectos de la dispersión secundaria dependen en gran medida de las características de los vectores animales y las plantas involucradas, así como de los hábitats que ocupan. Por lo tanto, se tiene que identificar características del proceso de dispersión que es probable que influyen en planta de la forma física a través de la germinación o el éxito de reclutamiento y el acceso a un hábitat adecuado.

Para que un carnívoro mejore los resultados de la dispersión de la semilla, la efectividad de dispersión por la diploendozoocoria debe ser naturalmente más alta que la dispersión por un solo vector (Schupp y Jordano, 2010). Los tipos de plantas y los dispersores primarios y secundarios involucrados influyen en la eficacia de la dispersión y la significación ecológica de la fase de dispersión secundaria. La participación de un carnívoro en la segunda fase del proceso de dispersión puede influir en planta de la forma física de tres maneras: mediante el transporte de las semillas, la alteración de su viabilidad y cambiando la cantidad que se dispersan.

Como los carnívoros tienden a recorrer mayores distancias que los frugívoros o herbívoros (Carbone *et al.*, 2005), la distancia de dispersión y el sitio de deposición

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(es decir, la ubicación en la que la semilla termina después de ser procesada por un dispersor, típicamente dentro de las heces o dentro de pellets regurgitados) pueden diferir dramáticamente para las semillas depositadas por dispersores primarios vs. secundarios (Dean y Milton, 1988; Nogales *et al*., 2007, 2012). La dispersión secundaria puede contribuir de manera significativa al rango de dispersión de la planta y a la dinámica de la población, especialmente cuando el dispersor primario tiene un home range relativamente pequeño y está restringido por movimiento o es un especialista en hábitat (Higgins y Richardson 1999; Nogales *et al*., 2012). Por ejemplo, después de consumir una presa con un home range muy pequeño (como un pequeño roedor), un pellet o excreta puede tardar hasta 22 horas en formarse.

La dispersión secundaria por parte de carnívoros de gran alcance puede permitir la colonización de hábitats recientemente adecuados bajo el cambio climático o áreas remotas como las islas (Nogales *et al*., 2012), o puede influir localmente en el número de semillas que ingresan al área. Una facilidad de incrementar la distancia de dispersión puede, sin embargo, reducir la dispersión de éxito o especies especializadas (Herrmann *et al*., 2016). Los dispersores secundarios pueden de este modo depositar semillas en lugares de mala adaptación o fuera de su zona de clima, pero la planta podría, sin embargo, beneficiarse de la dispersión de larga distancia y a través de este proceso colonizar parches de difícil acceso para los sistemas convencionales de dispersión (Nathan *et al*., 2008; Caughlin y Ferguson 2013).

El éxito de la germinación puede ser alterada a través del tratamiento de una semilla en el tracto digestivo de un carnívoro y puede ser afectada diferencialmente por varios dispersores secundarios. Las mejoras en el éxito de la germinación siguen si las semillas se benefician de una doble digestión debido a un tiempo de retención intestinal más largo (Nogales *et al*., 2015) o, posiblemente, si las heces de carnívoros son más ricas en nutrientes o incluye una menor cantidad de semillas competidoras que la del dispersor primario. La germinación o el reclutamiento de

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las plantas probablemente también se vean afectadas por una deposición en microhábitats inadecuados (por ejemplo, en una carretera, en un suelo pobre o en una vegetación densa) o en un sitio que eleve el riesgo de depredación de semillas posterior a la dispersión. Estos determinantes de la efectividad de la dispersión no son exclusivos de la dispersión mediada por carnívoros, pero su importancia en la diploendozoocoria aún no se ha estudiado. Un paso en esta dirección fue tomado por Culot *et al.*, (2015), quienes intentaron cuantificar la importancia relativa de la dispersión secundaria a escala micro por los escarabajos del estiércol, y un enfoque similar también podría ser útil para el estudio de las fases de este tipo de dispersión.

Efectos humanos: pérdida de hábitat y especies

Este mecanismo de dispersión puede influir en la adaptabilidad de la planta a los paisajes alterados por el ser humano y la resiliencia frente a las estructuras cambiantes de la comunidad a través de una mayor distancia de dispersión de semillas, alcanzando hábitats alternativos y el éxito de la germinación de semillas. La dispersión a larga distancia es de creciente importancia para la capacidad de recuperación de los ecosistemas ante los cambios ambientales, tales como la fragmentación del hábitat y el cambio climático, facilitando su rápida dispersión entre áreas desconectadas (Nathan *et al.*, 2008) y al aumentar el flujo de genes entre poblaciones (Bacles *et al.*, 2006). Algunos carnívoros incluso podrían estar creando corredores de semillas entre los fragmentos del hábitat cuando excretan con mayor frecuencia en las características lineales como los senderos (Suárez-Esteban *et al.*, 2013). Además, que tienen el potencial de influir en la magnitud de los efectos perjudiciales de la pérdida y fragmentación del hábitat en las comunidades vegetales. Así bien, la dispersión secundaria por este grupo de mamíferos tiene el potencial de aumentar efectivamente la velocidad potencial de los movimientos de las plantas (Naoe *et al.*, 2016) y la colonización del hábitat vacante. Esto puede

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convertirse en un proceso cada vez más importante a la vanguardia de un cambio de clima debido al cambio climático.

La estructura de las comunidades de carnívoros puede jugar un papel en la determinación de la utilidad de la dispersión secundaria de semillas. Con la excepción del puma (*Puma concolor*), todos los casos documentados de diploendozoocoria hasta la fecha involucraron un mesocarnívoro. La liberación de mesocarnívoros ha sido bien documentada cuando los carnívoros superiores han sido eliminados del paisaje mediante intervenciones humanas (Prugh *et al*., 2009). Los carnívoros también podrían desempeñar un papel en la estabilización de los cambios en la estructura de la comunidad a través de la selección de sus presas. Un estudio realizado en la República Democrática del Congo encontró que todos los dispersores de semillas en el sistema eran cazados por seres humanos, mientras que muy pocos depredadores de semillas experimentaron presión de caza (Beaune *et al*., 2013). Por lo anterior, se necesita más investigación para evaluar los efectos de los carnívoros en la resiliencia de las comunidades de plantas.

La dispersión secundaria por carnívoros no es en absoluto exclusiva de los tipos de diplocoria definido por Vander Wall y Longland (2004); más bien, es muy probable que el transporte adicional de semillas por las hormigas, los escarabajos del estiércol o los roedores dispersos a menudo ocurra después de que las semillas son depositadas por el dispersor secundario. Actualmente se desconoce cuán importante es este fenómeno ecológicamente, pero dada su posible prevalencia y las posibles implicaciones, es posible que ignorarlo pueda perjudicar la interpretación de amplios patrones ecológicos o dificulte los esfuerzos de conservación.

Por todo lo anterior y particularmente hablando de esta región, Aguascalientes ha tenido disturbios en los bosques del Área Natural Protegida (ANP) Sierra Fría (Minnich *et al*., 1994) esto ha ocasionado un proceso de recuperación de la vegetación donde los sistemas de dispersión, especialmente la zoocória que actúa

sobre estos, es clave para que el ecosistema logre su recuperación. Por lo cual es indispensable establecer el verdadero rol ecológico que tiene la fauna en los procesos de recuperación de la vegetación.

PLANTEAMIENTO DEL PROBLEMA

El ANP Sierra Fría ha tenido diferentes disturbios en décadas pasadas (Minnich *et al.*, 1994), lo que ha ocasionado un proceso de recuperación de la vegetación durante esos años hasta la actualidad. Durante este proceso de recuperación se ha observado como las especies pioneras vegetales son las que han tenido una mayor distribución y abundancia en los bosques templados de la sierra; donde anteriormente Díaz *et al.* (2016), estableció que las especies pioneras en este ecosistema son la Manzanita (*Arctostaphylos pungens*) y el Táscate (*Juniperus deppeana*). Debido a su amplia distribución, Rubalcava-Castillo (2017) confirmó que estas especies vegetales son dispersadas en el paisaje a través del proceso de endozoocoria por animales omnívoros, además de otro grupo de mamíferos implicados en la dispersión que podrían traer mayores beneficios al proceso de zoocória, al encontrar evidencias de dispersión de semilla por un carnívoro depredador: el Gato Montés (*Lynx rufus*), comprobándose que habría un proceso secundario de endozoocoria. De acuerdo con la investigación anterior, se encuentra un vacío de información referente al proceso de dispersión de semillas a través de la fauna, particularmente en el doble proceso de endozoocoria denominado diploendozoocoria (Hämäläinen, 2017), el cual, podría tener mayores beneficios para la regeneración de los ecosistemas que la endozoocoria convencional. Por lo anterior, es indispensable comprobar las aportaciones que tienen estos 2 procesos para establecer la función que realiza cada grupo de mamíferos en la zoocória; proceso que puede llegar a ser clave en la recuperación de los ecosistemas después de los disturbios.

JUSTIFICACIÓN

En Aguascalientes, la Sierra Fría representa el macizo montañoso más extenso del estado (cerca de 108,000 ha, Gobierno del Estado de Aguascalientes, 1994), sin embargo, esta zona ha sido objeto de un intenso disturbio relacionado a la deforestación para la extracción de leña en los años de 1920 a 1940; con el uso de los combustibles fósiles comenzó un periodo de recuperación de la vegetación y el ecosistema (Minnich, 1994); donde Díaz-Núñez *et al.* (2016) documento que las especies vegetales que han tenido una mayor recuperación en la zona son las pioneras. Particularmente, los bosques que alberga la Sierra Fría han sido ampliamente estudiados en el aspecto biológico y ecológico; sin embargo, son pocos los estudios que refieren el papel de la fauna en el proceso de recuperación a través de la dispersión de semillas forestales, sobre todo las pioneras (Rubalcava-Castillo, 2017). Por lo tanto, se tiene que identificar características del proceso de dispersión como la escarificación, germinación y el acceso a un hábitat adecuado (Hämäläinen, 2017), ya que, no solo inciden en la distribución espacial de las semillas sino también en la velocidad de germinación a causa del paso por el tracto digestivo; no obstante, el papel de los mamíferos carnívoros como agentes dispersores ha sido poco estudiado (Silverstein, 2005) en la Sierra Fría y en el estado.

OBJETIVOS E HIPÓTESIS

Objetivo general

Identificar la eficacia de la endozoocoria y la diploendozoocoria en mamíferos del orden carnívora en el bosque templado y el bosque tropical del ANP Sierra Fría.

Objetivos particulares

1. Definir las especies de mamíferos del orden carnívora que realizan la dispersión de semillas por endozoocoria y las que lo realizan por diploendozoocoria en los bosques templados y tropicales de la Sierra Fría.

2. Examinar la endozoocoria y diploendozoocoria de los mamíferos en el paisaje a través de los SIGs.

3. Comparar la eficiencia de la endozoocoria y la diploendozoocoria a través de la viabilidad, proceso de germinación y análisis del grosor (desgaste) de las testas por MEB.

Hipótesis de investigación

1. Los mamíferos de dieta omnívora serán los endozoocóricos (zorra gris, coyote y cacomixtle), mientras que los felinos (gato montés, puma y ocelote) serán los que realizarán el proceso de la diploendozoocoria. Debido a sus hábitos alimenticios, los diploendozoocóricos diseminarán con menor abundancia y riqueza de semillas que los endozoocóricos.

2. Existirá relación entre la vegetación y los sitios de excreción en el proceso de dispersión, lo que determinará la distribución potencial de los mamíferos en los sitios de estudio a través de corredores naturales en las áreas con poco disturbio, mientras que las áreas que presenten algún tipo de alteración como los caminos para vehículos tendrá una mayor distribución de excretas.

3. La viabilidad de las semillas que provienen de las excretas será menor que las provenientes de los doseles, sin embargo, el proceso de diploendozoocoria presentará mayor velocidad y porcentaje de germinación en semillas que el proceso por endozoocoria y las provenientes de los árboles. Esto se debe a que, las semillas de los doseles presentarán una composición estructural integra en sus testas sin modificación de sus elementos estructurales (sin desgaste en testas), mientras que las provenientes de excretas con proceso de endozoocoria presentarán desgaste superficial y aberturas entre sus estructuras para el paso de elementos que provoquen la germinación; las diploendozoocóricas tendrán un desgaste y aberturas mayores a las endozoocóricas.

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BIBLIOGRAFÍA

Bacles, C. F. E., A. J. Lowe y R. A. Ennos. 2006. Effective seed dispersal across a fragmented landscape. Science 311:628.

Baker, R. 1967. Distribution of recent mammals along the Pacific coastal lowlands of the western hemisphere. Systematic Zoology 16:28-37.

Beaune, D., F. Bretagnolle, L. Bollache, G. Hohmann, M. Surbeck, and B. Fruth. 2013. Seed dispersal strategies and the threat of defaunation in a Congo forest. Biodiversity and Conservation 22:225–238.

Benayas, J. M. R., Bullock, J. M. y Newton, A. C. 2008. Creating woodland islets to reconcile ecological restoration, conservation, and agricultural land use. *Frontiers in Ecology and the Environment, 6*(6), 329-336. doi:10.1890/070057.

Buitrón-Jurado, G. y N. Ramírez. 2014. Dispersal spectra, diaspore size and the importance of endozoochory in the equatorial Andean montane forests. Flora: Morphology, Distribution, Functional Ecology of Plants 209:299–311.

Bullock, S., H. Mooney y E. Medina (editores). 1995. Seasonally dry forests. Cambridge University Press, Cambridge.

Búrquez, A., A. Martínez-Yrízar, R.S. Felger y D. Yetman. 1999. Vegetation and habitat diversity at the southern edge of the Sonoran Desert. Pp. 36-67. En: Ecology of Sonoran Desert Plants and Plant Communities. R.H. Robichaux (ed.). University of Arizona Press, Tucson Az.

Carbone, C., G. Cowlishaw, N. J. B. Isaac y J. M. Rowcliffe. 2005. How far do animals go? Determinants of day range in mammals. American Naturalist 165:290–297.

Caughlin, T. y J. Ferguson. 2013. The importance of long distance seed dispersal for the demography and distribution of a canopy tree species. Ecology 95:952–962.

Ceballos, G. 1995. Vertebrate diversity, ecology, and conservation in Neotropical dry forests. Pp. 195-220. En: Seasonally dry tropical forests. S. H. Bullock, H. A. Mooney y E. Medina (eds.). Cambridge University Press, Cambridge.

Ceballos, G. y A. García. 1995. Conserving Neotropical biodiversity: the role of dry forest in western Mexico. Conservation Biology 9:1349-1356.

Ceballos, G. y L. Martínez. 2010. Mamíferos. En Ceballos, G., L. Martínez, A. García, E. Espinoza, J. B. Creel y R. Dirzo (Eds.), Diversidad, amenazas y áreas prioritarias para la conservación de las Selvas Secas del Pacífico de México (pp 124). D. F., México: Editorial Fondo de Cultura Económica y CONABIO.

Ceballos, G. y P. Rodríguez.1993. Patrones de endemicidad en los mamíferos de México. Pp. 75-99. En: Avances en el Estudio de los Mamíferos de México. R. A. Medellín y G. Ceballos (eds.) Asociación Mexicana de Mastozoología, México.

Chazdon, R. L. 2003. Tropical forest recovery: legacies of human impact and natural disturbances. Perspectives in Plant Ecology Evolution and Systematics, 6(1-2), 51-71. doi:10.1078/1433-8319-00042.

Correa, D., Álvarez, E. y Stevenson, P. 2015. Plant dispersal systems in Neotropical forests: availability of dispersal agents or availability of resources for constructing zoochorous fruits?. Global Ecology and Biogeography, 203–214. doi:10.1111/geb.12248.

Culot, L., M. C. Huynen y E. W. Heymann. 2015. Partitioning the relative contribution of one-phase and two-phase seed dispersal when evaluating seed dispersal effectiveness. Methods in Ecology and Evolution 6:178–186.

Cypher, B. y Cypher, E. 1999. Germination rates of tree seeds ingested by coyotes and raccoons. The American Midland Naturalist., 71-76. doi:http://dx.doi.org/10.1674/00030031(1999)142[0071:GROTSI]2.0.CO;2.

Darwin, C. R. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. First edition. John Murray, London, UK.

De Noir, F. A. 2002. Mecanismos de dispersión de algunas especies de leñosas nativas del Chaco Occidental y Serrano. ISSN: 0328-0543. *Revista de Ciencias Forestales*, 140-150.

Dean,W. R. J. y S. J. Milton. 1988. Dispersal of seeds by raptors. African Journal of Ecology 26:173–176.

Díaz-Núñez, V., J., Sosa-Ramírez e I. P., Macías-Medina. 2014. Diagnóstico fitosanitario de la vegetación en ecosistemas prioritarios de Aguascalientes, México. Comisión Nacional Forestal-Secretaría de Medio Ambiente del Estado de Aguascalientes. 84 p.

Díaz-Núñez, V., J. Sosa-Ramírez y D. R. Pérez-Salicrup. 2016. Vegetation patch dynamics and tree diversity in a diverse conifer and oak forest in central Mexico. Botanical Science. Vol. 94 (2): 229-240 pp.

Díaz, S., Wardle, D. A. y Andy, H. 2009. Incorporating biodiversity in climate change mitigation initiatives. En N. Shahid, D. E. Bunker. H. Andy, M. Loreau y C. Perrings, Biodiversity, Ecosystem Functioning, and Human Wellbeing (Capitulo 11). Oxford University Press, London, England.

Escribano-Avila, G., Calvino-Cancela, M., Pias, B., Virgos, E., Valladares, F. y Escudero, A. 2014. Diverse guilds provide complementary dispersal services in a woodland expansion process after land abandonment. *Journal of Applied Ecology, 51*(6), 1701-1711. doi:10.1111/1365-2664.12340.

Escribano-Ávila, G., Pías, B., Escudero, A. y Virgós, E. 2015. Importancia ecológica de los mamíferos frugívoros en la dinámica de regeneración de campos abandonados en ambientes mediterráneos. Asociación Española de Ecología Terrestre., 24(3), 35-42 pp. doi:10.7818/ECOS.2015.24-3.06.

Escribano-Avila, G., Pias, B., Sanz-Perez, V., Virgos, E., Escudero, A. y Valladares, F. 2013. Spanish juniper gain expansion opportunities by counting on a functionally diverse dispersal assemblage community. *Ecology and Evolution, 3*(11), 3751-3763. doi:10.1002/ece3.753.

Escribano-Avila, G., Sanz-Perez, V., Pias, B., Virgos, E., Escudero, A. y Valladares, F. 2012. Colonization of Abandoned Land by Juniperus thurifera Is Mediated by the Interaction of a Diverse Dispersal Assemblage and Environmental Heterogeneity. *Plos One, 7*(10). doi:10.1371/journal.pone.0046993.

Foley, J. A., DeFries, R., Asner, G. P., Barford , C., Bonan, G., Carpenter, S. R. y Snyder, P. K. 2005. Global consequences of land use. doi:10.1126/science.1111772. *Science,* 570–574.

Franklin, J. F., Mitchell, R. J. y Palik, B. 2007. Natural disturbance and stand development principles for ecological forestry. USDA for Serv N Res. US Department of Agriculture, Forest service, Northern research station. http://courses.washington.edu/esrm315/pdfs/Franklinetal2007.pdf. Obtenido de USDA for Serv N Res. US Department of Agriculture, Forest service, Northern research station. http://courses.washington.edu/esrm315/pdfs/Franklinetal2007.pdf.

Galindo, C., Sánchez, A. y R. Quijano. 2004. Population dynamics of a resident colony of Leptonycteris curasoae (Chiroptera:Phyllostomidae) in Central Mexico. Biotropica, 36:382-391.

Gentry, A.H. 1982. Patterns of Neotropical plant species diversity. Evolutionary Biology 15: 154.

Gobierno del Estado de Aguascalientes. 1994. Declaratoria del Área Natural protegida Sierra Fría, Aguascalientes, Diario Oficial Ags. Tomo LVII, No. 5. Órgano del Gobierno Constitucional del Estado, Aguascalientes. eservicios.aguascalientes.gob.mx/.../periodicooficial2009/usuario_webexplorer.as?

Hämäläinen, A., K. Broadley, A. Droghini, J. A. Haines, C. T. Lamb, S. Boutin, y S. Gilbert. (2017). The ecological significance of secondary seed dispersal by carnivores. *Ecosphere* 8(2). doi:e01685. 10.1002/ecs2.1685.

Herrera, C. M. 1985. Determinants of plant-animal coevolution - the case of mutualistic dispersal of seeds by vertebrates. Oikos, 44(1), 132-141. DOI:10.2307/3544054.

Herrera, C. M. 1989. Frugivory and seed dispersal by carnivorous mammals, and associated fruit characteristics, in undisturbed mediterranean habitats. Oikos, 55(2), 250-262. doi:10.2307/3565429.

Herrmann, J. D., T. A. Carlo, L. A. Brudvig, E. I. Damschen, N. M. Haddad, D. J. Levey, J. L. Orrock y J. J. Tewksbury. 2016. Connectivity from a different perspective: comparing seed dispersal kernels in connected vs. unfragmented landscapes. Ecology 97:1274–1282.

Hubbell, S.P. 1979. Tree dispersion, abundance, and diversity in a tropical rain forest. Science 203: 1299-1309.

Inventario Estatal y de Suelos para Aguascalientes (IEFyS). 2012. Inventario Estatal y de Suelos para el Estado de Aguascalientes. Gobierno del Estado de Aguascalientes-Comisión Nacional Forestal. 118p.

IUCN (International Union for Conservation of Nature) 2008. Red list of threatened species. Disponible en www.iucnredlist.org/

Janzen, D.H. 1982a. Removal of seeds from horse dung by tropical rodents: influence of habitat and amount of dung. Ecology 63:1887-1900.

Janzen, D.H. 1982b. Attraction of liomys mice to horse dung and the extintion of this response. Animal Behavior 30:483-489.

Janzen, D.H. (ed.) 1983. Costa Rica Natural History. Chicago University Press. Chicago. Jalisco, México. Fundación Ecológica Cuixmala y Universidad Nacional Autónoma de México.

Jenkins, M. 2003. Prospects for Biodiversity. *Science*, 1175–1177. doi: 10.1126/science.1088666.

Jordano, P. 2000. Fruits and frugivory. Pages 125–166 in M. Fenner, editor. Seeds: the ecology of regeneration in plant communities. Second edition. CABI Publishing, Wallingford, UK.

Jordano, P., Garcia, C., Godoy, J. A. y Garcia-Castano, J. L. 2007. Differential contribution of frugivores to complex seed dispersal patterns. Proceedings of the National Academy of Sciences of the United States of America, 104(9), 3278-3282. doi:10.1073/pnas.0606793104.

Lamb, D., Erskine, P. D. y Parrotta, J. A. 2005. Restoration of degraded tropical forest landscapes. *Science, 310*(5754), 1628-1632. doi:10.1126/science.1111773.

Laurance , W. F. y Williamson , G. B. 2002. Positive Feedbacks among Forest Fragmentation, Drought, and Climate Change in the Amazon. Conservation Biology, 1529–1535. doi:10.1046/j.1523-1739.2001.01093.x.

Lorenz , K. y Lal , R. 2010. Effects of disturbance, succession and management on carbon sequestration. Carbon sequestration in forest ecosystems. Springer, Berlin, 103–157 pp. doi:10.1007/978-90-481-3266-9_3.

Luna , I., Alcántara , O., Morrone , J. J. y Espinosa , D. 2000. Track analysis and conservation priorities in the cloud forests of Hidalgo, Mexico. Diversity Distribut, 137–143 pp. doi:10.1046/j.1472-4642.2000.00079.x.

Mascorro, V. S., Coops, N. C., Kurz, W. A. y Olguín, M. 2014. Attributing changes in land cover using independent disturbance datasets: a case study of the Yucatán Peninsula, Mexico. Reg Environ Change. doi:10.1007/s10113-014-0739-0.

Minnich, R., Sosa-Ramírez, J., Franco, V. y Barry, W. Y. 1994. Reconocimiento preliminar de la vegetación y de los impactos de las actividades humanas en la Sierra Fría, Aguascalientes. Investigación y Ciencia, 23-29.

Montiel, S. 2000. Vertebrate frugivory and seed dispersal of a Chihuahuan Desert cactus. *Plant Ecology*, 221-229. doi:10.1023/A:1009819419498.

Murray, K. G., Russell, S., Picone, C. M., Winnettmurray, K., Sherwood, W. y Kuhlmann, M. L. 1994. Fruit laxatives and seed passage rates in frugivores - consequences for plant reproductive success. Ecology, 75(4), 989-994. doi:10.2307/1939422.

Naoe, S., I. Tayasu, Y. Sakai, T. Masaki, K. Kobayashi, A. Nakajima, Y. Sato, K. Yamazaki, H. Kiyokawa y S. Koike. 2016. Mountain-climbing bears protect cherry species from global warming through vertical seed dispersal. Current Biology 26:R315–R316.

Nathan, R., F. M. Schurr, O. Spiegel, O. Steinitz, A. Trakhtenbrot y A. Tsoar. 2008. Mechanisms of long-distance seed dispersal. Trends in Ecology and Evolution 23:638–647.

Nogales, M. 1999. Some ecological implications of the broadening habitat and trophic niche of terrestrial vertebrates in the Canary Islands. Ecologia Illes 1:67–82.

Nogales, M., D. P. Padilla, C. Nieves, J. C. Illera y A. Traveset. 2007. Secondary seed dispersal systems, frugivorous lizards and predatory birds in insular volcanic badlands. Journal of Ecology 95:1394–1403.

Nogales, M., I. Castañeda, M. López-Darias, F. M. Medina y E. Bonnaud. 2015. The unnoticed effect of a top predator on complex mutualistic ecological interactions. Biological Invasions 17:1655–1665.

Nogales, M., R. Heleno, A. Traveset y P. Vargas. 2012. Evidence for overlooked mechanisms of long-distance seed dispersal to and between oceanic islands. New Phytologist 194:313–317.

Olson, D., E. Dinerstein, R. Abell, T. Allnutt, C. Carpenter, L. McClenachan, J. D'Amico, P. Hurley, K. Kassem, H. Strand, M. Taye y M. Thieme. 2000. The Global 200: A Representation Approach to Conserving the Earth's Distinctive Ecoregions. Word Wildlife Fund.

Ozinga, W. A., R. M. Bekker, J. J. Schaminée y J. M. van Groenendael. 2004. Dispersal potential in plant communities depends on environmental conditions. Journal of Ecology 92:767–777.

Padilla, D. P., A. González-Castro y M. Nogales. 2012. Significance and extent of secondary seed dispersal by predatory birds on oceanic islands: the case of the Canary archipelago. Journal of Ecology 100:416–427.

Padilla, D. P. y M. Nogales. 2009. Behavior of kestrels feeding on frugivorous lizards: implications for secondary seed dispersal. Behavioral Ecology 20:872–877.

Peters, E. B., Wythers, K. R., Bradford, J. B. y Peter, B. 2013. Influence of Disturbance on Temperate Forest Productivity. Ecosystems, 95–110. doi:10.1007/s10021-012-9599-y.

Pickett, S., T. A. y White, R., S. 1985. The ecology of natural disturbance and patchy dynamics. ISBN: 978-0-12-554520-4. Orlando: Academic Press, Orlando.

PNUMA-CEPAL. 2001. La sostenibilidad del desarrollo en América Latina y el Caribe: desafíos y oportunidades. LC/G2145 (CONF90/3).

Prugh, L. R., C. J. Stoner, C. W. Epps, W. T. Bean, W. J. Ripple, A. S. Laliberte y J. S. Brashares. 2009. The rise of the mesopredator. BioScience 59:779–791. Beaune, D., F. Bretagnolle, L. Bollache, G. Hohmann, M. Surbeck, and B. Fruth. 2013. Seed dispersal strategies and the threat of defaunation in a Congo forest. Biodiversity and Conservation 22:225–238.

Rocas, A. N. 1982. Estructuras y clasificación de semillas forestales mexicanas. *Revista de Ciencias Forestales.*

Rodrigues, R. R., Gandolfi, S., Nave, A. G., Aronson, J., Barreto, T. E., Vidal, C. Y. y Brancalion, P. H. S. 2011. Large-scale ecological restoration of high-diversity tropical forests in SE Brazil. Forest Ecology and Management, 261(10), 1605-1613. doi:10.1016/j.foreco.2010.07.005.

Rubalcava Castillo, F. A. 2017. Dispersión por endozoocoria y establecimiento de dos especies forestales pioneras en un bosque templado del ANP sierra fría, Aguascalientes. Tesis de Mestría, Universidad Autónoma de Aguascalientes.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R. y Wall, D. H. 2000. Biodiversity Global biodiversity scenarios for the year 2100. Science, 287(5459), 1770-1774 pp. doi:10.1126/science.287.5459.1770.

Sánchez-Martínez., G., C. J. Mehmel, N. E. Gillette, E. González Gaona, J. A. López Hernández, J. C. Monárrez González, J. L. García Rodríguez, S. R. Mori, H. E. Alanís Morales, J. M. Mejía Bojórquez, M. Cano Rodríguez, M. A. Cortés Chamorro, L. M. Torres Espinosa. 2012. Fundamentos para el control integral del descortezador Dendroctonus pseudotsugae barragani Furniss en México Folleto Técnico núm. 46. INIFAP-CIRNOC-Campo Experimental Pabellón, Aguascalientes México. ISBN: 978-607-425-797-7.

Schupp, E. W. y P. Jordano. 2010. Seed dispersal effectiveness revisited: a conceptual review. New Phytologist 188:333–353.

Semarnat. 2002. Norma Oficial Mexicana NOM-059-ECOL- 2001, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio- Lista de especies en riesgo. Diario Oficial de la Federación, 6 de marzo del 2002. México, D.F.

Silverstein, R. P. 2005. Germination of native and exotic plant seeds disperseed by coyotes (*Canis latrans*) in Southern California. doi: http://dx.doi.org/10.1894/0038-4909(2005)050[0472:GONAEP]2.0.CO;2. The Southwestern Naturalist., 472-478.

Sosa-Ramírez, J., V., Díaz-Núñez y A., Ponce-Montoya. 2015. Diversidad y Productividad del Estrato Herbáceo en una Sabana de la Sierra Fría, Aguascalientes. Áreas Naturales Protegidas Scripta, 1 (2): 51-66 pp. doi: 10.18242/ANPScripta.2015.01.01.02.0003.

Suárez-Esteban, A., M. Delibes y J. M. Fedriani. 2013. Barriers or corridors? The overlooked role of unpaved roads in endozoochorous seed dispersal. Journal of Applied Ecology 50:767–774.

Turner, M. G. 2010. Disturbance and landscape dynamics in a changing world. *Ecology*, 2833–2849. doi:10.1890/10-0097.1.

Vander Wall, S. B. y W. S. Longland. 2004. Diplochory: Are two seed dispersers better than one? Trends in Ecology and Evolution 19:155–161.

WCMC-World Conservation Monitoring Center. 2000. Statistical Analysis of Forests and Protection. (V3.1, July 2000), Global Forests Statistics. Disponible en:

Willson, M. F. 1993. Mammals as seed-dispersal mutualists in north-america. Oikos, *67*(1), 159-176. doi:10.2307/3545106.

World Resources, I. 2005. Ecosystems and human well-being: biodiversity synthesis. Washington, DC: World Resources Institute.

CAPÍTULO II

ENDOZOOCHOROUS DISPERSAL OF FOREST SEEDS BY CARNIVOROUS MAMMALS IN SIERRA FRÍA, AGUASCALIENTES, MEXICO

ABSTRACT

Some carnivorous mammals ingest fruit and disperse seeds of forest plant species capable of colonizing disturbed areas in ecosystems. The objective of the present study was to evaluate the dissemination of *Arctostaphylos pungens* and *Juniperus deppeana* seeds by the grey fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*) and other carnivores in the Protected Natural Area Sierra Fría, in Aguascalientes, Mexico. Scat collection was undertaken via transects using the direct search method, while the seasonal phenology of *A. pungens* and *J. deppeana* was evaluated by recording flower and fruit abundance on both the plant and the surrounding forest floor ground. Seed viability was assessed by optical densitometry via X-ray and a germination test. It was found that the grey fox, coyote, ringtail (*Bassariscus astutus*) and bobcat (*Lynx rufus*) disseminated seeds of *A. pungens* (212 ± 48.9 seeds/scat) and *J. deppeana* (23.6 ± 4.9 seeds/scat), since a large proportion of the collected scat of these species contained seeds (28/30 = 93.33%, 12/43 = 27.9%, 6/12 = 50% and 7/25 = 28% respectively). The grey fox, coyote, ringtail and bobcat presented an average of seed dispersion of both plant species of 185.4 ± 228.7, 4.0 ± 20.0, 12.1 ± 30.4, and 0.8 ± 1.5 per scat; the seed proportions in the grey fox, coyote, ringtail and bobcat were 89.6/10.4, 82.3/17.7, 90.4/9.6, and 38.1/61.9% for *A. pungens* and *J. deppeana*, respectively. The phenology indicated a finding related to the greater abundance of ripe fruit in autumn and winter ($P <$

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0.01). This coincided with the greater abundance of seeds found in scats during these seasons. Endozoochory and diploendozoochory enhanced the viability and germination of the seeds ($P > 0.05$), except in those of *A. pungens* dispersed by coyote. These results suggest that carnivores, particularly the grey fox, the coyote and the bobcat, play an important role in forest seed dissemination, and thus forest regeneration, by making both a quantitative and qualitative contribution to the dispersal of the two pioneer species under study.

Key words: *Canis latrans*; Endozoochory; scats; seed dispersal; *Urocyon cinereoargenteus*
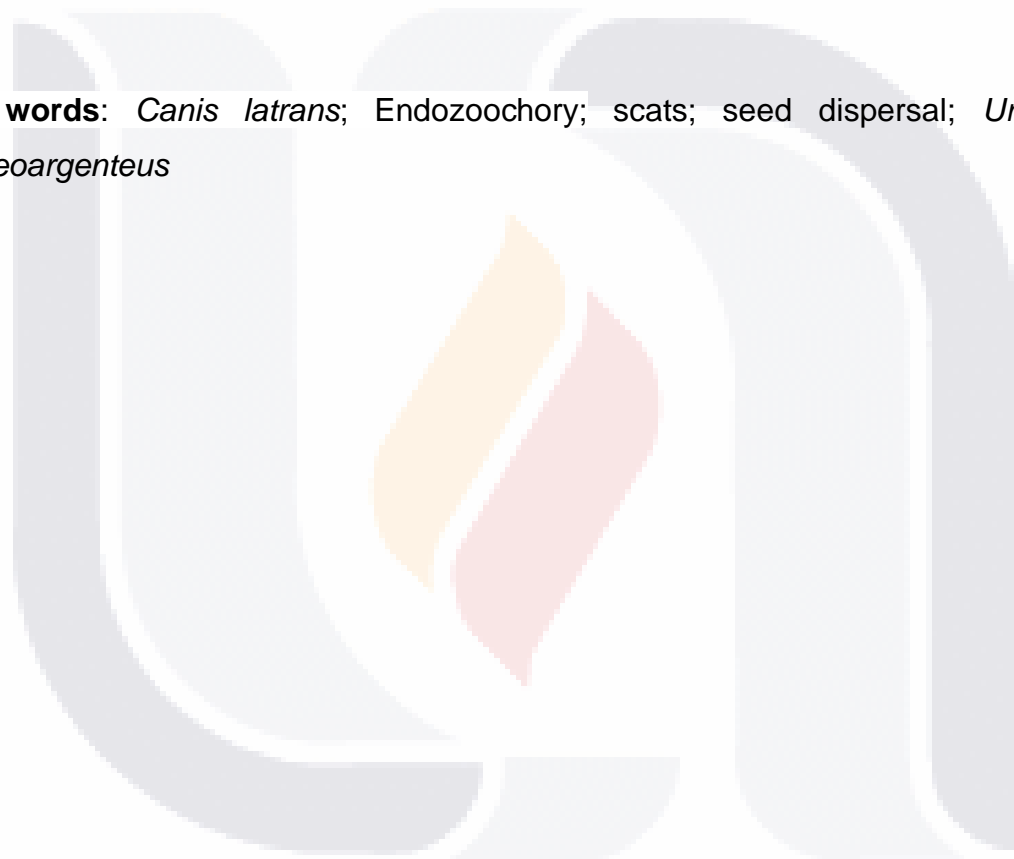
INTRODUCTION

In numerous plant species, seed dispersal is achieved via the process of endozoochory, in which plants produce fleshy nutritious fruits for consumption by animals, which then excrete the seeds at a distance from the parent plant (Cypher & Cypher, 1999). Various studies show that, in the ecological context of abandoned fields and degraded areas, frugivorous land mammals are principally responsible for seed dispersal and regeneration of vegetation (Escribano-Avila *et al*., 2012; Suárez-Esteban *et al*., 2013; Escribano-Avila *et al*., 2014). These studies reveal that scat distribution and deposition varies according to landscape, ecosystem and disperser, while previous studies suggest that carnivores often defecate along paths and trails used by human beings (Fedriani *et al*., 1999). Moreover, Suárez-Esteban *et al*. (2013) suggest that paths and trails are corridors for plant species dispersed by mammals through their selection of these sites for defecation, which may act to promote native seed dispersal.

In this context, Zúñiga *et al*. (2008) describe how, although carnivore species are opportunists that mainly consume rodents and lagomorphs, they diversify their diet with birds, arthropods, fish, reptiles and considerable quantities of fruit, the seeds of which are subsequently dispersed. For this reason, carnivorous species constitute an indispensable element of the dispersion guild of many plant species in highly anthropized environments (Lopez-Bao & González-Varo, 2011; Perea *et al*., 2013). Carnivorous mammals cover large areas and retain seeds for long periods in the intestine, making them key vectors for long-distance dispersal (Otani, 2002; Jordano *et al*., 2007). As a consequence, seed dispersal also benefits from the seed retention time and the transit time in the digestive tract (Cypher & Cypher, 1999), which may also have a beneficial effect on germination (Murray *et al*., 1994). Few studies explain the contribution of carnivores to the regeneration of vegetation; however, González-Varo *et al*. (2013) demonstrate how the carnivores red fox (*Vulpes vulpes*) and marten (*Martes martes*) play a crucial role in the long-distance

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dispersion of seeds in temperate ecosystems. Likewise, Nakashima *et al*. (2010) show that the carnivorous mammal palm civet (*Paradoxurus hermaphroditus*) plays a unique and important role in the regeneration of *Leea aculeata*.

There is increasing interest in diplochory (two-phase dispersion, also known as "secondary dispersion" or "indirect dispersion"), which involves a second phase of dispersal. However, relatively little attention has been given to "diploendozoochory"; i.e., seed dispersal involving ingestion of the seed by two or more different species of animals in sequence, generally a prey animal and its predator (Hämäläinen, 2017). Due to the complexity of the diploendozoochoric process, there is a shortage of worldwide studies of this mechanism. Of the few investigations in this area, one carried out by Sarasola *et al*. (2016) that involves a hypercarnivorous mammal recognized for its apex predator role: the puma (*Puma concolor*), which, usually ingested and dispersed over long distances, large quantities of seeds of herbaceous species initially consumed by its main prey: Dove (*Zenaida auriculata*), with this, it was possible to prove that this feline plays the role of secondary seed disperser and demonstrate that strictly carnivorous predators such as felines, could have extensive ecological functions.

The particular use of specific microhabitats and landscape elements by mammals, e.g., for sleeping, reproduction, hunting and shelter, determines seed deposition patterns (Russo *et al*., 2006), which greatly affect the probability of subsequent recruitment of the dispersed seeds (Escribano-Ávila *et al*., 2014). Escribano-Ávila *et al*., (2013) state that adaptation of a microhabitat for the germination and early survival of an animal-dispersed seed depends on its selection, handling and the effect of passage through the animal gut, in addition to the suitability of climatic conditions for triggering germination in the microsite, including temperature, humidity, light, oxygen and even the surface type (Guariguata, 1999).

In Aguascalientes, Mexico, the *Área Natural Protegida Sierra Fría* (the Sierra Fría Protected Natural Area, or ANP-SF by its Spanish acronym) includes a group of ecosystems belonging to three biogeographic provinces (Sosa Ramírez *et al*.,

2016). Díaz-Núñez *et al.* (2016) describe the recovery of these ecosystems since the 1990s, reporting that the pioneer species that present the greatest distribution and colonization of the area post-disturbance are the pointleaf manzanita or pingüica (*Arctostaphylos pungens* Kunth; Ericaceae), which is a fire-adapted plant that thrives in places where events have frequently destroyed holm oak trees (Rzedowski, 1978) and the checkerbark juniper or táscate (*Juniperus deppeana* Steud; Cupressaceae), which is a dioecious species that lives in temperate to semi-arid environments and is tolerant to alkaline and nutrient poor soils (Martínez, 1963; Batis *et al.*, 1999). Both of these plant species act as nurse species to other larger species. The objective of this study was to evaluate the dissemination of seeds of *A. pungens* and *J. deppeana* by the grey fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*) and other carnivores in the ANP-SF in order to determine whether these carnivores aid the regeneration of habitats. Specifically, we wished to determine whether the endozoochorous seed dispersal system in these mammals is one of the causes of the wide distribution in these plant species through the relation of the offer (abundance) of fruits in canopy with the abundance of seeds in scats, and the record of the type of surface where the scats are deposited. In addition, we wished to explain the role (dispersant, scarifier, germination promoter) of all mammals under study in the dispersion of the seeds of *A. pungens* and *J. deppeana* and whether bobcats also act as diploendozoochory vectors.

MATERIAL AND MÉTHODS

Study site

The study was carried out in an area of temperate forest in the ANP-SF, on the western border of the State of Aguascalientes, Mexico. The area presents a sub-humid temperate climate and summer rainfall (Rzedowski, 1978), with temperatures ranging from -3 °C to 18 °C and average annual rainfall fluctuating from 600 to 700 mm (SEDESO, 1995). The natural plant communities in the area are composed of *A. pungens*, *J. deppeana*, Oak (*Quercus potosina*), isolated elements of *Pinus leiophylla* var. *Chihuahuana* (a subspecies of the Chihuahua pine) and *Pinus teocote* (Díaz-Núñez *et al.*, 2012). Two areas were selected from within the ANP-SF for collection of scats: *Mesa del Aserradero* and *Mesa del Águila*. *Mesa del Aserradero* is located at the coordinates 22°11'55.51"N and 102°35'47.64"W, while *Mesa del Águila* is found at coordinates 22°12'1.52"N and 102°35'11.03"W. These two plateaus are separated by the ravine *Cañada de Piletas*. Based on pre-existing maps of the vegetation cover by Díaz-Núñez *et al.* (2016), where, for the estimation of forest coverage, a SPOT® 2013 satellite image of 5 meters resolution was used. The study area was divided into patches or fragments that form continuous mosaics within a landscape and four categories of coverage were established for the landscape classification: 1 = ≤10%, 2 = 11-30%, 3 = 31-50% and 4 ≥50%. Image analysis was performed using ArcGis® 10.5 software (ESRI, 2011) of the ArcMap module, thus generating vegetation maps in which sites with coverage < 40% were considered as open sites. Likewise, patches with coverage ≥ 40% were considered as closed sites. Based on the information on the previous maps and on direct field exploration of both plateaus, sites of closed vegetation (high cover) were identified, comprising numerous woody plants the canopies of which cover a large part of the forest floor, as well as open sites (low cover) with native grasses and very few woody plants (Figure 2.1).
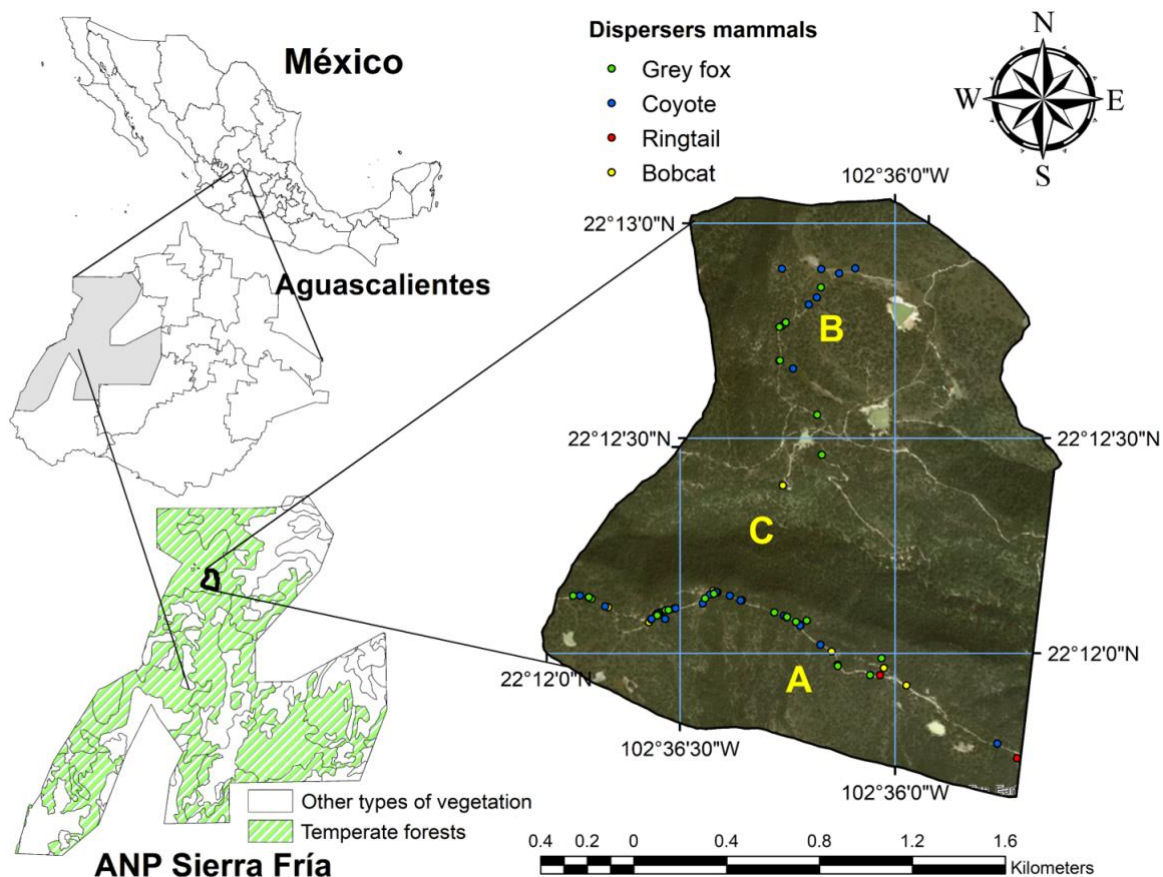
Figure 2.1 Geographical location of the study area in the temperate forest of the ANP-SF in the state of Aguascalientes, Mexico. Mesa del Aserradero (a), Mesa del Águila (b), and Cañada de Piletas (c).

Collection, identification, and location of scats

Visits were undertaken at the study sites during all four seasons of both 2015 and 2016. Scat collection was carried out in each site and season via transects, using the direct search method proposed by Nova (2012), which, consisted of making walking routes through the study area to locate scats at simple sight. Each transect consisted of a central line of 2 km in length, with two parallel lines located 20 m either side of the central line. The scat was then collected from the total area within the transect. The search for scats was performed in the entire study area dividing it by a

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grid of transects each separated by 20 m, trying to sample all types of surface. For the above, 72 transects were established on trails identified as routes for the movement of fauna, on dirt paths and among the vegetation in the area (both low and high cover), and the routes varied in order to cover as much of the plateaus as possible and to include all of the probable scat sites. Scat corresponding to each mammal species was identified based on the *Manual for Tracking the Wild Mammals of Mexico* (Aranda-Sánchez, 2012) and immediately labelled. The main characteristics for identification were the shape and dimensions of the scat: grey fox scats are cylindrical in shape and are present on the rocks, forming latrines; coyote scats are formed mainly by hair in the form of braids and finished in a long lock; ringtail scats are thin and elongated, consisting of fruits, seeds, hairs and feathers; bobcat scats are usually in a cylindrical form and divided into several packages (Figure 2.2). Once identified, the scats were georeferenced using a GPS (Garmin, eTrex®10, Taiwan) and placed in crepe paper bags, with the type of surface on which it was found recorded using the above-mentioned classification with the modification proposed by Matías *et al.* (2010), creating four distinct categories: (1) on-unpaved road on rocks (unpaved road/rock); (2) on-unpaved road on bare soil (unpaved road/bare soil); (3) off-unpaved road on bare soil (off-unpaved road/bare soil); and, (4) off- unpaved road on herbaceous vegetation (off-unpaved road/herbaceous).

	GREY FOX	COYOTE	RINGTAIL	BOBCAT
ANIMAL				
SCAT				
COLOR SIZE SHAPE	Dark brown. More or less cylindrical. Length 5.0 - 10.0 cm and width 1.0 - 2.0 cm.	Dark brown. More or less cylindrical. Length 10.0 - 20.0 cm and width 2.0 - 3.5 cm.	Depends on the content. More or less cylindrical. Length 5.0 - 10.0 cm and width 0. 5 - 1.5 cm.	Gray, greenish or blackish. Sectioned in packages. Length 10.0 - 15.0 cm and width 1.5 - 2.5 cm.
CONTENT	Mainly contain fruits and rodents in some cases. Mainly over rocks.	Mainly contain braided hair, skeletal remains and ending in strands.	Fruits and seeds, hairs or feathers.	Mainly contain hair, skeletal remains and keratin as claws.

Figure 2.2 Description of representative scat with defined characteristics for the identification of each carnivore included in the analyses. The measurements and descriptions are based on Aranda-Sánchez (2012).

To identify the potential prey of the bobcat (*Lynx rufus*) and determine the incidence of diploendozoochory, the guard hairs contained in the scats of this feline were identified by consulting the guides for identification of mammal guard hairs by Pech-Canche *et al.* (2009) and Monroy-vilchis & Rubio Rodríguez (2014). For this, prior to identification, the hairs were subjected to a cleaning, clarification and assembly process. The prey (species) was then identified with the use of an optical microscope (Leica Microsystems, DM LS2, Switzerland), considering patterns of hue, shape, maximum diameter and hair marrow structure.

Identification and abundance of seeds

The scat was left to dry at ambient temperature (23 °C) for 24 hours in Petri dishes, after which the seeds were extracted, washed under running water and left to dry for 24 hours for subsequent analysis and identification. The seeds from each scat

sample were quantified and then separated into three groups, according to plant species (*A. pungens*, *J. deppeana* or other species). Identification took into account the distinctive characteristics present in *A. pungens*, the seeds of which are wedge-shaped, an average of 3.2 mm in length and 2.6 mm in width and are sometimes united in groups of two to three (Márquez-Linares *et al*., 2006) and *J. deppeana*, the seeds of which are angular and irregular, 6 to 7 mm in length and 4 to 6 mm in width and light brown in colour (Rzedowski & Rzedowski, 2005). This process was conducted using a stereoscope (Leica Microsystems, MZ6, Switzerland), obtaining the total number of seeds per scat sample and thus the richness of the species present, from which the abundance per plant species and per disperser animal species was determined.

Phenological analysis

Six 32 x 32 m quadrants were installed in each vegetative cover and each plateau (three low and three high cover quadrants), i.e., six in the *Mesa del Aserradero* and six in *Mesa del Águila*. Five *A. pungens* and five *J. deppeana* individuals of basal diameter > 5 cm and height > 2 m were selected and quantified per quadrant. The trees selected under these criteria corresponded to adult plants of reproductive age and thus had a high probability of bearing fruit. In total, 120 examples were counted (60 *A. pungens* and 60 *J. deppeana*) between the two plateaus. From autumn 2015 to summer 2016, according to the calendar for northern meteorological seasons, two visits per season were conducted per each included tree or sampled individual, at the beginning of, and halfway through, each season. On each occasion, the phenological variables described below were recorded, for each sampled individual.

Initial – recently pollinated flower, from which the corollas had fallen for *A. pungens*, with an incipient development of a small green fruit (immature stage), Unripe – green fruit fully developed (intermediate stage of development) and Ripe –

fruit presenting red coloration typical of ripeness (reddish for *A. pungens* and brown for *J. deppeana*).

A modified version of the methodology proposed by Chapman *et al.* (1992) was applied to each of the sampled individuals in order to determine the abundance of flowers and fruit in the canopy. It is important to note that, for *J. deppeana*, only the abundance of fruits was determined since, as a gymnosperm, this species does not have flowers. Canopy volume was calculated for each sampled individual by measuring the longest axis from the crown and the axis perpendicular to this by extending a rope, marked at 1 m intervals, along the axis of the base of the tree. The height of the tree crown was measured using a clinometer. The canopy was then divided into four equal parts in the form of a Cartesian plane, on which imaginary lines were traced. Two 1 m^3 areas were selected per quadrant (8 m^3 in total), in which the number of flowers and fruit were counted separately for each estimated area. The average number of flowers and fruits for the two areas per quadrant was calculated, in this way, the general average for each of the four quadrants within an individual was calculated, in order to obtain a single general average of flowers and fruits for each sampled individual; finally, the general average per individual was used to calculate the total abundance of flowers and fruits based on the canopy volume previously calculated for each sampled individual.

The method described above was applied in order to determine the abundance of fruit on the forest floor, with the difference that the Cartesian plane generated here was applied on the forest floor surface below the canopy, calculating the average for the two areas, which this time comprised 1 m^2 per quadrant, thus obtaining an overall average per sampled individual. In order to obtain the percentage abundance, the same calculation of the total abundance of flowers and fruits was applied based on the area on the ground below the canopy previously calculated, which was obtained by calculating the area of a circle created using the

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rope marked at one metre intervals to cover the entire canopy. All of the fallen fruit found on the ground pertaining to the sampled individual were included in the count.

Viability and germination test

A control group was established with seeds taken from mature fruit collected in the forest canopy of the study area for both the viability and germination tests. For this, 12 random individuals with ripe fruits were selected. For *A. pungens*, six specimens were chosen at *Mesa del Aserradero* and six at *Mesa del Águila*. For *J. deppeana*, 12 individuals with fruits were selected from a nearby area, since the production of this species was incipient in our study area. From each individual, ten ripe fruits were randomly collected and 30 seeds were subsequently selected. In this way, 360 seeds were obtained, representing the 12 individuals selected for each plant species. The viability test was conducted via optical densitometry analysis using X-ray equipment (Faxitron X-Ray Corporation, Texas, U. S. A. at 10 s and 22 kv intensity) using the technique proposed by De La Garza and Nepamuceno (1986). Viable seeds, with well-developed embryos, were distinguished from non-viable seeds by the presence of mechanical damage and malformations, with the latter seed type either empty or without an embryo. The germination test was carried out in Parafilm-sealed Petri dishes (Antonio-Bautista, 2012), placed in an incubation chamber (Lab-Line, Model 310 Imperial III, Minnesota, U. S. A.) at a temperature of 25 ˚C for 63 days, with groups of 10 seeds per Petri dish, into which 6 ml of distilled water was poured prior to placement of the seeds.

Statistical analysis

The resulting data were captured in a database, based on which three statistical tests were performed using the StatGraphics (15.2, 2007) statistical program, with the values expressed as average ± *SD*. To analyse the seasonal dispersion by

mammals, an analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test were then conducted to determine differences in the contribution of each mammal to the dispersal of seeds of both species, abundance of seeds per season of the year and the total number of *A. pungens* and *J. deppeana* seeds dispersed separately. The *ab* averages with different literals in each variable presented statistically significant differences. Likewise, analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test were used to determine significant differences in the total seed dispersal for high and low vegetative cover, with a 95% significance level. In addition, the Chi-square test (X^2) of independence was conducted in order to test whether there is dependence on the dispersion carried out by mammals for dispersant plant species. To analyse the type of surface on which scat is deposited, the Chi-square test (X^2) of independence was also performed to verify whether there is dependence on the possible relationship between the deposition of scat (both on and off-unpaved roads) and the specific type of surface on which it is deposited (rocks, bare soil and herbaceous vegetation). Moreover, the Dunnett test was also conducted in order to determine significant differences in the viability and germination of seeds in the scat, compared to seeds collected from the canopy (control), to a 95% significance level. For the phenological analysis, analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) tests were also used to establish differences in the abundance of flowers and fruit in the canopy, as well as the fruit on the forest floor, per season and plant species, also at a 95% significance level.

RESULTS

Seasonal dispersal by mammals

Of the total mammalian scats found in the study area for a series of potential dispersers of, *A. pungens* and *J. deppeana*, only four mammal species pertaining to the carnivorous order and classified into three families (*Canidae*, *Procyonidae* and *Felidae*) were identified as potential dispersers by confirming the presence of seeds of these plant species in their scats. Seeds of *A. pungens* and *J. deppeana* were found in 110 scats of the total number of scats analysed in four species of mammals: coyote (43 scat samples = 39.1%); grey fox (30 scat samples = 27.3%); bobcat (25 scat samples = 22.7%); and ringtail (*Bassariscus astutus* Lichtenstein) (12 scat samples = 10.9) (Table 2.1). Of the scats of all four mammals, 22.7% contained seeds of *A. pungens* and 25.5% contained seeds of *J. deppeana*. It is important to note that, of the 25 scats found in wildcat, seven contained seeds of *A. pungens* (8 seeds) and *J. deppeana* (13 seeds). The seven scats with seeds all presented hairs and, through the use of the guide for identification of guard hairs, it was determined that the hairs of the prey belonged to the wild rabbit of the species *Sylvilagus floridanus.* Hairs of the genus of these rabbits have a multiserial medulla, a dark band and a shield shape.

In terms of seed abundance, the four mammals dispersed a total of 5,905 seeds of both plant species in the study area. Specifically, the grey fox presented significant differences ($P < 0.01$, $F_{6, 109} = 8.63$, $P = 0.00$) by dispersing 94.2% of the total seeds of *A. pungens* and *J.* deppeana (5,563/5,905 = 94.2%), where it disseminated the highest average number ($\bar{x} \pm SD$) of forest seeds per total scat sample (185.4 ± 228.7), while the coyote dispersed 3% (4.1 ± 20), the ringtail 2.5% (12.2 ± 30.5) and the bobcat 0.3% (0.84 ± 1.6) (Figure 2.3A).

Table 2.1 Data for each carnivore describing: number of scats analysed, total average number ($\bar{x} \pm SD$) of seeds found, average number of seeds ($\bar{x} \pm SD$) found belonging to each plant species, number of germinated seeds, and total number of scats on each type of surface.

Data	Dispersers				
	Grey fox	Coyote	Ringtail	Bobcat	Total
Scats analysed (N)	30	43	12	25	110
Total No. of seeds dispersed	185.4 ± 228.7	4.0 ± 20.0	12.1 ± 30.4	0.8 ± 1.5	5,905
Dispersed seeds					
A. pungens	356.2 ± 238.5	20.5 ± 48.7	66.0 ± 56.5	4.0 ± 1.4	5271
J. deppeana	41.1 ± 25.9	6.2 ± 6.3	3.5 ± 2.5	2.6 ± 1.6	634
Germination (N)					
A. pungens	0	0	0	0	0
J. deppeana	1	2	0	1	4
Surface on which scat is deposited (N)					
on-unpaved road on rocks	17	23	5	17	62
on-unpaved road on bare soil	10	14	6	5	35
off-unpaved road on bare soil	3	4	1	2	10
off-unpaved road on herbaceous vegetation	0	2	0	1	3

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Six scat samples from other potential dispersers of these plant species were collected, including the mammals hooded skunk (*Mephitis macroura* Lichtenstein) (4/116 = 3.5%), raccoon (*Procyon lotor* Linnaeus) (4/116 = 3.5%), raccoon (*Procyon lotor* Linnaeus) (1/116 = 0.86%) and weasel (*Mustela frenata* Lichtenstein) (1/116 = 0.86%). However, given that seeds of the plant species were not found in these scat samples, they were excluded from the analysis.

As in the case of disperser animals, a significant difference was found in general dispersal among the seasons of the year ($F_{3,112}$ = 6.2, P = 0.00). This significant difference was found in the autumn season (111 ± 196), which presented 89.5% of the total seed dispersal (Figure 2.3B).

With regard to the abundance of *A. pungens* and *J. deppeana* seeds, separately, in the scat, a statistically significant difference was found for *A. pungens* ($F_{2,113}$ = 32.1, P = 0.00). This was the most dispersed plant species in terms the total number of seeds per scat over the seasons, with 89.2% (211 ± 244.4), while *J. deppeana* recorded 10.8% (22.6 ± 26.2) (Figure 2.3C).

Statistically significant differences were observed in the Chi-squared independence tests (P < 0.05, $X^2{}_{12}$= 42.9, P = 0.00) conducted among the four carnivore species and dispersed plant species, indicating that there is no independence in the dispersion of these seeds. In this way, there are different preferences of carnivores associated with the species of fruit they eat. The above is corroborated by the differences in the number of seeds dispersed by each mammal in the previous results.

Finally, the influence of the vegetative coverage on the total seed dispersal by the four mammals in closed (high cover) and open (low cover) sites was recorded, with no statistically significant differences presented by either cover ($F_{1,114}$ = 3.39, P = 0.06), despite this, the highest average seed dispersal was recorded in the open sites (76.43 ± 190.8), compared to the closed sites (28.7 ± 68.1).
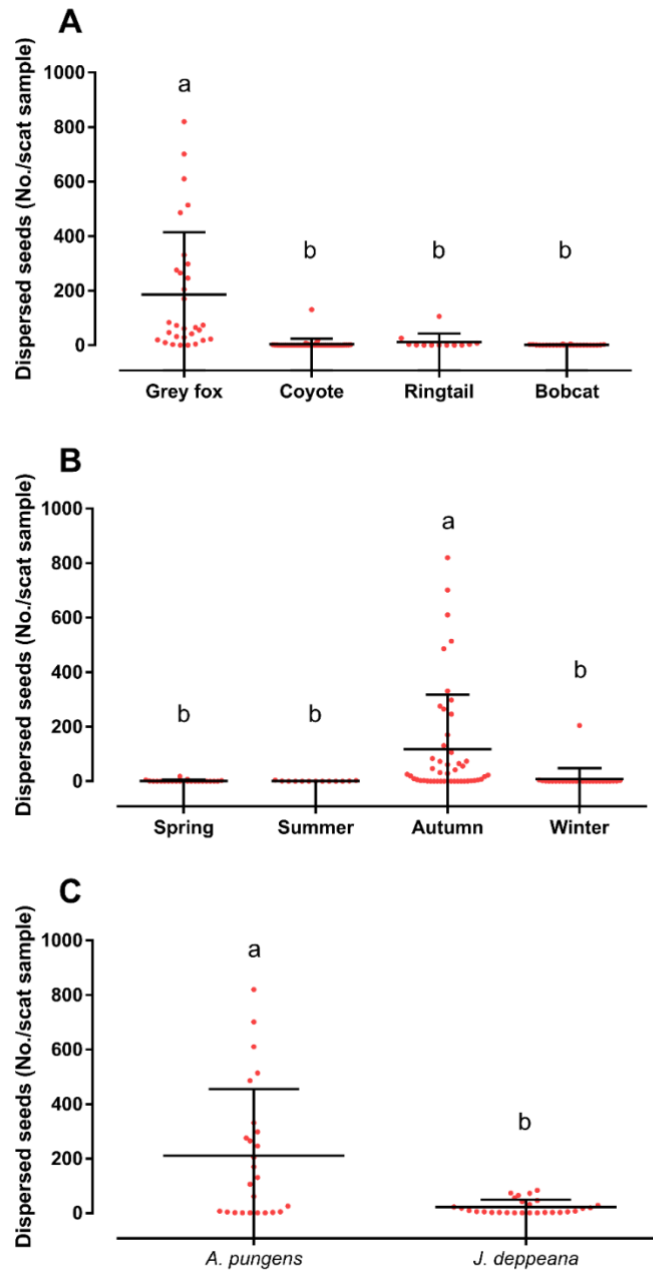
Figure 2.3 Dispersal of forest seeds in the ANP-SF. Average dispersal (± *SD*) by the grey fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), ringtail (*Bassariscus astutus*) and bobcat (*Lynx rufus*) (A). Average dispersal in each season (B). Average dispersal of the plant species *Arctostaphylos pungens* and *Juniperus deppeana* (C). [a-b] averages with different literals present statistically significant differences according to the Tukey HSD test ($P < 0.01$).

Phenological analysis

Arctostaphylos pungens presented two stages of flowering and fruiting during the study period. With regard to the abundance of flowers in the canopy, the seasons in which a higher average was presented (±*SD*) as well as statistically significant differences ($P < 0.05$, $F_{3,388} = 77.0$, $P = 0.00$), were summer (8089 ± 6808) and winter (6895 ± 6838) (Figure 2.4A). On completion, the two flowering periods were followed by two fruiting periods: spring (8616 ± 6547) and autumn (8347 ± 6348), and both seasons presented significant differences ($F_{3,388} = 38.5$, $P = 0.00$) and the highest average abundance of fruits (Figure 2.4B). Despite the presence of a large number of fruits in the canopies during the fruiting season, a low number of fruits was observed on the ground directly under the canopies, mainly in summer (333 ± 1041) and autumn (419 ± 1031), where significant differences were found in both seasons ($F_{3,388} = 7.5$, $P = 0.00$), (Figure 2.4C).
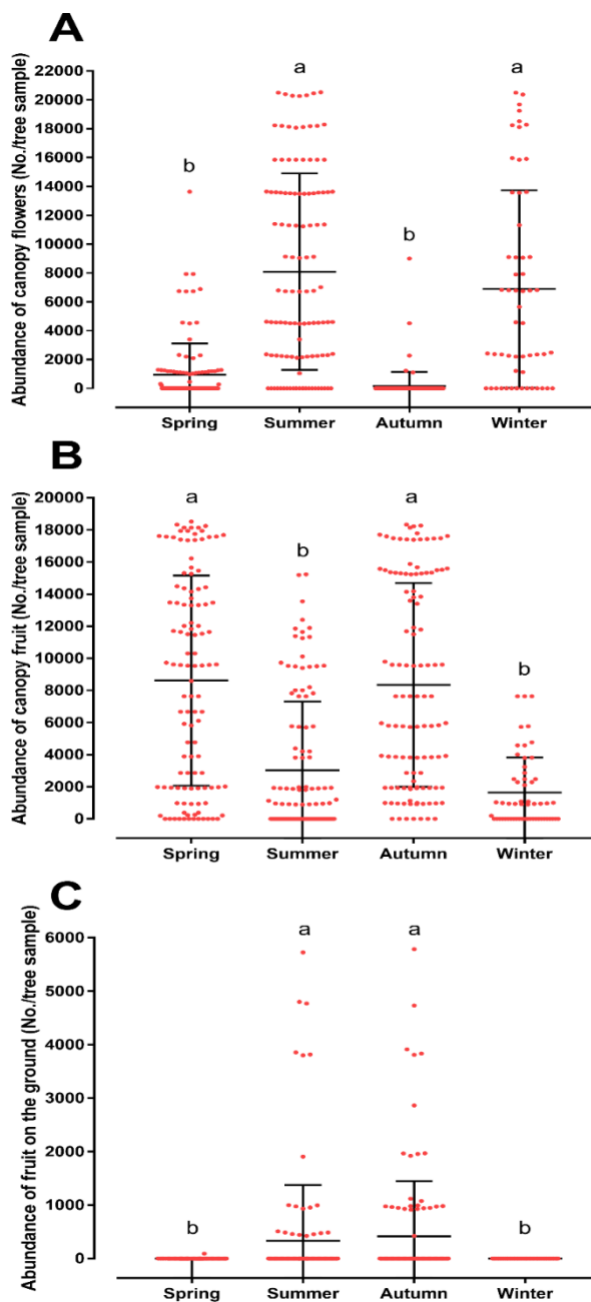
Figure 2.4 Phenological analysis of the *Arctostaphylos pungens* in the ANP-SF. Average abundance (± *SD*) of flowers (A), average abundance of fruit in canopy (B) and average abundance of *A. pungens* fruit on the forest floor (C), during the four seasons of the year. [a-b] averages with different literals present statistically significant differences according to the Tukey HSD test ($P < 0.05$).

Fruit production in the *J. deppeana* population was very low. However, the seasons in which there was a higher average abundance of fruits (±*SD*), as well as statistically significant differences ($F_{3,416} = 24.83$, $P = 0.00$), were spring (1.45 ± 2.57) and winter (3.41 ± 4.18) (Figure 2.5). No *J. deppeana* fruit were found on the forest floor. These results contrast with the abundance of *J. deppeana* fruit in the scat of the disperser species.
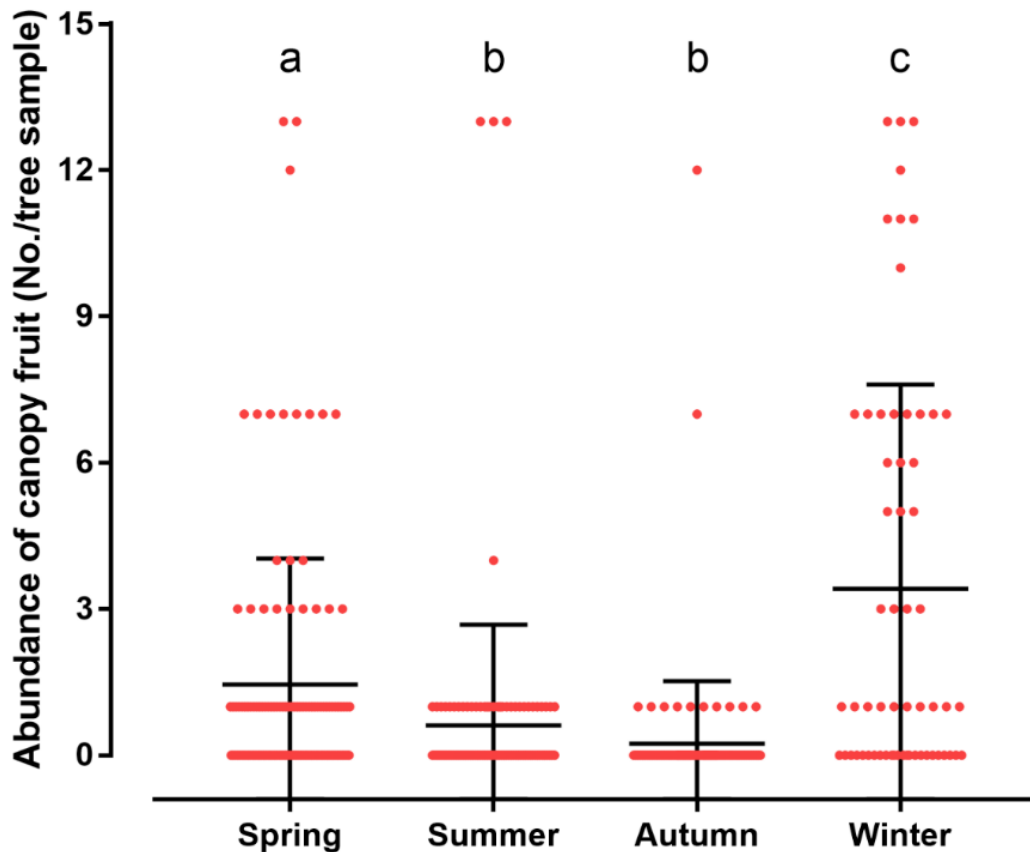
Figure 2.5 Phenological analysis of the *Juniperus deppeana* in the ANP-SF. Average abundance (± *SD*) of fruit in the *J. deppeana* canopy during the four seasons of the year. [a-c] averages with different literals present statistically significant differences according to the Tukey HSD test ($P < 0.05$).

Type of surface on which scat is deposited

The spatial distribution of the scat showed that 87.9% was deposited on unpaved road used by motor vehicles, as can be seen in figure 2.1. Of the scat samples collected on the unpaved road, 54.3% was found on rocks. Furthermore, statistically significant differences were revealed by the Chi-squared independence tests between the different types of surface on which the mammal scat was deposited and its placement on or off-unpaved road ($X^2_2= 41.0$, $P = 0.00$), showing that there is dependence between the deposition of scat (both on and off-unpaved road) and the specific type of surface on which it is deposited (rocks, bare soil and herbaceous vegetation); i.e., the dispersers deposit their scats on a particular type of surface, in this case, on rocks on unpaved road (Table 2.1).

Viability and germination

Endozoochory and diploendozoochory enhanced the viability of the *J. deppeana* seeds ($F_{4,29} = 1.39$, $P = 0.26$) since the passage of these seeds through the digestive tract of the grey fox (82.5 ± 13.6%), ringtail (84.1 ± 16.7%) and even bobcat (69.3 ± 41.3%) maintained the highest percentages of viability compared to seeds taken directly from the canopy (61.4 ± 12.3%). This is due to the fact that most of the canopy seeds presented perforations in their embryos caused by insects, lowering their viability compared to the seeds in the scats, which presented only minimal damage. The viability of *A. pungens* differed significantly between the seeds dispersed by the mammals and those taken from the canopy ($F_{4,17} = 3.51$, $P = 0.02$). This significance is reflected in the fact that the viability was only seen to be affected in the seeds obtained from coyote scat (45.2 ± 50.6%) (Table 2.2). The X-ray test showed that the *A. pungens* seeds taken from the canopy did not present damage or alterations (Figure 2.6A), while some of these seeds in the scat were either incomplete or presented some damage (Figure 2.6B). However, the seeds taken from the *J. deppeana* canopy presented a high level of damage caused by

screwworms in the embryo (Figure 2.6C), while the *J. deppeana* seeds dispersed by mammals were found to be intact or with minimal damage (Figure 2.6D).
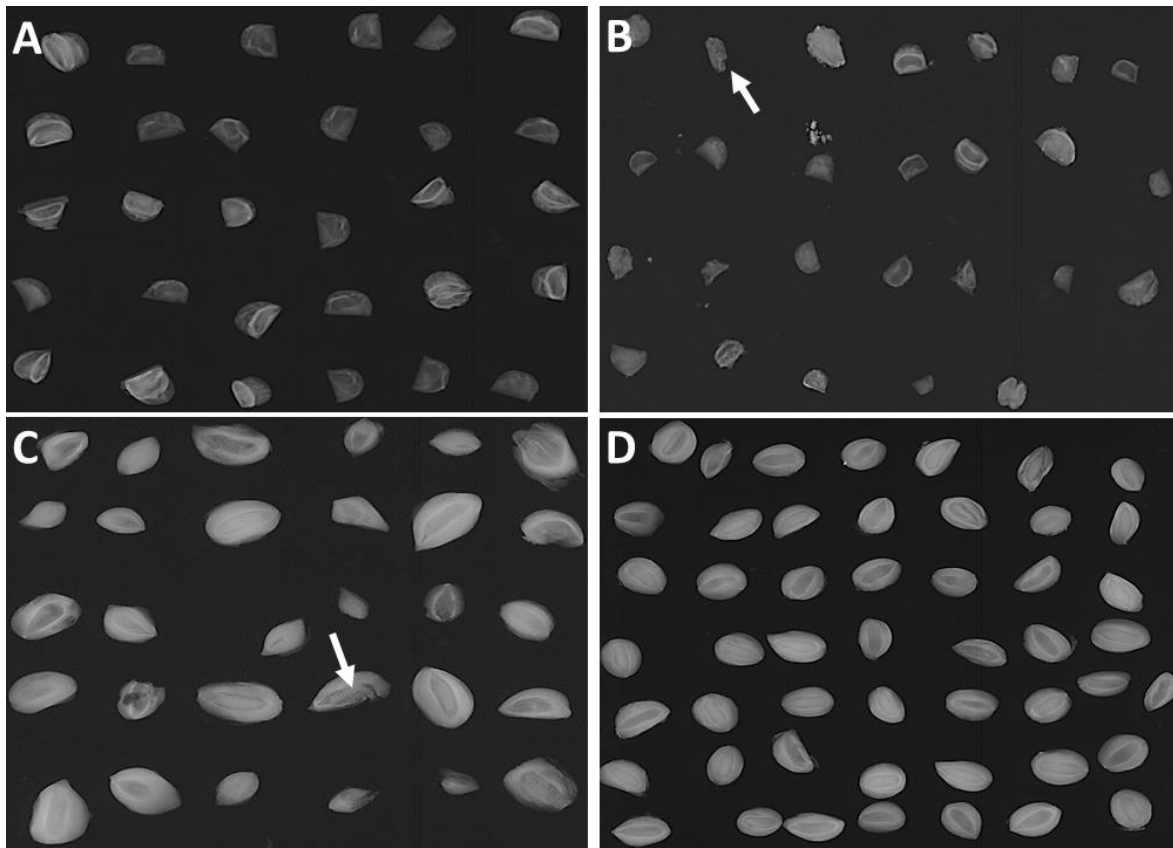
Figure 2.6 X-ray test conducted on *Arctostaphylos pungens* and *Juniperus deppeana* seeds taken from both canopy and scats. *Arctostaphylos pungens* seeds taken from canopy (A) and from the scat (B), with the arrow indicating an incomplete seed with damage. *Juniperus deppeana* seeds taken from the canopy (C), with the arrow indicating the invasion of the screwworm in the embryo, and from the scat (D).

Table 2.2 Average number of viable seeds ($\bar{x} \pm SD$) for the viability test conducted via X-ray in *A. pungens* and *J. deppeana* seeds taken from both the scat of the disperser mammals and the canopy.

Seed Species	Disperser Species	Seeds (N)	$\bar{x} \pm SD$
A. pungens	Grey fox	54	21.5 ± 30.4
	Coyote	13	1.2 ± 1.9*
	Ringtail	26	18.0 ± 0.0
	Bobcat	5	3.0 ± 0.0
	Canopy (control)	360	29.2 ± 0.7
J. deppeana	Grey fox	257	24.1 ± 16.3
	Coyote	31	3.8 ± 3.4
	Ringtail	13	3.7 ± 2.1
	Bobcat	13	2.0 ± 1.6
	Canopy (control)	360	18.4 ± 3.7

* Statistically significant differences according to the Dunnett test ($P < 0.05$).

In *A. pungens*, no germination occurred in either the canopy seeds or those recovered from scats, despite waiting for the maximum period of 63 days. This may be due to the fact that this species has a very hard coat that prevented the entry of water necessary to trigger germination. Evaluation of the *J. deppeana* seeds ($F_{4,29} = 0.89$, $P = 0.48$) revealed that the mammals coyote (8.0 ± 17.9%) and bobcat (6.67 ± 14.9%) enhanced germination, presenting higher germination percentages than those of the seeds taken from the canopy (1.67 ± 4.1%). It can therefore be stated that ingestion acted to improve germination in this case (Table 2.1).

DISCUSSION

Quantitative contribution of carnivores (to the dispersal of pioneer species)

The grey fox was found to be the most important species in terms of the dispersal of seeds during all seasons of the year, due to the fact that its diet is almost entirely composed of fruit (Villalobos-Escalante *et al*., 2014), including fruit of *A. pungens* and *J. deppeana*. The coyote was the species found to deposit the highest amount of scat, due to its wide distribution in the area. The ringtail, despite its inconsistent participation, also contributes to dispersal. However, while the diet of bobcat is considered essentially carnivorous (Sánchez-González *et al*., 2018), the present study found a constant level of dispersal of the seeds of both plant species (n = 21). This species of feline is thus a secondary disperser, consuming, as prey, rabbit (*S. floridanus.*) that has consumed the fruit of *A. pungens* and *J. deppeana*. Thus, these seeds undergo a second endozoochorous process, ingested first by the rabbit and then by the bobcat, which is considered a diploendozoochorous disperser (Hämäläinen *et al*., 2017), as described by Sarasola *et al*. (2016) when seeds of herbaceous plants previously ingested by the Eared Dove (*Zenaida auriculata*) were found in puma scats. Likewise, Nogales *et al*. (1996) and Nogales *et al*. (2015), report the secondary dispersal of seeds by the feral cat (*Felis silvestris catus*), in which these authors describe seeds of plant species combined with the remains of lizard prey (*Gallotia galloti*) in the scats of this feline. These studies coincide with our study, in which seeds were found in scats of the bobcat, indicating that this feline is another diploendozoochorous mammal. However, further research is required in order to closely observe feeding in this and other potential felines in the region to confirm this diploendozoochory.

Some species of fauna, such as the coyote and ringtail, may occasionally act as disperser agents, when the high energy requirements of the animals oblige them to consume large quantities of plant resources if they are unable to secure their

normal prey (Godínez-Álvarez *et al.*, 2002). This implies that fruit consumption by the animal is strongly influenced by seasonality (Campos and Ojeda, 1997), and their dietary habits present a preference for the consumption of rodents or larger prey (Rodríguez-Estrella *et al.*, 2000; Martínez-Vázquez *et al.*, 2010), although there is evidence of the consumption of seeds in their scat. For this reason, it is important to analyse the diet of species potentially involved in endozoochory in order to determine the quantity of seeds consumed. Such research would help to elucidate the trophic relationships that exist in the ecosystem, providing an approximation of the impact they could produce on the populations of plant species or the animals that consume them, in accordance with that established by Korschgen (1969).

The seasonality of the contribution of these mammals depends on prey and fruit availability. Therefore, with regard to the phenology of the plant species under study, we have proven statistically that the abundance of seeds in scat changes with the passing of the seasons, in which the highest numbers of dispersed *A. pungens* and *J. deppeana* seeds are presented in autumn. This abundance then decreases in winter due to the reduced presence of fruit in the canopy. It is likely that dispersal is related to the flowering and fruiting stages of the species analysed, although it is not necessarily equal between these stages, as seen in the two annual fruiting cycles of *A. pungens* and the lack of *J. deppeana* fruit in the year of observation, as established by the phenological study. This suggests the existence of other sites in Sierra Fria with a different phenology for these plant species on which the fauna feed and subsequently transport into the study area (Luna-Ruíz *et al.*, 2016). Similarly, our results contrast in seasonal terms, in that the phenology analysis strongly identifies two seasons (spring and autumn) that present an abundance of fruit, while the seeds contained in the collected scat are abundant in autumn only. This could indicate an abundance of other alternative prey in spring, for which reason the animals do not prioritize the consumption of fruit in this season. To verify the above, further research is recommended on parental assignment for dispersed seeds in scats in order to fully understand the seed flux.

Qualitative contribution of carnivores

It was observed that the viability of *J. deppeana* seeds remained intact during their passage through the digestive tract of the majority of the mammals studied, a finding similar to that of the study conducted by Aronne & Russo (1997), who conclude that *Myrtus communis* seeds dispersed by the red fox maintain their viability. Moreover, these authors also found that the low viability presented by the seeds taken from the canopy was attributable to the damage caused by parasitic insects, as reported by Martínez *et al*. (2007), who also observed damage by parasitic insects in *J. deppeana* seeds. Ingestion by the coyote particularly reduced the viability of *A. pungens* seeds, as reported by Matías *et al*. (2010), who determine a reduction in the viability of *Arctostaphylos uva-ursi* seeds dispersed by the red fox. Nevertheless, the reduced viability of another plant species after ingestion by a different mammal species does not necessarily explain the results found for the coyote in this study.

The present study found that the disperser mammals enhanced germination of *J. deppeana*, particularly in seeds dispersed by coyote and bobcat, compared to that of the seeds taken from the canopy. This finding is similar to that of Graae *et al*. (2004), who reported increased germination in the species *Cerastium alpinum* and *Stellaria longipes* after passing through the digestive tract of the artic fox (*Alopex lagopus*). In *A. pungens*, germination did not occur, a result also found by Márquez-Linares *et al*. (2006), who were unable to germinate this species from the scat of grey fox and coyote. However, these authors found that conditions mimicking those that occur during forest fires, such as certain temperature combinations (100 and 120 °C for five minutes), exposure to smoke and irrigation with water containing coal residues, can produce 29% germination.

The spatial distribution of scat over the landscape should also be noted, given that it has been shown that there are significant differences in the type of surface on which it is deposited, and that the majority was deposited on unpaved road with a preference for deposition on rocks. The coyote was the species that made the

highest number of deposits in this microhabitat. In second place, scat was found on bare soil on unpaved road, while a small percentage was found off-unpaved road, probably influenced by the ease of movement for dispersers in that microhabitat. Despite the fact that the majority was deposited on unpaved road, there is more opportunity for the germination of the seeds in the scat found on bare soil (both on and off-unpaved road, with 33.6% and 8.6% respectively) and on herbaceous vegetation (3.5%). We argue that 45.7% of the deposits via endozoochory are found in microsites suitable for the germination and establishment of plant species dispersed via this mechanism, provided there is no constant vehicle traffic over the unpaved road, which would eliminate the seeds that have germinated. Thus, the ideal scenario for germination would be if the seeds from the scat were deposited on bare earth (either on or off-unpaved road), than on rocks, given that seeds deposited on hard or easily-dried surfaces face factors that inhibit germination and establishment, with some seeds recalcitrant, finding obstacles for establishment or even being transported to other sites via the wind (Guariguata, 1999). Thus, despite having sampled from all types of surface of the study area, we found that about 88% of scats were found on-unpaved roads, however, it is clear that there is an establishment of these species of plants off-unpaved roads, therefore, it is necessary to carry out future research to understand if another dispersion system or factor is involved in carrying the seeds of scats that are on-unpaved roads out of these for its germination and establishment.

Given the above, there is the possibility that a secondary dispersal system, via hydrochory, anemochory (Correa *et al.*, 2015) or even predation by mice (Escribano-Ávila *et al.*, 2014), causes the seeds found on-unpaved roads to be transported beyond these paths to the surrounding areas of vegetation. In sites with bare soil (both on and off-unpaved road), and even beyond the unpaved road in our study area, numerous seedlings are observed at the beginning of ecological succession, possibly as a result of these dispersal mechanisms. This could be associated with that reported by Suárez-Esteban *et al.* (2013), who argue that

dispersers selecting unpaved roads can promote roadside restoration. Thus, further research is recommended in order to analyse the secondary seed dispersal of seeds deposited on unpaved roads.

While the results are not presented, because the statistical analysis did not reveal significant differences in the influence of vegetation cover on scat distribution, we found that the highest number of seeds was dispersed in sites with low cover, namely open sites (< 49% canopy). These results concur with that described by Matías *et al.* (2010) in that mammals involved in endozoochory, such as the red fox, stone marten (*Martes foina*), and wild boar (*Sus scrofa*), disperse even more seeds in degraded habitats, generating a window of opportunity for seeds and seedlings via lower levels of competition and increased nutrient availability (Keeley and Bond, 1999). The relatively higher percentage of seeds dispersed in open sites found in our study therefore suggests that grey foxes, coyotes, ringtails and bobcats might also be dispersing seeds to degenerated habitats. Again, further research analysing scats deposited within and beyond degenerated habitats of the area is required for confirmation.

The distribution and deposition of scat in the ANP-SF varies according to the region and ecosystem, for which reason our results differ from that established by Escribano-Ávila *et al.* (2014), who suggest that carnivores mainly deposit their scat under shrubs, thus generating a recruitment pattern for established Spanish junipers (*J. thurifera*). However, we do concur with this author in arguing that the excretion activity is the result of behaviour related to the marking of territory. The present study found statistically significant differences in that the majority of deposits were made on the unpaved road, which is no coincidence, given that these appear to be sites favoured by the mammals, as indicated by Suarez-Esteban *et al.* (2013) when reporting that unpaved roads are favoured by the arrival of seeds via the scats of mammals such as the red fox and the Eurasian badger (*Meles meles*). This conforms to the behaviour of this group of animals involving the use of linear structures (pathways or unpaved road) as sites for displacement and marking of territory. It is

worth mentioning that there is an underestimation of the number of scats and seeds found, due to the 20 m interval between each transect and the ease of finding scats on-unpaved roads, however, although there is an underestimation, it does not represent a bias, so the numbers presented here are representative of what happens with mammals throughout the area.

These animals have the potential to disperse over long distances, and further research should determine the species-specificity of seed dispersal distance (Gelmi-Candusso *et al*., 2019). These behaviours correspond to a landscape that receives the seeds of many fleshy fruit shrubs (Suárez-Esteban *et al*., 2013). It is probable that they do not always constitute a biological corridor factor; however, a high density of routes for the movement of fauna can act to increase or decrease the connectivity of habitats (Oliver and Larson, 1996).
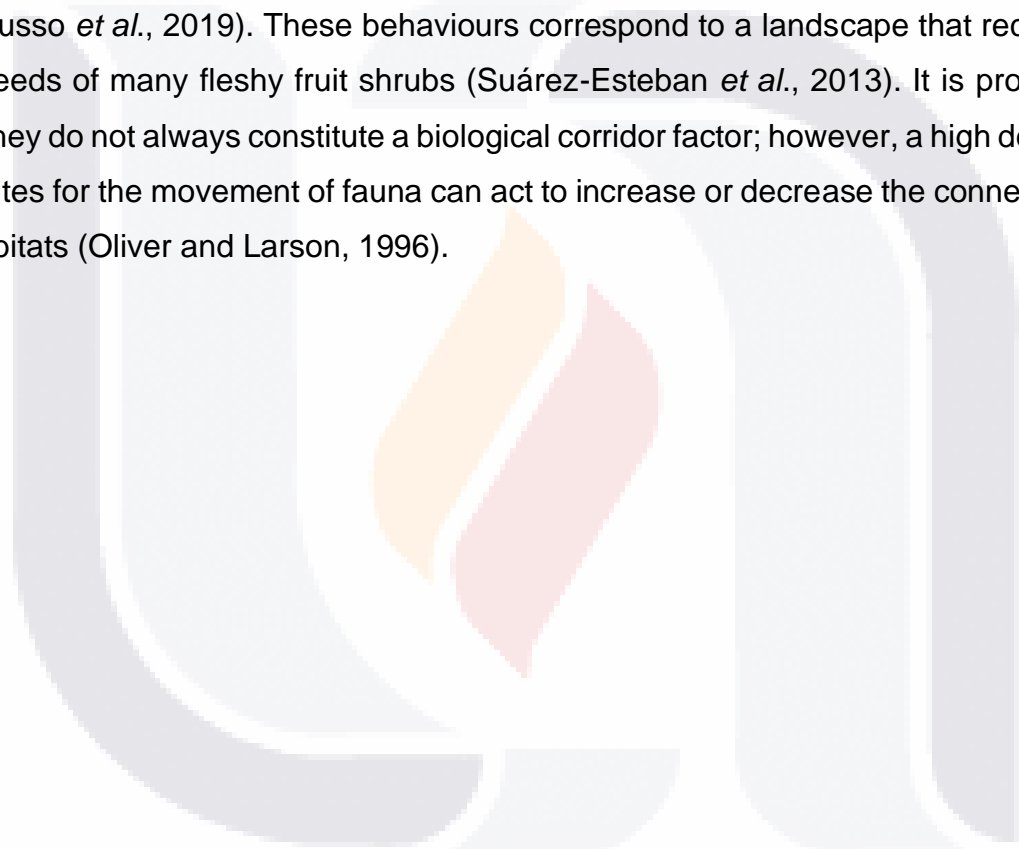
CONCLUSIONS

Our results show that carnivores, particularly the grey fox, seem to contribute both quantitatively and qualitatively to the dispersal of the two studied pioneer species. However, the quantitative contribution of all carnivores is dependent on prey availability and fruit availability, which presents a seasonal pattern, and the qualitative contribution in terms of viability and germination seems to be highly species-specific. Our results therefore support the hypothesis that carnivores are key for the dispersal of pioneer species, potentially even determining habitat regeneration. Future research should address seed dispersal distance by carnivore species in order to determine the spatial range of their influence.
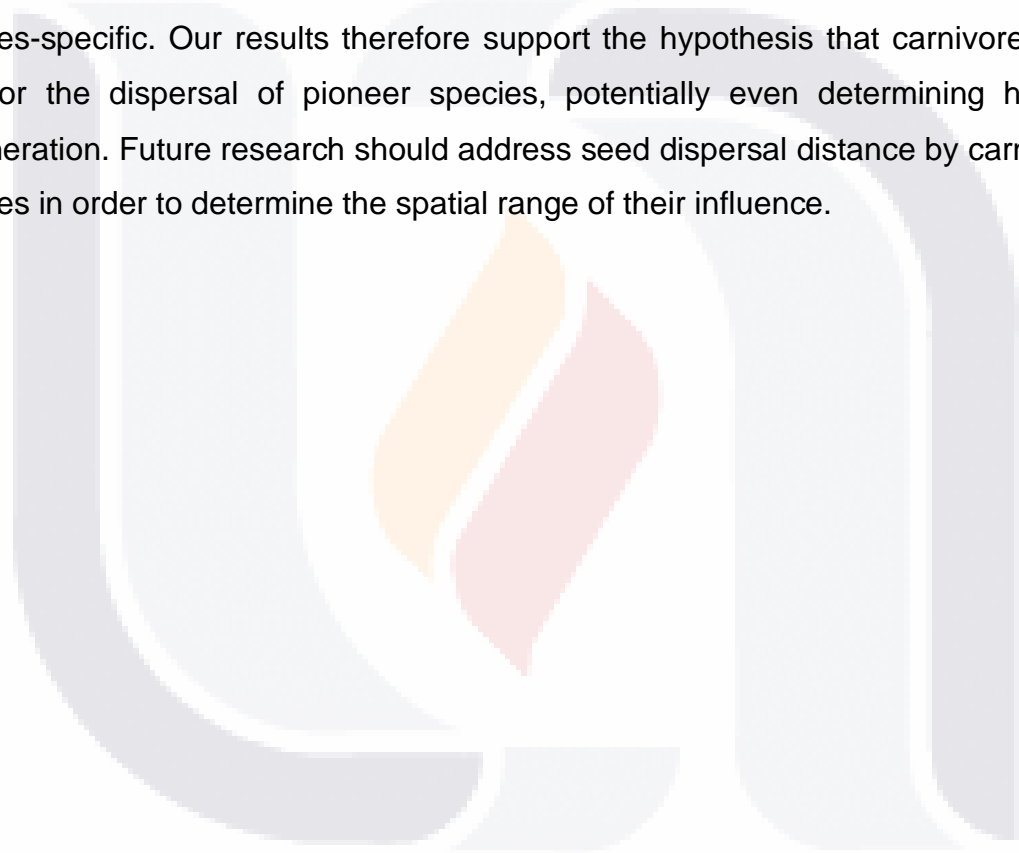
REFERENCES

Antonio-Bautista, A. (2012). Manual de Ensayos de Semillas Forestales Recopilación de Información. Coahuila. ISBN-978-607-95357.

Aranda-Sánchez, J. M. (2012). Manual para el Rastreo de Mamíferos Silvestres de México (C. N. p. e. C. y. U. d. l. B. (CONABIO). Ed, Primera ed. México, D.F. http://200.12.166.51/janium/Documentos/6800.pdf.

Aronne, G. & Russo, D. (1997). Carnivorous mammals as seed dispersers of *Myrtus communis* (Myrtaceae) in the Mediterranean shrublands. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, 131(3), 189-195 pp. doi:10.1080/11263504.1997.10654181.

Batis, A., Alcocer, M. I., Gual, M., Sánchez , C. & Vázquez-Yánez., C. (1999). Árboles y Arbustos Nativos Potencialmente Valiosas para la Restauración Ecológica y la Reforestación. México, D.F.: Instituto de Ecología, UNAM - Conabio.

Chapman, C., Chapman, L., Wangham, R., Hunt, K., Gebo, D., & Gardner, L. (1992). Estimators of Fruit Abundance of Tropical Trees. *Biotropica, 24*(4), 527-531. doi:10.2307/2389015

Campos, C. & Ojeda, R. (1997). Dispersal and germination of *Prosopis flexuosa* (Fabaceae) seeds by desert mammals in Argentina. *Journal of Arid Environments*, 707–714. doi:10.1006/jare.1996.0196.

Correa, D., Álvarez, E., & Stevenson, P. (2015). Plant dispersal systems in Neotropical forests: availability of dispersal agents or availability of resources for constructing zoochorous fruits?. *Global Ecology and Biogeography*, 203–214. doi:10.1111/geb.12248.

Cypher, B. & Cypher, E. (1999). Germination rates of tree seeds ingested by coyotes and raccoons. T*he American Midland Naturalist*., 71-76. doi:http://dx.doi.org/10.1674/00030031(1999)142[0071:GROTSI]2.0.CO;2

De la Garza, L.P. & Nepamuceno, M. F. (1986). Análisis radiográfico de semillas forestales en México. *Revista ciencia forestal*. 1-14 pp.

Diaz-Núñez, V., Sosa-Ramírez, J., & Pérez-Salicrup, D. R. (2012). Distribución y abundancia de las especies arbóreas y arbustivas en la Sierra Fría, Aguascalientes, México. ISSN 1405-2768. *Polibotánica*, (34), 99-126. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S140527682012000200004&lng=es&tlng=es.

Díaz-Núñez V., J. Sosa-Ramírez, & D. R. Pérez-Salicrup. (2016). Vegetation patch dynamics and tree diversity in a diverse conifer and oak forest in central Mexico. *Botanical Science*. Vol. 94 (2): 229-240. doi: http://dx.doi.org/10.17129/botsci.284

Escribano-Ávila, G., Calvino-Cancela, M., Pias, B., Virgos, E., Valladares, F., & Escudero, A. (2014). Diverse guilds provide complementary dispersal services in a woodland expansion process after land abandonment. *Journal of Applied Ecology*, 51(6), 1701-1711. doi:10.1111/1365-2664.12340.

Escribano-Avila, G., Pias, B., Sanz-Perez, V., Virgos, E., Escudero, A. & Valladares, F. (2013). Spanish juniper gain expansion opportunities by counting on a functionally diverse dispersal assemblage community. *Ecology and Evolution*, 3(11), 3751-3763 pp. doi:10.1002/ece3.753.

Escribano-Ávila, G., Sanz-Perez, V., Pias, B., Virgos, E., Escudero, A., & Valladares, F. (2012). Colonization of Abandoned Land by *Juniperus thurifera* Is Mediated by the Interaction of a Diverse Dispersal Assemblage and Environmental Heterogeneity. *Plos One*, 7(10). doi:10.1371/journal.pone.0046993.

ESRI [Environmental Systems Research Institute]. (2011). ArcGis version 10.5. Redlands, California. Available in: http://www.esri.com/software/arcgis/arcgisonline

Fedriani, J.M., Palomares, F., & Delibes, M. (1999). Niche relations among three sympatric Mediterranean carnivores. *Oecologia*, 121, 138–148.

Gelmi-Candusso, T. A., Bialozyt, R., Slana, D., Zárate Gómez, R., Heymann, E. W., & Heer, K. (2019). Estimating seed dispersal distance: A comparison of methods using animal movement and plant genetic data on two primate-dispersed Neotropical plant species. *Ecology and Evolution*, 9(16), 8965–8977. https://doi.org/10.1002/ece3.5422

Godínez-Álvarez , H., Valiente-Banuet , A. I., & Rojas-Martínez, A. (2002). The role of seed dispersers in the population dynamics of the columnar cactus *Neobuxbaumia tetetzo*. ISSN: 0012-9658. *Ecology*, 2617-2629. https://doi.org/10.1890/00129658(2002)083[2617:TROSDI]2.0.CO;2

González-Varo, J.P., López-Bao, J.V., & Guitian, J.M. (2013). Functional diversity among seed dispersal kernels generated by carnivorous mammals. *The Journal of animal ecology*, 82 3, 562-71.

Graae, B. J., Pagh, S., & Bruun, H. H. (2004). An Experimental Evaluation of the Arctic Fox (*Alopex lagopus*) as a Seed Disperser. *Arctic, Antarctic, and Alpine Research*, 36(4), 468-473 pp.

Guariguata, M. R. (1999). Early response of selected tree species to liberation thinning in a young secondary forest in Northeastern Costa Rica. *Forest Ecology and Management*, 124(2-3), 255-261. doi:10.1016/s0378-1127(99)00072-9.

Hämäläinen, A., K. Broadley, A. Droghini, J. A. Haines, C. T. Lamb, S. Boutin, & S. Gilbert. (2017). The ecological significance of secondary seed dispersal by carnivores. *Ecosphere* 8(2). doi:e01685. 10.1002/ecs2.1685.

Jordano, P., Garcia, C., Godoy, J. A., & Garcia-Castano, J. L. (2007). Differential contribution of frugivores to complex seed dispersal patterns. *Proceedings of the National Academy of Sciences of the United States of America*, 104(9), 3278-3282 pp. doi:10.1073/pnas.0606793104.

Keeley, J.E., M. Keeley, & W.J. Bond. (1999). Stem demography and post-fire recruitment of a resprouting serotinous conifer. *Journal of Vegetation Science*, 10: 69-76. https://doi.org/10.2307/3237162

Korschgen, L. J. (1969). Procedure for food-habits analyses. T*he wildlife Investigational Tenechniques*, 233-250. http://agris.fao.org/agrissearch/search.do?recordID=US19830915895.

López-Bao, J. V. & González-Varo, J. P. (2011). Frugivory and Spatial Patterns of Seed Deposition by Carnivorous Mammals in Anthropogenic Landscapes: A Multi-Scale Approach. *Plos One*, 6(1). doi:10.1371/journal.pone.0014569.

Luna-Ruíz, J. J., O. Moreno-Rico, Sosa-Ramírez J., & G. Sánchez-Martínez. (2016). Fenología y estrategias de propagación de la manzanita en la Sierra Fría, Aguascalientes. En Sosa-Ramírez J., O. Moreno-Rico, G. Sánchez-Martínez, J. J. Luna-Ruiz. & M. E. Siqueiros-Delgado. (2016). Contribución al conocimiento ecológico del chaparral de manzanita (*Arctostaphylos pungens* Kunth) en la Sierra Fría, Aguascalientes. Aguascalientes: Universidad Autónoma de Aguascalientes (UAA). Retrieved from https://www.uaa.mx/direcciones/dgdv/editorial/

Márquez-Linares, M. A., Jurado, E., & González-Elizondo, S. (2006). Algunos aspectos de la biología de la manzanita (*Arctostaphylos pungens* HBK) y su

papel en el desplazamiento de bosques templados por chaparrales. ISSN 1405-9177. *CIENCIA UANL*, 57-64 pp.

Martínez, A. J., Sainos, P., Lezama-Delgado, E., & Angeles-Álvarez, G. (2007). El tamaño sí importa: los frutos grandes de *Juniperus deppeana* Steud. (sabino) son más susceptibles a depredación por insectos. *Madera y Bosques*, 13(2), 65-81 pp.

Martínez, M. (1963). Las pináceas mexicanas. México, D. F.: Universidad Nacional Autónoma de México.

Martínez-Vázquez, J., María González-Monroy, R., & Díaz-Díaz, D. (2010). Hábitos alimentarios del Coyote en el parque nacional Pico de Orizaba. *Therya*, 1, 145–154. https://doi.org/10.12933/therya-10-4.

Matías, L., Zamora, R., Mendoza, I., & Hodar, J. A. (2010). Seed Dispersal Patterns by Large Frugivorous Mammals in a Degraded Mosaic Landscape. *Restoration Ecology*, 18(5), 619-627. doi:10.1111/j.1526-100X.2008.00475.x.

Monroy-vilchis, O., & Rubio Rodríguez, R. (2014). Guía de identificación de mamíferos terrestres del Estado de México, a través del pelo de guardia (1). México: Universidad Autónoma del estado de México. 115 pp.

Montiel, S. (2000). Vertebrate frugivory and seed dispersal of a Chihuahuan Desert cactus. doi: 10.1023/A:1009819419498. *Plant Ecology*, 221-229.

Murray, K. G., Russell, S., Picone, C. M., Winnettmurray, K., Sherwood, W., & Kuhlmann, M. L. (1994). Fruit laxatives and seed passage rates in frugivores - consequences for plant reproductive success. *Ecology*, 75(4), 989-994 pp. doi:10.2307/1939422.

Nakashima, Y., Inoue, E., Inoue-Murayama, M., & Rafiah Abd Sukor, J. (2010). Functional uniqueness of a small carnivore as seed dispersal agents: A case study of the common palm civets in the Tabin Wildlife Reserve, Sabah,

Malaysia. *Oecologia*, 164, 721–730. https://doi.org/10.1007/s00442-010-1714-1.

Nogales, M. , Medina, F. M. and Valido, A. (1996), Indirect seed dispersal by the feral cats Felis catus in island ecosystems (Canary Islands). *Ecography*, 19: 3-6. doi:10.1111/j.1600-0587.1996.tb00149.x

Nogales, M., I. Castañeda, M. López-Darias, F. M. Medina y E. Bonnaud. (2015). The unnoticed effect of a top predator on complex mutualistic ecological interactions. *Biological Invasions* 17:1655–1665. https://doi.org/10.1007/s10530-014-0823-x.

Nova J. S. (2012). The Wildlife Techniques Manual: Volume 1: Research. Volume 2: Management. Ed The Johns Hopkins University Press. 7ma edición. Baltimore, Maryland, USA. 686 y 414. https://books.google.com.mx/books?id=PL2IHTdzSeAC&printsec=frontcover &redir_esc=y#v=onepage&q&f=false

Oliver, C. D. & B. C., Larson. (1996). Forest Stand Dynamics. New York, USA. McGraw-Hill. 520 p. ISBN: 0471138339, 9780471138334. https://www.journals.uchicago.edu/doi/pdfplus/10.1086/419821

Otani, T. (2002). Seed dispersal by Japanese marten *Martes melampus* in the subalpine shrubland of northern Japan. *Ecological Research*, 17(1), 29-38 pp. doi:10.1046/j.1440-1703.2002.00460.x.

Pech-Canche, J., Sosa–Escalante, J., & Cruz, M. (2009). Guía para la identificacion de pelos de guardia de mamíferos no voladores del Estado de Yucatán, México.. *Revista Mexicana de Mastozoología (Nueva Época)*, 13(1), 7-33. doi:http://dx.doi.org/10.22201/ie.20074484e.2009.13.1.33.

Perea, R., Delibes, M., Polko, M., Suárez-Esteban, A. & Fedriani, J. M. (2013). Context-dependent fruit–frugivore interactions: partner identities and spatio-

temporal variations. *Oikos*, 122(6), 943-951 pp. doi:10.1111/j.1600-0706.2012.20940.x.

Rodríguez-Estrella, R., Moreno, A. R., & Tam, K. G. (2000). Spring diet of the endemic ring-tailed cat (*Bassariscus astutus* insulicola) population on an island in the Gulf of California, Mexico. Journal of Arid Environments, 44(2), 241–246. https://doi.org/https://doi.org/10.1006/jare.1999.0579.

Russo, S. E., Portnoy, S. & Augspurger, C. K. (2006). Incorporating animal behavior into seed dispersal models: Implications for seed shadows. *Ecology*, 87(12), 3160-3174 pp. doi:10.1890/0012-9658(2006)87[3160:iabisd]2.0.co;2.

Rzedowski, G. C. de, & Rzedowski, J. (2005). *Flora fanerogámica del Valle de México*. 2ª. ed, 1ª. reimp., Instituto de Ecología, A.C. y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pázcuaro, Michoacan, 1406 pp.

Rzedowski, J. (1978). La vegetación de México. México, D.F.: Limusa. http://www.academia.edu/9142430/VEGETACION_DE_MEXICO_Jerzy_Rz edowski.

Sánchez-González, R., Martin, A. D. H. S., Rosas-Rosas, O. C., & García-Chávez, J. (2018). Diet and abundance of bobcat (*Lynx rufus*) in the Potosino-Zacatecano Plateau, Mexico. *Therya*, 9(2). https://doi.org/10.12933/therya-18-498ISSN2007-3364.

Sarasola, J. H., Zanón-Martínez, J. I., Costán, A. S., & Ripple, W. J. (2016). Hypercarnivorous apex predator could provide ecosystem services by dispersing seeds. *Scientific Reports*, 6, 19647. doi:10.1038/srep19647.

SEDESO. (1995). Programa Integral de Manejo de la Zona Sujeta a Conservación Ecológica Sierra Fría. Aguascalientes: SEDESO.

Sosa-Ramírez J., A. Breceda-Solís, C. L. Jiménez-Sierra, L. I. Iñiguez-Dávalos y A. Ortega-Rubio, 2016. Capitulo XIX. Los ecosistemas de la Sierra Fría en Aguascalientes y su conservación. En: Ortega–Rubio, A., M. J. Pinkus-Rendón e I. C. Espitia- Moreno (Editores). Las Áreas Naturales Protegidas y la Investigación Científica en México. (pp. 447-471). Centro de Investigaciones Biológicas del Noroeste S. C., Universidad Autónoma de Yucatán y Universidad Michoacana de San Nicolás de Hidalgo. ISBN: 978-607-424-558-5

Suárez-Esteban, A., Delibes, M., & Fedriani, J. M. (2013). Barriers or corridors? The overlooked role of unpaved roads in endozoochorous seed dispersal. *Journal of Applied Ecology*, 50, 767–774. doi: 10.1111/1365-2664.12080.

Villalobos Escalante, A., Buenrostro, A., & Vega, G. (2014). Dieta de la zorra gris *Urocyon cinereoargenteus* y su contribución a la dispersión de semillas en la costa de Oaxaca, México. *Therya*, Vol.5, 355–363. https://doi.org/10.12933/therya-14-143.

Zúñiga, A., Muñoz-Pedreros, A., & Fierro., A. (2008). Dieta de *Lycalopex griseus* (GRAY, 1837) (Mammalia: Canidae) en la depresion intermedia del sur de Chile. *Gayana (Concepción)*, *72*(1), 113-116. https://dx.doi.org/10.4067/S071765382008000100013

CAPÍTULO III

SEED DISPERSAL BY CARNIVORES IN TEMPERATE AND TROPICAL DRY FORESTS

ABSTRACT

The seed dispersal mechanisms and regeneration of various forest ecosystems can benefit from the actions of carnivores via endozoochory. This study was aims to evaluate the role of carnivores in endozoochory and diploendozoochory, as well as their effect on seed viability, scarification, and germination in two forest ecosystems: temperate and tropical dry forest. We collected carnivore scat in the Protected Natural Area of Sierra Fría in Aguascalientes, Mexico, for two years to determine the abundance and richness of seeds dispersed by each carnivore species, through scat analysis. We assessed seed viability through optical densitometry using X-rays, analyzed seed scarification by measuring seed coat thickness using a scanning electron microscope, and evaluated seed germination in an experiment as the percentage of seeds germinated per carnivore disperser, plant species and forest type. In the temperate forest, four plant species (but mainly *Arctostaphylos pungens*) were dispersed by four mammal species. The gray fox dispersed the highest average number of seeds per scat (66.8 seeds). Bobcat dispersed seeds through diploendozoochory, which was inferred from rabbit (*Sylvilagus floridanus*) hair detected in their scats. The tropical dry forest presented higher abundance of seeds and richness of dispersed plant species (four species) than in the temperate forest, and the coati dispersed the highest number of seeds (8639 seeds). Endozoochory and diploendozoochory did not affect viability in thick testas seeds (1480μm) in temperate forest and thin testas seeds (281μm) in tropical dry forest. Endozoochory

improved the selective germination of seeds. Nine plant species were dispersed by endozoochory, but only one species (*Juniperus sp.*) by diploendozoochory. These results suggest that carnivores can perform an important ecological function by dispersing a great abundance of seeds, scarifying these seeds causing the formation of holes and cracks in the testas without affecting viability and promoting the selective germination of seeds.
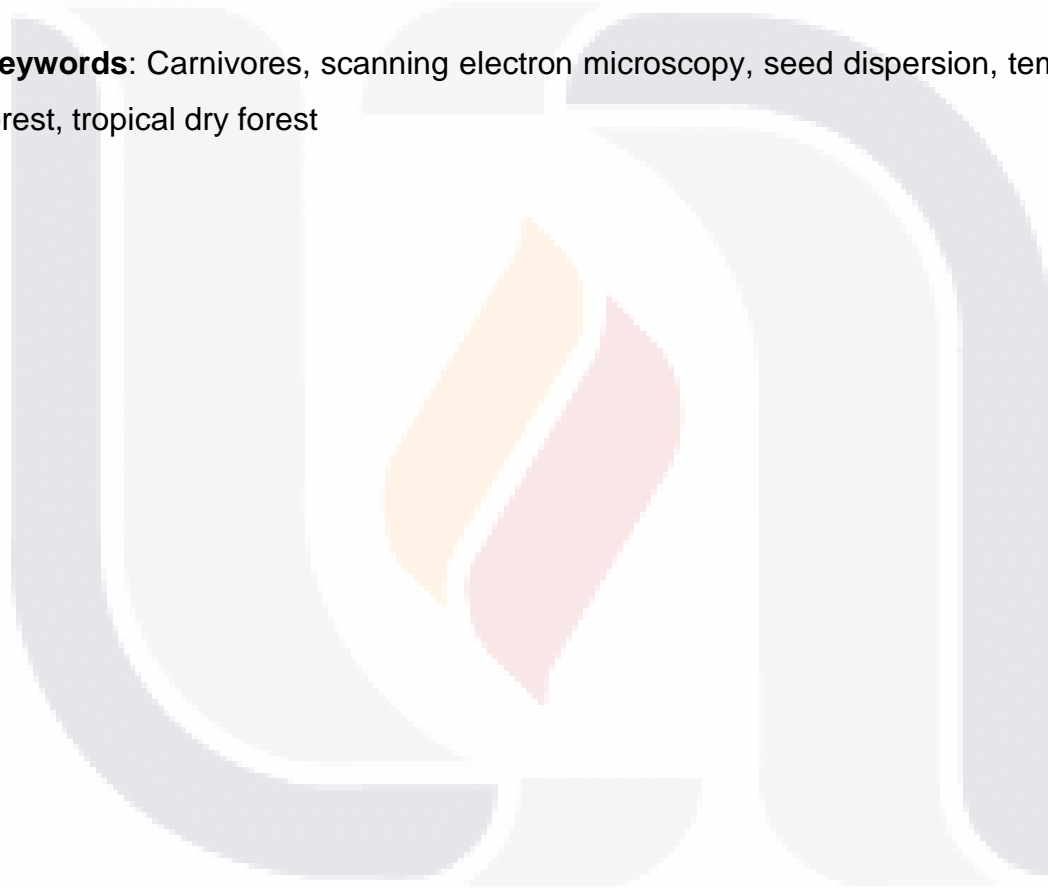
INTRODUCTION

Endozoochory is a seed dispersal process in which animals consume fruits and subsequently excrete the seeds at varying distances from the parent plant (Cypher & Cypher, 1999; Schaefer and Ruxton 2011). For seeds dispersed in this manner to survive and germinate, the seed coat must be capable of passing through the digestive tracts of the animals without damage to the embryo (Venier et al., 2012). In particular, carnivorous mammals can be involved in the endozoochory process; they are well known to consume large amounts of fleshy fruits (Koike, Morimoto, Goto, Kozakai, & Yamazaki, 2008; D'hondt & Hoffmann, 2011; Harrer & Levi, 2018) and are capable of dispersing viable seeds (with undamaged embryos) of a wide variety of plant species (Matias et al., 2010). Some carnivores can also disperse seeds via diploendozoochory, which involves the ingestion of seeds by two or more different species of animals in sequence, i.e., the seeds pass through a prey and then through a predator or carnivore (Hämäläinen, 2017).

Dispersion by endozoochory and diploendozoochory can influence plant distribution patterns (Haarmeyer et al., 2010) by facilitating the establishment of seeds in new habitats (Traveset et al., 2007). The success of both dispersal types and their influence on the recruitment of new plants depend on the number of seeds dispersed by the animals, the survival of these seeds following digestion and their probability of subsequent germination (Schupp et al., 2010; Venier et al., 2012). Therefore, although the absolute number of viable seeds dispersed is an important factor for endozoochory, analysis of the qualitative and quantitative components of dispersion is important to fully understand these processes (Schupp et al., 2010). In this sense, the process of diploendozoochory can be complex because the participation of the carnivore in the second phase of the dispersal process can influence the plant in three ways: transportation of the seeds, alteration of the quantity dispersed and modification of their viability and germination (Hämäläinen, 2017).

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Passage of the seeds through the digestive tracts of animals is a critical phase, during which they are subject to several processes that are potentially deleterious to seed viability and germination (Varela & Bucher, 2006; D'hondt & Hoffmann, 2011). These include wear of the testas and breaking of the physical dormancy period of the hard seeds of some plant species. Passage of the seeds through the digestive tract of animals can modify the seed coat and promote mechanical (in harder coats) or chemical (in softer coats) scarification, increasing the probability of either germination (Peco et al., 2006) or seed death if the embryo has been damaged (Campos et al., 2008). Although the hardness and thickness of a seed coat are important characteristics for seed survival and germination following passage through the digestive tract, the actual effects of this process on the structure of seed coats have been little explored in the literature (Venier et al., 2012). Recently, scanning electron microscopy has been used to observe changes in the seed coat as a consequence of passage through the digestive tract. Schaumann & Heinken (2002), used this technique to observe the testas of *Vaccinium myrtillus*, finding that control seeds presented intact cell walls, while those dispersed by martens (*Martes martes*) presented testas with damaged cell walls. Moreover, Costea et al. (2016) used scanning electron microscopy to observe how seeds of the species *Cuscuta pacifica*, dispersed by birds, presented fragmentation and even complete elimination of their outer layers.

In addition to the viability, change in seed coat and germination analysis in endozoochory, consideration must also be given to the ecosystem in which the seed dispersal takes place. The species of plants and animals that interact through seed dispersal could vary among different forest ecosystems, such as between temperate and tropical dry forests. Temperate forest are important biomes that provide ecosystem services worldwide and their characteristic vegetation type is represented by oaks and pines while, in the region of our study in the Sierra Fría, they are also dominated by *Juniperus sp.* and *Arctostaphylos pungens* (Díaz-Núñez et al., 2016). The most common seed dispersal modes found in this type of ecosystem are endozoochory (dispersal through consumption by animals),

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anemochory (dispersal by wind) and epizoochory (dispersal by adhering to mobile living beings) (Willson, Rice, & Westoby, 1990). Likewise, the seeds found in this type of forest are generally thick testas to protect their embryos from changes in temperature during the seasons and the passage through the digestive tract of dispersing animals (Ruprecht, Fenesi, Fodor, Kuhn, & Tökölyi, 2015; Rubalcava-Castillo et al., 2020). Tropical dry forests have canopy cover values greater than 30% and a great variety of flora and fauna (Olson et al., 2000). In particular, the vegetation in the region of the present study is dominated by communities of *Myrtillocactus geometrizans* and *Forestiera phillyreoides* (Argumedo-Espinoza et al., 2018). The most common dispersal systems found in this ecosystem are endozoochory, anemochory and hydrochory (dispersal by water) (Correa, Álvarez, & Stevenson, 2015). The seeds found in this type of forest are generally thin testas to adapt to the shadows under the canopies of the trees (Tiansawat, Davis, Berhow, Zalamea, & Dalling, 2014).

The role of carnivorous mammals as dispersal agents has been less studied than that of the birds, primates, and bats in temperate and tropical dry forests (Godínez-Alvarez et al., 2007; Stoner et al., 2007). The limited studies that focused on the dispersal of seeds by carnivores in forests include that of Rubalcava-Castillo et al. (2020), who showed the quantitative and qualitative contribution of mammals through endozoochory and diploendozoochory of seeds of *Arctostaphilos pungens* and *Juniperus deppeana* in temperate forest. In turn, Zarco-Mendoza et al. (2018) described numerous plant species that are dispersed by carnivores in the tropical dry forest. These authors found the seeds of 18 plant species in 384 scats of mammalian carnivores, concluding that carnivores can disperse seeds in abundance and that passage through the digestive tract had positive effects on the germination of two species, neutral effects on six species and negative effects on four species. However, due to the complexity of the diploendozoochoric process, there is a paucity of studies (greater than endozoochory studies) of this mechanism (Rubalcava-Castillo et al., 2020) in forests worldwide.

This study aims to complement and increase the knowledge of the role played by carnivores in seed dispersal through endozoochory and diploendozoochory by examining all the plant species found in carnivore scats to analyze abundance, viability, seed coat thickness and germination. Additionally, it aims to evaluate the role of carnivorous mammals in seed dispersal in the two different forest ecosystems: temperate and tropical dry forests. We hypothesize that the carnivorous mammals will perform important ecological functions by dispersing, scarifying, and favoring the germination of seeds with thick testas in temperate forest and seeds with thin testas in tropical dry forest.
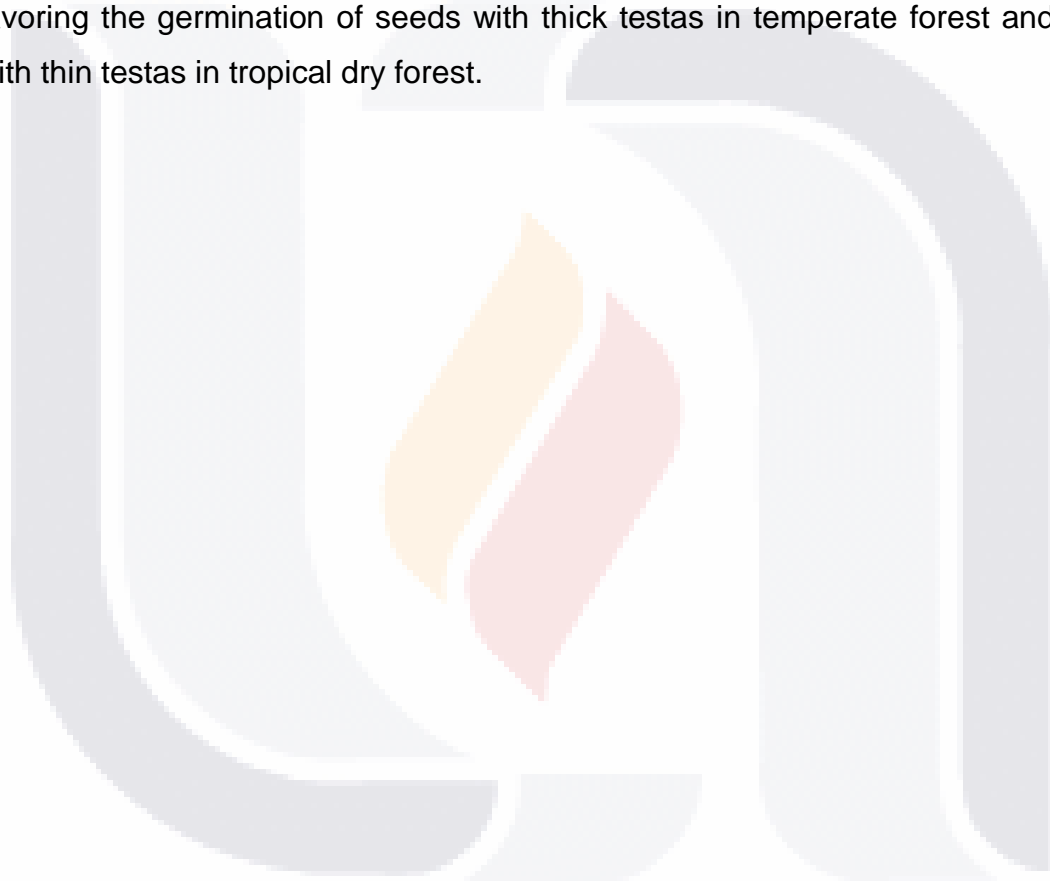
MATERIALS AND METHODS

Study site

We conducted the study in two temperate forest areas and one tropical dry forest area in the Protected Natural Area of Sierra Fría (PNA-SF) that host 14 species of carnivorous mammals (Chávez-Andrade et al., 2015), which is located in the western zone of the state of Aguascalientes, in Mexico (Figure 3.1). The temperate forest has a temperate subhumid climate and presents summer rainfall (Rzedowski, 1978) with an average annual precipitation of 650 mm (SEDESO, 1995). The natural plant communities in this forest are composed of pointleaf manzanita or pingüica (*Arctostaphylos pungens*), checkerbark juniper or táscate (*Juniperus deppeana*), strawberry tree or madroño (*Arbutus sp.*), oak (*Quercus potosina*), Chihuahua pine or ocote chino (*Pinus leiophylla* var. Chihuahuana) and twisted leaf pine or pino colorado (*Pinus teocote*), among others (Díaz-Núñez et al., 2016). On the other hand, the tropical dry forest has an average annual precipitation of 625 mm (Sosa-Ramírez, 1998), and the plant communities are composed of blue myrtle-cactus (*Myrtillocactus geometrizans*), palo bobo (*Ipomea murucoides*), kidneywood tree (*Eysenhardthya polystachya*), torchwood copal (*Bursera fagaroides*) and palo blanco (*Forestiera phillyreoides*), among others (Argumedo-Espinoza et al., 2018). We searched for scats in two temperate forests sites: "Monte Grande" (961 ha) and "Mesa del Águila y del Aserradero" (527 ha) and in one tropical dry forest site: "El Terrero de la Labor" (1,227 ha) (Figure 3.1). We sampled three different transects on each visit for each study area.
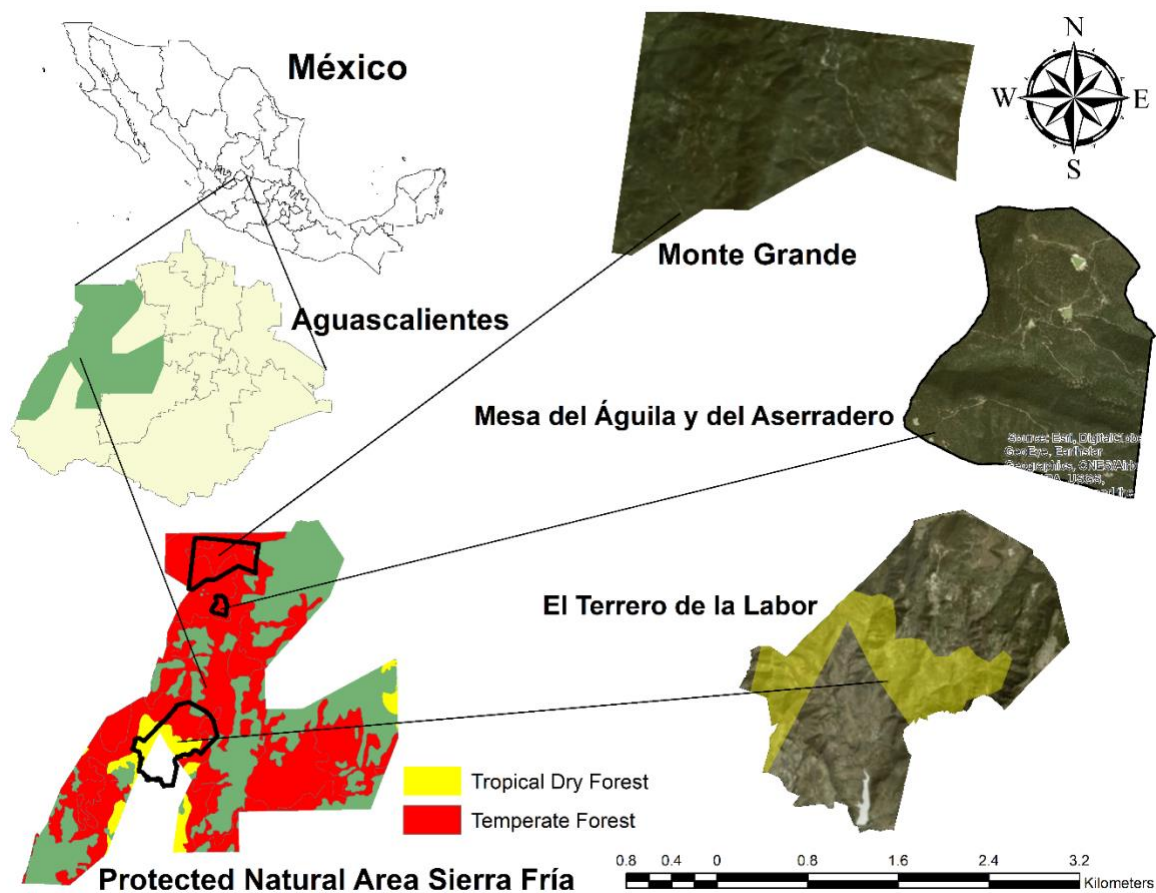
Figure 3.1 Location of the study areas Monte Grande, Mesa del Águila y del Aserradero, in the temperate forest, and El Terrero de la Labor, in the tropical dry forest of the Sierra Fría Protected Natural Area, Aguascalientes, Mexico.

Collection and identification of scats

We conducted field visits in each site once a month throughout the years of 2018 and 2019. Within each site, we collected scats located by sight within transects (Nova, 2012) of following walking routes through the study area to locate scats by sight. Each transect is delimited by a central line of 2 km in length, with two parallel lines located at a distance of 20 m on either side of the central line (Rubalcava-Castillo et al., 2020). We have established a total of 30 transects in each area on trails for the movement of fauna, on dirt roads and among vegetation, i.e., the scats

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were screened over a total area of 2 (temperate, tropical dry forest) x 30 (replicate transects) x 2000 m (transect length) x 40 m (transect width/buffer). We collected all of the scats found within the transect for identification, except for those that were dry and old, with a gray coloration, to avoid bias in the data related to the collection of old scats and a preference of collection towards specific animals. Thus, each transect was sampled twice during the study period. Scats corresponding to each mammalian species were identified based on the *Manual for Tracking the Wild Mammals of Mexico* (Aranda-Sánchez, 2012). Each scat associated with the mammalian species was classified into two categories according to the dietary habits of the animal: 1) mesocarnivorous/endozoochorous animals, the diet of which includes meat, insects, fruits, fungi, and other plant elements, e.g., the gray fox, and 2) hypercarnivorous/diploendozoochorous animals, the diet of which is based on meat (prey) and carrion, e.g., the felines.

Identification and abundance of seeds

We counted and identified all the seeds from the collected scats. We left the scats to dry at room temperature (23 °C) for 24 hours in Petri dishes, then extracted the seeds using a sieve (1mm mesh size) to retain the smallest seeds and wash them with running water. Once separated, the seeds were left to dry for 24 hours before analysis and identification. We used a stereoscopic microscope (Leica Microsystems, MZ6, Switzerland) to quantify the total number of seeds per scat. We also identified the seed species present in each scat to determine the richness using keys for each species (Rzedowski & Rzedowski, 2005) and comparisons with specimens from the Herbarium of the Autonomous University of Aguascalientes. We used these data to determine species richness, i.e., the number of seeds per scat per plant species, for each animal disperser and forest type (temperate or tropical). To infer diploendozoochory by the hypercarnivorous mammals, we also identified other elements contained in the scat. In the particular case of bobcats, which are fully carnivorous/predator (Sánchez-González, Martin, Rosas-Rosas, & García-

Chávez, 2018), if the presence of seeds associated with prey hairs is verified in the same scat, we identified the species of the seed and, in turn, the species of the prey, through the guard hairs. We then conducted a bibliographic review to verify whether the species of the seeds found form part of the diet of the prey. To determine the potential prey of the hypercarnivores, we identified the guard hairs of the prey contained in the scats of the predators by consulting the identification guide developed by Monroy-Vilchis and Rubio-Rodríguez (2014).

Three-step procedure

We used a three-step procedure to generate a robust evaluation of how ingestion by carnivores affects testa wear and seed viability and germination for each forest type and animal seed-disperser and dispersed plant species. Each step consists of a test: 1) a viability test, to determine whether the embryos from dispersed seeds show damage or remain intact, 2) a test of wear in the thickness of the testas using scanning electron microscopy and 3) a germination test to determine the impacts of the passage of seeds through the digestive tracts of the carnivores on seed germination. To carry out these tests, according to the abundance (seed per scat) per plant species, the seeds of five plant species (three temperate species and two tropical species) were used. Three replicates of 30 seeds (90 seeds) were used for each plant species with its respective disperser. In a few cases, the total of 30 seeds could not be collected, so only the available quantity was evaluated. The same batch of 90 seeds for each treatment was used in the viability and germination tests. For the scanning electron microscopy test, a representative plant species was selected for each forest, according to the abundance of seeds and the number of mammals that dispersed it. Thus, two seeds per mammal were analyzed.

Control groups

To check the effects of endozoochory and diploendozoochory on seeds from carnivores vs. seeds from the canopy, we established control groups based on the abundance of seeds found in the scats for each plant species. During the spring of 2019 (March-June), we collected 100 seeds from the canopy of 12 random individuals (trees) with ripe fruits for each plant species from the study areas to conduct compare viability, testa thickness and germination tests with the defecated seeds.

Viability test

We carried out viability tests for both the control and defecated seeds through optical densitometry analysis using X-ray equipment (Faxitron X-Ray Corporation, Texas, USA, at 10 s and 26 kv intensity). We performed densitometry analysis for each individual seed from the controls and the scats of each mammal, dispersed plant species and forest type, based on the technique proposed by Rubalcava-Castillo et al. (2020), which consists of observing the radiograph and distinguishing the viable seeds with undamaged testas and embryos from the non-viable seeds, by the presence of underdeveloped/incomplete embryos, empty seeds or no embryo.

Test of wear in testa thickness

We used whole and sagittal cut seeds for this test. We coated the seeds with yellow gold for 4 minutes in a Denton Vacuum apparatus (JFC-1100®, JEOL LTD, Tokyo, Japan). Once prepared, we placed the seeds inside the camera of a scanning electron microscope (JSM-35C®, JEOL LTD, Tokyo, Japan) (Dykstra and Reuss, 2003) in the Electron Microscopy Laboratory at the Autonomous University of Aguascalientes. We observed the seeds inside the camera and took thickness measurements on three parts of the testa at magnification 40x: (3) the micropyle

portion, (2) the central portion, and (1) the portion opposite the micropyle (Figure 3.2A), in which three measurements were taken per portion (Figure 3.2B). In addition, we recorded the qualitative characteristics of the surface and interior of the testas, including loss of superficial plant layers and the presence of holes and cracks in the external and internal parts of the testas.
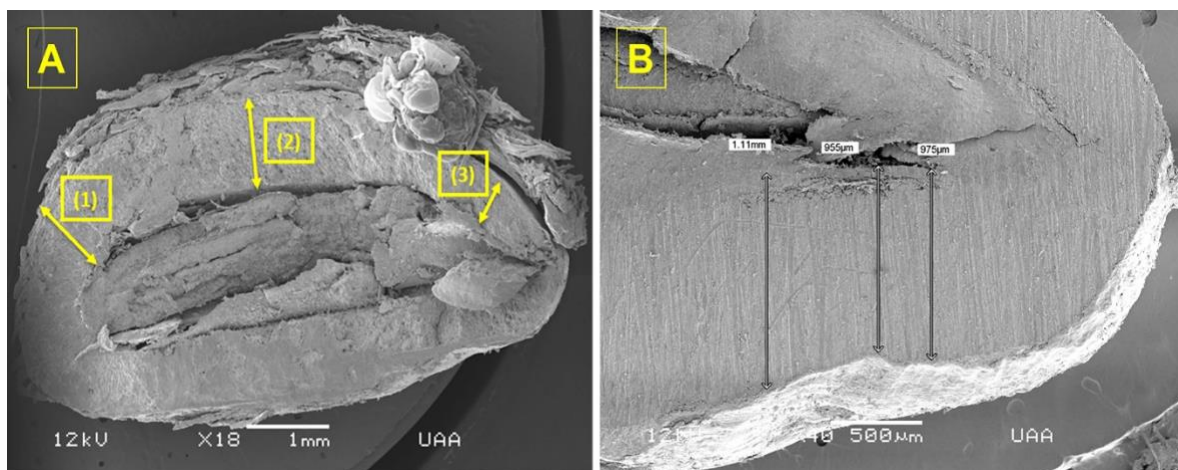

Figure 3.2 Image (x18) of a seed of *Juniperus sp*. taken with a scanning electron microscope. (A) Seed sections are shown: (1) section opposite the micropyle, (2) central section, and (3) the section on the side of the micropyle, (B) where the three measurements were taken for analysis.

Germination test

The control (canopy) seeds were stored for one month and subsequently used for the germination test. We washed the seeds from the scats and the canopy seeds in a 10% chlorine solution at a concentration of 50 ml. per 500 ml for 30 seconds in order to avoid absorption of the chlorine into the seed to clean the seeds of bacteria and fungi. Subsequently, we carried out three washes (30 seconds each wash) with distilled water to eliminate excess chlorine, and finally we applied fungicide to each seed (Interguzan 30-30, Int. Química de Cobre, Mexico) at a concentration of 1.2 g. per 100 ml to prevent fungal growth. We placed the seeds in Petri dishes, carrying out three replicates (one petri dish per replicate) of 30 seeds per treatment. The

experimental units consisted of 90 mm × 15 mm petri dishes, in which we sowed 30 seeds on filter paper and cotton, which were immediately moistened with 4 ml of distilled water (Antonio-Bautista, 2012). We placed the seeds inside a germination chamber (Lab Line, Imperial III, Melrose Park, IL, USA) at a controlled temperature of 25°C and a photoperiod of 12 hours for 61 days. All dishes were monitored every two days, recording the number of germinations per sample, in which germination was considered to have occurred when the radicles became visible (Herminio, 2003).

Statistical analysis

To analyze the contribution of each mammal to the dispersion of seeds (abundance of seeds) for each plant species in the temperate and tropical dry forest, we generated two different datasets for each forest type. Each dataset consisted of the number of seeds per scat for each carnivore and dispersed plant species, where the variable to be analyzed was the number of seeds per scat. Therefore, the sample size was dependent on the number of scats found for each animal species and plant. A multivariate GLM analysis was performed for each of the seed variables: abundance, species richness, viability, wear thickness and germination, to determine significance in the combination of factors: A) seed treatment (defecated vs control), B) animal species and C) type of forest (temperate vs tropical). The three factors were included in the same model as predictors. For temperate and tropical dry forest, we performed a Kruskal-Wallis test to determine significant differences among the average abundances of seeds per scat dispersed by each mammal under the null hypothesis that there will be no differences between the abundances of seeds per scat dispersed among carnivores. For the viability tests, testa thickness by SEM and germination, Dunnett's test was used, where the variables analyzed were the average percentage of viability and germination and the average testa thickness (µm). We conducted the above to determine significant differences between the average percentages/thicknesses in the seeds dispersed by each

carnivore compared to the seeds of the canopy (controls) for each plant species selected. We used the null hypothesis that there will be no differences between the average percentages/thicknesses of the seeds dispersed by each carnivore and their respective controls. We conducted all analyses at a significance level of 95%, using the Statgraphics program (16.1, 2012), with the values expressed as the average number of seeds per scat/% of viability or germination/testa thickness(µm): $\bar{x} \pm SD$.

RESULTS

Seed dispersal

According to the GLM analysis, the combined effect of the factors (mammal species and forest type) is significant for the abundance of seeds ($R^2 = 61.4\%$, $F_{13,86} = 10.55$, $p = 0.0001$). Four species of mammals dispersed seeds in the temperate forest: gray fox, coyote, ringtail, and bobcat. These species dispersed four species of plants: *Arbutus sp.*, *A. pungens*, *Juniperus sp.* and *Yucca sp.* (Table 3.1), with significant differences found in the average abundance of seeds (seeds per scat) dispersed in the scats among the four mammalian species ($X^2 = 14.73$, $p = 0.002$). The gray fox dispersed the highest average number of seeds (seeds per scat: $\bar{x} \pm SD = 66.8 \pm 68.2$), particularly those of the species *A. pungens* (241 ± 106) and, in turn, spread the highest number of scats, with the seeds of *Juniperus sp.* presenting the greatest frequency of appearance since they were found in 50 of the scats. The gray fox was also the most efficient mammal in seed dispersal in this system since 100% of its scats contained seeds. We identified the strictly carnivorous/hypercarnivorous bobcat as a diploendozoochorous species because all the seeds found in their scats were with the of hair of the rabbit species *Sylvilagus floridanus* (11.0 ± 0) (Table 3.1).

Four mammals dispersed seeds in the tropical dry forest – the gray fox and the ringtail, as in the temperate forest, as well as the coati and the badger. However, we found no diploendozoochoric mammals (Table 3.1), since it was not possible to find scats of hypercarnivores, and all the scats containing seeds in this forest were composed entirely of remains of fruits and their respective seeds. These were considered as endozoochoric according to the criterion proposed in the methodology and therefore diploendozoochory was discounted. We found no significant differences in the average abundance of seeds (seeds per scat) dispersed in the scats among the four mammalian species ($X^2 = 1.88$, $p = 0.59$), however, the coati dispersed the highest average abundance of seeds (seeds per scat: $\bar{x} \pm SD = 8639 \pm 12203$) in only three of their scats found. Thus, the tropical dry forest presented a

greater abundance and richness of dispersed species (R^2 = 15.41%, $F_{6,93}$ = 2.82, p = 0.01), since five plant species were dispersed. The gray fox and the ringtail dispersed four plant species in 83% and 81% of their scats, respectively (Table 3.1).

Table 3.1 Data for each forest type and carnivorous mammal species, describing the total number of scats, percentage of scats with seeds and average abundance of seeds ($\bar{x}$ ± SD) for each plant species. The occurrence of each plant species in the number of scats (N) is shown.

Forest	Disperser	Seed species	Scats (N)	Scats with seeds (%)	Abundance of seeds (seeds per scat: $\bar{x}$ ± SD)
Temperate	Gray fox		55	100.0	66.8 ± 68.2
		Arctostaphylos pungens	5		241.0 ± 106.0
		Juniperus sp.	50		51.3 ± 30.9
		Yucca sp.	2		18.5 ± 21.9
	Coyote		11	27.3	4.0 ± 2.7
		Arctostaphylos pungens	1		2.0 ± 2.8
		Juniperus sp.	2		3.0 ± 2.9
		Yucca sp.	1		8.0 ± 2.10
	Ringtail		9	77.8	43.4 ± 58.2
		Arbutus sp.	2		128.0 ± 10.0
		Juniperus sp.	3		13.67 ± 7.02
		Yucca sp.	2		3.5 ± 0.7
	Bobcat		9	11.1	11.0 ± 0.0
		Juniperus sp.	1		11.0 ± 0.0
Tropical	Gray fox		6	83.3	505 ± 1002.0
		Celtis sp.	1		84.0 ± 0.0
		Forestiera phillyreoides	2		44.5 ± 26.2
		Myrtillocactus geometrizans	1		2297.0 ± 0.0
		Prosopis laevigata	1		54.0 ± 0.0
	Ringtail		22	81.8	916.0 ± 1637.0
		Forestiera phillyreoides	5		23.2 ± 29.8
		Myrtillocactus geometrizans	13		1588.0 ± 1945.0
		Prosopis laevigata	1		12.0 ± 0.0
		Solanum sp.	4		72.3 ± 56.1
	Coati		3	66.7	8639.0 ± 12203.0
		Myrtillocactus geometrizans	1		17267.0 ± 0.0
		Prosopis laevigata	1		10.0 ± 0.0
	Badger		2	50.0	7.0 ± 0.0
		Prosopis laevigata	1		7.0 ± 0.0

Viability of seeds dispersed by carnivores

Five species of plants were selected for this analysis, based on the abundance of seeds found in the scats as specified in the methodology. For the temperate forest, we used seeds of *Arbutus sp.*, *A. pungens* and *Juniperus sp.*, while for the tropical dry forest, we used seeds of *F. phillyreoides* and *M. geometrizans*. According to the GLM analysis, the combined effect of the three factors (mammal species, forest type and seed treatment) was significant for the viability of seeds ($R^2 = 1.33\%$, $F_{6,3658} = 8.26$, $p < 0.0001$). In seeds of *Arbutus sp.* in the temperate forest, we found no significant differences ($F_{1,4} = 2.40$, $p = 0.19$) in the average percentage of viability ($\bar{x} \pm SD$) of the seeds dispersed by the ringtail (70.0 ± 19.8%), compared to those of the control (86.0 ± 7.7%). For *A. pungens*, we found significant differences ($F_{2,7} = 11.96$, $p < 0.0001$) in the average viability of the seeds dispersed by each mammal with respect to the control, particularly in those dispersed by the gray fox (91.6 ± 6.1%), which presented the highest percentage of viability compared to the control (76.0 ± 5.7%). In *Juniperus sp.*, we found no statistical differences ($F_{4,53} = 2.20$, $p = 0.08$) in seed viability for each mammal with respect to the control. Once again, however, the seeds dispersed by the gray fox (82.6 ± 12.4%) had a higher percentage of viability than those of the control (77.0 ± 6.0%), while practically half of the seeds dispersed by the bobcat were viable. In the tropical dry forest, for *F. phillyreoides* seeds, we recorded no significant differences in the average percentages of seed viability for each mammal, with respect to the control ($F_{2,8} = 0.57$, $p = 0.58$). However, the seeds dispersed by the gray fox (92.0 ± 11.3%) presented the highest percentage of viability compared to the control (79.0 ± 6.8%). Likewise, for *M. geometrizans*, we found no significant differences ($F_{3,14} = 1.10$, $p = 0.38$), although the viability of the seeds dispersed by most mammals achieved a higher percentage compared to the control (87.0 ± 3.8%), apart from those dispersed by the ringtail (75.3 ± 14.6%), which presented a lower percentage of viability (Table 3.2).

Table 3.2 Average viability percentages ($\bar{x} \pm SD$) from X-ray optical densitometry of seeds of *Arbutus sp.*, *Arctostaphylos pungens* and *Juniperus sp.*, with their respective animal dispersers, in the temperate forest; and of seeds of *Forestiera phillyreoides* and *Myrtillocactus geometrizans*, with their respective animal dispersers, in the tropical dry forest (both forests located at the Sierra Fría PNA in Aguascalientes, Mexico). N indicates the maximum number of seeds per treatment.

Forest	Seed species	Disperser species	Seeds (N)	Viability (%)
Temperate	*Arbutus sp.*	Ringtail	90	70.0 ± 19.8
		Control (canopy)	90	86.0 ± 7.7
	A. pungens	Gray fox	90	91.6 ± 6.1*
		Coyote	2	67.0 ± 0.0
		Control (canopy)	90	76.0 ± 5.7
	Juniperus sp.	Gray fox	90	82.6 ± 12.4
		Coyote	6	67.0 ± 0.0
		Ringtail	40	79.3 ± 15.1
		Bobcat	11	54.5 ± 0.0
		Control (canopy)	90	77.0 ± 6.0
Tropical	*F. phillyreoides*	Gray fox	90	92.0 ± 11.3
		Ringtail	90	84.0 ± 18.1
		Control (canopy)	90	79.0 ± 6.8
	M. geometrizans	Gray fox	90	82.0 ± 0.0
		Ringtail	90	75.3 ± 14.6
		Coati	90	90.0 ± 0.0
		Control (canopy)	90	87.0 ± 3.8

*Statistically significant differences according to the Dunnett test ($P < 0.05$).

As part of the seed viability analysis, physical changes were observed in the testas of the selected seeds, i.e., in the seeds with their respective dispersers (Table 1) of *Arbutus sp.*, *A. pungens* and *Juniperus sp.* for temperate forest and *F. phillyreoides* and *M. geometrizans* for tropical dry forest. When observing the radiographs for each selected plant species with their respective dispersers, particularly in the seeds of *Juniperus sp.*, we observed apparent changes to the seed testas due to mechanical damage during mastication, and due to passage through the digestive tracts of the gray fox (Figure 3.3A), coyote (Figure 3.3B), ringtail (Figure 3.3C), and bobcat (Figure 3.3D), but with no apparent damage to the seed embryos. Damage to the outer layers of the testa therefore had no influence on viability.
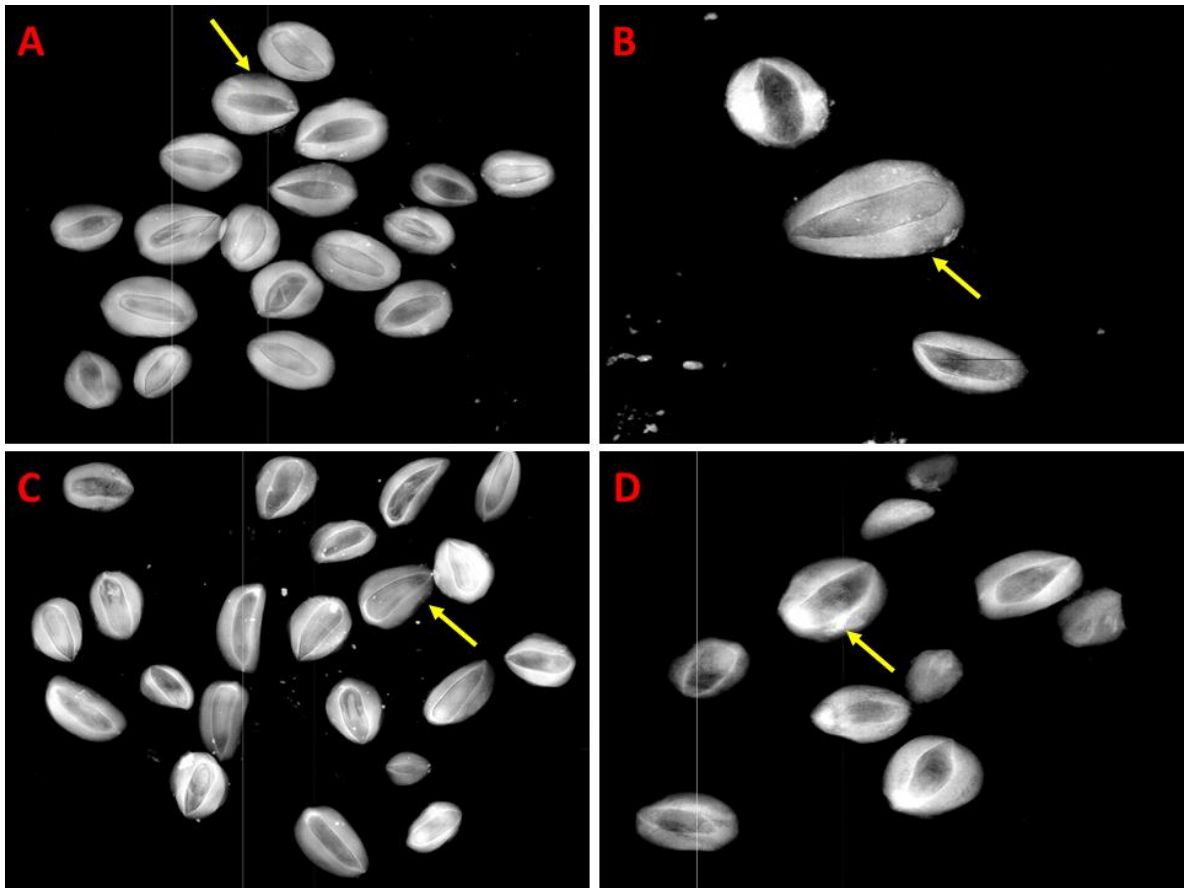
Figure 3.3 Seeds of *Juniperus sp.* from X-ray optical densitometry of the seeds from the scats of the different mammals in the temperate forest of the Sierra Fría PNA in Aguascalientes, Mexico. Seeds dispersed by: (A) gray fox, (B) coyote, (C) ringtail and (D) bobcat.

Wear in testa thickness

The thickness of the testas of seeds of *Juniperus sp.* from temperate forest and *F. phillyreoides* from tropical dry forest was analyzed. According to the GLM analysis, the combined effect of the three factors (treatment, mammals, and forest) was significant for the wear in the testa thickness of the seeds ($R^2 = 62.19\%$, $F_{6,61} = 16.72$, $p < 0.0001$). For *Juniperus sp.*, significant differences were found in the average thickness of the seed testas for each mammal, compared to the control

($F_{4.40}$ = 4.47, p = 0.00), since for all the mammals, the digested seeds presented greater thicknesses ($\bar{x} \pm SD$) than the control (731 ± 238µm), particularly in the ringtail (1480 ± 717µm), which presented the highest average. In tropical dry forest, the thickness values were lower in the control and for all the dispersers, compared to those of the temperate forest; however, significant differences were obtained between the seeds dispersed by each mammal and those of the control ($F_{2,20}$ = 4.30, p = 0.02), since the seeds dispersed by the ringtail had the highest average thickness (281 ± 50.6µm) relative to the control (215 ± 42.3µm) (Figure 3.4).
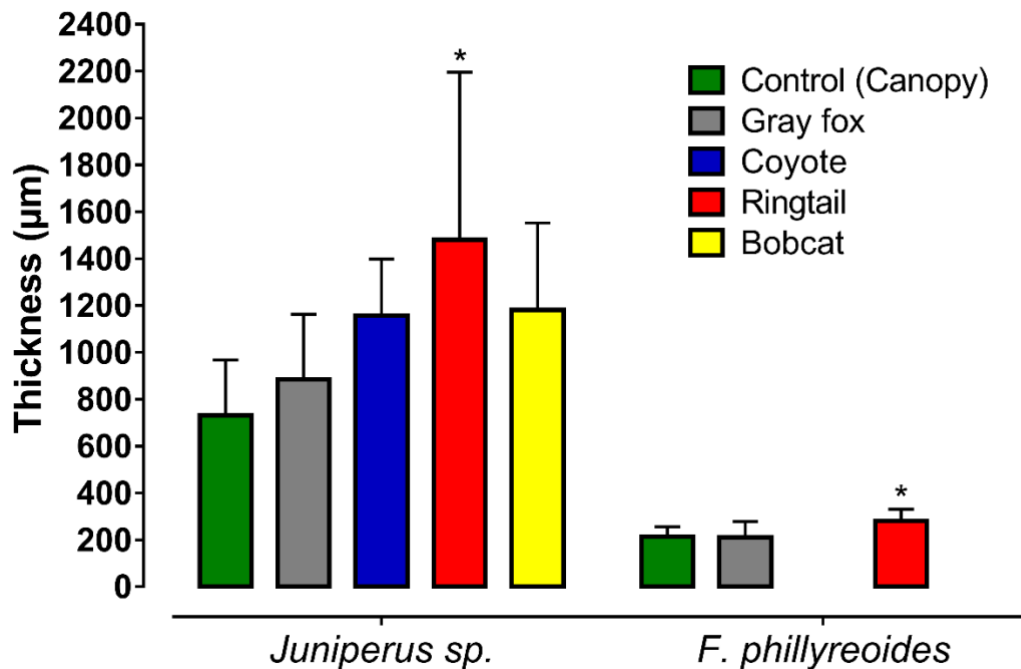
Figure 3.4 Measurements (µm) of the average thickness (± SD) of the seed testas of *Juniperus sp.* in the temperate forest and of the seed testas of *Forestiera phillyreoides* in the tropical dry forest, using seeds obtained in the field from scats of endozoochoric and diploendozoochoric mammals, and from the canopy in the Sierra Fría PNA in Aguascalientes, Mexico. * Statistically significant differences according to the Dunnett test ($P < 0.05$).

For the control in the temperate forest, the testa of the *Juniperus sp.* seed had a protective external vegetal fibrous layer (Figure 3.5A), which may have been removed when the seed passed in a first stage through the digestive tract of the

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rabbit and/or in a second stage through that of the bobcat (Figure 3.5B). This layer also presented wear or removal when the seed passed through the gray fox gut (Figure 3.5C) and the formation of cracks could be seen following passage through the tract of the ringtail (Figure 3.5D). These changes occurred in all of the seeds of each disperser although, due to time and budget, only two seeds per mammal were analyzed by scanning electron microscopy.
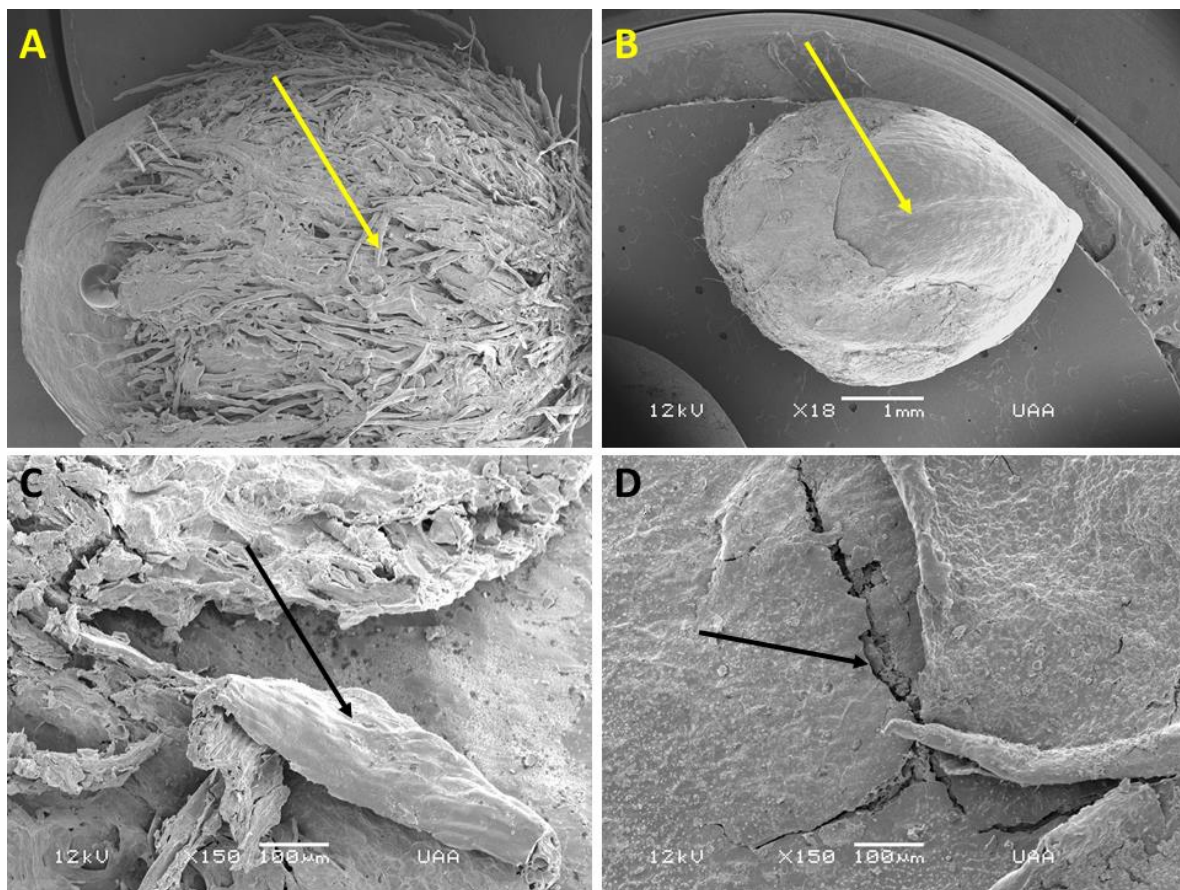
Figure 3.5 Images of seeds of *Juniperus sp.* of the temperate forest in the Sierra Fría PNA in Aguascalientes, Mexico, obtained using scanning electron microscopy. (A) Control seed with the protective outer fibrous layer intact (x19). (B) Seed dispersed by the bobcat with the outer layer removed (x18). (C) Seed dispersed by the gray fox with the testa surface detached (x150). (D) Seed dispersed by the ringtail with cracks across the testa (x150).

In the tropical dry forest, the seeds of *F. phillyreoides* did not have this protective layer, but only a line pattern presented on the surface (Figure 3.6A). There was a change in the pattern of these lines when they passed through the gray fox,

presenting cracks and holes (Figure 3.6B) but with no damage to the internal parts, and the endosperm and embryo therefore remained in good condition (Figure 3.6C). We observed large openings on the surfaces of the seeds from the ringtail scats (Figure 3.6D). As in the temperate forest, these characteristics were presented in all the seeds of each disperser, therefore, in four seeds from two mammals, these changes in the testas were seen.
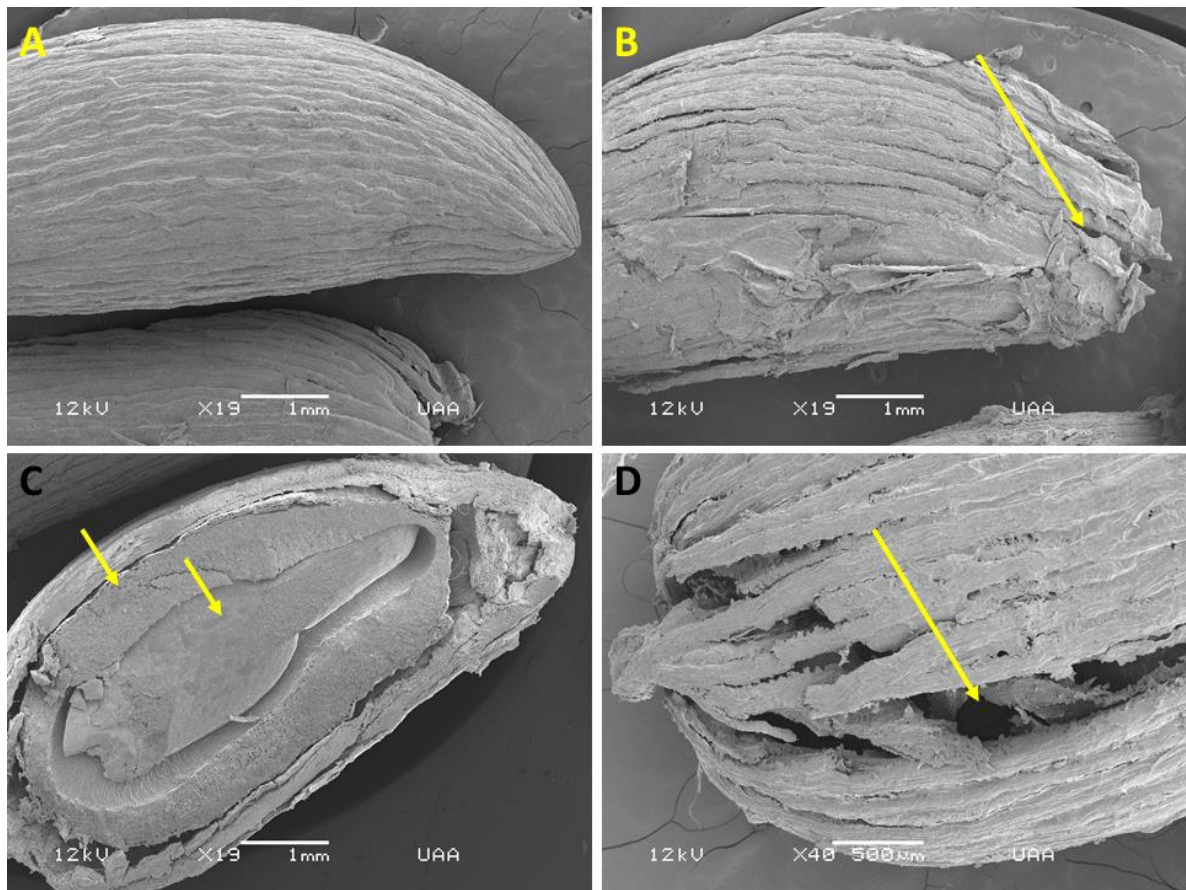
Figure 3.6 Images of seeds of *Forestiera phillyreoides* from the tropical dry forest in the Sierra Fría PNA in Aguascalientes, Mexico, obtained using scanning electron microscopy. (A) Control seed with a striated layer (x19). (B) Seed dispersed by the gray fox with cracks and holes on the outer layer (x19). (C) Seed dispersed by the gray fox with no damage to the endosperm or the embryo (x19). (D) Seed dispersed by the ringtail with large holes (x40).

Germination

The five plant species with their respective dispersers selected for the X-ray analysis were also used for the germination test. According to the GLM analysis and as happened in the variable seed passes, the combined effect of the three factors was significant for seed germination ($R^2 = 8.78\%$, $F_{6,1218} = 19.55$, $p < 0.0001$). The seeds obtained from the coyote and bobcat scats did not germinate, producing a germination rate of 0%. We therefore based the analysis on the three remaining mammal species (gray fox, ringtail, and coati) and the controls of the five plant species (Table 3.3). When performing the analysis for each plant species, we found no significant difference for *Arbutus sp.* in the temperate forest ($F_{1,4} = 1.58$, $p = 0.27$), since the average germination percentages ($\pm$ *SD*) of seeds from the control (70.0 $\pm$ 3.3%) and ringtail (62.2 $\pm$ 10.2%) were similar. For *A. pungens*, only the seeds dispersed by the gray fox germinated at a very low percentage (1.1 $\pm$ 1.9%) similar to the germination percentage in the control seeds (2.2 $\pm$ 1.9%). There were therefore no statistical differences found ($F_{2,4} = 0.57$, $p = 0.60$). Finally, in *Juniperus sp.*, we observed that the percentages of germination in seeds dispersed by the gray fox (12.2 $\pm$ 8.4%) and ringtail (17.5 $\pm$ 10.6%) were higher than that of the control (5.6 $\pm$ 1.9%), although these differences were not significant ($F_{4,5} = 1.79$, $p = 0.27$). In the tropical dry forest, for *F. phillyreoides*, we found significant differences in the average germination percentages of seeds dispersed by each mammal compared to the control ($F_{2,6} = 14.16$, $p < 0.0001$), with a higher average percentage for the control seeds (73.3 $\pm$ 3.3%) than in the seeds dispersed by any of the mammals, but particularly the ringtail, for which the seeds had the lowest germination percentage (27.8 $\pm$ 5.1%). On the other hand, for *M. geometrizans*, there were no significant differences between the average germination ($\pm$ *SD*) of the control and that of the seeds from the animals ($F_{3,8} = 0.65$, $p = 0.60$). However, the seeds dispersed by the gray fox presented greater germination (58.9 $\pm$ 20.4%) than the control seeds (44.4 $\pm$ 6.9%) (Table 3.3).

Table 3.3 Average germination percentages ($\bar{x} \pm SD$) of seeds of *Arbutus sp.*, *Arctostaphylos pungens* and *Juniperus sp.*, with their respective animal dispersers, in the temperate forest, and of seeds of *Forestiera phillyreoides* and *Myrtillocactus geometrizans*, with their respective animal dispersers, in the tropical dry forest (both forests located within the Sierra Fría PNA in Aguascalientes, Mexico). N indicates the maximum number of seeds per treatment.

Forest	Seed species	Disperser species	Seeds (N)	Germination (%)
Temperate	*Arbutus sp.*	Ringtail	90	62.2 ± 10.2
		Control (canopy)	90	70.0 ± 3.3
	A. pungens	Gray fox	90	1.1 ± 1.9
		Coyote	2	0.0
		Control (canopy)	90	2.2 ± 1.9
	Juniperus sp.	Gray fox	90	12.2 ± 8.4
		Coyote	6	0.0
		Ringtail	40	17.5 ± 10.6
		Bobcat	11	0.0
		Control (canopy)	90	5.6 ± 1.9
Tropical	*F. phillyreoides*	Gray fox	90	49.8 ± 17.1
		Ringtail	90	27.8 ± 5.1*
		Control (canopy)	90	73.3 ± 3.3
	M. geometrizans	Gray fox	90	58.9 ± 20.4
		Ringtail	90	52.2 ± 3.9
		Coati	90	50.0 ± 13.3
		Control (canopy)	90	44.4 ± 6.9

*Statistically significant differences according to the Dunnett test ($P < 0.05$).

DISCUSSION

During the study period, we found scats of gray fox, coyote, ringtail, coati and bobcat, which are associated with seed dispersal in the temperate and the tropical dry forest. While plants dispersed by mammals are widely distributed in the temperate zone of the PNA-SF (Díaz-Núñez et al., 2016). This is the case for *A. pungens*, the seeds of which were dispersed at a higher average abundance in the scats of gray fox, which corroborate previous findings for the same species (e.g., Rubalcava-Castillo et al., 2020). It is important to mention the role of the bobcat as a diploendozoochoric seed disperser (Hämäläinen et al., 2017), since *J. deppeana* seeds were found in its scats (Rubalcava-Castillo et al., 2020). In the tropical dry forest, the coati dispersed the highest abundance of seeds, with more than 8600 seeds in only three scats. Coatis can therefore play a key role in maintaining the dispersal service by spreading large amounts of seeds (Alves-Costa & Eterovick, 2007). The richness of the plant species found in the scats of the four dispersing carnivores for the tropical dry forest was higher than in the temperate forest. Although only five dispersed plant species were found in the tropical forest, suggesting that, despite the great richness of plant species in our study area (Argumedo-Espinoza et al., 2018), the mammals selectively feed on only a few plant species (Koike et al., 2008). Neither endozoochory nor diploendozoochory affected the viability or germination of the seed species in the two forest types. These results suggest that the viability of the seeds dispersed by the mammals under study was unaffected, which is crucial for retention (Nogales et al., 2015), the production of holes and cracks in the seed testas (Costea et al., 2016) and for improving the selective germination of thick-testa seeds in temperate forest and thin-testa seeds in tropical dry forest.

Seed dispersal

In the temperate forest, the gray fox was the most efficient mammal since 100% of its scats contained seeds of some plant species, demonstrating the ability of this animal to adapt its eating habits to consumption of fruits as an important resource in

its diet (Valkenburgh, 1996). However, seed dispersal depending may vary among different species of fox and the plant species. For instance, Bravo, Berrondo & Cueto (2019) report that 46% of the scats of the Andean fox (*Lycalopex culpaeus*) contained seeds of *Prunus cerasus* and *Malus domestica*. Nevertheless, foxes may be one of the main vectors of dispersal in forests.

While plants dispersed by mammals are widely distributed in the temperate of the PNA-SF (Díaz-Núñez et al., 2016), the abundance of dispersed or endozoochoric seeds may vary depending on region. In our case, the seeds of *A. pungens*, the seeds of which were dispersed at a higher average abundance in the scats of gray fox, which corroborate previous findings (e.g., Rubalcava-Castillo et al., 2020). However, Matías et al. (2010) reported a very low average abundance of *A. uva-ursi* seeds dispersed by red fox (*Vulpes vulpes*), marten (*Martes foina*) and wild boar (*Sus scrofa*). The seeds of *Arbutus sp.* were only dispersed by the ringtail, so dispersal of this plant species in the temperate forest of our region could be attributed solely to this mammal through selective feeding on the fruits of this plant (Koike et al., 2008). Long-term future studies are therefore important to our understanding of the variables that influence the dispersal of seeds in the different regions, the animal species involved and their preference for the ingestion of certain species of fruits.

It is important to mention the role of the bobcat as a diploendozoochoric seed disperser (Hämäläinen et al., 2017), since *J. deppeana* seeds were found in its scats, similar to the findings of Rubalcava-Castillo et al. (2020). Our results therefore reinforce those of other studies that demonstrate the diploendozoochory in some of these carnivores (Kurek & Holeksa, 2015; Sarasola et al., 2016). For this reason, it is important to consider hypercarnivores, such as bobcats, as an important component of the guild of seed dispersers.

In the tropical dry forest, the mammals under study actively participated in seed dispersal by spreading many seeds across the landscape. Such is the case of the coati, which dispersed the highest abundance of seeds, with more than 8600

seeds of the species *M. geometrizans* and *P. laevigata* found in only three scats. Coatis can therefore play a key role in maintaining the dispersal service by spreading large amounts of seeds (Alves-Costa & Eterovick, 2007). Likewise, the gray fox and the ringtail dispersed a large number of seeds of a greater variety of plant species and therefore play a role as alternative dispersers in forest landscapes such as that of the tropical dry forest (Alves-Costa & Eterovick, 2007).

The richness of the plant species found in the scats of the four seed-dispersing carnivores for tropical dry forest was higher than in temperate forest, as verified in the multivariate GLM analysis, although only five dispersed plant species were found. This is a low amount when compared to other areas of tropical forest where the richness of species dispersed by carnivores is higher (Alves-Costa & Eterovick, 2007; Zarco-Mendoza, Ríos & Godínez-Álvarez, 2018) suggesting that, despite the great richness of plant species in our study area (Argumedo-Espinoza et al., 2018), the gray fox, ringtail, coati and badger selectively feed on only a few plant species (Koike et al., 2008).

Viability

Viability is an essential property for seed germination, survival and establishment. In the temperate forest, most of the *A. pungens* seeds remained viable after being dispersed by the gray fox and ringtail, showing that passage of these seeds through the tract of these mammals did not affect their viability. In contrast, Rubalcava-Castillo et al. (2020) observed reduced viability of this plant species after being dispersed by the gray fox and ringtail. This contrasting finding suggests that the alterations or damage that the seeds undergo in the digestive tract may differ according to the year and study area, since these are the same plant and mammal species. Likewise, the seeds of *Arbutus sp.* dispersed by the ringtail seem to be unaffected by dispersion through endozoochory, since most of the seeds remained viable. However, there are other factors associated with dispersal as well as viability, such as the abundance, dispersal distance and germination of dispersed seeds, that

must be addressed before this mammal can be considered an effective agent for dispersal of this plant species.

Diploendozoochory produced a low percentage of viability in the seeds dispersed by the bobcat. Conversely, Nogales et al. (2015) verified that the viability of *J. turbinata* seeds by diploendozoochory in the *Galliota* lizard and its predator, the feline *Felis*, remained quite high. The decrease in viability of seeds associated with the bobcat could therefore be due to several factors: A) possible damage to the embryos as a consequence of the high retention times in the digestive tracts of the carnivores (Varela & Butcher, 2006), B) possible damage caused from the initial disperser/prey (in this case the rabbit), or C) the seeds collected from the canopy were defective.

In the tropical dry forest, the seeds of *F. phillyreoides* presented the highest viability in the scats of the gray fox, similar to the findings of Campos & Ojeda (1997) on the viability of *P. flexuosa* seeds from gray fox scats. This suggests that this mammal can disperse different forest species without negatively affecting the viability of the seeds. For *M. geometrizans*, the majority of seeds were able to remain viable in coati scats, similar to that observed by Alves-Costa & Eterovick (2007), who reported that the seeds can remain viable after passing through the digestive tract of this mammal. This establishes the role and importance of the coati as a mammal capable of dispersing a high quantity of *M. geometrizans* seeds without affecting viability.

Wear of the testa thickness

The result of the dispersal process involving passage through the animal gut could be aided by the seeds coming in contact with digestive tract acids, which cause changes in the internal and external structures of the testas, generally decreasing their thickness (Traveset et al. 2001; Nogales et al. 2007; Nogales et al., 2015). We had expected reduced thickness in the seeds dispersed by the animals; however, the opposite was found. The fact that the seed coat is thinner in the undigested seeds of the canopy relative to the seeds in the scats of all the dispersers could

indicate that the seed coat swells in some way while passing through the digestive tract. The thickness of the testas was thicker in the seeds of *Juniperus sp.* and *F. phillyreoides* that were found in the scats of all the animal species, especially those of the ringtail. In our case, the increase in thickness might be due to: a) the length of time that the seeds remained in the tracts, and the absorption of liquids in the intestines thus causing swelling of the seeds, b) the selection of fruits for the controls, i.e., seed thickness may vary according to the selected tree and even the year and time of collection, such that the seeds selected for this study for some reason may have had a thinner testa layer than the average, or c) the dispersers may select / transport seeds that have thicker testa layers on average. However, it was not possible to demonstrate this in the present study, and subsequent studies should aim to describe this absorption of liquids by seed coatings caused by the passage of the seeds through the tracts of mammals and perform an analysis of the controls through the seasons to establish whether such variation in thickness does in fact occur.

Through scanning electron microscopy, we are also possible observed the removal of the superficial vegetal layers, as well as cracks between the internal and external structures of the testa of seeds of some of the plant species that passed through the animal gut, such as *Juniperus sp.* and *F. phillyreoides*. This is similar to the case of *Cuscuta* seeds that passed through the digestive tracts of aquatic birds (Costea et al. 2016) as well as for *Vaccinium myrtillus* dispersed by the mammal *M. foina* (Schaumann & Heinken 2002). The removal and fragmentation of the testa probably benefitted the seeds by increasing their permeability to essential elements (water, light, oxygen) for germination. We can, thus, conclude that most of these seeds that passed through their digestive tracts of the carnivores remain viable and undergo a process of production of holes and cracks in the testas that can facilitate the entry of water and oxygen, which could benefit their subsequent germination.
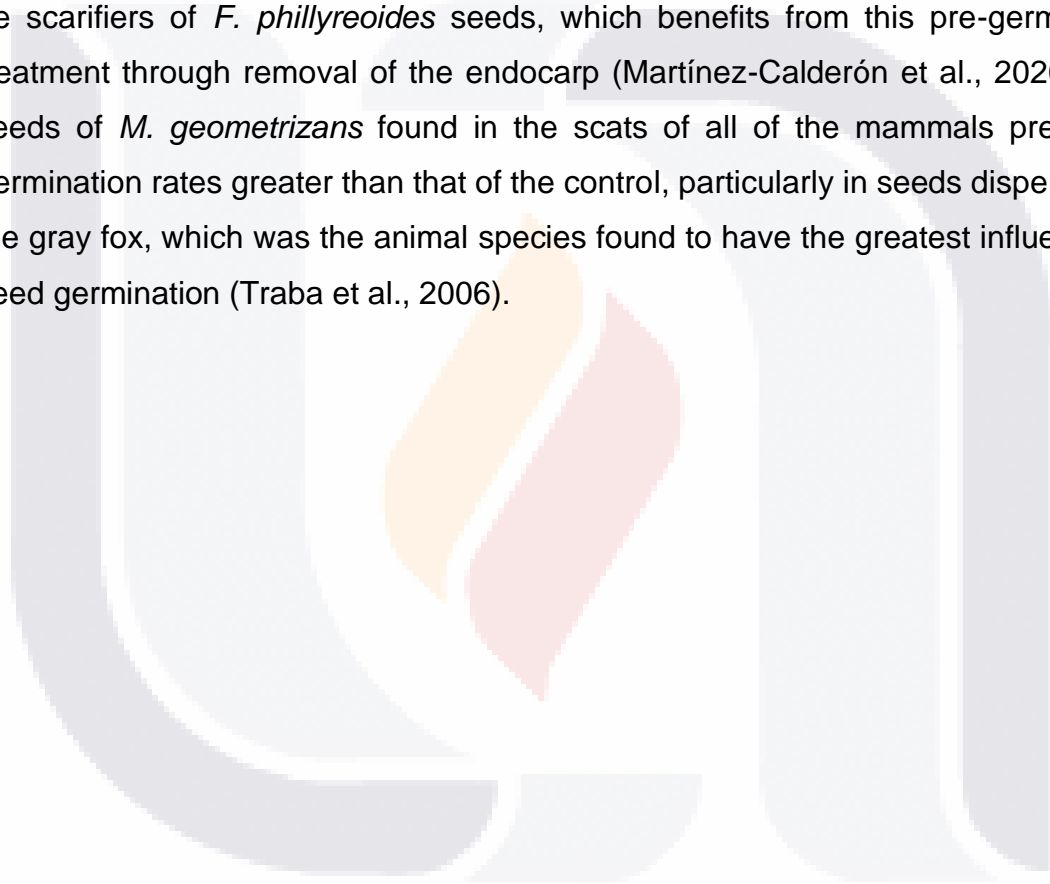
Germination

The carnivores in this study had varying impacts on the germination of the seeds they dispersed depending on the plant species. Although the action of the ringtail did not lead to a higher germination rate than that of the control in *Arbutus sp.* seeds, the percentages were very similar. This suggests that the passage of seeds of this plant species through the tract of ringtail is adapted to the process of endozoochory since the germination of the seeds was not affected. The seeds of this genus must be freed from the pulp of the fruit to successfully germinate (Narbona, Arista, & Ortiz, 2003), which is then enabled by endozoochory. The seeds of *A. pungens* presented very low germination in the gray fox scats, although the values are higher than the zero-germination observed by Rubalcava et al. (2020) for seeds planted under the same temperature conditions and incubation in a germination chamber. Likewise, Rubalcava et al. (2020) obtained lower germination rates in seeds of *J. deppeana* dispersed by gray fox, coyote, and bobcat, compared to the rates we report in the present study for gray fox and ringtail. This might be an indication of how the alterations to the testas of the *Juniperus sp.* seeds could have caused an increase in germination rates.

The seeds dispersed through diploendozoochory by the bobcat failed to germinate, possibly because some vertebrates with strong enzymatic digestion, such as the felines, actually damaged the seeds (Nogales et al., 2015). However, Rubalcava-Castillo et al. (2020) reported the germination of *Juniperus sp* seeds found in bobcat scats. Due to these variable rates of germination and high variation in seed viability of some *Juniperus* species (Rumeu et al. 2011), it is difficult to evaluate the effect of felines on their germination (Nogales et al., 2015) because the potential impacts of the primary disperser must also be considered, as well as the fact that the high latency recorded must be integrated with the low germination described for many *Juniperus* species (Adams 2008; Rumeu et al. 2009).

In the tropical dry forest, for the seeds of *F. phillyreoides*, the highest percentages of germination were presented by the control seeds. Despite this, the

seeds of *F. phillyreoides* dispersed by the gray fox presented a higher percentage compared with the other animals, and seeds dispersed by the gray fox can therefore remain viable without improving germination relative to the controls (Campos and Ojeda, 1997). Passage through the digestive tract can thus have a positive, neutral or negative effect on germination (Cypher & Cypher, 1999) and the adaptive importance of these mammals to trees such as *F. phillyreoides* is consequently related to dispersal (Peguero & Espelta, 2014). In addition, these animals can also be scarifiers of *F. phillyreoides* seeds, which benefits from this pre-germinative treatment through removal of the endocarp (Martínez-Calderón et al., 2020). The seeds of *M. geometrizans* found in the scats of all of the mammals presented germination rates greater than that of the control, particularly in seeds dispersed by the gray fox, which was the animal species found to have the greatest influence on seed germination (Traba et al., 2006).

CONCLUSIONS

The study showed that, in both the temperate and tropical dry forests, carnivores consuming fruits provide important seed dispersal services, by defecating viable seeds that are able germinate and thus can extend and reinforce the forest structure. However, the abundance and richness of dispersed seeds varies according the type of forest in which the dispersers are found. Seeds with thick testa in temperate forest and those with thin testa in tropical dry forest seem to be adapted to scarification by endozoochory, where mammals generate structural changes and openings in the testas, improving germination without affecting viability. In this study, the abundant and efficient participation of the gray fox and ringtail as dispersers of *Arctostaphylos pungens* in the temperate forest, and *Myrtillocactus geometrizans* in the tropical dry forest was highlighted. However, bobcat diploendozoochory acted to preserve seed viability, without improving germination. These results suggest that carnivores can perform an important ecological function by dispersing a great abundance of seeds, scarifying these seeds causing the formation of holes and cracks in the testas without affecting viability and promoting the selective germination of seeds.

REFERENCES

Adams, R. P. (2008). Junipers of the world: the genus *Juniperus*. Trafford Publishing, Vancouver.

Alves-Costa, C. P., & Eterovick, P. C. (2007). Seed dispersal services by coatis (*Nasua nasua*, Procyonidae) and their redundancy with other frugivores in southeastern Brazil. *Acta Oecologica*, 32(1), 77–92. https://doi.org/https://doi.org/10.1016/j.actao.2007.03.001.

Antonio-Bautista, A. (2012). Manual de Ensayos de Semillas Forestales Recopilación de Información. Coahuila. ISBN-978-607-95357.

Aranda-Sánchez, J. M. (2012). Manual para el Rastreo de Mamíferos Silvestres de México (C. N. p. e. C. y. U. d. l. B. (CONABIO) Ed. Primera ed.). México, D.F.

Argumedo-Espinoza, J., Sosa Ramírez, J., Díaz-Núñez, V., Pérez-Salicrup, D. R., & Siqueiros Delgado, M. E. (2018). Diversity, Distribution, and Abundance of Woody Plants in a Dry Tropical Forest: Recommendations for Its Management BT - Mexican Natural Resources Management and Biodiversity Conservation: Recent Case Studies. In A. Ortega-Rubio (Ed.) (pp. 479–500). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-90584-6_21.

Bravo, S. P., Berrondo, M. O., & Cueto, V. R. (2019). Are small abandoned plantations a threat for protected areas in Andean forests? The potential invasion of non-native cultivated species. *Acta Oecologica*, 95, 128–134. https://doi.org/https://doi.org/10.1016/j.actao.2018.11.002.

Campos, C., Peco, B., Campos, V., Malo, J., Giannoni, S., & Cardona, F. (2008). Endozoochory by native and exotic herbivores in dry areas: Consequences for germination and survival of *Prosopis* seeds. *Seed Science Research*, *18*, 91–100. https://doi.org/10.1017/S0960258508940344.

Campos, C. M., & Ojeda, R. A. (1997). Dispersal and germination of *Prosopis flexuosa* (Fabaceae) seeds by desert mammals in Argentina. *Journal of Arid Environments*, 35(4), 707–714. https://doi.org/https://doi.org/10.1006/jare.1996.0196.

Chávez-Andrade, M., Luévano-Esparza, J., Quintero-Díaz, G., Bárcenas, H. y Ceballos, G. (2015). Mamíferos de Aguascalientes. *Revista Mexicana de Mastozoología (Nueva Época)*, 5(2), 1–22. Retrieved from http://www.revmexmastozoologia.unam.mx/ojs/index.php/rmm/article/view/2 11.

Correa, D. F., Álvarez, E., & Stevenson, P. R. (2015). Plant dispersal systems in Neotropical forests: availability of dispersal agents or availability of resources for constructing zoochorous fruits? *Global Ecology and Biogeography*, 24(2), 203–214. https://doi.org/10.1111/geb.12248.

Costea, M., Stefanović, S., García, M. A., De La Cruz, S., Casazza, M. L., & Green, A. J. (2016). Waterfowl endozoochory: An overlooked long-distance dispersal mode for Cuscuta (dodder). *American Journal of Botany*, 103(5), 957–962. https://doi.org/10.3732/ajb.1500507.

Cypher, B. y Cypher, E. (1999). Germination rates of tree seeds ingested by coyotes and raccoons. *The American Midland Naturalist.*, 71-76. doi:http://dx.doi.org/10.1674/00030031(1999)142[0071:GROTSI]2.0.CO;2.

D'hondt, B., Vansteenbrugge, L., Van Den Berge, K., Bastiaens, J., & Hoffmann, M. (2011). Scat analysis reveals a wide set of plant species to be potentially dispersed by foxes. *Plant Ecology and Evolution*. Retrieved from https://www.ingentaconnect.com/content/botbel/plecevo/2011/00000144/000 00001/art00010.

Díaz-Núñez, V, Sosa-Ramirez J, Pérez-Salicrup. D. R. (2016). Vegetation patch dynamics and tree diversity in a diverse conifer and oak forest in central

Mexico. *Botanical Sciences*, 94(2), 12. https://doi.org/http://dx.doi.org/10.17129/botsci.284.

Dykstra, M. J, and Reuss L. (2003). Biological Electron Microscopy: Theory, Techniques and Troubleshooting. Kluwer Academic/Plenum, 2003. New York. 534 p.

Godínez-Alvarez, H., Rojas-Martínez, A., & Zarco-Mendoza, P. (2007). Dispersión de semillas por mamíferos: el caso del Valle de Tehuacán, una zona árida del centro de México. In G. Sánchez-Rojas & A. Rojas-Martínez (Eds.), Tópicos en sistemática, biogeografía, ecología y conservación de mamíferos (pp. 135–149). México: Universidad Autónoma del Estado de Hidalgo.

Haarmeyer, D. H., Bösing, B. M., Schmiedel, U., & Dengler, J. (2010). The role of domestic herbivores in endozoochorous plant dispersal in the arid Knersvlakte, South Africa. *South African Journal of Botany*, *76*(2), 359–364. https://doi.org/https://doi.org/10.1016/j.sajb.2009.12.001.

Hämäläinen, A., K. Broadley, A. Droghini, J. A. Haines, C. T. Lamb, S. Boutin, and S. Gilbert. (2017). The ecological significance of secondary seed dispersal by carnivores. *Ecosphere*, 8(2). doi:e01685. 10.1002/ecs2.1685.

Harrer, L. E. F., & Levi, T. (2018). The primacy of bears as seed dispersers in salmon-bearing ecosystems. *Ecosphere*, 9(1), e02076. https://doi.org/https://doi.org/10.1002/ecs2.2076.

Herminio, R. F. (2003). Estado del conocimiento de *Juniperus deppeana* Steud. Tesis de Ingeniería. Universidad autónoma Chapingo. 92 pp.

Koike, S., Morimoto, H., Goto, Y., Kozakai, C., & Yamazaki, K. (2008). Frugivory of carnivores and seed dispersal of fleshy fruits in cool-temperate deciduous forests. *Journal of Forest Research*, 13(4), 215–222. https://doi.org/10.1007/s10310-008-0069-5.

Kurek, P., & Holeksa, J. (2015). Grains in the diets of medium-sized carnivores -a case of diplochory? *Polish Journal of Ecology*, 63, 286–290. https://doi.org/10.3161/15052249PJE2015.63.2.012.

Martínez-Calderón, V., Sosa-Ramírez, J., González, J., Mendieta-Vázquez, A., & Sandoval Ortega, M. H. (2020). Propagation of *Forestiera phillyreoides*: A potential species for restoration in north-central Mexico. *Madera y Bosques*, 26, e2622052. https://doi.org/10.21829/myb.2020.2622052.

Matías, L., Zamora, R., Mendoza, I., & Hódar, J. A. (2010). Seed Dispersal Patterns by Large Frugivorous Mammals in a Degraded Mosaic Landscape. *Restoration Ecology*, 18(5), 619–627. https://doi.org/10.1111/j.1526-100X.2008.00475.x.

Monroy-vilchis, O., & Rubio Rodríguez, R. (2014). Guía de identificación de mamiferos terrestres del Estado de México, a través del pelo de guardia (1). México: Universidad Autónoma del estado de México. 115 pp.

Narbona, E., Arista, M., & Ortiz, P. (2003). Germinación de las semillas del madroño (*Arbutus Unedo* L., Ericaceae). *Acta Botánica Malacitana*, ISSN 0210-9506, No 28, 2003, Pags. 73-78, 28. https://doi.org/10.24310/abm.v28i0.7267.

Nogales M., Padilla D. P., Nieves C., Illera J. C., & Traveset A. (2007). Secondary seed dispersal systems, frugivorous lizards and predatory birds in insular volcanic badlands. *J Ecol*, 95:1394–1403.

Nogales, M., Castañeda, I., López-Darias, M., Medina, F. M., & Bonnaud, E. (2015). The unnoticed effect of a top predator on complex mutualistic ecological interactions. *Biological Invasions*, 17(6), 1655–1665. https://doi.org/10.1007/s10530-014-0823-x.

Nova J. S. (2012). The Wildlife Techniques Manual: Volume 1: Research. Volume 2: Management. Ed The Johns Hopkins University Press. 7ma edición. Baltimore, Maryland, USA. 686 y 414 pp.

Olson, D., E. Dinerstein, R. Abell, T. Allnutt, C. Carpenter, L. McClenachan, J. D'Amico, P. Hurley, K. Kassem, H. Strand, M. Taye y M. Thieme. (2000). The Global 200: A Representation Approach to Conserving the Earth's Distinctive Ecoregions. Word Wildlife Fund.

Peco, B., Lopez-Merino, L., & Alvir, M. R. (2006). Survival and germination of Mediterranean grassland species after simulated sheep ingestion: ecological correlates with seed traits. *Acta Oecologica*, *30*, 269–275. https://doi.org/10.1016/j.actao.2006.05.004.

Peguero, G., & Espelta, J. M. (2014). Endozoochory and Fire as Germination Triggers in Neotropical Dry Forests: an Experimental Test. *Biotropica*, 46(1), 83–89. https://doi.org/10.1111/btp.12076.

Rubalcava-Castillo, F. A., Sosa-Ramírez, J., Luna-Ruíz, J. J., Valdivia-Flores, A. G., Díaz-Núñez, V., & Íñiguez-Dávalos, L. I. (2020). Endozoochorous dispersal of forest seeds by carnivorous mammals in Sierra Fría, Aguascalientes, Mexico. *Ecology and Evolution*. https://doi.org/10.1002/ece3.6113.

Rumeu, B., Elias, R. B., Padilla, D. P., Melo, C., & Nogales, M. (2011). Differential seed dispersal systems of endemic junipers in two oceanic Macaronesian archipelagos: the influence of biogeographic and biological characteristics. *Plant Ecol*, 212:911–921.

Rumeu, B., Nogales, M., Elias, R. B., Padilla, D. P., Resendes, T., Rodríguez, A., Valdés, F., & Días, E. (2009). Contrasting phenology and female cone characteristics of the two Macaronesian island endemic cedars (*Juniperus cedrus* and J*. brevifolia*). *Eur J Forest Res*, 128:567–574.

Ruprecht, E., Fenesi, A., Fodor, E. I., Kuhn, T., & Tökölyi, J. (2015). Shape determines fire tolerance of seeds in temperate grasslands that are not prone to fire. *Perspectives in Plant Ecology, Evolution and Systematics*, 17(5), 397–404. https://doi.org/https://doi.org/10.1016/j.ppees.2015.07.001.

Rzedowski, C. G. de, & Rzedowski, J. (2005). *Flora fanerogámica del Valle de México*. (G. Rzedowski Calderón & J. Rzedowski, Eds.) (2a. ed). Pázcuaro, Michoacan: Instituto de Ecología A. C. y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Rzedowski, J. (1978). La vegetación de México. México, D.F.: Limusa.

Sánchez-González, R., Hernández-Saint Martin, A. D., Rosas-Rosas, O. C., & García-Chávez, J. (2018). Diet and Abundance of Bobcat (*Lynx rufus*) in the Potosino-Zacatecano Plateau, Mexico. *Therya*. scielomx.

Sarasola, J. H., Zanón-Martínez, J. I., Costán, A. S., & Ripple, W. J. (2016). Hypercarnivorous apex predator could provide ecosystem services by dispersing seeds. *Scientific Reports*, 6, 1–6. https://doi.org/10.1038/srep19647.

Schaefer, H. M., & Ruxton, G. D. (2011). *Plant-Animal Communication*. Oxford: Oxford University Press. https://doi.org/10.1093/acprof:osobl/9780199563609.001.0001.

Schaumann, F., & Heinken, T. (2002). Endozoochorous seed dispersal by martens (*Martes foina*, M. martes) in two woodland habitats. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 197(5), 370–378. https://doi.org/https://doi.org/10.1078/0367-2530-00053.

Schupp, E. W., Jordano, P., & Gómez, J. M. (2010). Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist*, *188*(2), 333–353. https://doi.org/10.1111/j.1469-8137.2010.03402.x.

SEDESO. (1995). Programa Integral de Manejo de la Zona Sujeta a Conservación Ecológica Sierra Fría. Aguascalientes: SEDESO.

Sosa-Ramírez, J. (1998). *Agua y sustentabilidad en Aguascalientes Tres ensayos*. Aguascalientes: Centro de Investigaciones y estudios Multidisciplinarios de Aguascalientes (CIEMA).

Stoner, K. E., Riba-Hernández, P., Vulinec, K., & Lambert, J. E. (2007). The Role of Mammals in Creating and Modifying Seedshadows in Tropical Forests and Some Possible Consequences of Their Elimination. *Biotropica*, *39*(3), 316–327. https://doi.org/10.1111/j.1744-7429.2007.00292.x.

Tiansawat, P., Davis, A., Berhow, M., Zalamea, P.-C., & Dalling, J. (2014). Investment in Seed Physical Defence Is Associated with Species' Light Requirement for Regeneration and Seed Persistence: Evidence from Macaranga Species in Borneo. *PloS One*, 9, e99691. https://doi.org/10.1371/journal.pone.0099691.

Traba, J., Arrieta, S., Jesús, H., & Cristina, C. (2006). Red fox (*Vulpes vulpes* L.) favour seed dispersal, germination and seedling survival of Mediterranean Hackberry (*Celtis australis* L.). *Acta Oecologica*, 30, 39–45. https://doi.org/10.1016/j.actao.2006.01.004.

Traveset A., Riera N., & Mas R. E. (2001). Passage through bird gut causes interespecific differences in seed germination characteristics. *Funct Ecol*, 15:669–675.

Traveset, A., Robertson, A.W., Rodríguez-Pérez, J. (2007). A review on the role of endozoochory in seed germination. In: Dennis, A.J., Green, R.J., Schupp, E.W., Westcott, D.A. (Eds.), Seed dispersal: theory and its application in a changing world. CABI Publishing, Wallingford, pp. 78–103.

Varela, O., & Bucher, E. H. (2006). Passage time, viability, and germination of seeds ingested by foxes. *Journal of Arid Environments*, *67*, 566–578. https://doi.org/10.1016/j.jaridenv.2006.03.013.

Valkenburgh BV (1996) Carnivore dental adaptations and diets: a study of trophic diversity within guilds. In: Gittleman JL (ed) Carnivore behavior, ecology, and evolution, vol 1. Cornell University Press, New York, pp 410–436.

Venier, P., Carrizo García, C., Cabido, M., & Funes, G. (2012). Survival and

germination of three hard-seeded Acacia species after simulated cattle ingestion: The importance of the seed coat structure. *South African Journal of Botany*, *79*, 19–24. https://doi.org/https://doi.org/10.1016/j.sajb.2011.11.005.

Willson, M. F., Rice, B. L., & Westoby, M. (1990). Seed dispersal spectra: a comparison of temperate plant communities. *Journal of Vegetation Science*, 1(4), 547–562. https://doi.org/10.2307/3235789.

Zarco-Mendoza, P., Ríos, L., & Godínez-Álvarez, H. (2018). Dispersal and germination of seeds ingested by carnivores in the Zapotitlan de las Salinas Valley, Mexico. *Polibotánica*, 139–147. https://doi.org/10.18387/polibotanica.46.7.

CAPÍTULO IV
CONCLUSIONES GENERALES

El Área Natural Protegida de Sierra Fría comprende bosques templados y bosques tropicales secos. Encontramos que la zorra gris, el coyote, el cacomixtle, el coatí y el gato montés son los dispersores en el bosque templado y bosque tropical seco, este último con mayor abundancia y riqueza de especies vegetales dispersas. Sin embargo, la dispersión de semillas de todos los carnívoros depende de la disponibilidad de presas y de frutos. En ambos bosques, la endozoocoria y la diploendozoocoria no afectaron la viabilidad o germinación de todas las especies de semillas. Estos resultados sugieren que los carnívoros en dispersión se adaptan a la abundancia y riqueza de semillas/frutos en los bosques donde habitan, para lo cual desarrollan importantes funciones ecológicas como la dispersión, escarificación, germinación selectiva de semillas de testa gruesa en bosques templados y semillas de testa delgada en bosques tropicales secos y determinando incluso la regeneración potencial del hábitat.

ANEXOS

Anexo A. Publicación del primer artículo en Ecology and Evolution en 2020.

Anexo B. Estatus para la aceptación del segundo artículo en Ecology and Evolution en 2020.

Anexo A. Publicación del primer artículo en Ecology and Evolution en 2020.

ORIGINAL RESEARCH

Endozoochorous dispersal of forest seeds by carnivorous mammals in Sierra Fría, Aguascalientes, Mexico

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Abstract

Some carnivorous mammals ingest fruit and disperse seeds of forest plant species capable of colonizing disturbed areas in ecosystems. The objective of the present study was to evaluate the dissemination of *Arctostaphylos pungens* and *Juniperus deppeana* seeds by the gray fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), and other carnivores in the Protected Natural Area Sierra Fría, in Aguascalientes, Mexico. Scat collection was undertaken via transects using the direct search method, while the seasonal phenology of *A. pungens* and *J. deppeana* was evaluated by recording flower and fruit abundance on both the plant and the surrounding forest floor ground. Seed viability was assessed by optical densitometry via X-ray and a germination test. It was found that the gray fox, coyote, ringtail (*Bassariscus astutus*), and bobcat (*Lynx rufus*) disseminated seeds of *A. pungens* (212 ± 48.9 seeds/scat) and *J. deppeana* (23.6 ± 4.9 seeds/scat), since a large proportion of the collected scat of these species contained seeds (28/30 = 93.33%, 12/43 = 27.9%, 6/12 = 50% and 7/25 = 28% respectively). The gray fox, coyote, ringtail, and bobcat presented an average of seed dispersion of both plant species of 185.4 ± 228.7, 4.0 ± 20.0, 12.1 ± 30.4, and 0.8 ± 1.5 per scat; the seed proportions in the gray fox, coyote, ringtail, and bobcat were 89.6/10.4%, 82.3/17.7%, 90.4/9.6%, and 38.1/61.9% for *A. pungens* and *J. deppeana*, respectively. The phenology indicated a finding related to the greater abundance of ripe fruit in autumn and winter ($p < .01$). This coincided with the greater abundance of seeds found in scats during these seasons. Endozoochory and diploendozoochory enhanced the viability and germination of the seeds ($p > .05$), except in those of *A. pungens* dispersed by coyote. These results suggest that carnivores, particularly the gray fox, the coyote, and the bobcat, play an important role in forest seed dissemination, and thus forest regeneration, by making both a quantitative and qualitative contribution to the dispersal of the two pioneer species under study.

KEYWORDS
Canis latrans, endozoochory, scats, seed dispersal, *Urocyon cinereoargenteus*

Anexo B. Publicación del segundo artículo en Ecology and Evolution en 2021.

ORIGINAL RESEARCH

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Seed dispersal by carnivores in temperate and tropical dry forests

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Abstract

The seed dispersal mechanisms and regeneration of various forest ecosystems can benefit from the actions of carnivores via endozoochory. This study was aimed to evaluate the role of carnivores in endozoochory and diploendozoochory, as well as their effect on seed viability, scarification, and germination in two forest ecosystems: temperate and tropical dry forest. We collected carnivore scat in the Protected Natural Area of Sierra Fría in Aguascalientes, Mexico, for 2 years to determine the abundance and richness of seeds dispersed by each carnivore species, through scat analysis. We assessed seed viability through optical densitometry using X-rays, analyzed seed scarification by measuring seed coat thickness using a scanning electron microscope, and evaluated seed germination in an experiment as the percentage of seeds germinated per carnivore disperser, plant species, and forest type. In the temperate forest, four plant species (but mainly *Arctostaphylos pungens*) were dispersed by four mammal species. The gray fox dispersed the highest average number of seeds per scat (66.8 seeds). Bobcat dispersed seeds through diploendozoochory, which was inferred from rabbit (*Sylvilagus floridanus*) hair detected in their scats. The tropical dry forest presented higher abundance of seeds and richness of dispersed plant species (four species) than in the temperate forest, and the coati dispersed the highest number of seeds (8,639 seeds). Endozoochory and diploendozoochory did not affect viability in thick-testa seeds (1,480 μm) in temperate forest and thin-testa seeds (281 μm) in tropical dry forest. Endozoochory improved the selective germination of seeds. Nine plant species were dispersed by endozoochory, but only one species (*Juniperus* sp.) by diploendozoochory. These results suggest that carnivores can perform an important ecological function by dispersing a great abundance of seeds, scarifying these seeds causing the formation of holes and cracks in the testas without affecting viability, and promoting the selective germination of seeds.

KEYWORDS

carnivores, scanning electron microscopy, seed dispersion, temperate forest, tropical dry forest