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Seed Dispersal of *Citharexylum tetramerum* and *Ziziphus penduculata* by Carnivorous in a Xerophilous Scrub at Tehuacan, Puebla, Mexico

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Abstract

Seed dispersion reduces the depredation rate and increases genetic flow. Some species of Carnivora consume fruits as a standard component of their diet, so they become potential seed dispersers. In Mexico, a few studies evaluated carnivorous as seed dispersers, especially in dry ecosystems. *Citharexylum tetramerum* and *Ziziphus pedunculata* are endemic plants from the Tehuacán-Cuicatlán's Biosphere Reserve (TCBR); however, there are very little data about dispersal seeds for both species. We assessed the germination of seeds ingested by carnivores obtained from feces. We compared them with seeds from fruits as a control group. At the Metropolitan University laboratory, we germinated seeds, from: plants, scats, and fruits, and we used Kruskall Wallis Test to compare percentage and germination rate. *C. tetramerum* seeds ingested by a carnivorous decreased in germination percentage and rate. In contrast, the percentage and rate of germination of *Z. pedunculata* seeds from carnivorous feces were higher than the control group. However, *Z. pedunculata* control seeds did not germinate during tests, but at the end of the tests, we found that 90% of control seeds were not dead, so they were dormant seeds. Carnivores interact as legitime dispersers for both plants because they are keeping the viability of seeds. Still, we only showed that *Z. pedunculata* gut carnivores broke with the dormancy of seeds. We consider that in future research, it could be essential to identify other animals that consume fruits of those plants and if it is possible to follow seeds after they have been removed. Those points could help to get better understanding of those endemic plants from Tehuacán.

Keywords

Seed Dispersal

1. Introduction

Plants benefit from seed dispersal because they decrease depredation rate and increase genetic flow and colonization of new habitats [1] [2] [3] [4] [5]. The quantity and quality components determine dispersal seeds' effectiveness; quality requires qualitative elements affecting the ability of the seed to find a safe place to germinate and how the disperser could influence the germination process [6].

Many plant species have evolved to use different media in order to reach longer distances for the seeds dispersal, these media frequently involved animals and have developed syndroms, or a group of special characteristic to attract animals in order to be a disperser [7]. Use of animals to disperse seeds is called zoochory, and when it is inside the animals body it is called endozoochory [8]. Arguybly endozoochory provides seeds with longer dispersal distances [8], which is an important feature in arid and semiarid lands. Plant that use endozoochory as a dispersion strategy normally produce fleshy fruits [9] with hard coated seeds which requires a chemmichal or physical treatment to germinate [7], it has be considered to prevent embryo damage in the digestive tract of animals. These fruits are normally consumed by birds or large herhervibores (horses or caws) who eat the fruit without actually chewing the seeds.

In general, species in the Carnivora mammalian order have anatomical structures specialized in consuming animal tissues [10], particularly the carnassials teeth. However, some include fruits and seeds as regular diet components [11] [12] [13] [14] [15]. For this reason, some carnivorous are potential seed dispersers [16] [17].

To be considered successfully dispersed, seeds must pass through the digestive system without being damaged and be deposited at a safe site far from the mother plant to reduce intraspecific competition and predation risk. Also, seeds must be deposited in conditions where it can germinate and establish such as good soil quality (not too hard or too soft and adecuate water suply [6]). However, there are different factors that influence the germination process; for example: carnivorous treatment in mouth and gut, herbivory, pathogens, competition and physological seed requirements [6].

Although seed dispersal by mammals is a very important topic in plant ecology [18], dispersal by non-frugivouros mammals have not received much attention [19]. Most of studies on seed loss by predation are focused on predispersal predation [20]. In Mexico, a few studies evaluated carnivorous as seed dispersers [15] [21]. Some of them were conducted at the Tehuacan-Cuicatlan Biosphere

Reserve (TCBR) [22] near to our working site.

Citharexylum tetramerum and *Ziziphus pedunculata* are endemic species from BRTC. *Citharexylum tetramerum* (Verbenaceae) (**Figure 1**) is a shrub: three meters high, with simple lanceolate or obovate leaves, flowers are axillary and white; fruits are green berries that change to black at maturity. On the other hand, *Ziziphus pedunculata* (Rhamneaceae) (**Figure 2**) is a shrub approximately five meters high. Its leaves are simple, most of them opposed, and their form could be obovate to oblong; flowers are aggregated and green; fruits are axillary, and shapes are oblong to orbicular and ginger color. It is considered as endargred by IUCN, Other names for this plant *Sarcomphalus pedunculatos and Condalia penduculata* are considered sinonimous [23].

We selected *Citharexylum tetramerum* and *Ziziphus pedunculata* because they are abundant in the southwest area of the TCBR. Also they are among the most abundant in mid-size carnivore feces like "coyotes" (*Canis latrans*), ring-tailed "cats" (*Basariscus astutus*), and gray "foxes" (*Urocyon cinereoargenteus*) and some others.

For this reason, we assessed the germination of seeds of *Citharexylum tetramerum* and *Ziziphus pedunculata* ingested by carnivores obtained from feces and compared them with seeds from fruits.

2. Materials and Methods

2.1. Study Area

Santo Tomas Otlaltepec (18˚17'22"N and 97˚45'37.8"W) is a small village in Atexcal county, of Puebla State, in Mexico. Otlaltepecs weather is tempered semiarid BS_1hw according to Köppen and modified by García [24] with a temperature range between 14˚C to 22˚C, and the rainfall season is from May through

Figure 1. *Citharexylum tetramerum*. https://inaturalist-open-data.s3.amazonaws.com/photos/5946883/original.jpeg.

Figure 2. *Ziziphus pedunculata*.
https://www.naturalista.mx/taxa/867219-Sarcomphalus-pedunculatus.

October [25]. The dominant plant community is xerophilous scrubland with columnar cacti, characterized by *Acacia farnesiana*, *A. cochliacantha* and *Prosopis laevigata*, associated with *Haematoxylon brasiletto*, *Caesalpinia melanadenia*, *Mimosa sp*, *Karwinskia mollis*, *Castela erecta* and *Bursera sp* [26].

2.2. Sample Collection and Preparation

We collected scats from *Canis latran*s (coyote), *Bassariscus astutus* (ringtail), and *Urocyon cinereoargenteus* (gray fox) along six one-kilometer-long pre-designated trails from February 2018 to January 2019. Trails were cleaned for scats one month before start the study. We identified feces at the species level according to morphological characteristics such as color, composition, shape, length, width, and deposition sites [27]. We removed seeds from scats and collected ripe fruits; also, we identified seeds to a species level with a photographic plants guide (TCBR 2010) and helped from local people.

In the laboratory, we assessed a study to compare the germination process of seeds from scats and fruits. We disinfected seeds with a 10% chlorine solution for 15 minutes and 70% ethyl alcohol for five minutes and rinsed with distilled water [28]. We placed *Z. pedunculata* seeds (n = 300, as a control 100, from *C. latrans*, 100 and *U. cinereargenteus* 100) in Petri dishes with 25 seeds in four replicas per carnivorous and control. Nevertheless, *C. tetramerum* seeds (n = 195 seeds, for control 75, *U. cinereargenteus* 90, *B. astutus* 30) were placed in, at least, 15 seeds per Petri dish with three to six replicates, depending on the availability of ingested seeds per carnivorous species. Petri dishes were placed in a growth chamber (EscLab-Line Instruments mod seat) at a constant temperature of 25˚C and a 12 hour photoperiod. Germination tests lasted 42 days, starting with the first record of germinated seeds. We considered a germinated seed at the radicle appearance [29]. At the end of germination tests, we selected seeds that did not germinated, and applied them a tetrazolium test to determine viability percentage—*Data analysis*—We used cumulative germination percentage and germination rate (germination speed per unit time) $V = \sum \frac{ni}{t}$, where ni is the number of germinated seeds, and t is the number of days since the first germinated seed [30].

We used the Kruskall-Wallis and multicompartions Dunn's Dunn test to compare percentage and germination rate because our data did not keep normality distribution. In Dunn's Test, multiple comparisons medians are significantly different if Z-value > 1.9600. We used chi-square to compare the viability percentage of seeds that did not germinate during germination tests. All yanalyses were run in NCSS [31] Statistical Software.

3. Results

We collected 193 scats during a year, 45 of *Canis latrans*, 104 of *Urocyon cinereargenteus*, and 44 of *Bassariscus astutus*. From those scats, organized from April to May 2018, we obtained 30 *C. tetramerum* seeds from ringtail scats and 108 from gray fox feces. From October 2018 to January 2019, we got 296 *Ziziphus pedunculata* seeds from coyote and gray fox feces. Furthermore, we got seeds directly from both species' parental plants simultaneously from scats.

C. tetramerum control seeds germinated 32 days after being sown; the germination percentage of the control group was constantly increasing, and most of the time, it was higher than the germination percentage of defecated seeds. Furthermore, the average control germination rate was higher than carnivorous's ingested seeds (**Figure 3**). We identified significant differences in germination percentage e (df = 2, H = 20.3845, p = 0.00004) (**Table 1**) and germination rate (df = 2, H = 44.6099, p > 0.0001), germination percentage and rate of the control group was significant (Z = 3.2073) higher than those from the defecated ones by gray fox and ringtail. The viability percentage of seeds that did not germinate was higher in control seeds (60.47%) than in seeds ingested by a gray fox (**Table 2**). Still, we could not identify significant differences between the control group and seeds from scats (χ^2 > 2.117, p > 0.0647).

Ziziphus pedunculata seeds defecated by coyote germinated eight days after they were sown; in contrast, ingested seeds by gray fox started germination 18 days after. Nevertheless, in the same laboratory conditions, the control group did not germinate during tests (**Figure 3**). We detected differences in germination percentage (df = 2, H = 60.1948, p > 0.0001) and in germination rate (df = 2, H = 62.6094, p > 0.0001), germination percentage and rate of seeds from carnivorous were different and higher than control seeds (Z > 3.2259). The viability percentage of non-germinated seeds was at least 45%; also, we found that 90% of control seeds were not dead, so they were dormant seeds (**Table 2**), therefore we could not identify significant differences in viability percentage between three treatments (χ^2 > 0.2850, p > 0.0643).

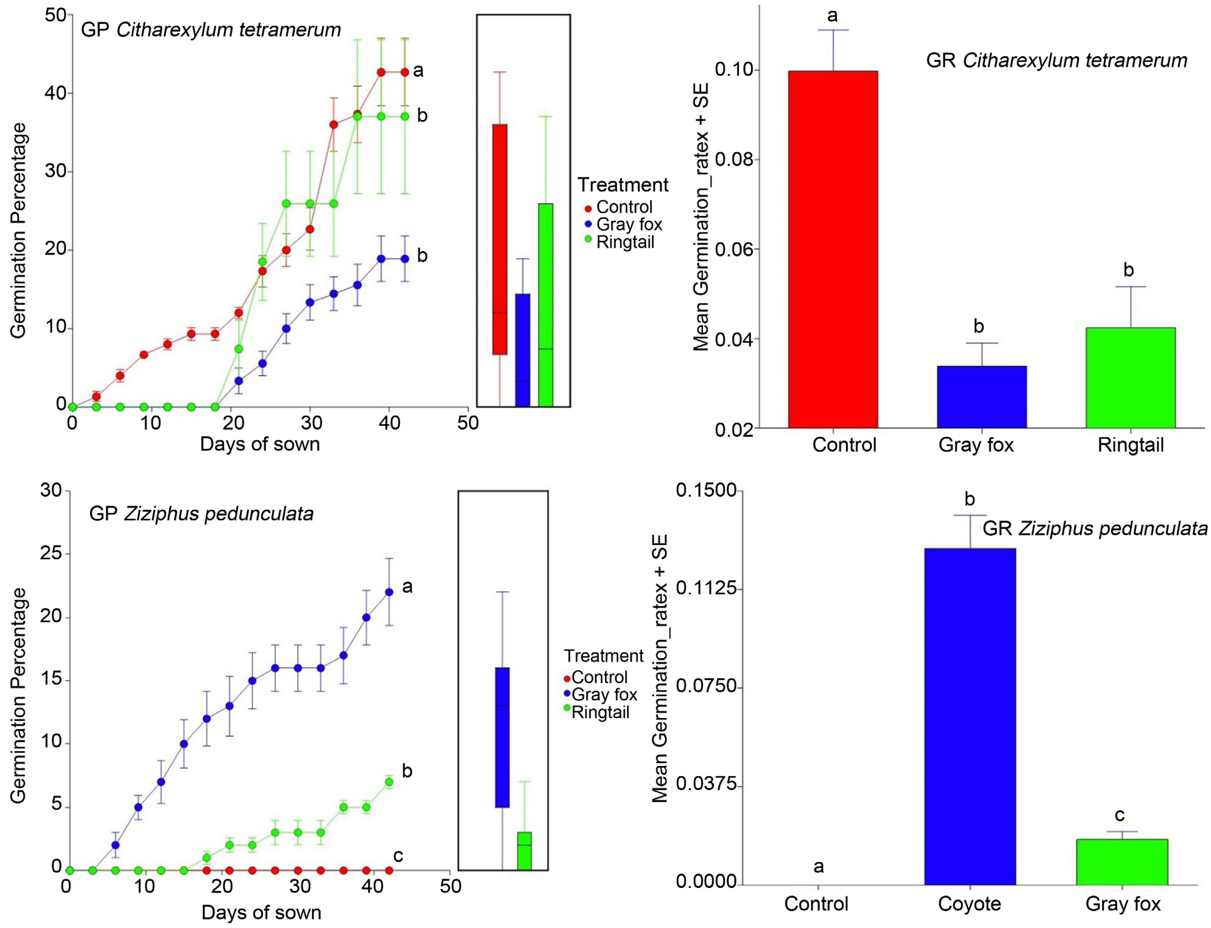

Figure 3. Germination rate (GR) of C. *tetramerum* (CT) and Z. *pedunculata* (ZP), within graphics; Same letters indicate no significant difference (p > 0.05)

Table 1. Kruskall-Wallis One-Way Test (H) and Dunn's Test multi multiple comparison. CT = Control, GF = Gray Fox, RT = Ringtail, CY = Coyote. Suppose Z-value > 1.96 medians are significantly different.

	Kruskall-Wallis One-Way				Dunn's Test (Multi-Comparison)	
Vegetal Species	Treatment	Median	H	Probabiity	Comparison	Z-Value
			Germination Percentage			
	Control	13.33			CT VS GF	5.2102
C. tetramerum	Gray fox	0	25.9974	<0.00001	GF VS RT	0.8377
	Ringtail	0			RT VS CT	3.5089
	Control	0			CT VS CY	8.8651
Z. pedunculata	Gray fox	0	60.1948	<0.00001	CY VS GF	5.5352
	Coyote	12			GF VS CT	3.3298
			Germiination Rate			
	Control	0.09523			CT VS GF	6.4563
C. tetramerum	Gray fox	0	44.4215	<0.00001	GF VS RT	0.2832
	Ringtail	0			RT VS CT	5.0791
	Control	0			CT VS CY	9.1797
Z. peduncuiata	Gray fox	0.0833	68.03	<0.00001	CY VS GF	6.0012
	Coyote	0.533			GF VS CT	3.1785

Table 2. Viability percentage of seeds that did not germinate during germination tests.

Vegetal Species	Treatments			
	Control	Gray Fox	Ringtail	Coyote
C. tetramerum	59.7 (26/44)	45.21 (33/73)	90 (9/10)	No tested
Z. pedunculata	90 (90/100)	82.8 (77/93)	No tested	92.31 (72/78)

Statistical analysis of using krusall-Wallis test and Dun's test to caompare individual differences between speciesis is shown in **Table 1**. In this table table is very clear that *C. tetramerun* germinates most from control, and there was no germination from seeds extracted from Gray fox or ringtail feces ($p < 0.01$). Opposite results came in the case of *Z. pendunculata* where seeds obtained from coyote and gray fox feces were the only one to germinate.

Finally, we can disregard the possibility that seeds were unviable because, in most cases, over 45% of non-germinated embryos were alive, as shown by the tetrazolium test (**Table 2**).

4. Discussion

C. tetramerum and *Z. pedunculata* are shrubs; both species are endemic to Puebla and Oaxaca State. *C. tetramerum* is endemic to a small area between the border of Puebla and Oaxaca [23]. For this reason, necessary dispersal seed studies are critical because there are data no for both species.

There were significant differences in germination between *C. tetramerum* seeds consumed by the carnivorous and the control group. In other species of *Citharexylum*, we found that seeds remain viable for an extended period, and a mechanic scarification was necessary to increase germination percentage [32]. Although, for our research, this increase in germination percentage did not occur after gut passage by carnivorous. In another study Soltani, *et al.* [33] found that germination percentage could decrease in -non-dormant seeds as compared to seeds that did not go through gut passage; also, the seed size of this plant could be a factor that caused some damage to seeds and directly affected germination since larger seeds have a better chance to be damaged by the disperser's tooth [34]. On the other hand larger seeds take a longer time to on germinate than smaller seeds [35].

Ziziphus pedunculata seeds ingested by carnivorous presented a higher germination percentage and rate than control seeds. The null germination of control seeds was reported in *Z. amole* seeds collected in Zapotitlan [22]. Furthermore, *Z.*, *mistol* seeds need physical and chemical scarification treatments to break dormancy imposed by the woody testa [36]. In our study, scarification of *Z. pedunculata* seeds was provided by carnivores, which helped to break dormancy.

From our results, we consider that those carnivorous are interacting with plants as legitimate seed dispersers because they keep the viability of seeds [37]. Seeds ingested by carnivorous travel distances between one to three kilometers

[13]. The longer the dispersion distance, the lesser the intraspecific competition for the seed and seedling, because of the reduction of dense dependent mortality that exists close to parental plants [38]. However, we do not know what happened with seeds after they were removed by carnivorous, and how many seeds survived to subsequent stages.

We could not find germination data of this species that could allow us to compare the effect of carnivores and not carnivorous animals (eg. birds) or more known dispersers such as Coati (*Nasua narica*).

5. Conclusions

In summary, *C. tetramerum* and *Z. pedunculata* seeds ingested by carnivorous coyotes, gray foxes, and ringtails maintain seed viability and can be considered as legitime dispersers. In the case of *Z. penduculata*, gut passage by carnivores broke seeds dormancy. Nevertheless, we do not know what happened with seeds after those animals removed them and if other dispersers contributed to "seeds" dispersal or if there are secondary dispersers of those plants. In future research, it could be essential to identify other vertebrates as birds or other mammals, that consume fruits from those plants and, if possible, follow seeds after they have been removed. Those points could give a better understanding of endemic plants and ecosystems of TCBR

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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