

NORWEGIAN UNIVERSITY OF LIFE SCIENCES



The role of goats in germination and dispersal of *Mimosa luisana* Brandegees (Leguminosae-Mimosoideae) seeds in Tehuacán-Cuicatlán valley, Puebla State, Mexico.



Mimosa luisana Brandegees (Leguminosae-Mimosoideae) pods

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Declaration

I, Luca Giordani, declare that this thesis is a result of my research investigations and findings. Sources of information other than my own have been acknowledged and a reference list has been appended. This work has not been previously submitted to any other university for award of any type of academic degree.

Signature.....

Date.....

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Abstract

Mimosa luisana Brandegees is a nurse plant of the Tehuacán-Cuicatlán valley. Despite its important role in the Tehuacán-Cuicatlán ecosystem, there is a paucity of relevant studies on the interaction between *M. luisana* and livestock. The endozoochory effect on *M. luisana* by domestic goats *Capra hircus* was evaluated in terms of excrement deposition, seed germination and survival. Four rectangular plots of 25m x 2m were placed randomly in a grazing area. Each transect was divided into three different habitat categories (open area, canopy area and boulder dominated area) and all the goat pellets were collected within the categories and checked for seeds. To evaluate the endozoochory effect on seeds germination and survival, 6 goats were fed with *M. luisana* seeds. Manure was collected at 8-hour intervals for 80 hours and samples of it were checked for the presence of seeds. The amounts of recovered seeds were used to estimate the total quantity of seeds in the total amount of pellets collected. Seeds from goat pellets (treatment) and seeds collected from pods in the field (control) were placed in a germination chamber for 24 days. Initiation time of germination, accumulative germination and final germination rate were compared between the treatment and the control. The results showed that goats deposit excrements and seeds in open areas which are optimal locations for *M. luisana* growth. Of the seeds that passed through the goats gut, 47.5% germinated while only 5.83% of the undigested seeds germinated. The passage through the goats' intestine not only affected the final germination rate, but it also shortened the germination time. After three days, 33.3% of digested seeds had germinated, while only the 1.6% of the seeds from the control germinated. The initiation time of germination among the Petri dishes was 2.75 days (± 2.37 S.D.) for digested seeds and 6.5 days (± 2.88 S.D.) for the control. Only one *M. luisana* seed was found in the 1958 field collected pellets and a low percentage (5.91%) of seeds in the feeding experiment survived after the passage through the goats gut. During the feeding experiment more than three

quarters of the seeds (77%) passed through goats intestine between 8 and 24 hours after ingestion. The study suggests that *M. luisana* seeds are potentially carried by goats for a considerable distance away from the mother plant. I conclude that goats can act both as an efficient and a legitimate disperser of *M. luisana* seeds. However, the low percentage of seeds passing through the goats intestine also shows that goats act as a predator of *M. luisana* seeds and may only occasionally function as disperser.

Keywords: Mimosa luisana; Goat; Endozoochory; Germination; Dry ecosystem.

1 Introduction

The Spanish conquerors introduced goats in the Tehuacán-Cuicatlán valley and subsequently goat raising has become one of the main subsistence activities for the local population (Hernández et al. 2001). In 1998 part of the valley was declared a Biosphere reserve in recognition of its high biodiversity (Davíla et al. 2002). This gave impetus to gather information on plant-herbivore interactions in order to make appropriate management decisions. As Baraza and Valiente-Baunet (in press) pointed out the mutualistic relationships between plants and ungulates as seed disperser are well known (Janzen 1984, Welch 1985) from different ecosystems like the temperate and Mediterranean region of Europe (Malo and Suárez 1995, Pakeman et al 2002, Couvreur et al. 2004), the tropical rain forests (Bodmer 1991) and the African savanna and bush dominated ecosystems (Miller 1996, Milton and Dean 2001). However, there is a paucity of relevant studies on the interaction between livestock and the Tehuacán vegetation.

In the Tehuacán-Cuicatlán ecosystem *Mimosa luisana* Brandegees plays an important role. Different studies defined *M. luisana* as a nurse plant of the Tehuacán-Cuicatlán ecosystem, as it is able to create favorable soil (Camargo-Ricalde and Dhillion 2003) and shade (Valiente-Baunet and Ezcurra 1991) conditions for the establishment of other species. Anyway very few

studies have focused on the ecology of *M. luisana* and to my knowledge none on its dispersion. Previous studies report that *M. luisana* is often browsed by goats (Dhillion et al. 2004) indicating that goats are a potential disperser of *M. luisana* seeds.

Dispersers can be categorized as either legitimate, efficient or as combination of both; where legitimacy is defined as the suitability of the deposition site for germination (Reid 1989) and efficiency is defined as the possibility to find seeds without damage in the disperser (Herrera 1989). The evaluation of goats in terms of legitimacy and efficiency for the dispersion of *M. luisana* seeds is the primary task when studying *M. luisana*'s reproductive cycle. Gut treatment on seeds is also an important aspect of endozoochory dispersion and it is strongly linked with legitimacy. Gut treatment may reduce legitimacy by destroying seeds and altering the speed of their germination (Schupp 1993, Traveset 1998). Another aspect is the role of goats as vehicles of seed deposition away from the mother plant; a role which is highly advantageous as supported by the Janzen and Connell hypothesis (Wang and Smith 2002). Distance that seeds are dispersed is connected with seeds retention time (Or and Ward 2003) and movement patterns (Vellend et al. 2003). Goats movement in the landscape are influenced by the shepherd, however an evaluation of seeds retention time becomes preliminary information on the role of goats in depositing the seeds away from the mother plant.

The objectives of this study were: a) to evaluate if goats are a legitimate disperser for *M. luisana* seeds; b) to evaluate if goats are efficient dispersers of *M. luisana* seeds; c) to evaluate if goat's gut treatment on *M. luisana* seeds alter seeds germination rate and d) to quantify the percentage of seeds able to pass through goats intestines.

2 Methods

2.1 Plant and herbivore species

Mimosa luisana (known as *uña de gato*) is a thorny shrub/tree endemic of Mexico and is distributed in Puebla and Oaxaca states (Grether et al. 2006). This species is common in several vegetation formations such as deciduous tropical forests, arid tropical scrubs, and thorny scrubs (Grether et al. 2006) as well as in abandoned agricultural fields (Camargo-Ricalde et al. 2002). *M. luisana*'s flowering occurs from April until November and fructification from September until December (Grether et al. 2006). The fruit is a one carpel pod, with each pod containing 2 to 8 seeds. Pods are grouped in bunches of 2 to 10. Seeds are small (2.7-3.5 mm long, 2.4-2.6 mm wide and 2.0-2.7 mm high), shiny dark brown and characterized by lentil form (Grether et al. 2006). *M. luisana* seeds are characterized by hard seed coats that impose seeds dormancy and determine low germinability; seeds dormancy might be broken through scarification (Camargo-Ricalde et al. 2004). Mimosa species are used by local people for fodder, firewood, living fences and constructions (Camargo-Ricalde et al. 2002).

The domestic goat, *Capra hircus*, was introduced in Mexico by the Spanish conquerors in the middle of the 16th century (Villegas-Durán et al. 2001) and subsequently goat herding became one of the most important subsistence activities for people living in the Tehuacán-Cuicatlán valleys (Hernández et al. 2001). Local people adopted an extensive grazing system which has been defined as one of the main reasons for environmental degradation in the area (Zavala-Hurtado and Hernández-Cárdenas 1998).

2.2 Study area

The Tehuacán-Cuicatlán valley is located between 17° 20' – 18° 53' N and 96° 55' – 97° 44' W and covers a surface of 11,050 km². The valley occupies territories of the States of Puebla and Oaxaca, Mexico and according to the classification of Reid (1964) (Rzedowski, 1978), is located in the Provincia Fisiográfica Sierra Madre del Sur. The region has a complex topography, with altitudes ranging from 500 to 3200 m a.s.l.; the dominant altitudinal range however is 1500-2400 m a.s.l. Fieldwork was conducted within the municipality of Zapotitlán Salinas, Puebla State, Mexico. The municipality, which is part of the Biosphere Reserve, lays at the altitudinal range of 1700-2000 m a.s.l.. The area has annual mean rainfall of 380 mm grouped in the summer months and mean annual temperature of 21° with rare frosts (García 1973). The vegetation is classified as matorral xerofilo (Rzedowski, 1978), which in the area is characterized by the presence of columnar cacti as *Neobuxbaumia mezcalaensis* Backeb., *Neobuxbaumia tetetzo* (F.A.C.Weber ex K.Schum.) Backeb. and *Cephalocereus columna-trajani* (Karw.) K.Schum.. The aforementioned columnar cacti constitute the dominant vegetation elements together with other cacti and other succulent species such as *Myrtillocactus geometrizans* Mart., *Opuntia* sp., *Agave* spp., *Mammillaria* sp., *Echinocactus* sp., *Ferocactus* sp., shrubs as *Mimosa luisana* Brandege, *Mimosa lacerata* Rose, *Acacia* sp., *Hechtia podantha* Mez., *Lippia graveolens* Kunth., *Calliandra eriophylla* Benth, *Euphorbia antisiphilitica* Zucc., and trees such as *Ipomoea arborescens* (Humb. et Bonpl.) G. Don., *Yucca periculosa* Baker, *Beaucarnea gracilis* Lem. and *Cercidium* sp..



Fig. 1 Map of the study area in Mexico

2.3 Spatial distribution of goat pellets

To evaluate goats' deposition patterns, 4 rectangular transects of 25m x 2m were placed randomly in a grazing area. In order to ensure randomness in selecting transects locations, the start of each transect was chosen after following a goat herd for 30 minutes and a new transect was started after 30 minutes. This method was objective and ensured that the transects were located in current grazing sites.

Each transect was divided into three different microhabitat categories: open areas, canopy areas, and boulder dominated areas. Areas not covered by trees, shrubs and cacti were defined as open areas. Areas under tree and shrub canopy or cacti were defined as canopy areas. Areas with surfacing parental material and boulders were defined as boulder dominated areas. For cactus species, only those having a dense branch structure as *Myrtillocactus geometrizans* Mart. were considered as contributing to soil coverage.

Canopy and boulder areas were measured and open areas were calculated by taking the difference between the aforementioned areas and the total area. Goat pellets were collected and the microhabitat categories in which the manure was found was recorded. Manure collection was repeated 3 times in the months of October and November (Table 1). In the first collection old pellets were also sampled in addition to the new pellets in the transects.

Table 1

Spatial distribution of goat pellets in the three microhabitat patches (open areas, canopy areas and boulder dominated areas) in the Tehuacán-Cuicatlán valley.

	open areas	canopy areas	boulder dominated areas	Total
10 Oct	778	192	24	994
30 Oct	450	125	15	590
20 Nov	290	65	19	374
Total	1518	382	58	1958

2.4 Endozoochory and bruchid effect on seeds survival

All goat pellets collected within the transects were checked for *M. luisana* seeds. In addition to this, six goats, all of them pregnant females and of the same type, were selected from a local herd. These six goats were placed in separate paddocks and fed at one time with 3000 locally collected *M. luisana* seeds which were mixed with alfalfa (*Medicago sativa* L.) and corn stubble (*Zea mais* L.). Eight hours after having fed the goats with *M. luisana* seeds, the troughs were emptied and the remaining seeds were counted in order to have the exact quantity of seeds eaten by each goat. The goats were fed continuously with alfalfa and corn stubble during the whole length of the experiment.

The seeds that were given to the goats were checked for infestation by the bruchids *Acanthoschelide mexicanus* SHARP and *Acanthochselides chiricahuae* FALL (Romero-Nápoles et al. 2005). A previous study reported that *M. luisana* seeds are widely infested by bruchids (Camargo-Ricalde et al. 2004) which has been shown to have a negative effect on seed recruitment. By tunneling into the seeds, bruchid larvae weaken the seeds and make them less resistant to mastication and digestive acids (Or and Ward 2003, Miller and Coe 1993, Coe and Coe 1987).

Goat pellets were collected every 8 hours over a period of 80 hours and put in separate numbered bags. The recollection period was defined based on the literature, which reports that goat retention time varies with the type of aliment, and is 38.2 h for particulate (Smuts et al 1995) and 47.6 h for alfalfa (Santini et al. 1992).

For each goat, a sample of 20 g of manure was extracted from the manure collected at each of the ten 8-hours intervals and checked for presence of *M. luisana* seeds. The following formula was used to estimate the amount of seeds passed through each goat's intestine:

$$\text{Tot seeds} = (\text{seeds found} * \text{total g})/200\text{g}$$

Estimated seeds quantities were subsequently transformed into percentages. Percentages of seeds survival were used to calculate the average seeds survival for the 6 goats.

2.5 Endozoochory effect on seeds germination

The effect of goat ingestion on seed germination was evaluated by collecting 120 seeds from the previously collected goat pellets. The control consisted of 120 seeds collected from pods of *M. luisana* found locally. Both the experimental seeds and control seeds were checked and those that were cracked or that floated in water were discarded.

The bruchids *A. mexicanus* and *A. chiricahuae* can have a scarification effect (Wiegand et al. 1999) on *M. luisana* seeds. In light of this, both digested and undigested seeds were checked for traces of bruchid infestation and when signs of infestation were discovered the seeds were excluded from the experiment.

Selected seeds were washed with a 3% soap solution and disinfected with a 5% chlorine solution (Camargo-Ricalde and Grether 1998). The seeds were washed with the solutions for only 5 seconds in order that they were disinfected but not chemically scarified.

Twenty-four Petri dishes, each containing five seeds, were randomly placed in a germination chamber with a 12 hour photoperiod for 24 days. All the seeds were placed on filter paper that had been wetted with sterile water. The temperature in the germination chamber was set at a constant 25°C since this is considered the optimal temperature to get the fastest and the highest rates of *Mimosa luisana* germination (Camargo-Ricalde et al. 2003). Germination was achieved when the radicle reached more than 1 mm in length (Bewley and Black 1978). Three out of the four germination indices identified by Castro et al. (in press) were used: initiation time of germination (ITG, the average time in registering the first germination among the dishes), accumulative germinations (AG, the cumulative frequency of

germination recorded daily) and final germination rate (FGR, the percentage of germination at the end of the experiment).

2.6 Data and statistical analysis

The proportion of each microhabitat in each of the four transects was determined and the obtained values were added in order to obtain the total proportion of area occupied by each microhabitat category. In order to get the total proportion of observed excrements per microhabitat, the number of excrements observed in each microhabitat was divided by the total amount of observed excrements. To evaluate whether observed data were significantly different from the expected ones (H_0 = goats excrements are distributed within each microhabitat in exact proportion of microhabitat's occurrence), a Goodness-of-fit comparison was used ($P < 0.05$). In addition, a chi-square test of independence on data in Table 1 ($P < 0.05$) was used to assess whether goat pellet deposition in the microhabitats was independent of time.

A Bonferroni z-test method ($P < 0.05$) (Neu et al. 1974) was also used to verify whether goats prefer defecating in some microhabitats.

In the germination experiment, the Petri dishes were the experimental units; initiation time of germination and final germination rate between experimental units of the different categories (digested and undigested) were compared using a t-test ($P < 0.05$). The t-test ($P < 0.05$) was also used to compare dishes of first germination rate between the two treatments. Statistical analyses were conducted with Minitab[®] 14, (Minitab[®] Statistical Software, 2007).

3 Results

3.1 Spatial distribution of goat pellets

The distribution of goat excrement in the three habitats differed from habitats' occurrence ($P < 0.05$) (Table 2). Pellet densities were higher in open areas and lower than expected under canopy or on boulder dominated areas (Table 2). There was no difference in pellet distribution between the three time periods ($P > 0.05$).

Table 2

Distribution of goat excrement in Open areas, Canopy areas and Boulder dominated areas compared with the microhabitat composition of the transect area (confidence interval 95%).

	Proportion of tot area (p_{i0})	Proportion observed in each area (\bar{p}_i)	Confidence interval of proportion of occurrence (p_i) (95% family confidence coefficient)
Open areas	0.543	0.775	$0.773 \leq P1 \leq 0.817$
Canopy areas	0.314	0.195	$0.174 \leq P2 \leq 0.216$
Boulder dominated areas	0.143	0.030	$0.021 \leq P3 \leq 0.039$

3.2 Endozoochory and bruchid effect on seeds survival

Pellets collected in the transects were manually crushed and only one *M. luisana* seed was found. The seed was found in an excrement belonging to the last collection, conducted on November 20th.

In the feeding experiment, in order to get a correct estimation of seeds' infestation rate, an infestation rate curve was built through recording infestation occurrence on a cumulative 5-

factorial number of seeds. A total of 330 seeds were checked for presence of bruchids' infestation and the value of 44.84% was considered a satisfactory estimation of the real infestation rate, since the last 3 infestation rates of the curve did not change appreciably from the value reported.

A total of 17469 seeds were eaten by the goats and 154 seeds were recovered out of the 60 samples.

Table 3

Observed and estimated number of *M. luisana* seeds in the pellets for each goat. Seeds survival average among goats: 5.91% (± 2.86 S.D.)

Goat no.	Total manure (g)	Seeds found in 100g	Total no. of seeds eaten	Estimated no. of seeds in total manure	% estimated seeds in manure
1	1507.52	7	2974	105	3.54
2	1284.31	18	2995	231	7.71
3	1291.12	12,5	2737	161	5.89
4	1419.39	5	2873	71	2.47
5	1290.59	23,5	2924	303	10.37
6	1473.87	11	2966	162	5.46
Mean: 5.91 (± 2.86 S.D)					

Only 5.9% (± 2.86 S.D.) of the ingested seeds were found in the pellets (Table 3) and of these, 77% were found between 8 and 24 hours after ingestion, but seeds were also found up to 72 hours after ingestion (Fig. 2).

No traces of either entrance or exit holes of bruchids were found on the collected seeds.

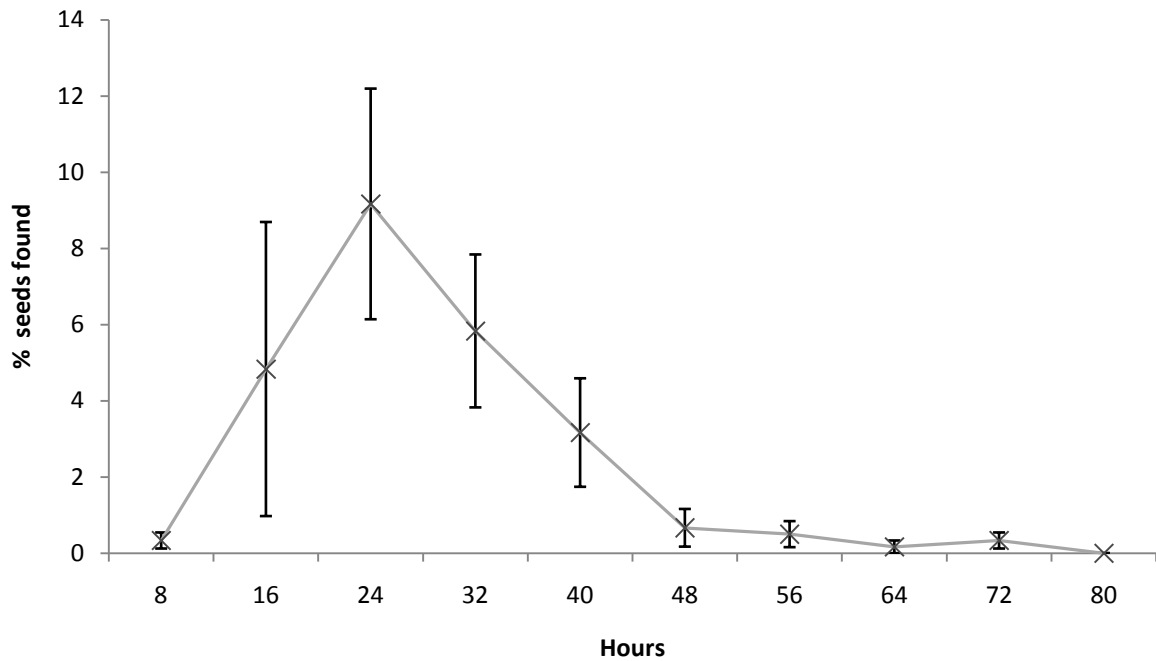


Fig 2. Gut retention curve: percent (\pm S.E.) seeds found in goat (n=6) manure as a function of number of hours since the seeds were ingested.

3.3 Endozoochory effect on seeds germination

Goats increased the germination rate of *M. luisana* seeds ($t = 6.11$; $P < 0.001$). Of the seeds that passed through the goats, 47.5% germinated while only 5.83% of the undigested seeds germinated. As shown in Figure 3, passage through the goats' intestine not only affects the quantity of seeds able to germinate, but it also shortens the germination time. After three days, 33.3% of digested seeds had germinated, while only the 1.6% of the seeds from the control germinated. The initiation time of germination (ITG) was 2.75 days (± 2.37 S.D.) for digested seeds and 6.5 days (± 2.88 S.D.) for the control. Initiation time of germination (ITG) was significantly different for digested seeds and control seeds ($t=-2.92$; $P<0.05$).

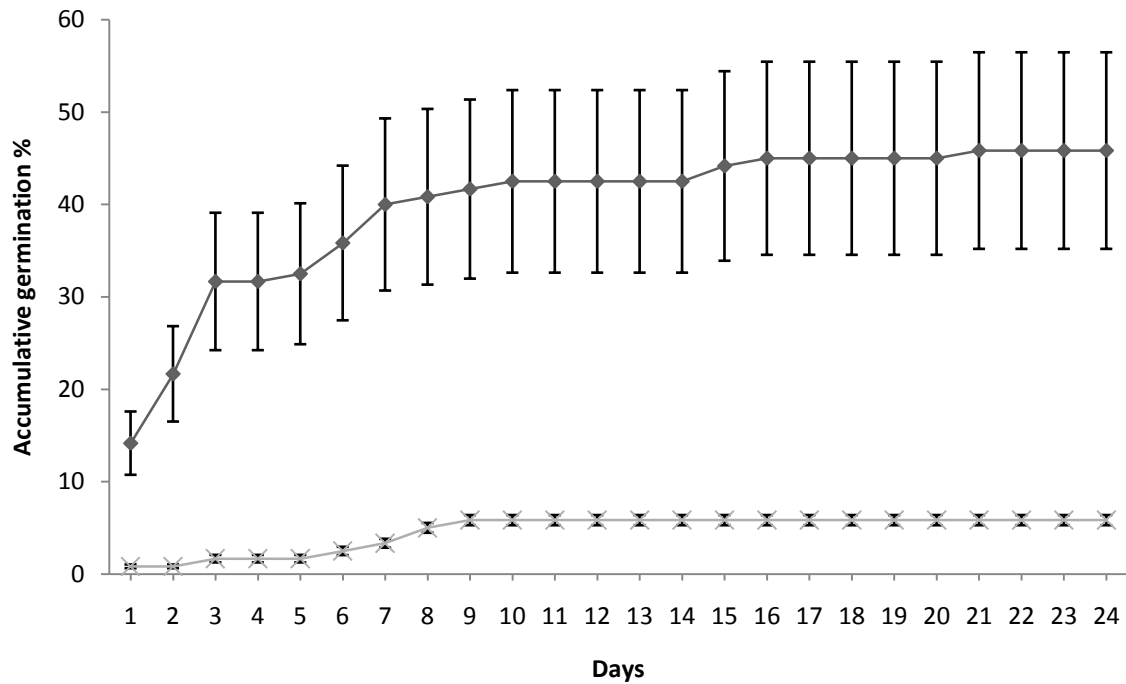


Fig 3. Accumulative germination (mean± S.E) of *M. luisana* seeds: ♦ digested seeds; x control

4 Discussion

Results on goat pellets spatial distribution revealed higher pellet densities in open areas and lower than expected under canopy or on boulder dominated areas. Evaluating whether this can be an advantage for *M. luisana* establishment and growth is complicated by the fact that the characteristics of a suitable site for plant establishment and growth are not fixed, but vary throughout plant development (Janzen 1983). Sites that are very suitable for seed germination may not be suitable for seedling growth and vice versa (Schupp 1993). No studies have focused on *M. luisana* germination rates in different microhabitats and it is therefore difficult to evaluate if it is an advantage for *M. luisana* to be deposited in open area. However, it has been recorded that *M. luisana* is common in abandoned fields and eroded soils (Camargo-Ricalde et al. 2003) suggesting that it does not require shade to germinate. In

a dynamic view of plant development and growth, open areas can therefore be associated with higher probability of survival. The suitability of the microhabitats where the deposition of pellets primarily occurred showed that goats are an efficient seed disperser of *M. luisana*.

The literature reports low seed survival after the passage through ungulate intestines (Jordano 1992, Schupp 1993). My results were similar in that only a small percentage of the *M. luisana* seeds were able to pass through the goats' intestines (5.91%). This is due primarily to the fact that goats chew and destroy most of *M. luisana* seeds, which was evident by seed fragments found in the feces. It is also important to underline that among the recruited seeds none showed any sign of bruchid infestation. This suggested that similarly to what has been found for *Acacia sp.* seeds (Or and Ward 2003, Schmidt 1988) *M. luisana* seeds infested by bruchids are easily destroyed by goats given their greater weakness due to bruchid infestation. The combination of high rate of bruchid infestation and the negative gut goats treatment concur to reduce the role of goats as legitimate dispersers of *M. luisana* seeds. No information are present regarding interannual variation of bruchids infestations of *M. luisana* seeds. Traveset (1991) pointed out that the population of two *Acacia*'s bruchids in Costa Rica seems to be influenced by severe weather conditions. The potential presence of exceptional years in which bruchids population and infestation decrease could influence the percentage of *M. luisana* seeds that are able to survive at the gut passage.

Only one seed was found in a total of 1958 collected pellets. Local shepherds considered the year in which the research was conducted a year of low production of *M. luisana*'s pods, which may contribute to the low discovery of *M. luisana* seeds. However, the almost complete absence of seeds in the field experiment is supported by the findings that only 5.91% of seeds were recovered within the feeding experiment. Under natural conditions goats are far from eating the amount of seeds used in the feeding experiment. Thus it is reasonable to expect very few seeds to be present in dispersed excrement, regardless of annual variation in seed production.

The seed germination experiment showed that *M. luisana* seeds are able to germinate after passing through goat intestines. This study indicated that goat gut treatment speeds up germination through the scarification of *M. luisana* seed coats. However, this scarification might not always be positive, since in dry ecosystems rains are rare events and seed dormancy could be an adaptation to ensure germination to occur only when enough resources are available (Or and Ward 2003). In years in which rains are more abundant, goats' scarification, which would result in faster germination, could be advantageous in terms of seedlings development and inter- and intra-specific seedlings competition (Izhaki and Safriel 1990).

More than three quarters of the seeds (77%) passed through goats intestine between 8 and 24 hours after ingestion. This suggests that *M. luisana* seeds are potentially carried by goats for a considerable distance away from the mother plant, since it has been demonstrated that goats' average speed in a similar landscape dominated by shrub in Texas is 5.1m/min (Etzenhouser et al. 1998). There are several advantages to this as outlined in the Janzen and Connell hypothesis such as the escape from high competition environments as well as the escape from areas of high density-dependent mortality caused by seed predators and pathogens that can occur directly under the mother plant (Wang and Smith 2002).

The results indicated that goats can act both as an efficient and a legitimate disperser although the low percentage of seeds that can pass through the goats intestine is undeniable and leads to the conclusion that goats act as a predator of *M. luisana* seeds and only occasionally as a disperser. Studies on long distance dispersion underlined that even a very small amount of seeds present in the dispersed excrements can be of great significance for vegetation dynamics and population genetic structure (Nathan 2006, Myers et al. 2004, Vellend et al. 2003). Myers et al. (2004) also suggested that rare long distance dispersion seems to be crucial for invasion dynamics and vegetation response to changes in land use. Even if goats do not move far away from shepherd settlement, they could contribute to *M. luisana* colonization of abandoned fields. This could be of particular interest since *M. luisana*

is a nurse plant (Valiente-Baunet and Ezcurra 1991, Camargo-Ricalde and Dhillion 2003) and was proposed as potential species within restoration projects in the Tehuacán-Cuicatlán valley (Camargo-Ricalde and Dhillion 2003).

The interaction between goats and *M. luisana* in the Tehuacán-Cuicatlán ecosystem should not be neglected and should be the object of future studies. New genetic techniques have lately been used for more in depth studies of long distance dispersion and vegetation dynamics (Wang and Smith 2002, Nathan 2006). It is also important to underline that goat pellets contained numerous seeds of other species, which suggests that goats might have an important role in the ecosystem that do not involve only *M. luisana*. Increasing the knowledge of the interaction between goats and Tehuacán-Cuicatlán vegetation is essential to develop an appropriate grazing management plan. It is of particular importance to couple the reserve's conservation goals with the subsistence need of the local people that depend largely on domestic goat herding.

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Fig 4. One of the goats during the feeding experiment



Fig 5. Landscape in Zapotitlán Salinas



Fig 6. *M. luisana* germinated seeds



Fig 7. *M. luisana* pods before being mixed with Alfalfa (*M. sativa*)