Effects of ingestion by sheep on the germination of seeds of the invasive *Prosopis juliflora* tree

Ahmed M. Abbas [a,b,*], *Lamiaa Mahfouz* [b], *Mohamed K. Ahmed* [b], *Mohammed A. Al-Kahtani* [b], *Graeme D. Ruxton* [c], *Adam M. Lambet* [d]

[a]*Department of Biology, Faculty of Science, King Khalid University, 61413 Abha, Saudi Arabia*

[b] *Department of Botany and Microbiology, Faculty of Science, South Valley University, 83523 Qena, Egypt*

[c] *School of Biology, University of St Andrews, St Andrews KY16 9TH, UK*

[d] *Marine Science Institute, University of California, Santa Barbara, California, USA 93106*

* Corresponding author. Tel.: +966540271385

E-mail addresses: ahhassan@kku.edu.sa, abbas@sci.svu.edu.eg (A.M. Abbas)

[1] Present address: 61413 Abha, Saudi Arabia.
[2]Present address: 83523 Qena, Egypt

ABSTRACT

Prosopis juliflora, a small mesquite tree, is native to Central and South America, and but is invasive in many tropical and subtropical regions, including North Africa and the Arab Gulf region. It has often been introduced for livestock forage but has often become a problematic weed. In Egypt the geographical pattern of its spread suggested that domestic sheep may be an important seed vector. The aim of this captive study was to analyse the effect of seed passage through the sheep digestive system on germination of *P. juliflora*. Only 7% of seeds fed to sheep passed through the digestive system intact. Larger seeds were more likely to pass through intact. Seeds that passed through a sheep intact were much more likely to germinate than untreated control seeds and germinated more quickly under artificial laboratory conditions. Taken together our results further strengthen evidence for the importance of sheep as vectors of this invasive pest. Thus, sheep must be considered in management strategies to prevent spread into protected areas, and further research to explore germination under natural conditions of seeds excreted in sheep dung is warranted.

Keywords: Invasive plant; Gebel Elba, Grazing; Prosopis; Ruminant; Seed dispersal

1. Introduction

Endozoochory, the dispersal of seeds through ingestion by vertebrates, is a biological mechanism used by diverse tree species, including many Fabaceae (legume) species common in arid ecosystems. Legumes provide crucial ecosystem services, including nitrogen fixation, high-quality forage for livestock and wildlife, wood products and food for humans (Felker, 1981). Several legume species have increased their distribution ranges because of human intervention and become invasive over the past two centuries, and this can be linked, in some cases, to dispersal by domestic and feral animals (Brown and Archer, 1989). The invasive mesquite, *Prosopsis juliflora* (Sw.) D.C, is often used and promoted as a food source for livestock over much of its invasive range (across much of Africa and the Middle East) due to its abundance, palatability and high protein content (Sawal et al., 2004; Chaturvedi and Sahoo, 2014).

Seeds of many species in the subfamily Mimosoideae, including genera such as *Prosopis*, *Acacia* and *Vachellia*, have physical dormancy requiring scarification of their seed coat. Further, these seeds are contained within indehiscent legumes (pericarp), which require decomposition, ingestion or other mechanisms to release the seeds (Livingston and Nials, 1990). Generally, scarification increases germination speed, although the mortality potential increases if the seed coat is weakened too much (Janzen, 1984). The sugary legumes of invasive *Prosopis* species are attractive to grazing and browsing animals, and at least some of their seeds remain intact after rumination and intestinal passage (Campos et al., 2011; Kramp et al., 1998; Robinson et al., 2008). For example, Peinetti et al. (1993), reported that *Prosopis caldenia* L. germination was increased by ingestion by cattle. Cattle increased the germination of *Prosopis flexuosa* D.C. but reduced viability, whereas seeds passing through horses maintained viability, but did not germinate more quickly (Campos and Ojeda, 1997). Among native mammals in the semi-arid areas of Argentina, the grey fox (*Urocyon cinereoargenteus*)maintained seed viability without increasing germination, whereas rodents reduced seed viability and enhanced germination rates (Campos and Ojeda, 1997). However, enhanced germination of seeds following passage through the herbivore gut may not always be beneficial (Lessa et al., 2013). The European wild boar (*Sus scrofa*) damaged all of the seeds it consumed (Campos and Ojeda, 1997). In general, the effects of herbivory are related to a number of factors, including gut retention period, seed size and the hardness of seed coats. (Janzen 1981, 1984).

Endozoochorous dispersers can enhance the fitness of host plants by moving seeds to new environments (colonization hypothesis; Howe and Smallwod, 1982) with lower predation, parasitism and competition risk compared to the environment near the mother plant (escape hypothesis; Janzen, 1970), but see (Hyatt et al., 2003). Dung can also provide a favorable environment for germination (Gokbulak and Call, 2004), although biotic interactions such as seed and seedling predation and intraspecific competition in the dung can modulate this relationship (Howe, 2008; Janzen, 1981). These studies show that the complexity of endozoochory interactions can elicit either benefical or detrimental effects on seed demographics depending on the species and abiotic factors involved (Lessa et al., 2013; Verdú and Traveset, 2005).

According to Schupp (1993), the quality of endozoochorous dispersal depends on how seeds are processed in the mouth and gut. Mechanical grinding during chewing and rumination by ungulates, as well as retention in the gut and exposure to digestive juices, may affect the survival and germination of seeds. Such effects vary among animal species ingesting the seeds (Kneuper et al., 2003; Traveset, 1998). The type of herbivores consuming seeds with physical dormancy determines the fraction of seeds germinating following gut passage (Jaganathan et al., 2016). In this context, determining whether endozoochory is advantageous to plant germination requires examining the cumulative effects of gut passage, deposition in faeces and seed dispersal patterns on germination and viability. Thus, understanding the functional role of domestic and wild animal species in seed dispersal of invasive species is central to determining how biotic interactions could be affected by anthropogenic drivers.

Prosopis juliflora, is native to Central and South America, and it is invasive in many tropical and subtropical regions, including North Africa and the Arab Gulf region (Abbas et al., 2018). Endozoochory may stimulate *P. juliflora* germination by liberating seeds from the fruit, while faeces may act as a source of nutrients and water during the early stages of seedling establishment and growth (Shiferaw et al., 2004). *Prosopis juliflora* can be dispersed by domestic animals such as goats, cattle and mules, and wild fauna such as gazelles and coyotes (Alvarez et al., 2017; Gonçalves et al., 2013). *Prosopis juliflora* was introduced in Egypt intentionally into Gebel Elba National Park during the late 1980s for agroforestry uses by local people of Old Hala'ib village. Eight years after its introduction, local people viewed it as an unwelcome, rapidly spreading, invasive species (Abbas et al., 2018). In Egypt, domestic sheep (*Ovis aries*) seem to be the main dispersal vector accelerating *P. juliflora* spread along trade routes and animal tracks into new areas (Shiferaw et al., 2004; Hundessa, 2016). Studies have found that ingestion of *Prosopis* seeds by mammal species (Alvarez et al., 2017; Campos and Ojeda, 1997; Gonçalves et al., 2013; Kramp et al., 1998). The aim of this study was to analyse the effect of seed passage through the sheep digestive system on germination of *P. juliflora*. We hypothesised that a portion of *P. juliflora* seeds would survive passing through the sheep's gut, and that germination rates of these excreted seeds would be increased by this process.

2. Materials and methods

2.1. Fruit collection

Fruits of *P. juliflora* were collected from multiple mature individuals in July 2018 in Wadi Merakwan, Gebel Elba National Park, south-east Egypt (22°1402″N–36°36030″E; Fig. 1) (See; Al-Gohary, 2008; Ball, 1912 ; for site description). Collected fruits were stored under dry and dark conditions in the laboratory at 25°C until the beginning of the experiment in October 2018. A subset of seeds was removed from pods, and weighed and measured (length, width and thickness) with an electronic vernier calliper (n = 50) to determine average seed mass and volume.

2.2. *Seeds retrieved after gut passage*

Four female adult sheeps (*Ovis aries*) of similar size and age (average of 40 kg and two years old) were individually housed and were fed at the Teaching and Experimental Farm of the faculty of veterinary medicine at the University of South Valley (26°11´8.8″ N – 32°43´56.4″ E). Four individuals is a typical sample size for Endozoochory experiments with animals such as goats (Alvarez et al., 2017). At the beginning of the experiment, 1000 *P. juliflora* seeds with pericarp were offered to each sheep. All seeds were consumed by sheep within 30 minutes. Afterwards, the sheep were fed with lucerne hay and sunflower pellets, and they had unrestricted access to water. All the dung pellets produced by each sheep were collected every 24 h for 4 days (0–24, 24–48, 48–72, 72–96 and 96-120 h after ingestion) and dried at 25°C for 72 h in a bell jar with silica gel to avoid seed fermentation and damage.

2.3. *Seed germination after gut passage*

Germination of seeds retrieved from sheep dung was compared with that of uneaten seeds. Seeds for the uneaten treatment were prepared in two ways: (i) seed pods (seed and pericarp) were broken into portions corresponding to individual seeds with pericarp fragments (control seeds) to simulate the effect of fruit fragmentation due to environmental breakdown/decomposition and rodent activity (Campos and Ojeda, 1997) or when sheep drop them when eating and fruits fall to the soil surface, or (ii) seeds were manually cleaned from pods to remove all pericarp and treated with 3.0% (v/v) sodium hypochlorite for 2 min as chemical scarification to break the exogenous physical dormancy of *P. juliflora* (Zare et al., 2011). Seeds for gut passage treatments were categorized as (i) seeds retrieved between 24 and 48 h after ingestion, (ii) seeds retrieved between 48 and 72 h after ingestion, (iii) seeds retrieved between 72 and 96 h after ingestion, and (iv) seeds retrieved between 96 and 102 h after ingestion. Seeds were removed from dung and only seeds with no evidence of apparent external damage were used for the germination experiment. Retrieved seeds were subjected to germination just after collecting them from the sheep pellets. Before germination treatments, all seeds were weighed and measured, and then disinfected by immersion in a 1% sodium hypochlorite solution for 2 min and thoroughly rinsed with sterile distilled water (10 min) before the germination treatments (Abbas et al., 2018). Seeds were then placed on filter paper in a 9-cm Petri dish (10 seeds per dish and four replicated dishes per treatment), and 5 ml of distilled water was added to each dish. Dishes were then wrapped with parafilm and placed in a germinator (ASL Aparatos CientificosM- 2004, Madrid, Spain) for 30 days under a photoperiod/temperature regime of 12 h light at 25°C (35 lmol m of wave length between 400 and 700 nm) and 12 h dark at 20°C. This temperature regime was chosen to mimic autumn temperatures in the south of Egypt when this species germinates (El-Keblawy and Al-Rawai, 2005). The dishes were inspected daily and germinated seeds were counted. A seed was considered to have germinated when its radicle emerged (Abbas et al., 2012). Final germination percent, the time required for the first seed to germinate, and mean time to germination were calculated. Mean time to germination

(MTG) is an index of seed germination speed was calculated as MTG = Σ (n × d)/N, where n is the number of seeds germinated at day d and N is the total number of seeds that germinated in the treatment. Seed viability of remaining non-germinating seeds was determined using the tetrazolium test (Mancilla-Leytón et al., 2011).

2.4. Data analyses

Variance estimates were calculated as the standard error of the mean (SE) and analyses were evaluated at a significance level of 0.05. The data series were tested for normality using a Kolmogorov–Smirnov test and for homogeneity of variance using Levene's test. Differences in biometric characteristic between uneaten and retrieved seeds were tested using a t-test. Differences in mean germination parameters and mean seed viability among uneaten and retrieved seeds at different times were tested using one-way analysis of variance ANOVA with Tukey's test for post hoc analysis. SPSS 23.0 for Windows (SPSS, Chicago, IL, USA) was used for all statistical analyses.

3. Results

On average, 7% (70 ± 21 seeds per 1000 seeds eaten) of *P. juliflora* seeds were retrieved intact from sheep dung. Most seeds were excreted during the first two collection periods, and relatively few were retrieving by day four (Table 1). Retrieved seeds were significantly longer, wider, thicker, heavier and had greater volume than uneaten seeds (*t*-test, $P < 0.001$) (Table 2).

Final germination rate was significantly higher for both manually scarified and sheep consumed seeds compared to uneaten seeds without pericarp removed (ANOVA, $F_{5,36}$= 5.34, HSD test, $P < 0.01$, Fig. 2). Final germination rate was the highest for seeds excreted between 24–48 h (70 ± 8 %) followed by uneaten seeds without pericarp and chemically scarified (65 ± 4 %) and seeds excreted after 48-72 h (64 ± 7 %). Uneaten seeds with pericarp had the lowest final germination rate (5 ± 4 %) (HSD test, $P < 0.05$) (Fig. 2A).

Time to first germination significantly differed among seed treatments and ranged from 2 to 11 days depending on seed treatment ($F_{5,32}$= 12.398, HSD test, $P < 0.0001$, Fig. 2B). The longest mean time to germination (38 ± 7 days) was recorded for seeds excreted at 24–48h and the lowest for uneaten chemically scarified seeds with pericarp (5 ± 4 days) ($F_{5,36}$= 3.27, HSD test, $P < 0.05$, Fig. 2C). All uneaten seeds (both those with and without pericarp) that did not germinate during the germination trials were viable, however no excreted, ungerminated seeds were viable.

4. Discussion

Our study provides a framework towards an understanding of endozoochory by sheep as a seed dispersal vector for the seeds of the invasive tree *P. juliflora*. Few seeds passed through the sheep gut intact: 7% of the consumed seeds were retrieved from faeces. Alvarez et al. (2017) reported that the recovery of ingested seeds of *P. juliflora* by goats and cattle was generally low (7% and 15% respectively), suggesting that most of them were destroyed by chewing and rumination processes. Seed recovery percent ages of 20% (goat) and 60% (cattle) have been reported from ingested fruits of several Mimosoideae species in Tanzania (Shayo and Uden, 1998). Seed recovery for *Prosopis glandulosa* Torr. was 92%, 11% and 9% in dung of cattle, sheep and goats respectively (Kneuper et al., 2003). Even so, enough seeds of *P. juliflora* can pass through the digesting system of sheep for them to be an effective dispersal mechanism, as described for the dispersion of different plant species by Darwin's finches in the Galapagos Islands (Guerrero and Tye, 2009). In order to assess the degree to which sheep naturally feed on *P. juliflora*, food preference tests should be carried out by offering sheep' fruits of *P. juliflora* and fruits of native plant species.

For *P. juliflora* and other species with indehiscent fruits and/or hard-coated seeds, seed liberation from fruits and seed scarification appears to be the main benefits of ingestion by animals, including camels (Abbas et al., 2018), goats (Baes et al., 2002; Kneuper et al., 2003), donkeys (Baes et al., 2002), horses (Janzen, 1981; Campos and Ojeda, 1997), cattle (Campos and Ojeda, 1997; Kneuper et al., 2003), sheep (Kneuper et al., 2003), rodents (Campos and Ojeda, 1997), foxes (Campos and Ojeda, 1997) and bears (Auger et al., 2002). Ruminal liquid contains proteolytic and cellulolytic enzymes that may help to remove the pericarp and to soften the seed coat (Robles and Castro, 2002). Additionally, seeds are exposed to an acidic medium and enzymatic activity in the abomasum and duodenum, which could further erode the pericarp and the seed coat (Gardener et al., 1993). Once freed from fruits during digestion by sheep, retrieved seeds of *P. juliflora* showed faster and higher final germination compared with uneaten seeds covered with the pericarp. The pericarp may prevent hydration of the seed, which is essential for its germination. This result is supported by retrieved seeds being larger and with darker colour than uneaten seeds, reflecting the seed hydration process. Moreover, uneaten chemically scarified seeds without pericarp showed similar final germination to retrieved seeds between 48 and 72 h after ingestion. This suggests that gut retention times lower than 48 h limit the necessary chemical scarification of *P. juliflora* seeds, which are required for germination (Traveset, 1998).

Our results are consistent with the occurrence of primary physical seed dormancy (Baskin et al., 2000) in *P. juliflora* related to the fruit's cover that restricts the germination of unliberated seeds to about 5%, though 100% of them were viable. In addition, it has been shown that *P. juliflora* seeds can maintain some viability after multiple years in the soil seed bank, even under conditions suitable for germination (Martin, 2006). Passage through the gut of large herbivores also enhances seed germination, for example, in some Mediterranean species, such as *Retama sphae- rocarpa* (L.) Boiss., with physical dormancy imposed by a hard seed coat (Manzano et al., 2005; Ramos et al., 2006).

High seed germination rates together with Low seed viability of ungerminated seeds after gut passage suggested that sheep may be an effective disperser of *P. juliflora*, whose invasion in south-east Egypt is along sheep grazing routes (Abbas et al., 2016). Although passage through the gut freed *P. juliflora* seeds from their pericarps, increasing and accelerating their germination, it also effecting their viability in comparison with uneaten seeds. This was probably caused by damage to the embryo by acidic conditions and enzymes during digestion (Blackshaw and Rode, 1991).

Once on the soil surface, sheep faeces may provide moisture necessary for the germination of *P. juliflora*, as well as nutrients for its seedlings (Ocumpaugh et al., 1996). In the field, about 20% of excreted *P. juliflora* seeds can be preserved for longer than 6 months with viability in the faeces of mules (Gonçalves et al., 2013). However, the survival of *P. flexuosa* seedlings from dung-germinated seeds did not exceed 1 week, whereas those seeds that did not remain in dung germinated during the rainy season showing higher survivorship (Campos et al., 2011). Clearly, more research is needed to determine whether secondary dispersal moves seeds into suitable microsites for germination (Jaganathan et al., 2016).

The ability of sheep to disperse the seeds of the invasive neophyte *P. juliflora* should be taken into account when designing conservation and management plans in order to prevent its spread, for example by regulating the use of sheep along commercial routes, especially in nature conservation areas such as the Gebel Elba National Park.

5. Conclusion

Sheep can potentially disperse seeds of the P. juliflora that they eat. This should be taken into account when designing management strategies for *P. juliflora* in order to prevent its invasion into undesired areas. It could also be used as a management tool for spreading populations of desirable shrub species.

Conflicts of interest

Authors declare that there are no conflicts of interest.

Acknowledgment

The authors would like to express their gratitude to King Khalid University, Saudi Arabia for providing administrative and technical support

References

Abbas, A.M., Soliman, W.S., Ahmed, M.E., Ibrahim, N.H., Mohamed, M., Mahmoud, F. Y., Mansour, H.M., Mohamed A. 2016. Predicting the spatial spread of invasive *Prosopis juliflora* (SW.) D.C along environmental gradients in Gabel Elba National Park, Egypt. Int. J. Sci. Eng. Res. 7, 596–599.

Abbas, A.M., Mancilla-Leytón, J.M., Castillo, J.M., 2018. Can camels disperse seeds of the invasive tree *Prosopis juliflora* ? Weed Res. 58, 221–228.

Abbas, A.M., Rubio-Casal, A.E., De Cires, A., Figueroa, M.E., Lambert, A.M., Castillo, J.M. 2012. Effects of flooding on germination and establishment of the invasive cordgrass *Spartina densiflora*. Weed Res. 52, 269–276.

Al-Gohary, I.H. 2008. Floristic Composition of Eleven Wadis in Gebel Elba , Egypt. Int. J. Agric. Biol. 10, 151–160.

Ahmed S.A., Tudsri, S., Rungmekarat, S., Kaewtrakulpong, K. 2012. Effect of feeding

Prosopis juliflora pods and leaves on performance and carcass characteristics of Afar sheep *Kasetsart Journal.* Nat. Sci. 46, 871-88

Alvarez, M., Leparmarai, P., Heller, G., Becker, M. 2017. Recovery and germination of *Prosopis juliflora* (Sw.) DC seeds after ingestion by goats and cattle. Arid Land Res. a Manag. 31, 71–80.

Auger, J., Meyer, S.E., Black, H.L. 2002. Are American black bears (*Ursus americanus*) legitimate seed dispersers for fleshy-fruited shrubs? Am. Midl. Nat. 147, 352–367.

Baes, P.O, DE Viana, M.L., Suhring, S. 2002. Germination in *Prosopis ferox* seeds: effects of mechanical, chemical and biological scarificators. J. Arid Environ. 50, 185–189.

Ball, J. 1912. The Geography and Geology of South-Eastern Egypt, p: 394. Ministry of Finance", Survey Department, Covert Press, Egypt, 1912 (Ministry o). Cairo: Cairo: Government Press.

Baskin, J.M., Baskin, C.C., LI, X. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. Plant Species Biol. 15, 139–152.

Blackshaw, R.E., Rode, L.M. 1991. Effect of ensiling and rumen digestion by cattle on weed seed viability. Weed Science Society of America Allen Press Stable 39, 104–108.

Brown, J. R., Archer, S. 1989. Woody plant invasion of grasslands: establishment of honey mesquite (*Prosopis glandulosa* var.*glandulosa*) on sites differing in herbaceous biomass and grazing history. Oecologia 80, 19–26.

Campos, C., Campos, V., Mongeaud, A., Borghi, C., Los Rios, C., Giannoni, S.M. 2011.

Relationships between *Prosopis flexuosa* (Fabaceae) and cattle in the Monte desert: Seeds, seedlings and saplings on cattle-use site classes. Rev. Chil. Hist. Nat. 84, 289–299.

Campos, C.M., Ojeda, R.A. 1997. Dispersal and germination of *Prosopis flexuosa* (Fabaceae) seeds by desert mammals in Argentina. J. Arid Environ. 35, 707–714.

Chaturvedi, O.H., Sahoo, A. 2013. Nutrient utilization and rumen metabolism in sheep fed *Prosopis juliflora* pods and *Cenchrus* grass. SpringerPlus 598, 2-7.

El-Keblawy, A., Al-Rawai, A. 2005. Effects of salinity , temperature and light on germination of invasive *Prosopis juliflora*. J. Arid Environ. 61, 555–565.

Felker, P. 1981. Uses of tree legumes in semiarid regions. Econ. Bot. 35, 174–186.

Gardener, C.J., Mcivor, J.G. Jansen, A. 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. J. Appl. Ecol. 30, 63–74.

Gokbulak, F., Call, C.A., 2004. Grass seedling recruitment in cattle dungpats. J. Range Manag. 57, 649-655

Gonçalves, G.S., De Andrade, L.A., Gonçalves, E.P., De Oliveira, L.S.B., Dias, J.T. 2013. Qualidade fisiológica de sementes de algaroba recuperadas de excrementos de muares. Semin. Cienc. Agrar. 34, 593–602.

Guerrero, A.M.,Tye, A. 2009. Darwin's finches as seed predators and dispersers. Wilson J. Ornithol. 121, 752–764.

Howe, H.F. 2008. Specialized and Generalized Dispersal Systems Where Does "The Paradigm" Stand, 1–12

Howe, H.F., Smallwod, J. 1982. Ecology of Seed dispersal. Annu. Rev. Ecol. Evol. Syst. 13, 201–228.

Hyatt, L.A., Rosenberg, M.S., Howard, T.G., Bole, G., Fang, W., Anastasia, J., Gurevitch, J. 2003. The distance dependence prediction of the Janzen-Connell hypothesis: A meta-analysis. Oikos 103, 590–602.

Jaganathan, G.K., Yule, K., Liu, B. 2016. On the evolutionary and ecological value of breaking physical dormancy by endozoochory. Perspect. Plant Ecol. 22, 11-22.

Janzen, D.H. 1970. Herbivores and the Number of Tree Species in Tropical Forests. Am. Nat. 104, 501–528.

Janzen, D.H. 1981. Enterolobium Cyclocarpum Seed Passage Rate and Survival in Horses, Costa Rican Pleistocene Seed Dispersal. Agents Ecology 62, 593–601.

Janzen, D.H. 1984. Dispersal of Small Seeds by Big Herbivores: Foliage is the Fruit Am. Nat. 123, 338–353.

Janzen, D.H. 1983. Seed and pollen dispersal by animals: convergence in the ecology of contamination and sloppy harvest. Biol. J. Linn. Soc. 20, 103–113.

Kneuper, C.L., Scott, C.B., Pinchak, W.E. 2003. Consumption and dispersion of mesquite seeds by ruminants J. Range Manage. 56, 255–259.

Kramp, B.A., Ansley, R.J., Tunnell, T.R. 1998. Survival of mesquite seedlings emerging from cattle and wildlife feces in a semi-arid grassland. Southwest. Nat. 43, 300–312.

Lessa, L.G., Geise, L., Costa, F.N. 2013. Effects of gut passage on the germination of seeds ingested by didelphid marsupials in a neotropical savanna. Acta bot. bras., 27, 519–525.

Livingston, S.D., Nials, F.L. 1990. Archaeological and paleoenvironmental investigations in the Ash Meadows National Wildlife Refuge, Nye County, Nevada, Technical Report No. 70. Desert Research Institute, University of Nevada System, Quaternary Sciences Center.

Mancilla-Leytón, J.m., Fernández-Alés, R., Vicente, A.M. 2011. Plant-ungulate interaction: goat gut passage effect on survival and germination of Mediterranean shrub seeds. J. Veg. Sci. 22, 1031–1037.

Manzano, P., Manzano, P., Malo, J.E., Peco, B. 2005. Sheep gut passage and survival of Mediterranean shrub seeds. Seed Sci. Res. 15, 21–28.

Martin, S.C. 2006. Longevity of Velvet Mesquite seed in the soil. Rangeland Ecol. Manag. Arch. 23, 69–70.

Ocumpaugh, W.R., Archer S., Stuth, J.W. 1996. Switchgrass recruitment from broadcast seed vs. seed fed to cattle. J. Range Manag. 49, 368–371.

Peinetti, R., Pereyra, M., Kin, A., Sosa, A. 1993. Effects of Cattle Ingestion on Viability and Germination Rate of Calden (*Prosopis Caldenia*) Seeds. J. Range Manag. 46, 483-486.

Ramos, M.E., Robles, A.B., Castro, J. 2006. Efficiency of endozoochorous seed dispersal in six dry-fruited species (Cistaceae): from seed ingestion to early seedling establishment. Plant Ecol. 185, 97–106.

Robinson, T.P., van Klinken, R.D., Metternicht, G. 2008. Spatial and temporal rates and patterns of mesquite (*Prosopis* species) invasion in Western Australia. J. Arid Environ. 72, 175–188.

Robles, A.B.,Castro, J. 2002. Effect of thermal shock and ruminal incubation on seed germination in *Helianthemum apenninum* (L.) Mill. (Cistaceae). Acta Bot. Malacit. 27, 41–47.

Sawal, R., Ratan, R., Yadav, S. 2004. Mesquite (*Prosopis juliflora*) Pods as a Feed Resource for Livestock - A Review. Asian-Australas J. Anim. Sci. 17, 719-725.

Schupp, E.W. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. Vegetatio 107, 15–29.

Shayo, C.M., Uden, P. 1998. Recovery of seed of four African browse shrubs ingested by cattle, sheep and goats and the effect of ingestion, hot water and acid treatment on the viability of the seeds. Trop. Grasslands 32, 195–200.

Shiferaw, H., Teketay, D., Nemomissa, S., Assefa, F. 2004. Some biological characteristics that foster the invasion of *Prosopis juliflora* (Sw.) DC. at Middle Awash Rift Valley Area, north-eastern Ethiopia. J. Arid Environ. 58, 135–154.

Traveset, A. 1998. Effect of seed passage through vertebrate frugivores' guts on germination : a review 1, 151–190.

Verdú, M., Traveset, A. 2005. Early emergence enhances plant fitness: A phylogenetically controlled meta-analysis. Ecology. 1385-1394.

Zare, S., Tavili, A., Darini, M.J. 2011. Effects of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis Juliflora*. J. For. Res., 22, 35–38.

Table 1

Number of retrieved seeds by four sheep 24– 48, 48–72, 72–96, and 96-120 h after ingestion, seeds subjected to germination, germinated seeds and viable seeds for the invasive tree *Prosopis juliflora* from south-east Egypt

		Sheep				
Period	Treatment	1	2	3	4	Total
24–48 h	Retrieved seeds	16	20	30	23	89
	Germinated seeds	8	15	22	18	63
	Viable ungerminated seeds	0	0	0	0	0
48–72 h	Retrieved seeds	20	35	46	28	129
	Germinated seeds	15	23	25	20	83
	Viable ungerminated seeds	0	0	0	0	0
72–96 h	Retrieved seeds	8	11	12	9	40
	Germinated seeds	1	2	2	1	6
	Viable ungerminated seeds	0	0	0	0	0
96-120 h	Retrieved seeds	4	6	8	5	23
	Germinated seeds	2	3	5	2	12
	Viable ungerminated seeds	0	0	0	0	0

Table 2

Morphological characteristics for uneaten and retrieved seeds by sheep for the invasive tree species *Prosopis juliflora* in South-east Egypt

	Length (mm)	Width (mm)	Thickness (mm)	Mass (g)	Volume (mm3)
Uneaten seeds	6.2 ± 0.0[b]	3.9 ± 0.1[b]	2.4 ± 0.0[a]	0.042 ± 0.001[a]	58.8 ± 0.8[b]
Retrieved seeds	6.7 ± 0.1[a]	4.7 ± 0.1[a]	2.2 ± 0.1[b]	0.040 ± 0.001[b]	69.4 ± 3.2[a]

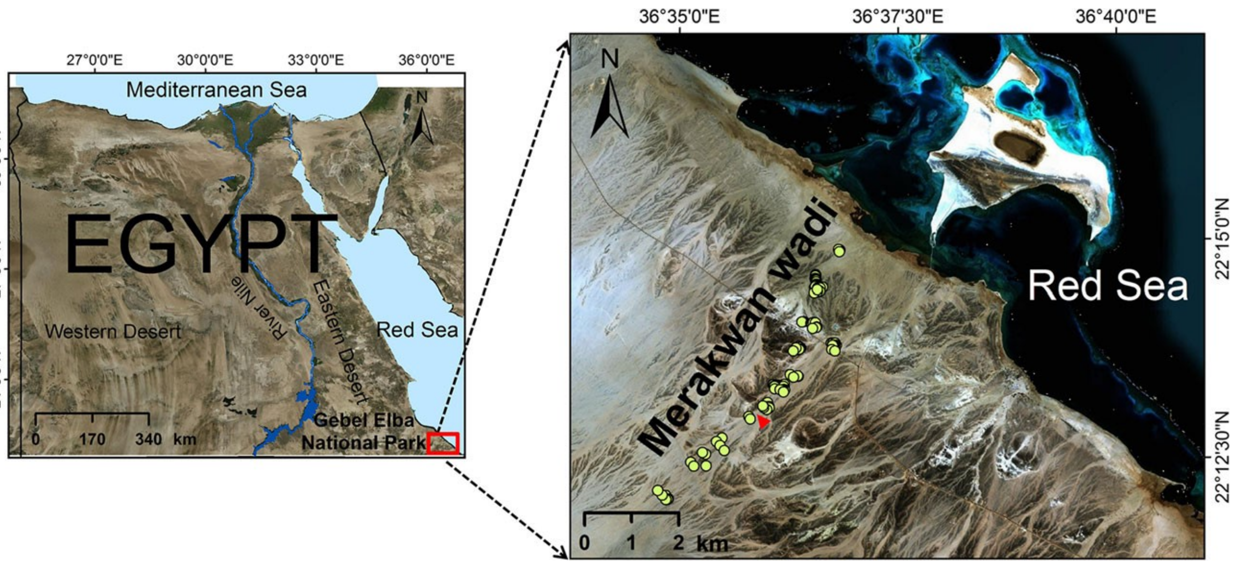
Different letters in the same column indicate significant differences between uneaten and retrieved seeds (*t*-test, $P \leq 0.001$). Values are mean ± SE ($n = 50$).

Figure legend

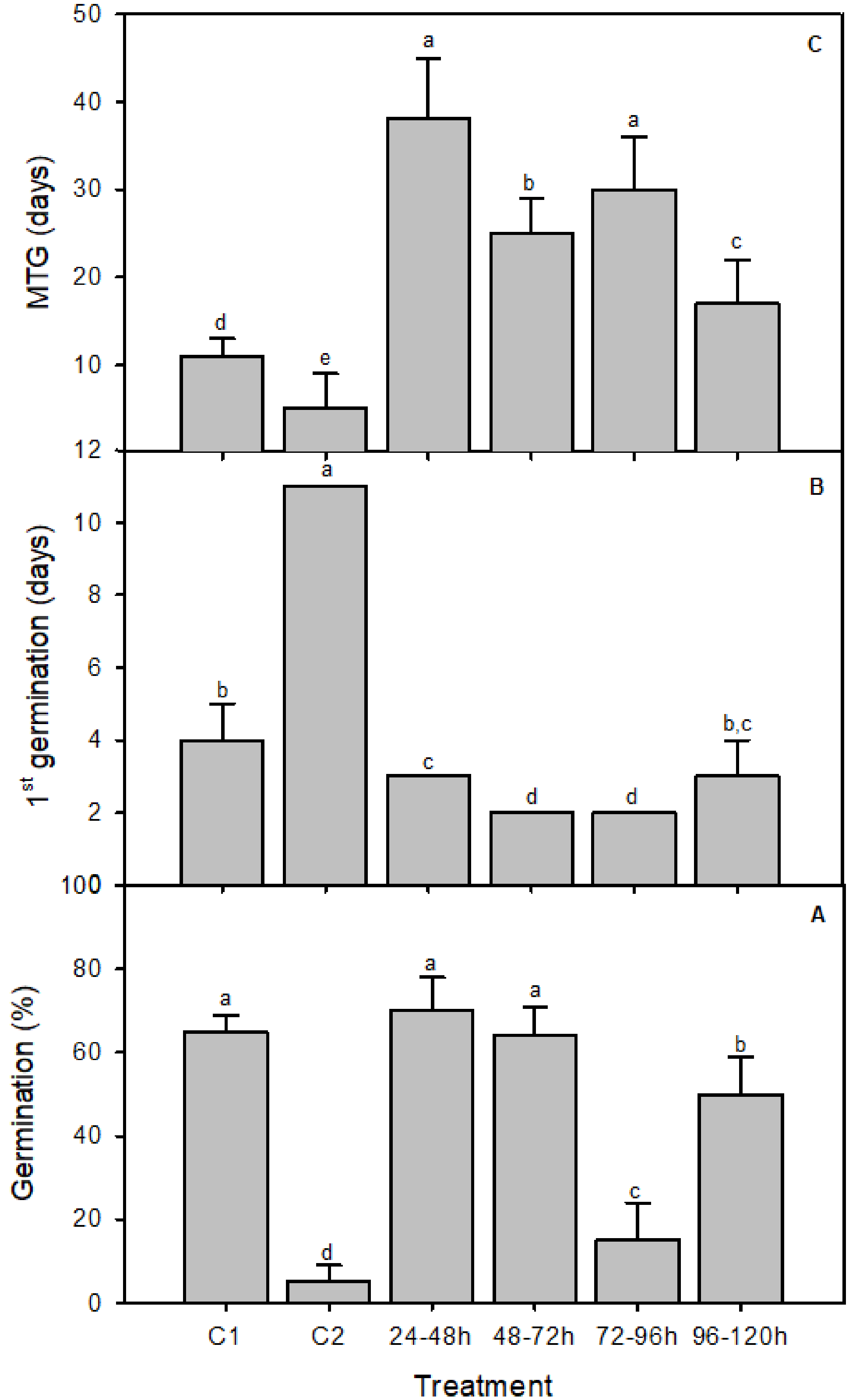
Fig. 1. Location of Gebel Elba National Park in south-eastern Egypt, distribution of the invasive tree *Prosopis juliflora* (yellow circles), and location where its seeds were collected to feed the sheep (triangle) in Wadi Merakuan in the Park.

Fig. 2. Percentage of final germination, number of days to first germination, mean time to germination (MTG), for uneaten seeds without pericarp (C1), uneaten seeds with pericarp (C2), and seeds retrieved from sheep's dung at 24–48 h, 48–72 h, 72-96 h, and 96-120 h for the invasive tree *Prosopis juliflora* from South-east Egypt.

Fig. 1.


27°0'0"E
30°0'0"E
33°0'0"E
36°0'0"E
Mediterranean Sea
30°0'0"N
27°0'0"N
24°0'0"N
EGYPT
Western Desert
River Nile
Eastern Desert
Red Sea
0 170 340 km
Gebel Elba
National Park
N
36°35'0"E
36°37'30"E
36°40'0"E
22°15'0"N
22°12'30"N
Merakwan wadi
Red Sea
0 1 2 km

423 **Fig. 2.**



MTG (days)
C
a
b
a
c
d
e
1st germination (days)
B
a
b
b,c
c
d
d
Germination (%)
A
a
a
a
b
c
d
C1
C2
24-48h
48-72h
72-96h
96-120h
Treatment