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Original article

Seed dispersal by serrated tortoises (*Psammobates Oculiferus*) and the effect of their gut passage on seed germination

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ABSTRACT

Some tortoises are frugivores and can disperse seeds away from the parent plant to areas favourable for their establishments through gut passage. We investigated the plant species dispersed by the serrated tortoise (*Psammobates oculiferus*) in the wild, the effect of gut passage on the germination percentage and the retention time of the ingested food. The tortoises used in the study were captured in the non-wildlife protected areas of Botswana and were kept at the animals' outdoor enclosure at Botswana College of Agriculture (BCA), where experiments were conducted. Faeces of tortoises passed out before being fed in captivity were collected and analysed for the presence of seeds which were later identified at BCA herbarium. At BCA, the tortoises were maintained on indigenous vegetation and supplemented on various fruits and vegetables. The gut passed seeds, seeds extracted directly from fruits as well as the whole fruits were planted at the end of the experiment and number of germinated seeds recorded daily. The food retention time was recorded. The tortoises dispersed Grewia flavescence in the wild. Gut passage significantly enhanced germination percentage of tomatoes while the food retention time ranged from three to seven days. This study demonstrates the importance of tortoises in the ecosystem.

1. Introduction

Zoochory is one of the several methods of plant seed dispersal (Corlett, 1995; Wunderle, 1997; Corlett, 1998). Seed dispersal is an ecological process that enables a plant to disperse from the parent plant to places that favour their establishment. The spreading of seeds away from the parent plant may lead to new sprouting grounds. The falling of seeds beneath parent plants may result in seedlings facing competition, risks of inbreeding and being infected with diseases (Fragoso et al., 2003).

Among vertebrates, mammals and birds are the mostly studied seed dispersers (Wilson, 1989; Traveset, 1998; Herrera, 2002; Cochrane, 2003; Tiffney, 2004; Valenta and Fedigan, 2009) while other vertebrates are less studied. Saurochory (seeds dispersal by reptiles), has received little attention (Liu et al., 2003). According to Hailey (1997), most of the extant tortoise species of Africa are herbivorous while a few are omnivorous. The tortoises may disperse seeds either by epizoochory or endozoochory. The frugivorous tortoises may disperse seeds through gut passage which may either enhance or delay seed germination in some plant species.

Gut retention time impacts digestive efficiency and seed dispersal. The period the seeds stay in the gut of the animal may lead to the damage of the seed or to the improvement of the seed for faster germination. The type of animal which ingests the seeds also matters, for example Davis (2007) reported significantly lower seed mortality during the passage through the digestive tract of an elephant compared to the goat (*Capra hircus*). If the animal moves far away from the parent plant after ingestion, the seeds may be dispersed far away. Sadeghayobi (2011) stated that survival and quality of the ingested seeds depend on the treatment that the seeds receive in the mouth and digestive tract of the animal, as well as on the environmental suitability at the location where they are deposited. Tortoises subject seeds to less severe digestive processes in their guts hence they may be good seed dispersers.

Our study investigated the plant species dispersed by *Psammobates oculiferus* in the wild, the gut retention time and the effects of food passage through the digestive tract of *P. oculiferus* on the germination percentage of tomato seeds. These have important ecological implications for plant establishment and for biodiversity conservation.

2. Materials and methods

2.1. Study animal

Psammobates oculiferus is commonly known as serrated tent tortoise because of its ray-like shell pattern. The adults have an average shell length of range 12-15 cm. The *P oculiferus* is distinguished from its relatives in the genus Psammobates by its relatively low-domed shell which is strongly serrated along its margins at the front and back. Each scute has a radiating star-pattern of black rays on a tan background. The tortoises are endemic to southern Africa. They occur in Botswana, Namibia, and South Africa (Boycott and Bourquin, 2000).

Four (4) adult female serrated tent tortoises (*P. oculiferus*) of average mass 235.55 ± 1g were used in the current study. They were treated in accordance with the guidelines of animal care. The tortoises were captured at Sejanah cattle post and Mmaphoroka fields which are non-protected wildlife areas of Botswana. The permit to capture tortoises was obtained from the Ministry of Wildlife, Tourism and Environment, Botswana using a research permit: EWT8/36/4 XXV (41). The tortoises were transported to Botswana College of Agriculture (BCA) in Sebele where they were kept in an animal outdoor enclosure. They were provided with fresh vegetables and fruits daily. Water was provided at all times. The study was carried out from January to May 2014.

2.2. Plant species dispersed by *Psammobates oculiferus* in the wild

To find out the plant species dispersed by *P. oculiferus* in the wild, the tortoises were given no food but water for the first three days after being captured from the wild. Their faeces were collected and sent to the herbarium for analysis. The seeds found in the faeces were collected and identified.

2.3. Food retention time of *P. sammobates oculiferus*

To determine the retention time of food in the digestive tracts of tortoises, the tortoises were provided with feeds mixed with small colourful beads of diameter 3mm. According to Sadeghayobi et al. (2011) seed size has no effect on retention time. A total of twelve colourful beads were mixed with tomatoes and lettuce and then offered

to tortoises. In the current study, the retention time was calculated by subtracting the time in which the colourful beads appeared in the faeces from the time the feeds containing beads were ingested by the tortoises.

2.4. Effects of gut passage of *Psammobates oculiferus* on seed germination percentage

In order to determine the effects of gut passage of *P. oculiferus* on seed germination percentage, the tortoises were fed on various fruits containing seeds including tomatoes. The faeces of the tortoises were later collected and searched for seeds. A total of twenty tomato seeds were found in collected faeces. All the recovered seeds were not physically badly damaged. The gut passed (GP) seeds, mechanically extracted (ME) seeds and the intact fruits (IF) of tomatoes were planted to determine the germination percentage of the seeds. Ten seeds from each treatment were planted into black plant plastic bags except for intact fruits where two whole tomatoes were used. The plant plastic bags were kept at the BCA green house and watered daily. Germination was recorded daily until there was no germination at all. The results were analysed using analysis of variance (ANOVA). Mechanically extracted seeds were used as the control.

2.5. Data analysis

The data were analysed using Statistical Analysis System (SAS) software. Tukey test was used to compare the means of the treatments. All significant tests were computed at 5% level of probability (Zar, 1996).

3. Results

3.1. Plant species dispersed by *Psammobates oculiferus* in the wild

The faeces of the tortoises composed mostly of leafy vegetation. The faeces of tortoises from Sajenah cattle post contained Grewia flavescence fruit pulp. No seeds were found in faeces of tortoises from Mmaphoroka fields in Kweneng West district.

3.2. The effects of gut passage of *Psammobates oculiferus* on seed germination percentage

The analysis of variance (ANOVA) showed a statistically significant difference between treatments ($P < 0.05$). The Tukey test revealed that means of seed germination percentage for gut passed seeds and mechanically extracted seeds were not significantly different from each other but statistically significantly different from that of seeds in intact fruits. The cumulative number of germinated seeds for gut passed seeds (GP), mechanically extracted seeds (ME) and intact fruit seeds (IF) over 15 days is shown in Figure 1.

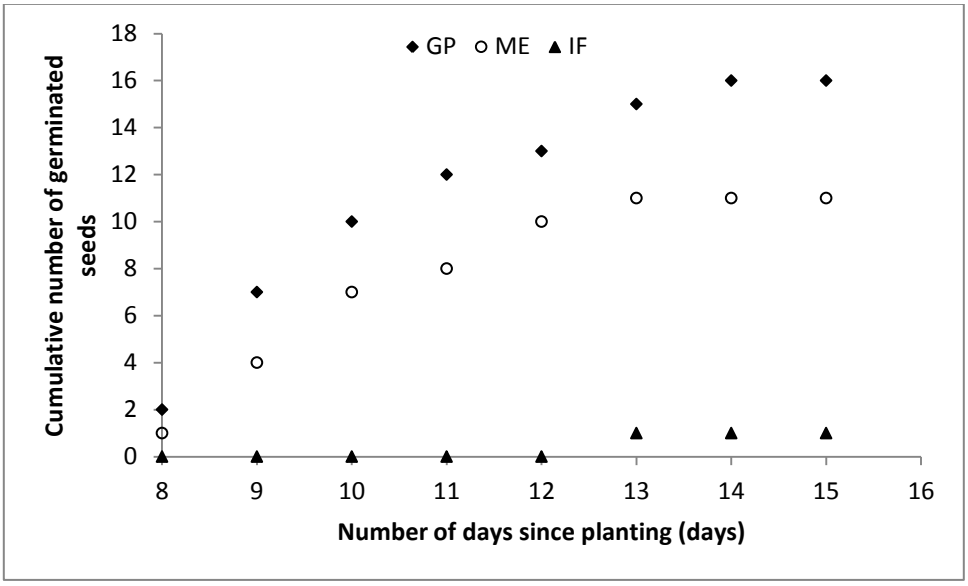

Fig. 1. Cumulative number of germinated seeds of gut passed seeds (GP), mechanically extracted seeds (ME) and intact fruit seeds (IF) of tomatoes over a period of fifteen days.

The germination percentage summary of tomato seeds in different treatments is shown in Table 1. Seed germination percentage of gut passed (GP) and mechanically extracted (ME) seeds over fifteen days experimental period were 80 and 55 respectively. For intact fruit (IF), only one seed germinated over fifteen days and the percentage could not be calculated since the exact number of seeds in the fruit was not known, but many.

Table 1
Seed germination percentage of gut passed (GP) seeds, mechanically extracted (ME) seeds and intact fruits (IF) of tomatoes over fifteen days experimental period.

Treatment	Germination (%)	Number of days since planting
GP	80.00	15
ME	55.00	15
IF	-	15

3.3. Food retention time in the gut of *Psammobates oculiferus*

Table 2 shows the time taken by food to pass through the digestive tract of P. oculiferus.

Table 2
The retention time of food inside the gut of *P oculiferus* measured in days as shown by the passing of colourful beads in faeces.

Food retention time (days)	Number of beads released in faeces per day after ingestion
1	0
2	0
3	3
4	3
5	1
6	1
7	0
8	0

4. Discussion

4.1. Plant species dispersed by *Psammobates oculiferus*

The *Psammobates oculiferus* used in the current study dispersed *Grewia flavescence* plant species in Sajenah cattle post because the seeds were found in the faeces of the tortoises collected from there. However, the effect of gut passage on the germination rate and success of the seeds was not tested. Several studies have reported seed dispersal by chelonids [for example (Hnatiuk, 1978; Waibel et al., 2012). Seed dispersal away from parent plants is important in that seeds may escape distance-and density-dependent predation below parental trees, and therefore have higher survival probabilities [Connell, 1970; Janzen, 1970).

4.2. Food retention time

Beads were recovered in the faeces from day 3 to day 7 after ingestion (Table 2). Sadeghayobi (2011) stated that the retention time of Galapagos tortoises ranged from six (6) to thirty-three (33) days while Jerozolimski et al. (2009) reported that the seed passage time through the digestive tract of adult *Chelonoidis denticulata* varied from 3 to 17 days in south eastern Amazon. The difference in the retention times may be attributed to the different species of tortoises used in the studies, the type of food ingested and the season and ambient temperature at which each study was conducted. The longer retention time may result in a longer distance in which the seeds can be dispersed away from the parent plant.

4.3. The effects of gut passage of *Psammobates oculiferus* on seed germination percentage

Gut passed seeds had a higher percentage of germination as compared to mechanically extracted seeds and whole planted fruit. The germination percentage of mechanically extracted seeds was 55 while that of gut passed

seeds was 80 (Table 2). The current study showed that tortoise gut passage significantly enhances seed germination percentage. This is in agreement with the findings of Rick and Bowman (1961), who reported the improvement of the germination of Galapagos tomato seeds which passed through the gut of the Galapagos tortoises. Liu et al. (2003) also reported enhanced germination percentage and rate of *Serenoa repens* seeds recovered from faeces of Florida box turtle (*Terrapene carolina bauri*). The study by Moolna (2007) showed that giant tortoise ingestion improves seed germination for an endemic ebony species in Mauritius. However, the study by Hansen et al. (2008) showed that gut passage of seeds by Aldabra tortoise decreased the germination success of Syzygium mamillatum. Waibel et al. (2012) reported that gut passage by A*ldabrachelys gigantea* increased the percentage germination of seeds of *Mimusops coriacea* and *Lantana camara*, but not of *Veitchia merrillii* or *Wikstroemia indica*. The differences in the results of the current study and other studies may be due to the differences in the species of tortoises used as well as the food ingested or the seeds of different plant species used in the different studies. The age of the tortoises used in each study may also have attributed to the differences in the results as Waibel et al. (2012) observed shorter gut passage time for sub-adults than adults of *A. gigantea*.

5. Conclusion

The findings of our study suggest that *Psammobates oculiferus* disperse *Grewia flavescence* seeds in the wild in Sejanah cattle post in Botswana. The retention time of seeds in the tortoise ranged from three to seven days and that can enable seeds to be dispersed far away from the parent plant to reduce competition and thus improve the ecosystem. The passage of food through the digestive tract of *P. oculiferus* has a positive effect on the germination percentage of tomato seeds. In future we recommend the fruits to be fed to sub-adult and adult *P. oculiferus* to test how variation in age and size of the tortoises would affect seed germination after ingestion and egestion. We recommend that the distance at which the tortoises disperse seeds from the parent plant to new areas be measured. The influence of seasonal changes in the dispersal distance should also be investigated. It is also imperative to determine the effect of season and ambient temperature on food retention time on *P. oculiferus.*

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