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SPITTING TYPE SEED DISPERSAL BY DOMESTIC GOAT IN THE ZOOCHORIAL PROCESS OF BLACKTHORN PLUM

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Abstract - The blackthorn plum, *Prunus spinosa* L., has fruits with a stony endocarp. It is known that the germination of the *Prunus* species is long-lasting and challenging. Endozoochory, which is seed dispersal via ingestion by vertebrate animals, has been reported to break the dormancy in many plant species. In this study, a special zoochorial relationship, which was perceived to occur between domestic goats (*Capra hircus*) and *P. spinosa*, was investigated with regard to its various aspects. The pits spat by goats during rumination were examined microscopically and compared to the intact pits. The endocarp of pits from goats was seen to be superficially eroded, and the lignified cells were fragmented and broken. The average germination rates were detected in the five experimental groups for the 2018 and 2019 years, respectively, as it follows: 3.85% and 42.3% in the samples from goats with endocarp; 11.4% and 6.8% from goats without endocarp; 2.3% and 59.2% from the tree with endocarp; 3.9% and 9.6% from the tree without endocarp; and 0.8% and 80.0% in the samples sowed as whole dried fruit. A high germination rate and the shortness of the germination period for the first year reveal that the goats, as spitting type seed disperser, play an important supportive zoochorial role for the *P. spinosa* in nature.

Keywords: goat; blackthorn; *Prunus spinosa*; germination; zoochory.

Introduction

Seed dispersal into environment, which promotes germination and survival, is a key process in plant communities for the seedling recruitment to occur. In nature, a wide range of dispersal mechanisms have been described for many plant species, such as dispersal by wind, water, gravity, and animals (Groom & Lamont, 2015). It has been obviously inspected that a large number of animal species, including mammals, birds, reptiles, fish, and invertebrates, play an important role in this process. In fact, between 65% and 90% of woody species in tropical and subtropical Asia were noted to be dispersed by vertebrates (Corlett, 2017). Zoochory, which is seed-dispersing by animals, occurs among vertebrates predominantly in two ways, namely endozoochory (seeds are eaten by animals) and epizoochory (seeds are carried by fur, feathers, or feet). The benefits of the zoochory for plant species are multiple. For example, the dispersal away from the parent plant reduces intraspecific competition and may diminish the pressure of species-specific herbivores and pathogens (Rojas-Aréchiga & Vázquez-Yanes, 2000; Corlett, 2017). Seeds can increase the opportunity to find a more appropriate germination site by means of possible long-distance dispersal. Furthermore, while scarification of the seed coat in alimentary system of animals can break dormancy and enhance seed germination rate, the removal of pulp surrounding the seeds may rescue the seeds from its possible inhibiting effects on germination (Traveset et al., 2007), as well as the seed predators which may inhabit the pulp (Janzen, 1985).

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Up to the present, many factors influencing the effectiveness of zoochory have been described, among which the species of the plants, their size, and shape, as well as the hardness of seeds, their chemical composition, and color of fruits, species, and feeding habits of the animals. Primarily, the small, rounded, hard seeds covered with colorful pulp other than green constitute the main issue in zoochory (Pakeman et al., 2002; Groom & Lamont, 2015). During the endozoochory, the main route of seed dispersal is faeces for most of the animal species, such as ruminants. Many kinds of seeds, especially relatively soft and larger ones, were reported to be crushed by chewing during eating and by further mastication during ruminating by these animals (Harrington et al., 2011). Furthermore, the critical size of feed required to leave the rumen is known to be of < 1.0 mm for goat and sheep, and these animals must break up the large portion of feed by chewing (Domingue et al., 1991). As a result of this physiological feature, the sizes of the seeds recovered from the ruminant faeces were mostly less than 3-4 mm, which is shown in the related studies (Baraza & Valiente-Banuet, 2008; Mancilla-Leytón et al., 2012; Benthien et al., 2016). Actually, relatively bigger and softer seeds can be also excreted by regurgitation, which represents another important spreading way in endozoochory. This behavior has been reported especially in birds (Balgooyen & Moe, 1973; Kleyheeg & van Leeuwen, 2015). Even though the most well-known route of endozoochorous seed dispersal by ungulates is faeces, some recent studies conducted on goats (Delibes et al., 2017) and red deer (Castañeda et al., 2018) have revealed that spitting viable seeds from the cud during rumination is also important, at least for the seeds of some plant species. As indicated by these researchers, although spitting of seeds by ruminants has been stated in some publications (Janzen, 1985; Feer, 1995), none of them argues about the role of this aspect in endozoochorous process of ungulates.

Seed dormancy, which is evaluated mostly as a kind of evolutional adaptation to harsh environmental conditions, is a well-known issue in many plant species. In this respect, different types of dormancy have been described, such as physiological, morphological, morpho-physiological, physical or the combination of some (Baskin & Baskin, 2014). The most common cause of dormancy in woody plant species was reported to be the mechanical reasoned circumstance consequences from the water-impermeable, restrictive hard seed coat. Related studies have revealed that some applications, such as mechanical scarification, pretreatment with acid, hot water, dry heat, dipping in boiling water for a determined period, may promote germination. Furthermore, it has been indicated that the success of these processes is closely related to various factors such as plant species and treatment characteristics (Shiferaw et al., 2004; Nasr et al., 2013). In fact, although dormancy can be broken in some species by improving proper environmental conditions, such as temperature, water, air, and light, this is not enough in some others. It has been underlined that seed dormancy is a complex issue which can be controlled by many different factors, including genetics. Generally, this sophistication is the reason why the results of the studies conducted on some species to break dormancy are inconsistent (Baskin & Baskin, 2014).

Prunus species, including blackthorn, can be described as one-stoned fruit called "drupe" because of the seed, which has a though endocarp. The studies related to the species of the genus have shown that most of them exhibit strong dormancy. Restriction of the embryo by hard and impermeable endocarp as a mechanical barrier and some endogenous growth regulators, such as gibberellins, abscisic acid, and cytokinins, have been mostly postulated to have a possible role in dormancy among the genus. Even though dormancy is a well-known issue for many *Prunus* spp., among them, *P. spinosa* has been generally indicated as the most rigid species of the genus in this respect. However, there is relatively little information about the mechanism of seed dormancy in this species (Iakovoglou & Radoglou, 2015; Afroze & O'Reilly, 2017). It was mentioned that *P. spinosa* has double dormancy as embryonal- and mechanical-dormancy arising from the hard seed coat. Therefore, direct autumn sowing of the seeds results mostly in no germination during the following spring, and the dormancy may continue until next spring in nature, as encountered in most other species of the genus (Takos & Efthimiou, 2003; Afroze & O'Reilly, 2017). To overcome dormancy and to maximize germination in *Prunus* species, various pretreatments have been applied, among which gibberellic acid, hydrogen peroxide, citric acid, and sulfuric acid scarification, endocarp removal, and stratification (warm and cold application). In this respect, especially warm and cold treatments, mostly applied in succession as warm plus cold for a relatively long period, have been of special importance (Eşen et al., 2006; Zeinalabedini et al., 2009; Pipinis et al., 2012).

The present study investigates from different perspectives the domestic goats (*Capra hircus*) mediating endozoochory of *Prunus spinosa* L. (Rosaceae) (sloe, blackthorn), which naturally occurs in the fields of Turkish Thrace. This relationship between goats and blackthorn, which is widely growing wild in uncultivated areas of Europe, West Asia, and the Mediterranean (Sabatini et al., 2020), was revealed for the first time in this study, and the obtained data were discussed in order to shed light on possible similar relationships which occur in nature between blackthorn, or plants with similar seeds, and domestic goats, or similar wild ruminants such as cervids.

Materials and Methods

Description of the study area

The study was performed in Turkish Thrace, the European part of Turkey, where *Prunus spinosa* has natural distribution. GPS coordinates in the center of the area (23.764 km^2) are 41°19'N and 27°12'E with altitude, which ranges from s.l. to 1031 m. Turkish Thrace is bounded on the west by Greece, on the east by Bosphorus strait and the Black Sea, on the north by the Black Sea and Bulgaria, on the south by the Sea of Marmara, and on the southwest by the Aegean Sea. The diverse and transitional weather conditions are the result of the interplay of the different climatic zones in the region. Vegetation is composed predominantly of steppe and maquis in places and forests in highlands, which are mostly located on the sea costs. According to the State Meteorological Service data related to the study area, the temperature (°C) values generally vary between -15.8 and 42.5 in a year, and monthly average temperature (min-max) (°C) and precipitation (mm = kg/m^2) values from January to December are given in Table 1.

Table 1. Monthly average temperature (with min-max values in parentheses) and monthly total precipitation data obtained from Turkish State Meteorological Service for the study area from January to December.

Months	Average temperature (ºC)	Total precipitation (mm)
Jan	4.7 (1.9-7.9)	68.5
Feb	5.4 (2.4-8.9)	54.4
Mar	7.2 (4.1-11.0)	54.0
Apr	11.7 (8.1-15.7)	40.8
May	16.6 (12.7-20.6)	36.6
Jun	21.1 (16.6-25.3)	37.9
Jul	23.6 (18.9-28.0)	24.2
Aug	23.8 (19.3-28.2)	15.4
Sep	20.1 (16.0-24.4)	33.8
Oct	15.5 (12.0-19.4)	60.6
Nov	11.2 (8.1-14.7)	74.2
Dec	7.1 (4.2-10.3)	81.1

General characteristics of the study material *Prunus spinosa*

The species is widely distributed in the northern hemisphere, including Turkey, and has many intra-specific variants which have many similar traits among themselves (Dönmez & Yıldırımlı, 2000). *P. spinosa*, which has recently gained more attention due to its pharmaceutical properties, such as diuretic, laxative, antispasmodic, antiinflammatory, antioxidant, antibacterial and antifungal activities (Pinacho et al., 2015; Sabatini et al., 2020), is a spiny shrub with weaker thorns in deep soils and humid areas, up to 1-2 (-3) m; twigs hairy. Leaves 10-50 × 8-25 mm, obovate to elliptic, margins crenate-serrate, glandular, with a pair of large glands present at the base of the lamina. Flowers are white, single or in pairs, flowering before the leaves, 10-15 mm in diameter (Dönmez & Yıldırımlı, 2000). Drupes globose, 8-15 mm in diameter. Exocarp is bluish-black, glaucous, mesocarp green, sour; endocarp is stone adherent to mesocarp, smooth or slightly scabrous, 8-14 × 8-10.5 mm. Seed 9-10 × 5-6.5 mm. The species flowers in spring and produces fruits (ripe) in autumn.

Field study and collection of the material

Field studies were carried out in a wide range of different areas of Turkish Thrace, predominantly in two provinces – Tekirdağ and Kırklareli – during which, some distribution characteristics on the field and ripening period of the fruit of *P. spinosa* were recorded by repetitive field researches conducted predominantly in autumn. Feeding behavior of 250 goats and 80 sheep from 6 different pens grazing on the fruit was observed in these periods. These animals were also observed in the pens to see the further processing of the fruits during rumination or any other processes. Bedding materials in the pens were inspected in detail for the presence of any seeds.

For further research, the fruits from the field and seeds from the pens were collected in the same localities coordinately during three-week period at the end of the autumn when fruits were fully ripened and consumed by the goats. In this stage, in order to be sure that the daily seeds processed by goats are collected, beddings of the pens were checked out in detail, and owners were asked about the removing regime of the beddings. In order to eliminate the possible effects of lot (Afroze & O'Reilly, 2017) and herds on the germination or on some other features of seeds, samples were collected from more than ten localities scattered throughout the study area, then mixed as homogeneously as possible. Measurements and sowings were conducted on the samples selected randomly from these.

In order to observe the eating and chewing habits of goats and sheep on *P. spinosa*, ripe fruits collected from the field were supplied to animals in the pens and related behaviors were inspected and recorded.

Preparation and sowing of the material

Some of the fruits were washed vigorously to eliminate pulp as much as possible and then dried. The other fruits were dried with pulp. Endocarps were broken by nippers and removed in some parts of the seeds collected from the pens and those from the fruits. The seeds were inspected and photographed under stereo - and scanning electron - microscope in detail. All the samples were weighed in precision scales, and the width, length and height (W, L, H) of seeds were measured using a caliper. Furthermore, the pressure resistance of the seeds (100 seeds from goat, 100 seeds from the tree) was determined by compressive strength analyses using TA.HD *Plus* Texture Analyzer.

The seeds, which had been selected randomly from the groups were sowed individually in the viols (D: 3.5 cm, h: 5.5 cm) using peat (pH 5.5-6.8) at the end of January 2018, and all the germinations occurring in the pots were recorded until the end of the 2019. In this stage, 626 samples were sowed in five groups constituted as follows: i) 130 seeds from pens (from goat) with endocarp; ii) 132 seeds from pens (from goat) without endocarp (removed by using nippers); iii) 130 seeds from tree with endocarp (washed seeds); iv) 104 seeds from the tree without endocarp (removed by using nippers); and v) 130 seeds within the dried pulp. Throughout the whole study period, all the samples and sowed materials were held in the open area and under the natural climatic conditions which are natural for *P. spinosa*. In this way, the seasonal temperature regime, to which local plant has been evolutionarily adapted and which may be effective on the germination (Iakovoglou & Radoglou, 2015; Afroze & O'Reilly, 2017; Pipinis et al., 2018), was provided. Meanwhile, sowed samples were watered occasionally due to the quick-drying tendency of the peat in the viols.

The data obtained from the study of the five experimental groups are given as the mean value, together with the minimum-maximum values. Also, the percentages of germinated seeds for all groups for the first (2018) and second (2019) years were presented. Mikro Sistem Z650 stereomicroscope was used for the surface examination of the seeds from goats and from nature, and of their coating endocarps; micrographs were taken using Kameram 5 camera and its imaging software. Detailed surface morphology micrographs were obtained with JEOL 6335F JSM scanning electron microscope at a magnification of ×200-1000.

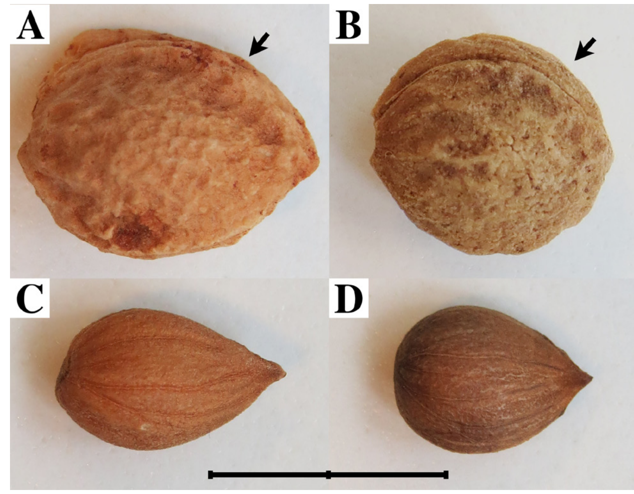

Figure 1. Seeds with (a, b) or without (c, d) endocarp from nature (a, c) and from goat (b, d). Arrows indicate the ventral fusion line of the endocarp. Scale bar: 10 mm.

Results

It was noticed that the fruit ripening of the *P. spinosa* begins in early autumn in Thrace. Defoliation is mostly completed until the beginning of winter; however, unlike most of the other *Prunus* species, such as the plum tree, ripe fruits mostly stay on the shrub until around mid-winter. It was observed that browsing goats do not prefer crude fruits to eat, but can feed on the ripe fruits with appetite and easily as the result of the suitable morphological traits of the tree; this is not a routine issue for regular ground feeders, such as sheep and cattle. In fact, because ripe fruits do not tend to drop on the ground, the probability of encounter between the fruits and the animal species is limited in nature.

In the feeding trials, it was observed that both goats and sheep can eat ripe fruits of *P. spinosa* under captive conditions. Few seeds were ejected before ingestion and some seeds were cracked during chewing as it was understood from the breaking sounds. Although exact numerical data could not be obtained in both types of animals, similar behaviors were inspected in both goats and sheep. Due to the active and fast feeding habits of the goats in the field (Agrawal et al., 2014), the indicated losses of the seeds during eating the fruits were evaluated to be much less in nature than in captive condition. Furthermore, in the pens in which goats and sheep were kept together, it was seen that goats spit the seeds from cud, whereas sheep take the intact spat seeds from the ground, crack it and eat, which is a type of behavior not seen in goats.

In the field studies, shrubs of *P. spinosa* were seen predominantly on the field borders and along the field roads. This distribution pattern is likely to be due to the transportation of the seeds containing goat beddings from pens to the fields as organic fertilizer.

The average value of compressive strength was measured to be of 35918.5 g (16620.1-62147.2) in spat seeds by the goats, and 35117.1 g (18097.9-60796.6) in seeds gained directly from fruits. This low level of difference was evaluated as the result of cracking the relatively weak seeds by chewing during eating and/or ruminating. Related tests also indicated that average dimensions (L×W×H) were less in the spat seeds (11.1×9.1×6.6 mm) than in natural seeds (12.1×9.5×6.6 mm). However, our microscopic examination showed that the main reason for this reduction is related to the loss of the

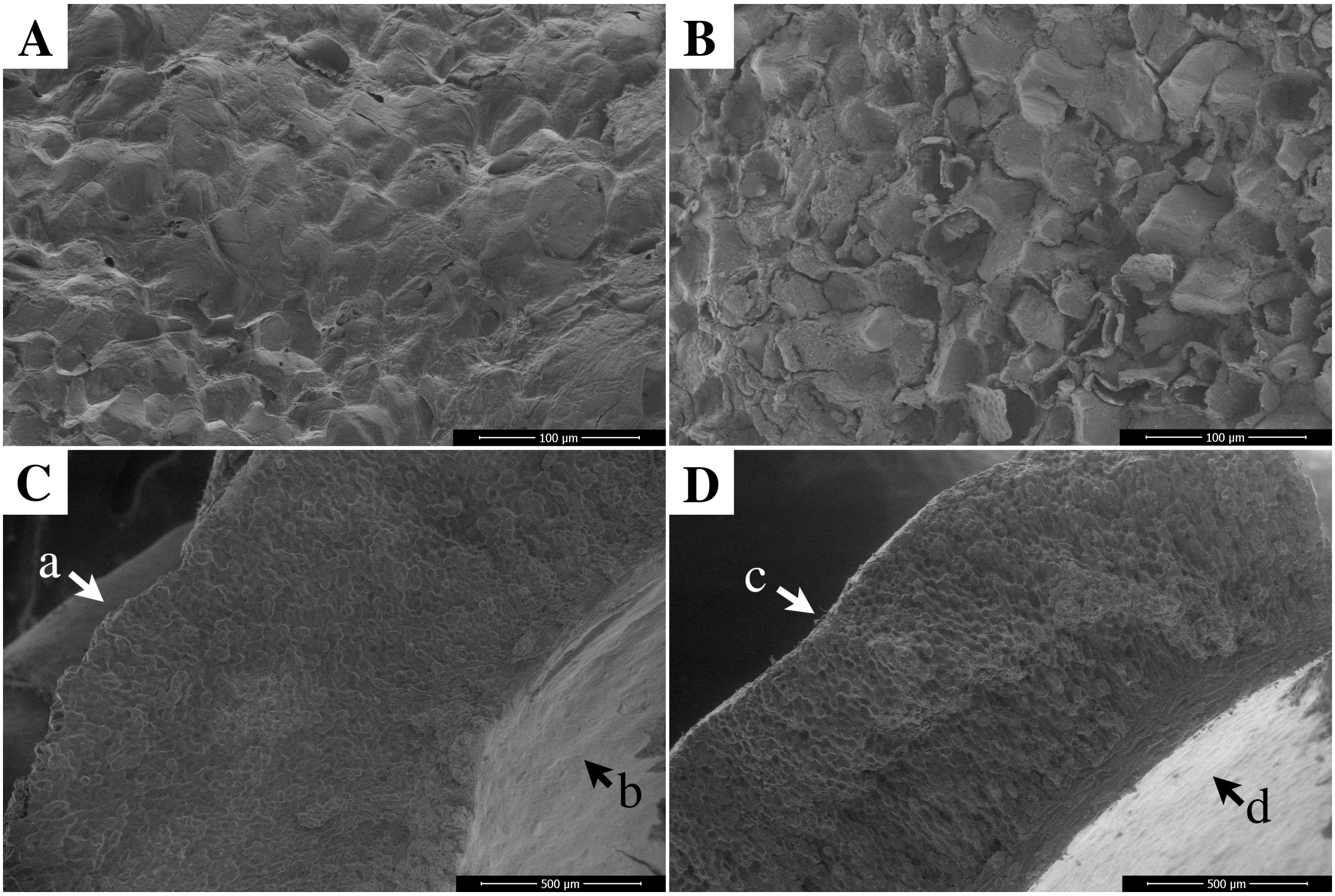

Figure 2. SEM views of endocarp from natural (a, c) and spat (b, d) seeds; a and b external surface, c and d cross-section at the ventral fusion line of the endocarp. White arrows external surface, black arrows internal surface.

endocarp projection, which appeared predominantly at the basal side, and the ventral fusion line of seeds (Figure 1). In fact, no obvious thinning other than superficial corrosion (Figure 2) was seen on the outer surface of endocarp, as it was also understood from the equal results of measures related to the height of seeds.

Furthermore, spat seeds were relatively lighter with or without endocarp (Table 2), possibly because of the indicated partial loss of endocarp and loss of water during and just after the digestive process in the seed which seemed darker, smaller and more rounded at the samples spat by goats (Figure 1). This might be the consequence of the increase in the possible endocarp permeability of *P. spinosa* (Afroze & O'Reilly, 2017).

In this study, which has been conducted to cover the two-year germination period, the total counts of germinated samples of the groups are as it follows: 60 (46.2%) and 24 (18.2%)

Table 2. Results of compressive strength analyses in the seeds.

	Seeds from goats	**Seeds from tree**
Counts of seed examined	100	100
Average value of compressive strength (g) (min-max)	35918.5 (16620.1-62147.2)	35117.1 (18097.9-60796.6)
Average dimensions with endocarp (mm) (L × W × H)	11.1 × 9.1 × 6.6	12.1 × 9.5 × 6.6
Average weights with endocarp (mg) (min-max)	318.5 (195-480)	338.9 (240-426)
Average weights without endocarp (mg) (min-max)	91.8 (56-124)	97.5 (72-130)

Table 3. Results related to germinated seeds in the groups.

Measured data	Seed Groups				
	From goat (endochorial)		From fruit (naïve)		Whole dried fruit
	with endocarp	without endocarp	with endocarp	without endocarp	
Counts of sowed samples	130	132	130	104	130
Average dimensions (mm) (L × W × H) (min-max)	10.5 × 9.0 × 6.4	8.1 × 5.7 × 3.5	12.1 × 9.3 × 6.7	9.4 × 5.9 × 4.0	11.9 (10-14)
Average weights (mg) (min-max)	287.3 (135-440)	85.7 (58-129)	345.4 (191-478)	98.3 (60-131)	1,150.5 (640-1,939)
Germinated samples in 2018					
Counts (%)	5 (3.9%)	15 (11.4%)	3 (2.3%)	4 (3.9%)	1 (0.8%)
Average duration of germination (min-max)	64.8 (34-89)	58.8 (34-92)	71.4 (60-77)	70.8 (60-78)	74
Average dimensions (mm) (L × W × H)	10.8x9.8x6.7	8.4x5.6x3.9	13.5x9.5x7.0	9.3x6.0x4.0	11
Average weights (mg) (min-max)	346.0 (303-392)	92.0 (58-129)	406.7 (396-416)	106.0 (97-120)	1,174.0
Germinated samples in 2019					
Counts (%)	55 (42.3%)	9 (6.8%)	77 (59.2 %)	10 (9.6%)	104 (80.0%)
Average duration of germination (min-max)	30.0 (7-60)	19.6 (7-33)	10 (9.62 %)	30.2 (20-52)	22.9 (1-60)
Average dimensions (mm) (L × W × H)	10.7 × 9.0 × 6.4	8.8 × 6.0 × 4.5	12.0 × 9.3 × 6.6	9.4 × 5.9 × 3.9	12.0 (11-14)
Average weights (mg) (min-max)	293.2 (162-440)	109.0 (68-129)	337.6 (191-456)	100.6 (65-119)	1,161.9 (640-1,939)
TOTAL Counts of germination	60 (46.2%)	24 (18.2%)	80 (61.5%)	14 (13.5%)	105 (80.8%)

in the samples from goats with and without endocarp; 80 (61.5%) and 14 (13.5%) in the samples from the tree with and without endocarp; and 105 (80.8%) in the samples which were sowed as dried seeds within the pulp. Counts of germinated samples of the groups in 2018 and 2019, respectively are as it follows: 5 (3.85%) and 55 (42.31%) in the samples from goats with endocarp; 15 (11.36%) and 9 (6.82%) in the samples from goats without endocarp; 3 (2.31%) and 77 (59.23 %) in the samples from the tree with endocarp; 4 (3.85%) and 10 (9.6%) in the samples from the tree without endocarp; and 1 (0.77 %) and 104 (80.0%) in the samples which were sowed as dried seeds within pulp (Table 3, Figure 3).

In the present study, the first germination was inspected on the 15th of March, 2018, in one sample from seed groups from goats both with and without endocarp, and the last germination was seen on the 8th of June, 2018, in one sample in the group from goats without endocarp. In 2019, the first germination was observed on the 9th of March, 2018, in one sample of dried seeds within the pulp, and the last germination was seen on the 7th of May, 2019, in one sample from seed groups from goats with endocarp and in dried seeds within the pulp. Actually, the sowed seeds are expected to germinate when the air warms up in spring, independently of sowing time. The durations of germinations, which have occurred in the spring seasons during the two-year study period, were compared

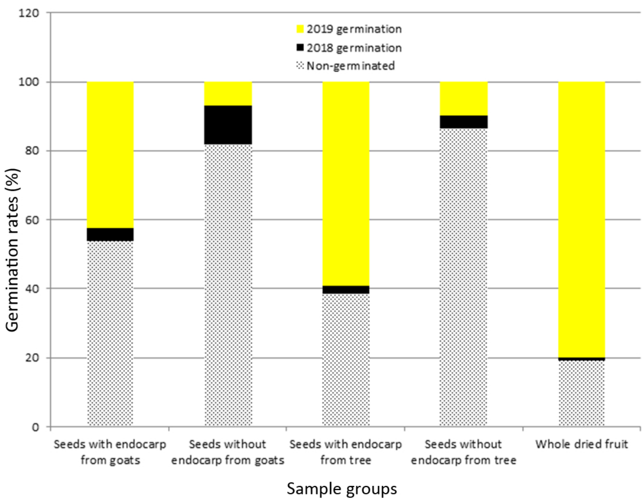

Figure 3. Germination performance of the sample groups according to the years.

among the groups and between the years. In this process, the duration for first germinated seed (9 March) was accepted as one day regardless of the year, and this was followed by the others, which germinated after 9 March.

In this respect, in the spring/germination season of the 2018, average durations of germinations were calculated as 64.8 and 58.8 days for seeds from goats with and without endocarp; 71.4 and 70.8 days for seeds from three with and without endocarp; and 74 days for dried seeds within the pulp. As for the 2019, these values were as it follows: 30.0 and 19.6 days for seeds from goats with and without endocarp; 10.0 and 30.2 days for seeds from three with and without endocarp; and 22.9 days for dried seeds within pulp (Table 3).

During the screening of the beddings in the goat pens, some different seeds, such as plum seeds and acorn, were also collected. Even though it was not observed directly, they have been evaluated to be spat from cud due to their big size and affected endocarp or pericarp in different degrees. It was revealed that these differences of endocarp in plum seeds, both microscopically and macroscopically, were similar to those in *P. spinosa*.

Discussion

In this study, average percentages of germination for the first year are of 3.85%, 11.36%, 2.31%, and 3.85% in the seeds sowed as endochorial (from goat) with and without endocarp, and manually extracted seeds from pulp with and without endocarp, respectively. The seeds of *P. spinosa* are known to germinate at a low rate, and the percentage of germination could not be enhanced over 50% even in the sowing trials after pretreatments such as warm, chilling, and exogenous hormone (Afroze & O'Reilly, 2017). For example, the maximum percentage of germination was reported as 26% when seeds were warm-stratified for two weeks and continuously altered for eight weeks of cold stratification (Iakovoglou & Radoglou, 2015). As for this species, double dormancy was stated for the seeds; of those, the mechanical dormancy was claimed to be weak, and embryonal dormancy was major (Afroze & O'Reilly, 2017). It was reported that the removal of endocarp to eliminate mechanical dormancy in the seeds of the *Prunus mahaleb* improved germination significantly, whereas the pretreatment with gibberellic acid followed by cold stratification for 1 month resulted in higher germination percentages (Pipinis et al., 2012).

The procession of seeds in the animal digestive tract during zoochory is known to break dormancy at different levels (Rojas-Aréchiga & Vázquez-Yanes, 2000). This effect depends on the species of animals, conditions of the digestive tract, type of seeds, and type of dormancy (Khurana & Singh, 2001; Razanamandranto et al., 2004). For example, the importance of the differences in animal species has been demonstrated in all stages of zoochorial process. It is reported that bats and birds tend to have highly positive effects on seed germination, whereas non-flying mammals have positive but small effects, and this was suspected to be a result of longer food retention times in the digestive tract of non-flying mammals (Traveset et al., 2007). This issue was stated in literature as "the longer the seeds are in the animal, the higher the seed mortality" (Janzen, 1984). In this respect, although more research is needed to obtain more exact data, spitting the seeds just after the ruminal process before getting into the intestine seems to be the appropriate way of spreading for some seeds, at least to survive with higher germination potential. Concerning the ruminants, which have longer food retention times in the digestive tract, it has been reported that these animals can spread many kinds of seeds by defecation, which may occur in different viability levels according to seed and animal species. Besides, a negative correlation was indicated between the rate of recovery from the faeces and the size of the seeds (Simao Neto et al., 1987; Pakeman et al., 2002). Among the ruminants, goats are a well-known seed dispersal vector for many kinds of grass and shrubs, such as cacti, legume, acacia, carob, spurge, and fescue species (Khurana & Singh, 2001; Baraza & Valiente-Banuet, 2008; Mancilla-Leytón et al., 2012). As for the zoochorial process in goats, it was reported that the shape and size of the seeds are important traits on the level of the passage of the gastrointestinal tract without digestion and germination after scattered by faeces. Fundamentally, the plant species with rounded, hard-textured seeds smaller than 3-5 mm are inspected in the endozoochory in goats (Lacey et al., 1992; Holst & Allan, 1996; Robles et al., 2005; Baraza & Valiente-Banuet, 2008; Harrington et al., 2011; Mancilla-Leytón et al., 2012; Grande et al., 2013). Although faecal endozoochory was reported to be the principal way in the zoochorial process of the ruminants (Khurana & Singh, 2001; Baraza & Valiente-Banuet, 2008; Mancilla-Leytón et al., 2012), some species are known to spit some relatively big sized seeds during rumination, such as white-tailed deer (*Odocoileus virginianus*) (for *Spondias mombin* seed) (Janzen, 1985), fallow deer (*Dama dama*) (Delibes et al., 2017), and red deer (*Cervus elaphus*) (for *Chamaerops humilis, Crataegus monogyna*, *Celtis australis,* and *Ceratonia siliqua* seeds) (Castañeda et al., 2018). It was observed that goats could spit relatively big (6.1 × 4.8 mm – 22 × 12 mm) seeds with high viability (up to 71.5%) from their cud during rumination, such as olive (*Olea europaea*), dwarf palm (*Chamaerops humilis*), argan tree (*Argania spinosa*), *Chamaerops humilis*, *Crataegus monogyna*, *Celtis australis*, *Olea europaea* var. *sylvestris*, *O europaea* var. *domestica* (13.3 × 5.8 mm), and *Ceratonia siliqua* (7.5 × 5.3 mm). In this last study, it was also pointed out that the seeds fed from 10% to 45%, were spat again during this period, and the percentage of viability was up to 71.5% in the seeds spat (Delibes et al., 2017).

Ruminants are able to utilize the lignocellulosic fibrous materials in favor of their special digestive physiology. It is concluded that there are only minor differences between the rumen activities of sheep and goats. However, sheep are grazing animals, whereas goats are essentially browser. Goats have a competitive advantage over sheep in woodland and shrubland; they are generally more active, selective, and walk long distances in search of food and relish a variety of food, and may select vegetation at various heights when foraging. Goats can distinguish between bitter, sweet, salty and sour tastes, and show a higher tolerance for bitter taste than other types of livestock. Furthermore, they can tolerate negative digestive effects of tannins and some secondary plant metabolites by means of special saliva secretion and relatively bigger liver tissue for their body as a detoxifier, which allow them to feed greatly on fruits and leaves which contain a high amount of tannins and others alike. Sheep can evaluate concentrated food, which is rich in seeds, better than goats, and they continue feeding on forage with a different fibrous level in different seasons (NRC, 2007; Agrawal et al., 2014; Ferreira et al., 2017). Goats, however, appear to be opportunistic. They can exhibit seasonal adaptations to changes in forage quality, and when forage plants lignify, these animals switch to browse or fruits (Hofmann, 1989). All these differences in behavior can be useful to explain the differences between goats and sheep with respect to the concern of our study. Although sheep can eat the seeds of *P. spinosa* spat by goats in the fold, their possible negative pressure on this plant seems to be ignored because of their grazing habits in the field.

In our attempts to determine the appetite of the animals against fresh fruity seeds of blackthorn, it has been revealed that both captive animals can eat this fruit, and both animals, especially sheep, can crack some seeds during chewing. Some smaller seeds belonging to other plant species were determined in the faeces of goats, but we could not meet seeds of blackthorn, at least during our investigation periods. This event, spreading by faeces, is possible to occur, but not very commonly because of the size of the blackthorn seeds. In a related study (Castañeda et al., 2018), seeds belonging to four different plant species were fed to captive red deer, and, as a result, 77.9% of the seeds disappeared (i.e., digested or missed), 13.8% were defecated, and 8.3% were spat during rumination. The researchers indicate that the seeds contained in the multi-seeded pods, like carob, were more frequently dropped earlier during eating. In contrast, smaller ones were rarely ejected, and larger seeds (dwarf palm, 16.3 × 11.0 mm) were delivered more frequently from the cud, while smaller ones (hawthorn, 6.1 × 4.8 mm) were mostly defecated (Castañeda et al., 2018). Goats are generally more active and, selective, and they can walk long distances in search of food (Agrawal et al., 2014), and, when considering such a rush feeding habit, cracking the seeds in the field during eating is less possible than under captive condition.

In this study, for the first year, the lowest germination rate (0.79%) was obtained from the seeds sowed as whole dried fruit with pulp. Actually, the fruit pulp is known to decrease or even preclude germination through some inhibitor contents or by altering the microenvironment of the seeds (Traveset et al., 2007). However, during the first year experiments, it was recorded that the average percentages of germination of the endochorial seeds with and without endocarp were higher than the similar groups of the seeds whose pulps were mechanically removed. This result indicates some further factors which affect germination related to spitting type endozoochory in goats, in addition to removing the pulp. It is known that seeds are exposed to severe mechanical and/or chemical scarification when passing through the digestive tract of the frugivores (Traveset et al., 2007). At this point, some factors were evaluated to be likely responsible, such as mechanical effects of chewing during eating and ruminating (cud-chewing), temperature, watery environment, pH, and some other chemical contents of the rumen. In ruminants, food is mixed with saliva in rumen and reticulum, *fore-compartments of the stomach,* which contain specialized, dense, and diverse microbial population belonging to diverse families from bacteria, protozoa, fungi, and phages. As a result of the microbial digestion of nutrients, the ruminal environment is anaerobic and rich in CO_2, volatile fatty acids, oxidized reducing factors, and methane. In the rumen, values of pH, temperature and oxidation-reduction potential of rumen vary between 5.5-7.2, 38-42 °C and (-)250 – (-)450 mV, respectively, depending on animal species, feeding habits and some other physiological status more or less (NRC, 2007; Agrawal et al., 2014). Conversely, in the second year, the highest germination rate (80.0%) was obtained from the seeds which had been sowed as whole dried fruit with pulp. Although there are studies that claim that the fruit pulp definitely suppresses the germination (Traveset et al., 2007), in our study, this is valid for the first year, whereas in the second year, it is the opposite. Hence, in plants with this type of fruit/seeds, the germination attempts should be dropped to the second year because fruit pulp is retardant but not a permanent suppressant.

The results from the germination process for the second year show some significant differences compared to the first year (Figure 3). The average percentages of germination for the second year are of 42.31%, 6.82%, 59.23%, and 9.6% in the seeds sowed as endochorial (from goat) with and without endocarp, and manually extracted seeds from pulp with and without endocarp, respectively. This is most likely associated with the fact that the goats have provided some activation of the seeds. In addition, the results also reveal that, although the endocarp delays the germination process, it may provide protection in the long-term waiting period. One of the most striking results obtained in the second year was the highest rate of germination (80%) observed from fruit-dried seeds. When all the data of our study are evaluated together, it is understood that the pulp suppresses effectively the first-year germination (rate of germination 0.8%), but it also seems to provide some kind of protection in the long term period or provides a significant advantage in the second year germination. In addition, considering the possible benefits of the pulp, at least on the long-term germination, it seems possible to mention some of the other possible benefits for the tree. For example, the fruit-dried seed is higher in volume and lower in mass, making it to be transported easier by wind or water. Therefore, fruit dried with seeds tend to wait, but their germination performance is higher in the second year. Apparently, the plant endeavor primarily on its long-distance propagation, it is either waiting to dry out with its fruit and somehow getting away in nature, or with goat or similar group animals; germination occurs immediately as it is already guaranteed to be taken away by the goat. It seems that the goat's selection of potential stout seeds is an additional benefit. All this appears to be an evolutionary adaptation which targets the propagation of the plant.

Indeed, our observations can be interpreted as a possible evolutional relationship between goats, or caprine, and blackthorn concerning the following aspects: **i)** goats can crack some seeds, possibly those which are weak and this may play a role in the selection of stronger seeds; perhaps, as a result of this selection, the average value of compressive strength in seeds from goat is at least slightly higher than those seeds gained from the tree; **ii)** the ripe fruits wait on the tree for a long period, mostly longer than leaves in autumn and winter, as if they wait to be taken far from the mother tree; **iii)** the general morphology of the blackthorn tree (Dönmez & Yıldırımlı, 2000) is proper for goats to feed at almost every level; and **iv)** even though goats can tolerate many kinds of taste and metabolites in the fruit (Agrawal et al., 2014), they do not eat crude fruit of blackthorn containing immature seeds that are weak and not yet suitable for germination, having instead a good appetite for ripe fruits with endocarps that are stronger and more resistant to the zoochorial process (Kneuper et al., 2003). Actually, such a possible evolutionary relationship should not be surprising. Zoochory is well known for its positive effect on the selection of high-quality seeds and fruits (Corlett, 2017), and a large fraction of living plants produce fruits which attract animals. It is known that for millions of years both the pulp and seeds of the fruit have been subjected to selective pressures exerted by frugivores. In this context, the specific seed traits in an endozoochorous plant were expected to be a result of the combined selection imposed by frugivores and other biotic and abiotic factors. It was indicated that the nature of the relationships between fruit chemistry and morphology and the type of disperser was crucial for the understanding of the co-evolution of plant–frugivore interactions (Traveset et al., 2007). As a result, the two-year research on the zoochorial process of *P. spinosa* by domestic goats shows that **i)** goats significantly stimulate earlier (first year) germination in the seed of *P. spinosa*; **ii)** goats seem to be an effective factor in the spread of this species in nature; **iii)** goats do not contribute positively to the long-term (two-year) total germination rate of the seeds of this plant; however, **iv)** compressive strength analyses of the seeds show that goats provide durable seed selection eliminating the weak ones, possibly by breaking them during chewing and/or by inactivating them in the digestive tract. Loses in the diversity and regeneration of the vegetation in nature have been associated predominantly with the pollution and changes in the availability of seed dispersal animals over the world (Ozinga et al., 2009; Corlett, 2017). This reality reveals the importance of any zoochory in terms of nature. Our study indicates the importance of goats for the ecosystem, as similar roles have been mentioned in many publications as well (e.g., Baraza & Valiente-Banuet, 2008; Iakovoglou & Radoglou, 2015; Benthien et al., 2016), which evaluate the positive effect of the goats on the vegetation, which is higher than their possible pressure on some seedlings in nature, and they cannot be compensated by any other livestock with regard to the zoochorial aspect.

References

Afroze F., O'Reilly C., 2017. Effects of seed moisture content, warm, chilling, and exogenous hormone treatments and germination temperature on the germination of blackthorn seeds. Plant Biosystems 151, 474-483.

Agrawal A.R., Karim S.A., Kumar R., Sahoo A., John P.J., 2014. Sheep and goat production: basic differences, impact on climate and molecular tools for rumen microbiome study. International Journal of Current Microbiology and Applied Sciences 3, 684-706.

Balygooyen T.G., Moe L.M., 1973. Dispersal of grass fruits-an example of endornithochory. The American Midland Naturalist 90, 454-455.

Baraza E., Valiente-Banuet A., 2008. Seed dispersal by domestic goats in a semiarid thorn scrub of Mexico. Journal of Arid Environments 72, 1973-1976.

Baskin C.C., Baskin J.M., 2014. Seeds: Ecology, biography, and evolution of dormancy and germination, 2nd ed., Academic Press, Kentucky, USA.

Benthien O., Bober J., Castens J., Stolter C., 2016. Seed dispersal capacity of sheep and goats in a near-coastal dry grassland habitat. Basic and Applied Ecology 17, 508-515.

Castañeda I., Fedriani J.M., Delibes M., 2018. Potential of red deer (*Cervus elaphus*) to disperse viable seeds by spitting them from the cud. Mammalian Biology 90, 89-91.

Corlett R.T., 2017. Frugivory and seed dispersal by vertebrates in tropical and subtropical Asia: An update. Global Ecology and Conservation 11, 1-22.

Delibes M., Castañeda I., Fedriani J.M., 2017. Tree-climbing goats disperse seeds during rumination. Frontiers in Ecology and the Environment 15, 222-223.

Domingue B.M.F., Dellow D.W., Barry T.N., 1991. The efficiency of chewing during eating and ruminating in goats and sheep. British Journal of Nutrition 65, 355-363.

Dönmez A.A., Yıldırımlı S., 2000. Taxonomy of the genus *Prunus* L. (Rosaceae) in Turkey. Turkish Journal of Botany 24, 187-202.

Eşen D., Yıldız O., Çiçek E., Kulaç Ş., Kutsal Ç., 2006. Effects of different pretreatments on the germination of different wild cherry (*Prunus avium* L.) seed sources. Pakistan Journal of Botany 38, 735-743.

Feer F., 1995. Seed dispersal in African forest ruminants. Journal of Tropical Ecology 11, 683-689.

Ferreira L.M.M., Hervás G., Belenguer A., Celaya R., Rodrigues M.A.M., García U., Frutos P., Osoro K., 2017. Comparison of feed intake, digestion and rumen function among domestic ruminant species grazing in upland vegetation communities. Journal of Animal Physiology and Animal Nutrition 101, 846-856.

Grande D., Mancilla-Leytón J.M., Delgado-Pertiñez M., Martin-Vicente A., 2013. Endozoochorus seed dispersal by goats: recovery, germinability and emergence of five Mediterranean shrub species. Spanish Journal of Agricultural Research 11, 347-355.

Groom P.K., Lamont B.B., 2015. Seed release and dispersal mechanisms. In: K. Michalczyk (Ed). Plant life of Southwestern Australia: Adaptations for survival, pp. 172-188. De Gruyter Open Ltd., Berlin, Germany.

Harrington K.C., Beskow W.B., Hodgson J., 2011. Recovery and viability of seeds ingested by goats. New Zealand Plant Protection 64, 75-80.

Hofmann R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78, 443-457.

Holst P.J., Allan C.J., 1996. Targeted grazing of thistles using sheep and goats. Plant Protection Quarterly 11 (Supplement 2), 271-273.

Iakovoglou V., Radoglou K., 2015. Breaking seed dormancy of three orthodox Mediterranean Rosaceae species. Journal of Environmental Biology 36, 345-349.

Janzen D.H., 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. American Naturalist 123, 338-353.

Janzen D.H., 1985. *Spondias mombin* is culturally deprived in megafauna-free forest. Journal of Tropical Ecology 1, 131-155.

Khurana E., Singh J.S., 2001. Ecology of tree seed and seedlings: implications for tropical forest conservation and restoration. Current Science 80, 748-757.

Kleyheeg E., van Leeuwen C.H.A., 2015. Regurgitation by waterfowl: An overlooked mechanism for long-distance dispersal of wetland plant seeds. Aquatic Botany 127, 1-5.

Kneuper C.L., Scott C.B., Pinchak W.E., 2003. Consumption and dispersion of mesquite seeds by ruminants. Journal of Range Management 56, 255-259.

Lacey J.R., Wallander R., Olson-Rutz K., 1992. Recovery, germinability, and viability of Leafy Spurge (*Euphorbia esula*) seeds ingested by sheep and goats. Weed Technology 6, 599-602.

Mancilla-Leytón J.M., Fernández-Alés R., Martín Vicente A., 2012. Low viability and germinability of commercial pasture seeds ingested by goats. Small Ruminant Research 107, 12-15.

Nasr S.M.H., Savadkoohi S.K., Ahmadi E., 2013. Effect of different seed treatments on dormancy breaking and germination in three species in arid and semi-arid lands. Forest Science and Practice 15, 130-136.

N.R.C., 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. National Research Council, The National Academies Press, Washington, USA.

Ozinga W.A., Römermann C., Bekker R.M., Prinzing A., Tamis W.L., Schaminée J.H., Hennekens S.M., Thompson K., Poschlod P., Kleyer M., Bakker J.P., van Groenendael J.M., 2009. Dispersal failure contributes to plant losses in NW Europe. Ecology Letters 12, 66-74.

Pakeman R.J., Digneffe G., Small J.L., 2002. Ecological correlates of endozoochory by herbivores. Functional Ecology 16, 296-304.

Pinacho R., Cavero R.Y., Astiasarán I., Ansorena D., Calvo M.I., 2015. Phenolic compounds of blackthorn (*Prunus spinosa* L.) and influence of in vitro digestion on their antioxidant capacity. Journal of Functional Foods 19, 49-62.

Pipinis E., Milios E., Mavrokordopoulou O., Gkanatsiou C., Aslanidou M., Smiris P., 2012. Effect of pretreatments on seed germination of *Prunus mahaleb* L. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 40, 183-189.

Pipinis E., Milios E., Mavrokordopoulou O., Smiris P., 2018. Effect of sowing date on seedling emergence of species with seeds enclosed in a stony endocarp. Journal of Sustainable Forestry 37, 375-388.

Razanamandranto S., Tigabu M., Neya S., Odén P.C., 2004. Effects of gut treatment on recovery and germinability of bovine and ovine ingested seeds of four woody species from the Sudanian savanna in West Africa. Flora 199, 389-397.

Robles A.B., Castro J., González Miras E., Ramos M.E., 2005. Effects of ruminal incubation and goat's ingestion on seed germination of two legume shrubs: *Adenocarpus decorticans* Boiss. and *Retamasphaero carpa* (L) Boiss. Options Méditerranéennes 67, 111-115.

Rojas-Aréchiga M., Vázquez-Yanes C., 2000. Cactus seed germination: a review. Journal of Arid Environments 44, 85-104.

Sabatini L., Fraternale D., Di Giacomo B., Mari M., Albertini M.C., Gordillo B., Luigi Rocchi M.B., Sisti D., Coppari S., Semprucci F., Guidi L., Colomba M., 2020. Chemical composition, antioxidant, antimicrobial and anti-inflammatory activity of *Prunus spinosa* L. fruit ethanol extract. Journal of Functional Foods 67, 103885.

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. Journal of Arid Environments 58, 135-154.

Simao Neto M., Jones R.M., Ratcliff D., 1987. Recovery of pasture seed ingested by ruminants. 1. Seed of six tropical pasture species fed to cattle, sheep and goats. Australian Journal of Experimental Agriculture 27, 239-246.

Takos I.A., Efthimiou G.S.P., 2003. Germination results on dormant seeds of fifteen tree species autumn sown in a Northern Greek nursery. Silvae Genetica 52, 67-71.

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

Zeinalabedini M., Majourhat K., Khayam-Nekoui M,. Hernández J.A., Martínez-Gómez P., 2009. Breaking seed dormancy in long-term stored seeds from Iranian wild almond species. Seed Science and Technology 37, 267-275.