

Differential germination of two closely related species of *Solanum* in response to bird ingestion

A. Barnea, Y. Yom-Tov and J. Friedman

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This study evaluated the effect which ingestion of seeds of *Solanum nigrum* and *S. luteum* by bulbuls (*Pycnonotus xanthopygos*) and blackbirds (*Turdus merula*) had on their germination, and tried to reveal the ecological implications of such endozoochory. Those closely related plant species are often sympatric but whereas *S. nigrum* is a noxious weed invariably associated with human disturbed habitats, *S. luteum* may also occur in natural plant associations. Our data indicated that germination of seeds of *S. nigrum* was not affected by ingestion by either bulbuls or blackbirds. On the contrary, germination of the seeds of *S. luteum* was significantly ($p < 0.01$) improved by their ingestion by either bird species. Comparative scanning electron micrographs of the seeds showed that seed coat sculpture of *S. nigrum* was not affected by ingestion. In contrast, in ingested seeds of *S. luteum*, the sculpture of the seed coat was abraded compared with the uningested seeds; here, also, in the seeds ingested by the bulbuls, the intracellular contents disappeared, and in most of those ingested by blackbirds, there was destruction of the cell wall as well. Assumedly abrasion of the seed coat sculpture of *S. luteum* increases the seed coat permeability and consequently germination. It would seem that germination and dispersal of this more arid species which grows in unstable habitats is mediated largely by birds, whereas the germination of *S. nigrum* seeds is not affected by avian ingestion; for this weed, birds serve only as dispersal agents.

A. Barnea and Y. Yom-Tov, Dept of Zoology; J. Friedman, Dept of Botany; George S. Wise Faculty of Life Sci., Tel Aviv Univ., Ramat Aviv 69978, Israel.

Introduction

Ornithochory is a process of seed dispersal in which a frugivorous bird consumes the pulp and regurgitates or excretes all or some of the seeds unharmed (Ridley 1930). It has been suggested that this process is advantageous, as, among other effects, it enhances germination (Turcek 1963, Van der Pijl 1972). Modification of the coat structure of these seeds due to ingestion is considered among the factors which enhance germination (McKey 1975, Janzen 1983, Agami and Waisel 1988).

There are few works which follow the effect of ingestion on seed germination (Lieberman et al. 1979, Debussche 1985, Agami and Waisel 1986, Lieberman and Lieberman 1986). These studies indicate a wide range of responses to ingestion, and germination enhancement was not always confirmed. Additionally,

there is no evidence about the actual causes or mechanisms of the observed differences in germinability. Therefore, we reasoned that a study of two closely related plant species might better reveal the ecological implications of endozoochory.

Solanum nigrum L. and *S. luteum* Miller are similar species which are genetically and morphologically distinct, and do not interbreed. *S. nigrum*, with dark berries, is hexaploid ($n = 36$), while *S. luteum*, with orange or yellow fruits is tetraploid ($n = 24$) (Karschon et al. 1978). Fruit color is a major diagnostic characteristic which allows easy discrimination between the species (Saarisalo-Taubert 1967). Both species produce fruits most of the year, except for the winter season (December–March) in which fruits are rare. Bulbuls (*Pycnonotus xanthopygos*) and blackbirds (*Turdus merula*), consume the fruits of both plant species and excrete the

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Tab. 1. Cumulative germination percentages (% $\bar{X} \pm SE$) of *Solanum luteum* and the significance of differences in germination percentage of ingested and uningested seeds.

Mean cumulative germination (% $\bar{X} \pm SE$) of:	Date of collection	1984			1985		
		Jul	Oct	Nov	Jun	Sep	Nov
Seeds ingested by bulbuls		2 \pm 16 (10;300)	30 \pm 15 (10;300)	84 \pm 8 (10;200)	64 \pm 22 (5;100)	26 \pm 32 (5;100)	87 \pm 21 (5;100)
Seeds ingested by blackbirds		40 \pm 10 (10;300)	39 \pm 19 (5;135)	81 \pm 11 (10;200)	84 \pm 9 (5;100)	84 \pm 2 (5;100)	87 \pm 9 (5;100)
Uningested (control) seeds		0 (5;150)	0 (4;120)	73 \pm 13 (5;100)	24 \pm 7 (5;100)	8 \pm 10 (5;100)	58 \pm 18 (5;100)
Difference between ingested and uningested seeds		*	*	ns	*	*	ns

* = significant ($p < 0.01$); ns = not significant (one way analysis of variance, ANOVA).

seeds in their feces (Danin 1983). The bulbul is a tropical species and common in most parts of Israel, while the blackbird is a palearctic bird, found in northern and central parts of the country.

Both plant species are annual or biennial and ruderal, which occasionally grow in the Mediterranean phytogeographical territory of Israel. However, despite the fact that they are closely related and may grow sympatrically, these species differ in their ecological requirements. Very sparse populations of *S. luteum* occur along the coast within indigenous plant communities, e.g. in association with *Ceratonia siliqua* or *Pistacia lentiscus*. Plants of *S. luteum* are often restricted to the shade of *Pistacia* where they survive the long dry Mediterranean summer. Compared with *S. nigrum*, they are deemed better adapted to drought conditions. The latter species is a very common noxious weed in irrigated lands in Israel, during the summer (Givelberg et al. 1984).

To better understand the differential ecological response of these plant species, we deemed it of interest to study the effect of bird ingestion on the germination of their seeds.

The aims of this work were:

- 1) To evaluate the effect which two different frugivorous bird species exert on germination of the two closely related *Solanum nigrum* and *S. luteum*.
- 2) To compare the effect of ingestion on seeds which were produced during different seasons of the year.
- 3) To elucidate the mechanisms possibly involved in this effect and to assess the ecological implications of such endozoochory.

Materials and methods

Observations on *S. nigrum* and *S. luteum* growing along the edges of fish-ponds in northern Israel (33°40'N, 35°35'E) were made once every two months during 1984

Tab. 2. Cumulative germination percentages (% $\bar{X} \pm SE$) of *Solanum nigrum* and the significance of differences in germination percentage of ingested and uningested seeds.

Mean cumulative germination (% $\bar{X} \pm SE$) of:	Date of collection	1984		1985		
		Oct	Nov	Jun	Sep	Nov
Seeds ingested by bulbuls		23 \pm 17 (12;360)	86 \pm 9 (10;200)	96 \pm 5 (5;100)	90 \pm 12 (5;100)	100 \pm 0 (5;100)
Seeds ingested by blackbirds		92 \pm 11 (12;360)	77 \pm 17 (10;200)	79 \pm 14 (5;100)	94 \pm 4 (5;100)	96 \pm 4 (5;100)
Uningested (control) seeds		61 \pm 9 (4;120)	66 \pm 5 (5;100)	81 \pm 11 (5;100)	76 \pm 5 (5;100)	97 \pm 3 (5;100)
Difference between ingested and uningested seeds		ns	ns	ns	ns	ns

ns = not significant (one way analysis of variance, ANOVA).

(each observation lasted at least 5 h). These observations established that ripe fruits of both species are ingested by bulbuls and blackbirds.

Six collections of ripe fruits of each species (each at the same date) were made in 1984 (17 July, 2 October, 10 November) and 1985 (15 June, 17 September and 15 November). The collected fruits were kept in the shade and within a day or two were offered to captive bulbuls and blackbirds. The birds were kept separately in a cage (100 × 30 × 50 cm). At least three birds of each species were randomly chosen for the experiments. Each bird was given no less than 3 d of acclimatization before and between experiments. Food included fruits (oranges, grapes, etc.), eggs, maggots and bird-food, plus water ad lib.

On the day of the experiment, each bird was offered only 10 ripe fruits of one of the two plant species, plus water. To ascertain the retention time of the seeds within the digestive track, we counted unconsumed fruits every two minutes and noted the first appearance of the seeds of pulp in the feces. At the end of this experiment each bird was offered ripe fruits ad lib., and on the next day the feces of each bird were collected and allowed to dry in the shade in room temperature, so that it would be possible to separate the seeds from the feces and count them. As a control, seeds were extracted from complete uningested berries and were stored identically to seeds which were found in the feces.

Each bird participated in no more than four experiments in a row and was then replaced by another bird from the collective cage. Three preliminary experiments for each plant species had been carried out to find out whether ingestion of seeds by different individuals from the same bird species had any effect on their germination success. Each experiment included six lots, each of 100 ingested seeds, ingested by six birds (three for each bird species). Each lot of seeds was set to germinate in five replicates. The results showed no significant differences between germination of seeds which were ingested by different individuals from the same species, hence the feces from all the birds which participated in a certain trial were kept together. Due to the long season (April–November) of fruit setting of these two species, experiments included fruits from different collections in 1984 and 1985.

After the drying period (2–3 d) seeds were rinsed under running water for 10 min. Then, seeds were set to germinate in Petri dishes, 9 cm diameter, on two filter papers with 5 ml distilled water, in $25 \pm 2^\circ\text{C}$, under continuous illumination (see Givelberg et al. 1984).

The number of seedlings in each dish was recorded every morning until none appeared during two consecutive observations (usually a period of 10 d). The mean cumulative germination percentage and standard error ($\% \bar{X} \pm \text{SE}$) were calculated. The significance of any difference between the treatments and the control in each series and for each tested plant species was determined by a one-way analysis of variance (ANOVA) after angular transformation of the data.

Additionally, seed coats of 10 ingested and uningested seeds from the experiments carried out in November 1984 and in September 1985 were randomly checked for scanning electron microscope (SEM) examination. These were gold-coated in vacuum, photographed, and seed-coat sculptures were compared.

Results

The minimum retention time of seeds

The mean time of first void of *S. luteum* seeds from the alimentary tract of bulbuls was 5.6 ± 0.6 min ($n = 8$), and from that of blackbirds 4.5 min ± 0.3 ($n = 6$). The corresponding values for seeds of *S. nigrum* were 4.8 min ± 0.4 ($n = 6$) and 4.5 min ± 0.8 ($n = 6$), respectively. A two-way analysis of variance showed no significant differences in minimum retention time of seeds between bird or plant species (accordingly, F_s values were 1.785 and 0.639, $p < 0.05$).

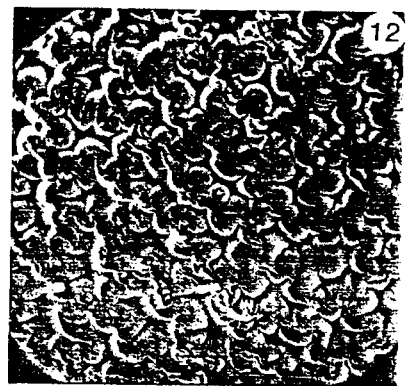
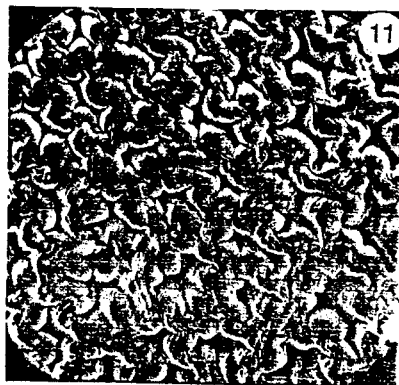
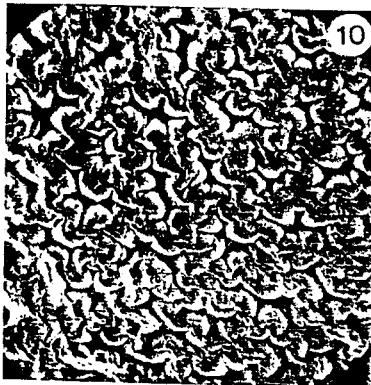
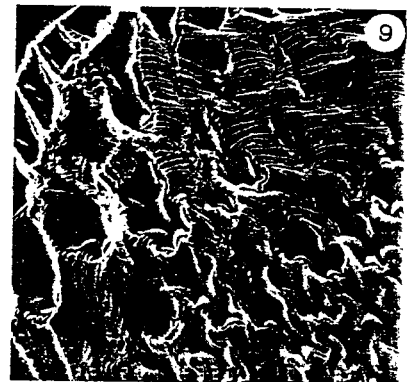
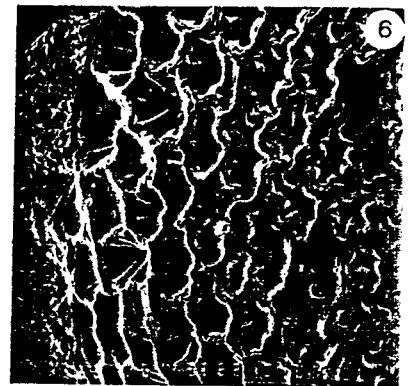
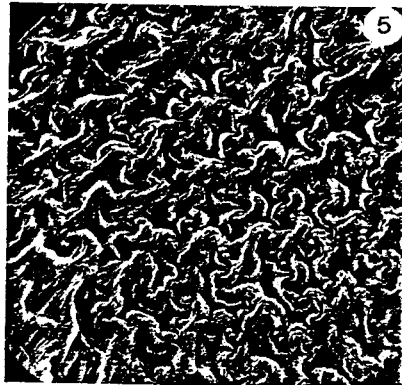
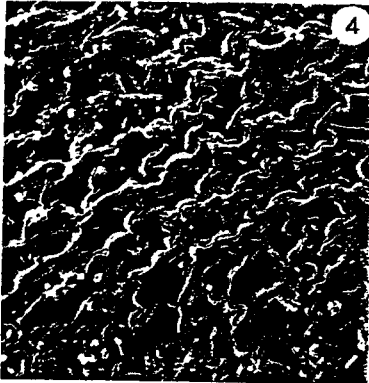
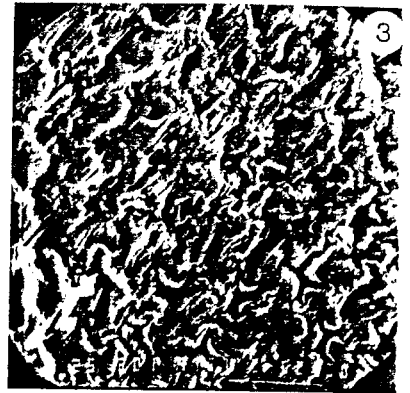
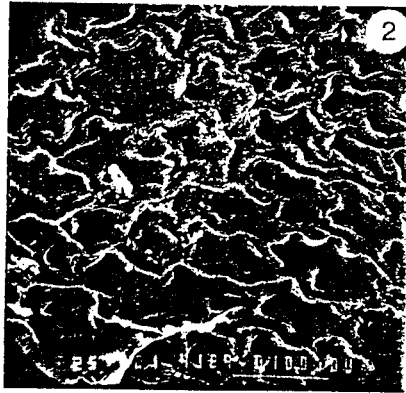
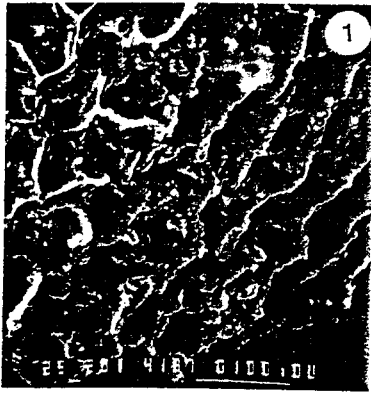
Germination in response to bird ingestion

Preliminary trials showed no significant differences between germination in soil and in Petri dishes, hence our experiments were carried out for convenience in Petri dishes.

Germination of ingested seeds of *S. luteum* from most collections was significantly enhanced compared with that of the control (Tab. 1). Compared with bulbuls, ingestion by blackbirds yielded significantly higher germination percentages ($p < 0.05$). However, seeds collected in November (in 1984 and in 1985) were not affected by birds' ingestion, either by blackbirds or bulbuls.

In none of the experiments did control seeds of *S. luteum* attain germination percentages higher than 50%, excluding control seeds of November collections

Fig. 1. Micrographs of seed coat of *Solanum luteum* and *S. nigrum*. Micrographs are magnified 130 times. From left to right: seeds ingested by blackbirds (nos. 1; 4; 7; 10), by bulbuls (nos. 2; 5; 8; 11) and uningested control seeds (nos. 3; 6; 9; 12). Micrographs of seed coats of *S. luteum*, September, 1985: 1–3. Micrographs of seed coats of *S. luteum*, November, 1985: 4–9. Micrographs of seed coats of *S. nigrum*, September, 1984: 10–12.



(of both 1984 and 1985). After November 1984 there was a steady decline in the germination percentage of control seeds, reaching very low percentages (8%) in the collections from mid until late summer (June and September 1985). Over the study period seeds of *S. nigrum* were indifferent to bird ingestion. In none of the experiments was there any significant difference in germination between ingested and uningested (control) seeds (Tab. 2). Cumulative germination percentages for all treatments as well as for all seed collections throughout the year, were never less than 60%, barring one instance of seeds collected in October, 1984 and ingested by bulbuls.

The effect of ingestion by birds on the sculpture of seed coats

Fig. 1: 1-3 show that there is a difference in seed coat sculpture of *S. luteum* between seeds ingested by either blackbirds or bulbuls and control seeds, respectively. In the coat of an uningested seed (Fig. 1: 3) the cell walls and the waxy substance which fills the cells are evident, whereas in the coat of a seed that has passed through a bulbul gut (Fig. 1: 2), the filling substance is missing even though the anticlinal cell walls are still noticeable. In a seed which has passed through a blackbird gut, most of the cell walls are also destroyed so that the 'beehive' shape of the seed coat typical after passage through a bulbul gut is no longer evident, except in small areas.

Since the germination percentages of seed of *S. luteum* collected in November 1984, and subsequently ingested by birds, did not differ from those of uningested seeds, micrographs of their coat sculpture were also taken. As can be seen from Fig. 1: 4-9, the coat sculpture of these seeds had been only slightly impaired by the avian ingestion. This is apparent on comparison with Fig. 1: 1-3 which, we believe, display the sculpture of seed coat extant throughout the year. Such comparison reveals only slight disparity between control, uningested seeds and ingested ones.

Among seeds which ripened in November, two types were detectable in each of the three treatments (ingestion by blackbirds, by bulbuls or uningested control). The first, and greatly predominant type, comprised seeds whose cells contained the filling substance (Fig. 1: 4-6), while the second and rather rare type displayed a gradual erosion of the seed coat in ingested compared with control seeds (Fig. 1: 7-9). It is noteworthy that in both experiments carried out in November (1984 and 1985), the germination percentages under the three treatments were not significantly different, unlike those in the four experiments carried out during other months of the year (Tab. 1).

Fig. 1: 10-12 reveal no difference in seed coat sculpture of *S. nigrum* between seeds ingested by birds (bulbuls or blackbirds) and uningested control seeds.

Discussion

Differential dependence of the two tested plant species on birds as dispersal agents and germination mediators

During most of the year, seeds of *S. luteum* showed considerable dependence on avian ingestion for their germination. Thus, in 4 out of 6 tests germination of ingested seeds was significantly improved ($p < 0.01$) over that of the control, uningested seeds. This finding is consistent with the fact that unlike in ingested seeds of *S. nigrum* the coat sculpture in ingested *S. luteum* seeds was considerably reduced (Fig. 1: 1-3).

From the data presented in Tab. 2 it is clear that the germination rate of *S. nigrum* seeds is not affected by avian ingestion, which is contrary to previous opinion (Danin 1983). In our observations the uningested seeds of this species yielded a germination rate similarly high to that of seeds which had passed through an avian gut. This finding clearly suggests that the germination rate of *S. nigrum* seeds is not affected by their seed coat sculpture (Fig. 1: 10-12).

Since the gut retention time of seeds of the two plant species did not differ significantly, this leaves us with the possibility that the different coat sculpture of *S. luteum* and *S. nigrum* seeds accounts for the different effect which ingestion exerted on them. It is therefore suggested that the birds, particularly blackbirds, enhance germination only of those seeds with mechanisms to arrest germination. So far as seeds of the more arid *S. luteum* are concerned, passage through avian gut enhances germination. This enhancement is important since in the habitat where *S. luteum* grows, the ambient conditions (as temperature and precipitation) are variable, and plants of *S. luteum* are successful mostly under the shade of the small *Pistacia* bushes. Due to the birds' habit to excrete their feces mostly when they stand on the bushes, ingestion, in addition to other possible vectors of dispersal, enables the seeds to germinate in the site where establishment is safer. On the contrary, *S. nigrum* is very common in irrigated fields, where water conditions are relatively stable. Its seeds readily germinate and have no means to keep germination under control. For this species birds serve only as dispersal agents, but not as germination mediators.

Differential effect of ingestion by the two investigated bird species on seed coat sculpture and germination by *S. luteum*

In all the experiments, except for those testing germination of seeds collected in November, seeds ingested by blackbirds yielded significantly ($p < 0.05$) higher germination percentages than did seeds ingested by bulbuls. The explanation for this is provided by the SEM micrographs which reveal a greater change in the coat sculpture of seeds ingested by blackbirds than by bulbuls, namely, a destruction of the anticlinal walls, as well as depletion of the filling substance (Fig. 1: 1-3).

Indeed, this work shows a clear correlation between

germination success and the reduction of seed coat sculpture. However, our knowledge at this stage both about the mode of action of seed ingestion on germination control, as well as the causes for differential effect caused by the two bird species on seed coat sculpture of the same plant species, is poorly understood.

As for the mode of action, in laboratory experiments scarification of seed coat is a rather common practice for inducing germination. This can be achieved either mechanically (e.g., by clipping the seeds, piercing or decoating the seed-coat, etc.) or chemically (e.g., by treating the seeds with acids; Justice 1972, Mayer and Poljakoff-Mayber 1982). It is reasonable to assume that either one, or both mechanisms can exist in the digestive system of the birds. However, this question remains open, and is subject to further study. The same can be argued for differences in germination which were noticed in response to ingestion by blackbirds and by bulbuls.

Nevertheless, some indirect evidence supports that scarification of seed coat by ingestion enhances seed germination: Decoated seeds of *Solanum incanum*, which grow in Nigeria, germinated faster than did non-decoated seeds (Joshua 1978). Also, a clear correlation has been found between germination improvement and abrasion of the seed coat sculpture (Barnea 1988). This when seeds of some Mediterranean perennials (*Arum hygrophilum*, *Asparagus aphyllus*, *Ephreda campylopoda*, *Rubia tenuifolia*, *Myrtus communis*, *Rubus sanctus*, *Rhamnus palaestinus*, *R. alaternus*, and *Morus nigra*) were subjected to birds' ingestion. It is reasonable to assume that ingestion enhances seed coat permeability, thereby probably improving gas exchange and water intake by the seed, and thus, leading to a higher rate of germination.

Differential germination of ingested seeds of *S. luteum* as a function of season

Seeds of *S. luteum* were differently affected by avian ingestion throughout the year. Thus, a very significant dependence of germination on ingestion by birds was found for seeds collected during the summer (June–October), but in November, the seeds from all three treatments (ingestion by bulbuls, ingestion by blackbirds and no ingestion at all) yielded similar high germination percentages independent of ingestion. This suggests that seeds with different qualities of seed coat are produced during the year by the same plant. The phenomenon is not new, and has been reported in other species (e.g., Howe 1986, Agami and Waisel 1988). Ambient conditions which existed at the time of seed ripening (e.g., photoperiodicity or thermoperiodicity) can affect future germination ability of those seeds (Karssen 1970, Gutterman and Heydecker 1973, Kigel et al. 1979). The ecological meaning of the existence of various types of seeds is not always clear. In the case of *S. luteum* it might be that in the summer, which is the

dry season in Israel, seed dispersal to adequate niches is most important for subsequent germination. This can be obtained by high dependence on dispersal by birds, that pick a few fruits and fly away to perching places (Howe 1979, Debussche et al. 1982). On the other hand, during the rainy season, which starts in November, seed germination is not so critically dependent on ingestion because conditions suitable for germination are favorable not only under the canopy of the bushes.

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