

## ***Podarcis lilfordi* from the Balearic islands as a potential disperser of the rare Mediterranean plant *Withania frutescens***

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**Abstract** – This study examines whether the lizard *Podarcis lilfordi* is a legitimate disperser of the rare Mediterranean plant *Withania frutescens* by using the biochemical test of triphenyl-2H-tetrazolium chloride for testing seed viability. This lizard eats the fresh fruits of the plants and defecates intact seeds which have been retained 1 to 3 d in their gut. Viability of seeds recovered from faeces was very high and comparable to the viability of fresh seeds. Seed dispersal by this lizard in the Balearic islands may facilitate population expansion of this rare plant in the Balearics. © Elsevier, Paris

**Plant-animal interactions / endemic lizard / Mediterranean islands / *Podarcis lilfordi* / *Withania frutescens***

### **1. INTRODUCTION**

Seed consumers have an important effect on plant distribution, plant fitness [24] and plant invasions [17, 21]. They can also influence the evolution of fruit characteristics [7, 13, 22]. Although the study of interactions between fruits and their vertebrate consumers has received great attention [11, 14, 29, 33], very little is known on fruit consumption and potential dispersal of seeds by lizards (see [9, 12, 25, 27, 31, 32]). The effect of seed passage through lizard's guts on germination in twelve plant species has been only examined in four lizard species of the genus *Gallotia* (Lacertidae), *Liolaemus*, *Tropidurus* (Tropiduridae) and *Ctenosaura* (Iguanidae). Three of the lizards are considered potential dispersers of some of the plants examined [26]. Because lizards are extremely abundant in islands (e.g. [5, 16]), they may potentially have important effects on plant communities and these should be examined.

In this study, I explore whether the lizard *Podarcis lilfordi* consumes fruits of the Solanaceae plant *Withania frutescens* in the field and I examined whether this lizard is a legitimate disperser of this plant (i.e. the seeds remain viable after passing through the digestive tract of lizards).

*Withania frutescens* is a rare plant with an extremely restricted distribution in the Mediterranean, although it is abundant in some small islands of the archipelago of Cabrera (Balearics) [19]. In these islands, the endemic

lizard *Podarcis lilfordi* is the most abundant terrestrial vertebrate [1].

### **2. MATERIALS AND METHODS**

The study was conducted during spring 1998 in the island of Na Redona (Cabrera National Park, Balearics, Mediterranean, Spain), where *Withania frutescens* is quite abundant [19]. To examine whether *Podarcis lilfordi* actively consumes fruits of *W. frutescens*, I collected 43 adult lizards in baited cans on May 2, 1998. They were maintained individually in cotton bags until they defecated. Lizards were released at the site of capture and faeces examined for the presence of seeds.

To examine viability of seeds consumed by lizards, I collected fresh mature fruits of *W. frutescens* from bushes and stored them at 5–8 °C for about two months. On June 24, fruits were depulped and seeds removed by hand. Firm seeds of yellow colour and without deformities were recorded as sound. They were checked again for soundness using the common horticulturist's method of 'emerging seeds' in water: seeds that sink are considered as viable, whereas seeds that float on the water surface are regarded as non-viable. Only seeds that were judged as sound were used to examine seed viability of ingested and uningested (control) seeds. Fifty seeds were supplied to five adult lizards (snout-vent length: 59–67 mm;

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mass: 5–8 g), together with other food items such as meal-worms, vitamins and water. Lizards were kept in a terrarium (80 × 40 cm) where they could maintain body temperatures that are within the optimal range for food digestion in lizards [28] during ca. 12 h per day.

Seed viability of ingested ( $n = 50$ ) and non-ingested control ( $n = 50$ ) seeds was tested using 2,3,5-triphenyl-2H-tetrazolium chloride (TTC, 1 %) [15]. The pH of the solution in distilled water was adjusted to 6.5 with 1 M NaOH [30]. The solution was prepared and maintained under dark conditions at all time.

Untreated seeds immersed in TTC at 30 °C for 2 d did not stain the embryo, probably because of the thick and hard seed coat in this species. Therefore, all seeds were bisected longitudinally and immersed in the tetrazolium solution for 3 h at room temperature. Viable embryos stained pink and non-viable ones remained white.

To obtain micrographs of seed coats, seeds were first washed in 75 % ethanol for 15 min and gold-coated during 6 min in a vacuum (Polarom E 5400 SEM coating system). Micrographs were taken with a scanning electron microscope (SEM, HITACHI S530). The image was digitalized and processed with 'Adobe PhotoShop' PC-software.

### 3. RESULTS

#### 3.1. Field observations

Pellets from adult lizards of both sexes collected in the field contained seeds of *W. frutescens* (24 % males and 29 % females) along with other food items (Coleoptera, Hymenoptera, plant material and sand) (table I). Only in three (17 %) pellets with seeds were

the seeds entirely surrounded by fruit pulp, whereas no or very small pieces of pulp were present in the remaining pellets with seeds (table I).

#### 3.2. Effect of gut passage on seeds

Differences in seed coats between ingested and non-ingested seeds have been observed in the electronic microscope. Seed coat sculpture of digested seeds was abraded (figure 1b) compared to control uningested seeds (figure 1a).

All seeds ( $n = 50$ , 100 %) retained for 1–3 d in the lizard's guts and further defecated in the laboratory, along with meal-worms (soft food), looked like uningested seeds (e.g. yellow colour, kidney shape, no deformities). In contrast, seeds obtained from pellets of field-fed (soft and hard food) lizards, for which retention time was unknown, the external appearance was sound in 26 out of 32 seeds (81 %). Two seeds (6 %) were broken and four (13 %) were soft.

#### 3.3. Seed viability

The TTC test showed that the proportion of viable seeds was very high and similar in ingested (48 out of 50, 96 %) and non-ingested seeds (50, 100 %) ( $\chi^2$ ,  $P > 0.80$ ). This result indicates that *P. lilfordi* is a legitimate disperser of this plant.

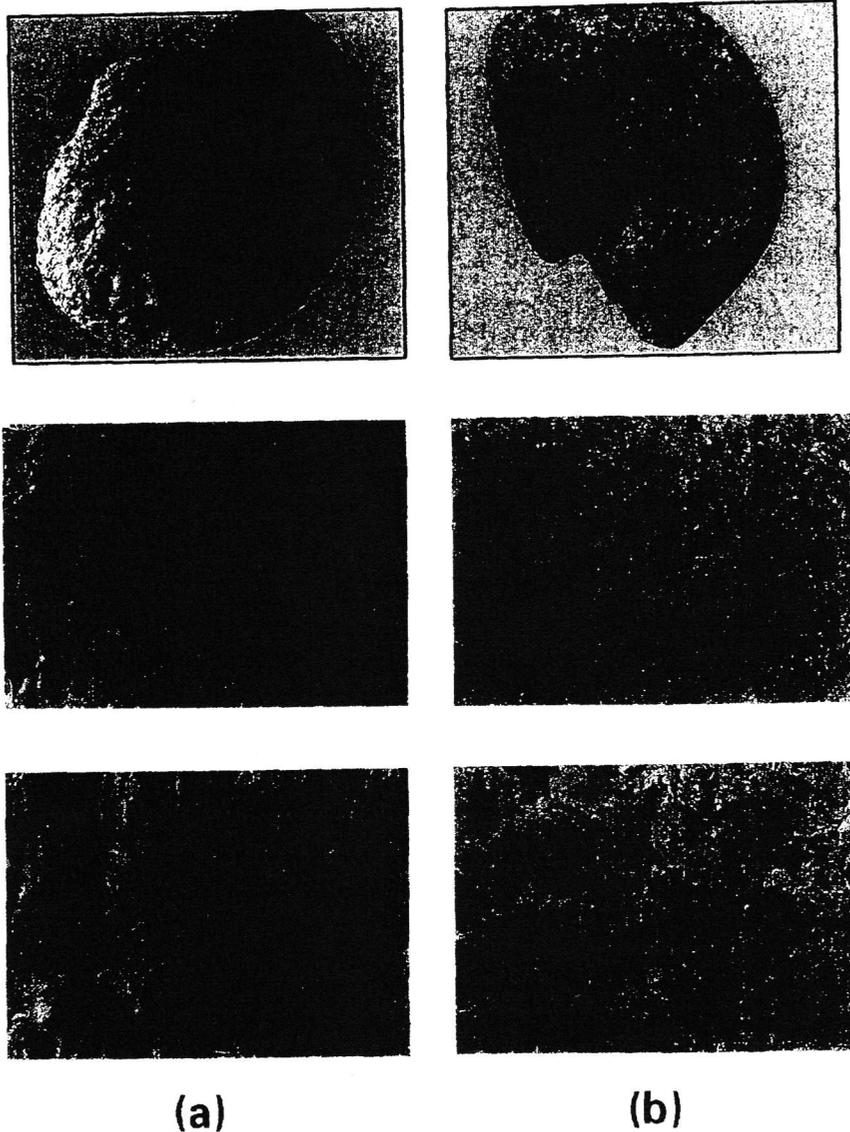
## 4. DISCUSSION

#### 4.1. Field observations

The lizard *P. lilfordi* actively consumes fruits of the rare Mediterranean plant, *W. frutescens*. Although

**Table I.** Summary data of the number and percentage of seeds collected in the scats defecated by field-captured adult males and females *P. lilfordi* in Na Redona island. Also indicated is whether fruit pulp was present with seeds in each scat.

|   | Males<br>( $n = 32$ ) | Females<br>( $n = 11$ ) | Total<br>( $n = 43$ ) |
|---|-----------------------|-------------------------|-----------------------|
| No. scats collected                             | 49                    | 21                      | 70                    |
| No. scats with seeds                            | $n = 12$<br>24 %      | $n = 6$<br>29 %         | $n = 18$<br>26 %      |
| No. seeds per scat                              | 1–5                   | 1–4                     | 1–5                   |
| Total No. seeds obtained                        | 23                    | 9                       | 32                    |
| Scats with seeds without pulp                   | 42 %<br>$n = 5$       | 50 %<br>$n = 3$         | 44 %<br>$n = 8$       |
| Scats with seeds and with little pieces of pulp | 42 %<br>$n = 5$       | 33 %<br>$n = 2$         | 39 %<br>$n = 7$       |
| Scats with seeds surrounded by pulp             | 17 %<br>$n = 2$       | 17 %<br>$n = 1$         | 17 %<br>$n = 3$       |



**Figure 1.** Micrographs (scanning electron microscope) of seed coats from *Withania frutescens* that were taken fresh from the fruit (a) or ingested by *P. lilfordi* (b). Seed size is ca. 5 × 4 mm and photographs magnified × 20, 50 and 100.

only 26% of faecal pellets contained seeds, this finding is remarkable considering that the study was conducted at the end of the fruiting period, when relatively few fruits were available on the plants (1–10 fruits per plant; a single plant can have up to 700 fruits; Castilla, unpubl. data). It is thus not unlikely that the ingestion of fruits may be higher

during the peak of the fruiting period. Ingestion of fruits by the lizard *P. lilfordi* does not seem to be incidental to the consumption of insects or other plants, because fruits are hanging from branches and are inside a receptacle. Therefore, these results indicate that adult males and females *P. lilfordi* actively consume fruits of *W. frutescens* in the Balearic islands.

In addition during the digestive process, lizards appear to separate the seeds from the pulp. Given that fruit pulp affects the retention time of seeds in the disperser's gut [8, 18], and that seed retention time is an important factor determining germination success [26], it would be interesting to collect more data on the way lizards treat the fruits and to examine its consequences on germination performance.

#### 4.2. Effect of gut passage on seeds

Observations with a scanning electron microscope showed differences in seed coats between ingested and non-ingested seeds. In ingested seeds, the waxy and oily particles inside the wall cavities were removed when compared to uningested seeds. This is a common process that may facilitate water absorption and gas exchange, and ultimately enhance seed germination.

The percentage of sound seeds was high in both laboratory (along with soft prey) or field (with soft and hard prey) digested conditions. However, broken and soft seeds were only found in the field sample. Differences in the type of food that is ingested along with the fruits and in retention time may affect the chemical and mechanical abrasion of the seeds, their appearance and the seed's coat sculpture. However, lizards may have also consumed fruits that were in bad conditions and that contained damaged seeds. Given that differences in seed retention time may have a great influence on the percentage and rate of seed germination [2, 3], and that digestion is a temperature-dependent process in lizards [28], more detailed studies should be conducted with the Balearic lizards to fully understand the effect of gut passage on seed performance.

#### 4.3. Seed viability

Viability of ingested seeds was extremely high. The high percentage of viable seeds may be biased by the fact that I used only sound seeds for this experiment. Thus, this number may not represent the real percentage of seed viability in fruits of *W. frutescens*. However, this result indicates that *P. lilfordi* does not have a deleterious effect on the viability of *W. frutescens* seeds after consumption and retention in the gut for at least 3 d.

Frugivores that produce only negligible external damage to seeds and that treat seeds such that they remain germinable, are considered as true legitimate seed dispersers [10]. Thus, *P. lilfordi* is a legitimate disperser of *W. frutescens*, and most probably may facilitate population expansion of this plant in the Balearics. However, to be a potential disperser, the herbivorous have to be not only legitimate but also effective (account for most seedlings produced) and

efficient (i.e. disperse seeds in good germination sites). In this study, I did not examine these two aspects, but recent data indicate that *P. lilfordi* is an effective disperser of this plant (Castilla, unpubl.). The effectiveness of this lizard species is not known; however, most *Podarcis* lizards are continuously moving among different microhabitats, which is an important thermoregulatory mechanism [4]. Also, *Podarcis* lizards have a preference for rocky areas [4-6], and plants of *W. frutescens* appear to be successful in rocky areas and under the shade of other bushes. The home range of this lizard may probably exceed 1 500 m<sup>2</sup>. In a closely related insular *Podarcis* lizard, the home range is of 1 323 m<sup>2</sup> [23]. All these characteristics suggest that *P. lilfordi* may be a good vector of dispersal that is able of carrying seeds away from the parent plant and that enables the seeds of *W. frutescens* to germinate in a site where establishment is safe. Future studies should quantitatively examine the efficiency of lizards as seed dispersers.

*P. lilfordi* has already been cited as a potential disperser of *Cneorum tricoccon* [25]. In addition, this lizard consumes fruits and nectar of sixteen plant species in the archipelago of Cabrera [20]. The previous results, together with the fact that *P. lilfordi* is the only terrestrial vertebrate in most islands of the Cabrera archipelago, suggest that this lizard may have an important impact on the distribution and abundance of *W. frutescens* and other natural populations of endemic and rare insular Mediterranean plants. However, this has to be examined in order to confirm such prediction.

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