Acta Oecologica **21** (2) (2000) 119−124 /
S1146609X0000120X/FLA

Does passage time through the lizard *Podarcis lilfordi*'s guts affect germination performance in the plant *Withania frutescens*?

Aurora M. Castilla[§]

Instituto Mediterráneo de Estudios Avanzados (CSIC-UIB), Km 7.5, Ctra. Valldemossa, 07071 Palma De Mallorca, Spain.
Corresponding author (e-mail: castilla@uia.ac.be)

Received 20 May 1999; revised 1 December 1999; accepted 4 January 2000

Abstract — I tested predictions of the hypothesis that a longer seed passage time through the gut of the lizard *Podarcis lilfordi* enhances germination performance in the plant *Withania frutescens*. I compared germination success and germination time between seeds that were ingested by lizards and control seeds that were not. I also explored relationships between natural variation in seed passage time and germination performance. Germination success did not differ between ingested (63 %) and non-ingested seeds (56 %); there was no significant relationship between germinability and retention time. Germination time did not differ between ingested and non-ingested seeds, and was unrelated to retention time. Hence, I found no support for the hypothesis that prolonged retention times should improve germination performance. In addition, this is a pilot study stressing the importance of reptiles as seed dispersers, at least in certain habitats. © 2000 Éditions scientifiques et médicales Elsevier SAS

Endemic lizard / Mediterranean islands / plant-animal interactions / *Podarcis lilfordi* / seed retention time / *Withania frutescens*

1. INTRODUCTION

The effect of seed ingestion by frugivores on the subsequent germinability of seeds is an important topic for understanding the ecological and evolutionary aspects of plant-frugivore interactions. A recent review [18] concluded that half of the published studies found that consumption of seeds affected the percentage of germination (defined as germination success in this study) and/or germination rate (defined as germination time in this study); and that seed ingestion enhanced germination (i.e. resulted in an increased percentage or increased 'rate' of germination) about twice as often as it inhibited germination performance [18]. A major mechanical cause for germination enhancement is the abrasive effect that passage through the frugivore's gut has on the seed coat, inducing an enhanced permeability to water and gases. Because seed coat abrasion should be more intense the longer the seeds are retained in the digestive tracts of herbivores, it is reasonable to expect that variation in passage time will influence germination performance [23]. Passage time indeed affects germinability and explains part of the interspecific variation in the responses of seeds to ingestion [18]. However, few studies have examined the effect of variation in passage time on differences in seed germination performance within single plant-frugivore systems (but see [2, 14, 16, 23]), and to my knowledge this issue has not been addressed for lizards.

This study tests the hypothesis that a longer seed passage time through the guts of the lizard *Podarcis lilfordi* enhances germination performance of the plant *Withania frutescens*. I choose this system for two main reasons. First, reptiles retain seeds in their guts for several days (e.g. turtles: 2–20 d, [16]; snakes: 2–30 d, [8]; lizards: 2–4 d, [15, 24]), which is an order of magnitude longer than in small mammals and birds, which retain seeds from several minutes to hours (e.g. [11, 12, 18]). It is reasonable to expect that variation in passage time, and its putative effects, will be more easily detected when average passage time is longer. Second, in a previous study [5], I have shown that abrasion of the coat of *W. frutescens* seeds occurred

[§] Present address: Department of Biology, University of Antwerp, B-2610 Wilrijk, Belgium.

after passage through the lizard's gut, without reducing seed viability. These observations substantiate the mechanistic basis of the hypothesis in my study system. I will test two predictions of the hypothesis that a longer gut passage time should intensify seed coat abrasion and hence speed up germination. First, germination success (measured in percentages) should increase with passage time. Second, germination time (defined as the time elapsed between the start of the experiment and the emergence of the radicle from the seed) should decrease with gut retention time. To test these predictions, I first compared the performance of seeds that were ingested and excreted by lizards to that of control (i.e. non-ingested) seeds. Second, I analysed germination performance as a function of observed natural variation in seed retention time.

2. MATERIALS AND METHODS

2.1. Seed collection

I collected fresh mature fruits of *Withania frutescens* on Na Redona island (Cabrera National Park, 39º09' N, 2º56' E, Balearic Islands, Spain) from 24 April to 2 May 1998. *W. frutescens* is a Solanaceae plant with a restricted distribution in the Mediterranean area [19]. However, it is very abundant on some small islands of the Cabrera archipelago, where the endemic lizard *Podarcis lilfordi* (Lacertidae) is the most abundant terrestrial vertebrate [1]. This lizard is small (snout-vent length < 90 mm), heliothermic and mainly insectivorous (details in [3]), and also consumes fruits and nectar of sixteen plant species in the archipelago of Cabrera [17]. Lizards also consume *W. frutescens*, even when the availability of fruits is very low [5]. Fruits of *W. frutescens* are round (5–8 mm diameter), orange, red or purple when mature, and contain from one to eleven seeds (3–5 mm diameter) (Castilla, unpubl). Collected fruits were stored cold (5–8 °C) during ca. 50 d before they were depulped and seeds removed by hand.

2.2. Feeding experiment

I captured five adult lizards (snout-vent length: 59–67 mm, mass: 5–8 g) on Cabrera island and housed in the laboratory at the University of Antwerp (Belgium). Lizards were kept in two terraria (50×40 cm) that were placed in a temperature-light controlled room (lights switched on 900–1 800 h; day temperature: 32–35 °C; night temperature: ca. 20–25 °C). Day temperature was maintained within the range of preferred body temperatures in lacertid lizards [4]. Within a short time period (ca. 1 h), all lizards were force-fed ten big (ca. 4–5 mm diameter) and depulped seeds each. Mealworms and vitamin-enriched water were continuously available ad libitum.

I only used seeds of approximately the same size, because size and mass of a seed usually influences the speed at which it passes through the digestive tract of frugivores [9]. I selected the biggest seeds, because a larger number of them were available. Also, I fed lizards only with depulped seeds because feeding whole fruits to lizards would not allow me to control for variation in seed size and number, and because fruit pulp may influence seed retention time [14]. The small sample of fruits available impeded me to conduct a combined (pulped vs. depulped seeds) experiment.

Terraria were inspected every 3 h from 700 to 2 200 h to collect lizard faeces. No defecations occurred during this interval. The collection of seeds ended when the fifty seeds which were ingested had already been defecated. Faeces were subsequently examined for the presence of excreted seeds. Seeds were washed with running water for 5 min, and with distilled water for 30 min. They were dried with paper and stored in paper bags at room temperature (ca. 22 °C) during ca. 10 d before the start of the germination experiment. All seeds (excreted and non-ingested) were treated and kept under similar conditions before germination.

2.3. Germination experiment

Of fifty excreted seeds, only 43 were available for the germination experiment. The retention time of seven seeds was accidentally mistaken in the laboratory. I therefore used an equal number of 43 non-ingested seeds in this experiment. On 29 June 1998, 43 ingested and 43 non-ingested (i.e. control; retention time = 0) seeds were placed in Petri dishes (3 cm diameter) on a filter paper immersed in sterile distilled water. Two to four randomly selected seeds were placed in each dish; seeds that experienced equal retention times were distributed over different dishes. The dishes were placed in a room with controlled light and temperature. Light regime consisted of 16 h of white fluorescent light and 8 h of darkness. Ambient temperature was monitored throughout the experiment at 15-min intervals with temperature data loggers (Stowaway, Onset Computer Corporation, Idaho, USA). The mean ± 1 SE of the daily average temperatures was 28.7 ± 0.1 °C. The mean amplitude of the daily temperature fluctuations was 6.9 ± 0.1 °C (mean min-max temperatures: 18.6–35.5 °C).

Individual seeds were inspected every 2–3 d until all seeds had germinated (radicle present) or died (fully infected with fungi, soft and without endosperm). At each inspection, I recorded which individual seeds had germinated. Seeds infected by fungi were removed from the dish to reduce their possible effect on the remaining sound seeds. Emerging seedlings were removed from the dish when the cotyledon and hypocotyl were visible.

2.4. Statistical tests

For the correlation (Pearson) tests, I provide information about the statistical power ($1 - \beta$) of the test and the least significant number (LSN), which is the minimum sample size for obtaining a significant result at $\alpha = 0.05$ and $1 - \beta = 0.95$ [6].

3. RESULTS

Of fifty seeds ingested by lizards, seven were accidentally mixed in the laboratory and no retention time could be assigned. I therefore reduced the sample size to 43 excreted and a similar number of non-ingested control seeds.

The lizard *P. lilfordi* retained the seeds of *W. frutescens* for periods of 18 to 92 h (mean = 43 h, $n = 43$). Most seeds (86 %, $n = 43$) were retained for less than 48 h (*table I*).

Germination success did not differ between non-ingested (56 %) or excreted (63 %) seeds ($Chi^2 = 0.43$, df = 1, $P > 0.50$), or among seeds that were retained for different time (G-test = 3.452, df = 5, $P > 0.60$) (*table I*). Although germination success tended to increase with seed retention time, the correlation was not significant ($r = 0.550$, $P > 0.20$; one-tailed test; correlation weighted for sample size; $n = 7$; $1 - \beta = 0.44$; LSN = 27) (*figure 1*).

Table I. Germination performance of *W. frutescens* seeds as a function of seed retention time in the guts of *P. lilfordi*. A retention time of 0 corresponds to seeds that were not ingested by lizards. Shown are the sample size (*n*), the percentage of seeds that germinated and the number of days until the onset of germination (median, minimum and maximun).

Retention time (h)	*n*	Percentage germination	Germination rate (d)	
			Median	Min-max
0	43	56	69	40–118
18	8	63	48	43–102
27	2	50	–	102
43	13	69	57	27–118
47	14	50	100	53–120
66	4	75	91	53–118
92	2	100	94	92–102

All seeds germinated within 27 to 120 d. The distribution of germination time did not differ significantly between excreted and non-ingested seeds (Kolmogorov-Smirnov two-sample test: D = 0.153, $P > 0.50$) (*figure 2*). I did not detect the predicted negative correlation between seed retention time and germination time ($r = 0.154$, $P > 0.20$; $n = 51$; $1 - \beta = 0.29$; LSN = 447) (*figure 3*).

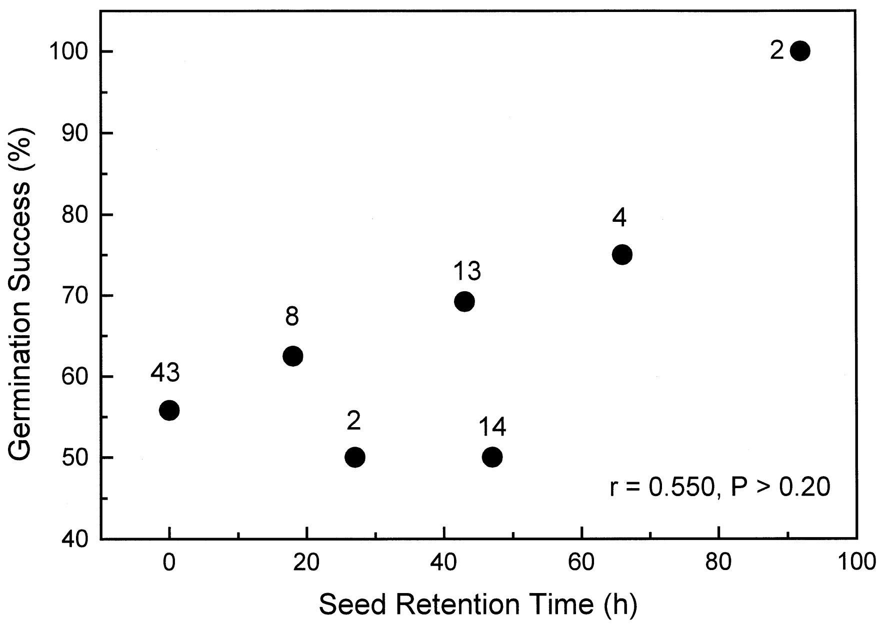

Figure 1. Germination success (in percentages) of *W. frutescens* as a function of seed retention time (in hours). Numbers above dots indicate sample size.

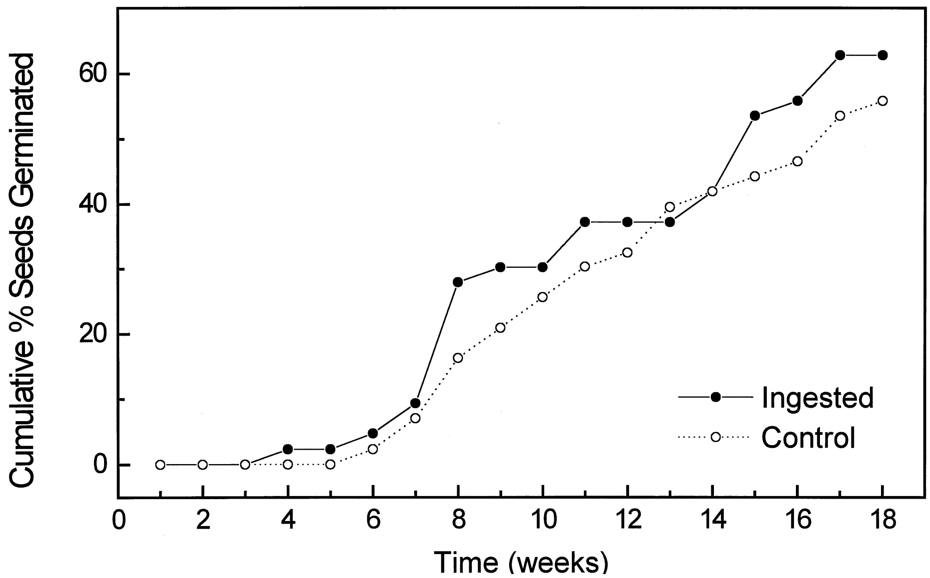

Figure 2. Cumulative percentage of *W. frutescens* seeds that germinated at different times, for seeds that were retained in the lizard guts for 18 to 92 d (ingested) and for non-ingested (control) seeds.

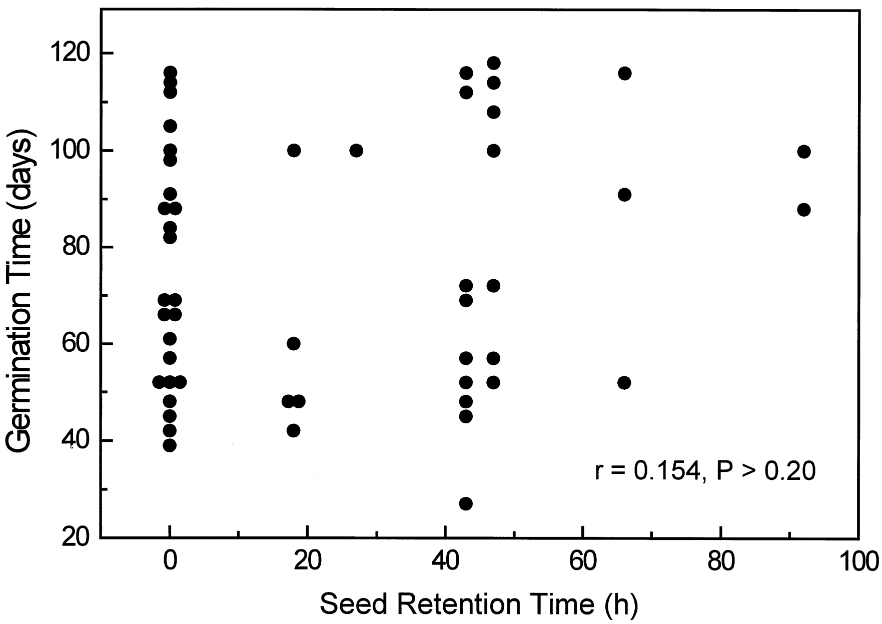

Figure 3. Germination time (in days) of *W. frutescens* seeds as a function of seed retention time (in hours). Retention time of 0 corresponds to control seeds which were not ingested by lizards.

4. DISCUSSION

This is the first study to examine the effects of variation in seed retention time by a lizard on germination performance of plants. Lizards are a taxonomic group that has been largely ignored in studies of plant-animal interactions [18].

The results of this study do not support the hypothesis that a longer gut passage time should improve germination success and germination time of *W. frutescens*. Although it is known that mechanical and enzymatic actions within the guts of frugivores affect the germination ability of seeds [10], the strength of these effects may not be a simple function of time, or other factors may overrule the effects associated with increasing retention time.

Germination success of seeds ingested by lizards was not significantly higher than that of control seeds, whose seed coats were not abraded by digestive processes. This result is consistent with those reported elsewhere (i.e. seed ingestion by frugivores usually does not affect germination success; [18]). However, some lizards have been shown to incur a positive (*Tropidurus torquatus* [7]) or a negative (*Gallotia galloti* [21]) effect on germination success of some plants.

Ingestion by *P. lilfordi* did not significantly improve germination time of *W. frutescens*. Similarly, the canary lizard *Gallotia galloti* has no detectable effect on germination time of the plant *Lycium intrincatum* (Solanaceae) [21]. However, the same lizard species (*G. galloti*) has a positive effect on germination time of *W. aristata* (Solanaceae) [21]. Other studies conducted with representatives of different families of lizards (Tropiduridae, Iguanidae) and plants (Cactaceae, Anacardiaceae, Rubiaceae, Cneoraceae) reported that seed passage through the lizard gut had either no effect (*Ctenosaura pectinata* [13]; *Liolaemus pictus* [25]) or improved germination time (*Tropidurus torquatus* [7]; *L. pictus* [25]). Hence, there seems to be considerable variation in the effect of gut passage on germination performance. The responses of seeds may be specific for different plant species and/or for the animal species which are involved.

My results do not allow me to reject the null hypothesis that there is not an effect of variation in seed retention time on germination performance. Although germination success tended to increase with gut passage time, as predicted by the alternative hypothesis, I found no statistical support for such a relationship. This could be attributed to the small sample size used, and hence to the low statistical power of the test. The minimum sample size (LSN) necessary to judge the observed correlation ($r = 0.55$) as significant at $\alpha = 0.05$ with $1 - \beta = 0.95$, is 27 (instead of the actual seven). That means that I would need to increase the number of samples at different retention times by a factor of 4. This was unfeasible for my study. Indeed, the experimental set-up used attempted to reduce variation in retention time due to other factors (i.e. lizard body size, ambient temperatures, seed size, supplementary diet) because this may potentially confound the hypothesized relationships. Experimental rigorousness [20] inevitably trades off with sample size. I conclude that under the experimental condition used in this study, there is no indication for an effect of retention time on germination success.

I found no indication at all for the predicted negative correlation between germination time and seed retention time. Even though the power of the test was low, it seems rather unlikely to detect such a correlation, given that it requires increasing sample size by a factor of 10 (i.e. 447, instead of 51). In addition, the biological relevance of a significant correlation of $r = 0.15$ ($R^2 = 0.01$) is very questionable. Thus, variation in seed retention time does not appear to have an effect on germination time, at least not within the range of retention times observed in this study (18–92 h).

I have previously shown that abrasion of the seed coat occurs during gut passage in the study system [5]. However, I was unable here to detect any effect on germination performance of putative differences in degree of seed coat abrasion due to variation in seed retention time in the lizards' guts. Because *W. frutescens* seeds have a relatively thick seed coat (Castilla, unpubl.), it is possible that the abrasive effects during gut passage are not sufficiently strong to affect permeability of the seed coat and hence germination performance, at least not within the range of retention times observed in this study. Longer retention times may be required to detect any effect; this would imply that the degree of seed coat abrasion does not increase linearly with time.

The available data are not sufficient to test these possibilities. However, future studies could help to clarify these issues. For instance, it is possible to experimentally manipulate the time lizards retain seeds in their guts, because digestion rate is temperature dependent in reptiles [22]. Preferred body temperatures for activity, and optimal temperatures for organism function are rather high in *Podarcis* lizards (33–36 °C [4]), such that we can expect that gut passage would be considerably delayed at low body temperatures (e.g. 20–25 °C). Another avenue would be to exploit natural variation in the thickness of the seed coat, for instance by using seeds of different size and from fruits produced in different seasons, to further explore the effects of variation in retention times on germination performance of *W. frutescens*.

This is a pilot study stressing the importance of reptiles as seed dispersers, at least in certain habitats, such as small islands, where in addition, many of the flora and fauna are endemic or endangered. Besides, results here are a valuable pilot to design a more complete experiment to look at the effect of the retention time on germination performance of plants using a wide array of seed sizes and pulp type.

Acknowledgments

I thank J. Moreno, director of the Cabrera National Park, and P. Pino for giving permission to work on the island, and J. Mayol for providing permission to work with the endemic lizard. I thank the military commanders (especially J. Padilla) and the wardens (especially Mercé and Oriol) for field assistance, and Juan 'El Payés' for transport to Na Redona islet. Dr A. Traveset gave access to her personal bibliography. M. Verstappen and A. Herrel helped to feed and take care of the lizards. G. Olyslaegers offered help in setting up the germination conditions for *W. frutescens*. D. Bauwens made the figures and improved the English of the manuscript. Laboratory climatic chambers were provided by the Research Group of Plant Physiology (Prof. J.P. Verbelen) and Functional Morphology (Prof. F. De Vree) at the University of Antwerp (UA). This work was supported by the Project DGICYT, PB96-0860 and PB97-1174 (to Dr A. Traveset), and by contracts of the Spanish National Science Foundation (IMEDEA, C.S.I.C.) and MEC.

REFERENCES

[1] Alcover J.A., Ballesteros E., Fornós J.J., Historia Natural de L'Archipelag de Cabrera, Moll-CSIC, Mallorca, 1993, 777 p.

[2] Barnea A., Yom-Tov Y., Friedman J., Does ingestion by birds affect seed germination?Funct. Ecol. 5 (1991) 394–402.

[3] Bauwens D., Castilla A.M., Ontogenetic, sexual and microgeographic variation in color pattern within a population of the lizard *Podarcis lilfordi*, J. Herpetol. 32 (1998) 581–586.

[4] Bauwens D., Garland T., Castilla A.M., Van Damme R., Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation, Evolution 49 (1995) 848–863.

[5] Castilla A.M., *Podarcis lilfordi* from the Balearic Islands as a potential disperser of the rare Mediterranean plant *Withania frutescens*, Acta Oecol. 20 (1999) 103–107.

[6] Cohen J., Statistical PowerAnalysis for the Behavioral Sciences, 2nd ed., Lawrence Erlbaum Associates, Hillsdale, NJ, 1988.

[7] Cortes-Figueira J.E., Saurocory in *Melocactus violaceous* (Cactaceae), Biotropica 26 (1994) 295–301.

[8] Engel T.R., Seed dispersal and plant regeneration by snakes? Ecotropica 3 (1997) 33–41.

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

[10] Janzen D.H., *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rican Pleistocene seed dispersal agents, Ecology 62 (1981) 593–601.

[11] Jordano P., Fruits and frugivory, in: Fenner M. (Ed.), Seeds, the Ecology of Regeneration in Plant Communities, Commonwealth Agricultural Bureau International, Wallingford, 1992, pp. 105–156.

[12] Laska M., Food transit times and carbohydrate use in three Phyllostomid bat species, Z. Saugetierkd. 55 (1990) 49–54.

[13] Mandujano S., Gallina S., Bullock S.H., Frugivory and dispersal of *Spondias purpurea* (Anacardiaceae) in a tropical deciduous forest in Mexico, Rev. Biol. Trop. 42 (1994) 107–114.

[14] Murray K.G., Russell S., Picone C.M., Winnett-Murray K., Sherwood W., Kuhlmann M.L., Fruit laxatives and seed passage rates in frugivores: consequences for plant reproductive success, Ecology 75 (1994) 989–994.

[15] Nogales M., Delgado J.D., Medina F.M., Shrikes, lizards and *Lycium intricatum* (Solanaceae) fruits: a case of indirect seed dispersal on an oceanic island (Alegranza, Canary Islands), J. Ecol. 86 (1998) 866–871.

[16] Rick C.M., Bowman R.I., Galápagos, tomatoes and tortoises, Evolution 15 (1961) 407–417.

[17] Sáez E., Traveset A., Fruit and nectar feeding by *Podarcis lilfordi* (Lacertidae) on Cabrera archipelago (Balearic islands), Herpetol. Rev. 26 (1995) 121–123.

[18] Traveset A., Effect of seed passage through vertebrate frugivore's guts on germination: a review, in: Edwards P.J. (Ed.), Perspectives in Plant Ecology Evolution and Systematics, vol. 1, Rubel Foundation ETM, Zurich, 1998, pp. 151–190.

[19] Tutin T.G., Heywood V.H., Burges N.A., Moore M.M., Valentine D.H., Walters S.M., Webb D.A., Flora Europea, vol. 3, Cambridge University Press, 1972, pp. 195–196.

[20] Underwood A.J., Experiments in Ecology. Their Logical Design and Interpretation Using Analysis of Variance, Cambridge University Press, UK, 1997, 504 p.

[21] Valido A., Nogales M., Frugivory and seed dispersal by the lizard *Gallotia galloti* (Lacertidae) in a xeric habitat of the Canary Islands, Oikos 70 (1994) 403–411.

[22] Van Damme R., Bauwens D., Verheyen R.F., The thermal dependence of foraging behaviour, gut passage rate and food consumption in the lizard *Lacerta vivipara*, Funct. Ecol. 5 (1991) 507–517.

[23] Wahaj S.A., Levey D.J., Sanders A.K., Cipollini M.L., Control of gut retention time by secondary metabolites in ripe *Solanum* fruits, Ecology 79 (1998) 2309–2319.

[24] Whitaker A.H., The roles of lizards in New Zealand plant reproductive strategies, N. Z. J. Bot. 25 (1987) 315–328.

[25] Willson M.F., Sabag C., Figueroa J., Armesto J.J., Caviedes M., Seed dispersal by lizards in Chilean rainforest, Rev. Chil. Hist. Nat. 69 (1996) 339–342.