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Regeneration Ecology of the Rare Plant Species *Verbascum dingleri*: Implications for Species Conservation

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Abstract: *Verbascum dingleri* Mattf and Stef. is a Greek endemic plant species belonging to the family of Scrophulariaceae that only occurs in northeastern Greece, east of the city of Kavala. Knowledge of species distribution, habitat requirements, reproduction, ecology, and population characteristics is limited in the literature. In this study, habitat characteristics, population counts, fruit and seed diversity, and germination were studied for the first time. The results indicate that the species geographical distribution is very restricted, lying in the Mediterranean floristic zone at a low altitude (100–200 m asl) and on very shallow soils. The habitat of this species is characterized by the Csa climate type, with a mean annual precipitation of 602 mm and a mean annual temperature of 14.6 °C. The species occurs in the area lying between the geographical coordinates 40°58′16.59″ N, 24°27′54.93 E, and 41°05′7.2″ N, 24° 47′17.2″ E. The species thrives in degraded shrub communities, dominated by the shrub species *Paliurus spina-cristi* Mill., *Olea europea* L. ssp. *europaea*, and *Quercus coccifera* L. Only a very small number of individuals were found (less than 200) at a density considered too small for long-term persistence of the species. The fruits of the species contained a high number (mean value 58.2) of minute seeds. The seeds exhibited high germination (up to 80.0% in laboratory and up to 30% in ambient conditions). We conclude that in situ and ex situ species conservation and habitat restoration are feasible through the introduction of seedlings produced from seeds collected from local populations.

Keywords: endangered plant; ex situ conservation; plant reintroduction; seed germination; seedling propagation

1. Introduction

Over the last decades, many plant species have been threatened as a consequence of several anthropogenic disturbances and climate change that resulted in species habitat loss. Especially, species with very limited distribution ranges, specific site requirements, and small populations are at high risk of extinction [1].

Unfavorable site conditions for species self-reproduction and seedling development in natural or human-disturbed habitats may cause difficulties in species ecological status and future prospects [2–4]. Thus, data on seed ecology and species regeneration requirements are of great importance.

Early phases of plant life such as germination, seedling development, and early growth are very important stages in plant life cycle and greatly vary in space and time, especially for species of limited populations [5,6]. These stages are commonly affected by many endogenous (lethargy or desiccation tolerance) and exogenous (e.g., site conditions) parameters that greatly threaten species life [7,8]. Successful seed germination and seedling propagation are key factors for ex situ species conservation and in situ species restoration [9,10].

Verbascum dingleri Mattf and Stef. is a rare Greek plant species that belongs to the family Scrophulariaceae, with extremely narrow geographical distribution in northern Greece [11–14]. According to the available information, its presence worldwide has been recorded only three times [12] in two localities, as reported in the Global Biodiversity Information Facility (GBIF) Backbone Taxonomy Checklist Dataset (https://doi.org/10.15468/39omeiassessed via GBIF.org): one time, near Chrysoupolis in 1936, in a location with coordinates 40,98 N, 24,70 E, at an altitude of 150 m above sea level, and two times, in Toxotes, close to the first record (at a distance of 15 km) and at the same elevation, on limestone. The first species record was described by Mattfeld in 1926 [11]. Very few further records have been found in the Greek "grey" literature, but these were omitted in the manuscript. The high frequency of anthropogenic disturbances in the area where the species lives seems to affect the species conservation status. The target species population was located adjacent to a linear construction project which had commitments to mitigate biodiversity impacts. The commitments included seed collection for post construction habitat restoration initiatives.

In this study, the autecology of *V. dingleri* was investigated to provide a better understanding of the conservation of this species. The research was focused on determining the species distribution range and habitat area, community characteristics, population information, and reproduction abilities for in situ and ex situ conservation. More specifically, the study aimed at:

(i) Determining the environmental drivers forcing species distribution;
(ii) Studying species fruit and seed diversity;
(iii) Investigating seed germination behavior in laboratory (controlled environment) and ambient conditions (nursery), including the effect of fruit characteristics on seed germination;
(iv) Developing a scientific approach for the species and its habitat conservation by producing seedlings from seeds in a nursery.

2. Materials and Methods

2.1. Species Description

V. dingleri is [11] a perennial herb species that grows up to approximately 60 cm. The stem is erect, slender, with parallel lines or grooves. The inferior part of the stem is greyish with stellate hairs, without glands, and the upper part is smooth and without hairs. The basal leaves (rosette) are lax with stellate hairs on both sides, denser on the lower side. The shape of the basal leaves is oblong, inversely ovate, the inferior part pinnate, with a length of approximately 12 cm and a width of 3–5 cm. Basal leaves with peduncles have a length of about 3 cm and a width of about 0.5 cm. Leaves' base is flat above and convex underneath. The wings on either side of the petiole inflorescence with simple or complex panicles more or less branched, open, erect and slender, 15–30 cm long, racemose. The flowers with peduncles can be solitary, 5–7 mm long, with a little bract that appears ovate-lanceolate, acute, without hairs. The calyx is more or less conic, with a length of 3–3.5 mm and a diameter of 2.5 mm, without hairs. The calyx lobes are linear-lanceolate, acute or sub-obtuse, with a length of 2.8–3 mm and a width of 0.8–1 mm, and present margins with minutely sparse glands, with obscure three-nerved and middle-nerve keel. The corolla is yellow, about 1.5–2 cm in diameter, without hairs on both sides; the corolla tube is about 3 mm long, with rounded inversely ovate lobes. The anthers are reniform, about 1.5–1.8 mm long, the stamens filament are about 3 mm long and 2.5 mm wide. The filaments are pale-yellow (sometimes whitish), dry, and dense with clavate apex. The bauds are globose with slender, dense, stellate greyish hairs. The style apex is flattened-clavate, about 8–9 mm long. The mature capsule is ovate-globose, about 7 mm long and 5.5 mm in diameter, partly glabrous, the style is persistent. The seeds are ovate-turbinate, intensively warty and gibbous, about 1–1.2 mm long and

0.6–0.7 mm wide [11]. Flowering lasts from late May to the end of June, and fruiting from mid-July to early September.

2.2. Species Distribution

Based on the available information, a survey for species appearance was carried out in 2016 and 2017 in a wide area around the three locations recorded in the GBIF (2018) in northeastern Greece. The surveys were carried out on foot by two people in the locations mentioned in the GBIF and in nearby similar areas on the basis of site-related similarities. Special emphasis was given to locations with topographical and ecological characteristics similar to those of the suggested locations for the species, such as altitude, slope aspect, topography, geological and edaphic characteristics, vegetation type and land uses. It should be noted that the reported coordinates of the first species record (in Chrysoupolis) correspond to a flat, agricultural land, 20 m above sea level. Probably, some correction of the coordinates is possible taking into consideration the year of the report (1936).

2.3. Estimation of Environmental Drivers Limiting the Species Distribution

In the locations where the species was found, we recorded the geographical coordination by GPS, the altitude, the topographical characteristics, the slope aspect and inclination, and the soil depth. For local climate estimation, the meteorological data of the nearest meteorological station of Kavala were used, which is at a distance of 5 km from the western area of species distribution and 25 km from the eastern limits, at similar altitude, latitude, and distance from the sea. To estimate the specific soil conditions of the species habitat, a soil sampling was carried out in 2017. Four surface soil samplings were made from four locations, with three replicate samples per location. The soil properties were measured (texture, pH—using the 1:5 (weight/volume) method—total nitrogen, phosphorus, and potassium concentrations) by standard methods for soil analysis [15,16]. In addition, because of the extensive rock presence on the soil surface, a visual estimation of rock percentage covering the sampling area was carried out.

2.4. Community Characteristics and Estimation of Possible Biotic Interactions

To gain a good understanding of the vegetation existing in the species habitat, we used the sampling method of Braun–Blanquet and a specific, modified abundance/dominant scale [17]. Thus, a full record of phytosociological data was made in five plots, sized 100 m^2 [18], in the summers of 2016 and 2018. Floristic elements where collected in the field, while plant taxa identification was made at species level in the laboratory. The plant species found were analyzed according to their functional attitudes, life form, and any possible interactions with *V. dingleri*.

2.5. Fruit and Seed Collection and Laboratory Analysis

We measured the percentage of individuals bearing fruits among 100 randomly selected individual plants and measured the number of fruits for 30 individuals. In early July 2016, only the minimum number of most of the mature fruits was collected (approximately 60–70 fruits from the most productive plants), since in the case of seeds and fruits belonging to rare and protected species, their collection and use in experiments should be limited to minimum [19]. The collected fruits were put in sealed plastic bags, transported to the laboratory (Aristotle University of Thessaloniki) on the same day of collection, and put in a refrigerator. The size (diameter) of all collected fruits was measured using a digital caliper with accuracy of 0.1 mm. Then, they were classified in three size classes, according to their diameter: a large class, with diameter (d) over 3.5 mm, a medium class, with 3.5 mm > d > 3.0 mm, and a small class, with d < 3.0 mm. Afterwards, the seeds were carefully extracted from the fruits and separated from the peel, and the amount of seeds per fruit was measured. The seeds of each fruit were then set separately in small paper bags.

The morphological characteristics of seeds (length, weight, and water content) were determined in a sampling of 15 seeds of five randomly selected fruits per fruit class (225 seeds in total). The seed number per capsule was counted using a stereomicroscope (magnification range 6.7–45×). The floating method was used for seed purity estimation; only high-quality mature seeds were selected for the test. Then, the length and the fresh weight of fully developed seeds were measured in each fruit class. The seed water content was determined following standard laboratory procedures. The seeds were gravimetrically dried at 72 ± 2 °C for 72 h [20], then the final seed water content was calculated on a dry mass basis (%). All seeds were stored in the refrigerator at 4 °C up to the initiation of the germination examinations (three months later).

2.6. Seed Germination under Controlled and Ambient Environmental Conditions

Before assessments, the seeds were surface-sterilized using 0.85% sodium hypochlorite for 1 min, after which they were washed with distilled water. Four replicates of 25 seeds for each of the three fruit classes were placed in glass Petri dishes (9 cm diameter) containing a layer of filter Whatman paper wetted with distilled water. Parafilm M® was used for wrapping the Petri dishes to restrict any moisture loss, while distilled water was added as needed to provide seeds with an adequate moisture level. The Petri dishes were placed in a plant growth chamber at a constant temperature of 20 °C. This temperature was selected on the basis of the existing data for other species of the genus *Verbascum* [21,22]. The fruit size effect on seed germinability was studied by testing the seeds of four fruits per fruit class (1st, 2nd, and 3rd). Seed germination was checked every two days; water was added as needed during the period of the germination test. The criterion for establishing germination was the emergence of a radicle with length of approximately 2 mm [23]. The experiment was terminated when no seeds germinated for one week. The cumulative germination percentage was evaluated every two days, and the final germination after 28 days. The germination percentage was calculated as the average of four replicates of 25 seeds according to Equation (1), and the mean germination time (MGT) was calculated according to Equation (2) [24,25].

$$GP\ (\%) = (number\ of\ germinated\ seeds/total\ seeds\ per\ sample) \times 100 \quad (1)$$

$$MGT = \Sigma(t.n)/\Sigma n \quad (2)$$

where t is the time (days) from the beginning of the test to the end of the assessment, and n is the number of germinated seeds on day t.

2.7. Seed Germination and Seedling Emergence at Nursery Conditions

Nine fruits were randomly selected (three from each size class), and a random sample of 15 seeds was taken from each of them (in total, 135 seeds). The seeds were planted in plastic pots (Quick pots of 24 cavities with cell volume of 330 cm^3 and depth of 16 cm) in an open-air nursery (research forest nursery of the Laboratory of Silviculture of Aristotle University of Thessaloniki), under relatively similar climatic conditions (similar type of climate, same latitude, closed to the sea), in March 2017. The pots were filled with a common growing medium consisting of peat/perlite in a ratio of 3:1 v/v. The position of the pots in the nursery was changed periodically. All pots were watered to field capacity. After one month, the number of fully developed seedlings (shoot with leaves) per fruit was recorded.

2.8. Statistical Analysis

Statistical analysis of the data was performed using the SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA). Before the analysis, the percentage values of seed germination and seedling emergence were arcsine-transformed to cover the normality and homogeneity assumptions. Seed morphological data as well as the transformed values of seed germination and seedling emergence were subjected to one-way analysis of variance to detect any differences between fruit classes. Comparison of the means followed the least significant differences (LSD) criterion (0.05 level of probability).

3. Results

3.1. Species Geographical Distribution

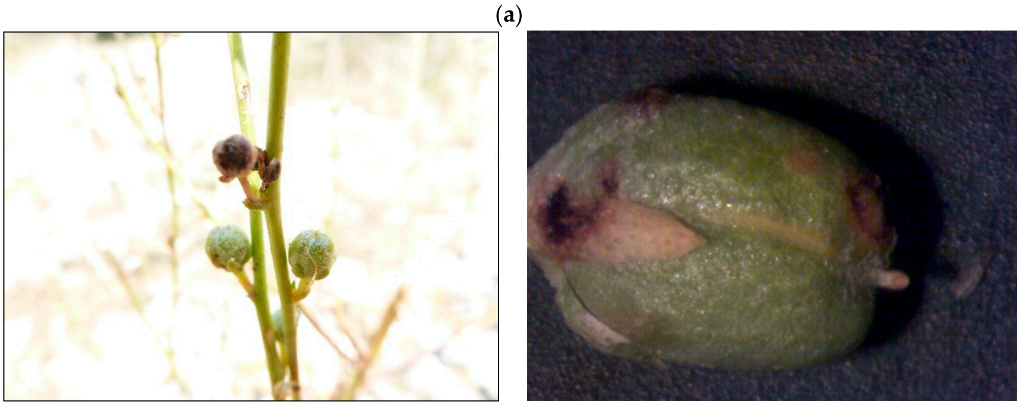
All locations where the species was observed lie along south-facing, very rocky slopes, at the cliff foot of the mountains Symvolo and Rhodope, at low altitudes (100–150 m asl), approximately 3–15 km from the Aegean Sea, just over the plain (agricultural) area lying between the sea and the mountains. The area of the species appearance is restricted to this altitudinal zone, suggesting that these specific ecological conditions favor its thriving. However, the core population of the species was found more westward with respect to the location indicated in the previous records, at 40°58′16.59′′ N, 24°27′54.93′′ E, near the village Chalkero, close to the city of Kavala, approximately 150 km from Thessaloniki (Figure 1), in areas exposed to several anthropogenic actions, such as grazing production, livestock raising, agricultural crops cultivation, presence of vehicles, and generally, in degraded habitats.

Figure 1. Map showing the location of the species *Verbascum dingleri* occurrence. Geographical coordinates: 40°58′16.59′′ N, 24°27′54.93′′ E.

The climate of this area belongs to the type Csa according to the Koeppen classification system. On the basis of the available data of the nearest meteorological station of Kavala city, the mean annual temperature is 14.6 °C, and the mean annual rainfall is 602 mm. The mean monthly temperature during the coldest month (January) is 4.2 °C, and that during the warmest month (August) is 26.0 °C, while the prevailing wind is from the southeast (SE) [26].

From a geological point of view, the study area is part of the Rhodope massif that consists of metamorphic and plutonic-eruptive rocks. The specific rock types where the species appears are marbles and limestone. The soils are very shallow (depth range 5–19 cm), with an extremely high rock presence (80–85% of the total area) (Figure 2a, Table 1). The soil is alkaline, and the pH value was found to be almost constant (7.8) in all four sampling points, with very slight differences. However, even though the soil is very shallow, it was found to be relatively rich in organic matter (7.18%), with adequate total nitrogen content (0.42%), as well as phosphorous and potassium contents (8.55 ppm and 336.5 ppm, respectively). Other similar habitats near the species area were investigated during the species flowering period, but we did not locate any additional populations.

(**a**)



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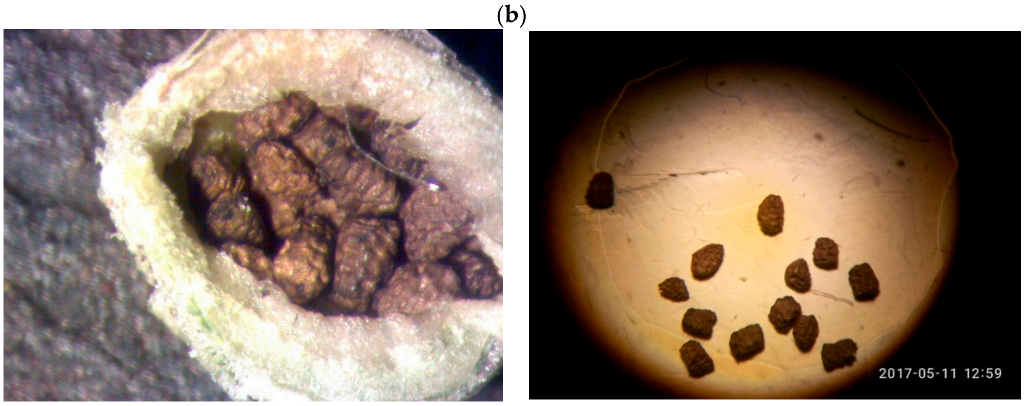

(**c**)

Figure 2. Photo of the species *V. dingleri:* (**a**) in situ photo near Chalkero village, showing the dominant site characteristics; (**b**) fruits of *V. dingleri*; (**c**) seeds of *V. dingleri* (photos from a stereomicroscope).

Table 1. Soil characteristics of the habitat of *V. dingleri*.

Soil Sample	Depth Cm	Rock Appearance (%)	Soil Texture	pH	Organic Matter (%)	C (%)	Total N (%)	C/N	P ppm	K ppm
1	13	85	SL	7.76	6.28	3.64	0.315	11.6	4.08	165
2	18	85	SL	7.83	6.86	3.98	0.356	11.2	9.09	320
3	14	80	SL	7.78	5.93	3.44	0.450	7.70	13.75	602
4	19	85	L	7.84	9.63	5.58	0.559	10.0	7.29	259
Mean ± std error	16 ± 1.47	83.8 ± 1.25	SL	7.80 ± 0.02	7.18 ± 0.84	4.16 ± 0.49	0.42 ± 0.054	10.1 ± 0.88	8.55 ± 2.02	336.5 ± 94.07

The site topography provides a habitat that suffers from hot and dry weather conditions during the dry summer season. As summer approaches, the sunrays fall almost vertically, causing the soil to dry and exposing the vegetation to high sunlight. This, in turn, results in high summer evaporation rates, causing the drying of *V. dingleri* and other annual plants and eventually their disappearance during the late summer season.

The spatial data analysis showed that the species niche is determined by the afore-mentioned specific site characteristics that favor the species survival and thriving. The species distribution is probably constrained by hard dispersal obstacles or physiological thresholds along environmental gradients rather than by interactions with other species. Thus, the species appears only in dry, rocky, south-faced slopes, with medium inclination, on shallow soils and limestone, under the climate type Csa, and at a short distance from the sea, in the location at 40°58′16.59′′ N, 24°27′54.93′′ E.

3.2. Community Characteristics

The vegetation of the area belongs to the Ostryo-Carpinion floristic zone. However, on the basis of the plot where the plant data analysis was made, *V. dingleri* occurs in specific degraded shrub communities, dominated by the woody species *Paliurus spina-christi*, which is a tree species with limited diffusion. These communities consist of a loose shrub layer, dominated by the species *P. spina-christi*, *Olea europea* ssp. *europaea*, and *Quercus coccifera*, with a canopy cover of approximately 30% and a herb layer consisting of the species *Euphorbia dendroides*, *V. dingleri*, *Plantago bellardii*, and many species common in open, degraded, grazing areas of the Mediterranean zone, such as *Avena barbata*, *Allium sphaerocephalon*, *Capsella bursa-pastoris*, and *Bromus tectorum* (the full record of plant abundance/dominance [15] is shown in Table 2). All the recorded species are native, and no exotic species were found. On the basis of the above-mentioned vegetation data, the habitat type where *V. dingleri* was found could be classified as that of the eastern Balkan association *Euphorbio–Paliuretum* or *Oleo sylvestris–Paliuretum spinae christi* [27]. Further data are required for a full phytosociological analysis.

Table 2. Full list of flora taxa recorded in the communities where the species *V. dingleri* was found. The modified abundance/dominant scale according to the Braun–Blanquet method [17] was used.

Species	Family	Dominance				
		Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Euphorbia dendroides	Euphorbiaceae	2a	2m	2m	2b	2a
Paliurus spina-christi	Rhamnaceae	2a	3	2a	2a	2b
Helianthemum sp.	Cistaceae	+		+	r	+
Avena barbata	Poaceae	1	1	+	2m	1
Crupina vulgaris	Asteraceae	R	+			+
Allium sphaerocephalon	Amaryllidaceae	1	1	+	+	1
Planta gobellardii	Plantaginaceae	2m	+	1	1	2m
V. dingleri	Scrophulariaceae	1	+	1	1	1
Asparagus acutifolius	Asparagaceae	2m	1	+	+	1
Melica ciliata	Poaceae	1	+	1	1	1
Capsella bursa-pastoris	Brassicaceae	+	1	+	+	+
Oleaeuropea ssp. europaea	Oleaceae	2b	2a	2b	2b	2a
Quercus coccifera	Fagaceae	2b	2a	2b	2a	2b
Dracunculus vulgaris	Araceae	R				r
Hordeum sp.	Poaceae	1	+		1	+
Bromustectorum	Poaceae	+	+	+	+	+
Vulpia sp.	Poaceae	1	2m		+	1

3.3. Fruit and Seed Diversity

The fruit of *V. dingleri* is a capsule with a diameter of 3–4 mm (mean value 3.24 ± 0.06 mm). The fruits ripen mature from late July to August and then turn green to brown-yellowish. The capsule is generally globose to sub-globose in shape (Figure 2b), and usually two-loculed, with each locule containing approximately half of the fruit's numerous seeds. The average number of fully developed seeds per fruit is 58.18 ± 3.37 (n = 64 fruits), ranging from 15 to 114 (Table 3). Few seeds (3.04 ± 0.86 per fruit) are abnormal (or not fully developed). Statistical analysis revealed significant differences between the three fruit classes in the number of normal seeds and abnormal (or not fully developed) seeds per fruit. In addition, a correlation procedure showed that the number of normal seeds per fruit was significantly positively correlated with the fruit size, i.e., the larger fruit, the higher the number of normal seeds and the lower the number of abnormal seeds (Tables 3 and 4).

Table 3. Fruit and seed characteristics of *V. dingleri* per fruit class. The values are means ± standard error of the mean. Fruit class: Class 1, fruits with diameter (d) > 3.5 mm; Class 2, fruits with 3.5 mm > d > 3.0 mm; Class 3, fruits with d < 3 mm. Means of the same columns followed by different letters are significantly different; ns, non-significant differences.

	Fruit Traits			Seed Traits			
Fruit Class	**Mean Fruit Diameter/mm**	**Number of Mature Seeds**	**Number of Immature Seeds**	**Seed Length/Mm**	**Seed Fresh Weight/gr**	**Seed Dry Weight/gr**	**Seed Moisture Content (%) of Dry Mass**
1st	3.66 ± 0.02a	79.05 ± 4.50a	0.42 ± 0.23c	0.644 ± 0.018 ns	5.0×10^{-4}	3.9×10^{-4}	22.9
2nd	3.26 ± 0.03b	57.04 ± 3.51b	3.92 ± 1.48b	0.626 ± 0.020 ns	4.8×10^{-4}	3.6×10^{-4}	27.8
3rd	2.61 ± 0.08c	29.77 ± 3.88c	5.61 ± 1.43a	0.627 ± 0.033 ns	4.6×10^{-4}	3.4×10^{-4}	29.4
Mean	3.24 ± 0.06	58.18 ± 3.37	3.04 ± 0.86	0.633 ± 0.014	4.8×10^{-4}	3.6×10^{-4}	26.7

Table 4. Results of the statistical analysis for fruit and seeds of *V. dingleri*.

Correlations						
		Fruit Diameter	**Fruit Class**	**N of Normal Seeds**	**N of Abnormal Seeds**	**Germination Final %**
Fruit diameter	Pearson Correlation	1.0	−0.928 **	0.544	0.0 [b]	−0.364
	Sig. (2-tailed)		0.000	0.130		0.335
	N		64	64	64	12
Fruit class	Pearson Correlation			−0.532	0.0 [b]	0.105
	Sig. (2-tailed)			0.141	.	0.787
	N			64	64	12
N of normal seeds	Pearson Correlation				0.0 [b]	0.051
	Sig. (2-tailed)				.	0.896
	N				64	12

Note: ** Correlation is significant at the 0.01 level (two-tailed); [b] cannot be computed because at least one of the variables is constant.

The seeds are very small (minute), ovoid to polygonal, with surface ripples. Mature seeds are dark brown in color (Figure 2c), and their average length is 0.633 ± 0.014 mm. Their mean fresh weight is 4.8×10^{-4} g, which defines them as small seeds. No significant differences were found in seed size between the three fruit classes. The average moisture content of fresh seeds was 26.7% of dry mass, and there was a tendency toward slightly higher values in the larger fruit classes. The seed dispersion of the species is barochory and ornithochory in nature; thus, considering the light seed mass, it is anticipated that species dispersion occurs to some distance from the mother plants.

3.4. Seed Germination Behavior

The germination percentage of *V. dingleri* seeds varied across different fruit classed in laboratory conditions. The best final germination percentage (40%) was observed for smaller seeds belonging to Class 3 (fruit diameter < 3 mm), (Figure 3 and Table 5). Seeds from Classes 1 and 2 showed a quite similar germination pattern, and their final germination percentage was 32% and 26.7%, respectively. However, the fruit size, more specifically, the fruit diameter, does not affect the seed germination percentage of the species. The statistical analysis did not reveal any significant differences among germination percentages. The seeds from all fruit classes started to germinate seven days after sowing, and the germination progress stopped after six weeks. However, most seeds germinated within a period of four weeks, a common period for many species. The mean time to complete germination (MGT) ranged from 17.8 to 24.7 days and was not significantly affected by the fruit class.

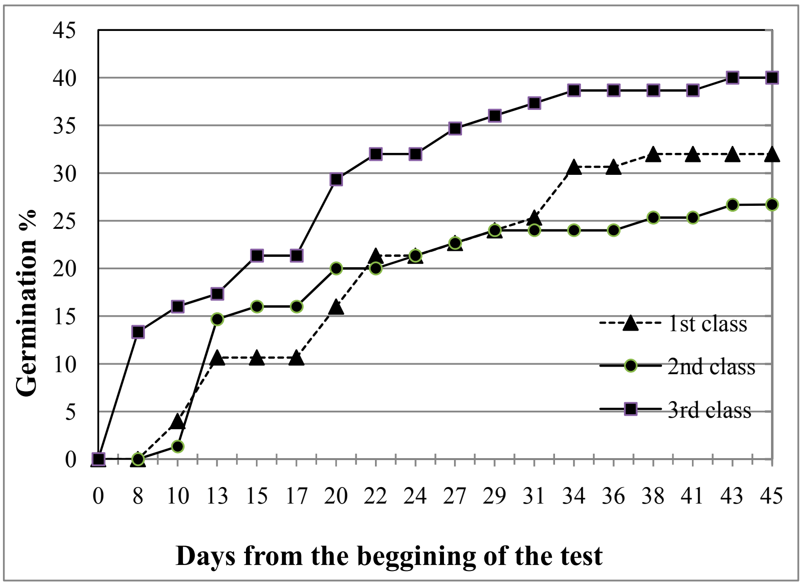

Figure 3. Cumulative germination percentage curves of *V. dingleri* seeds as a function of time for the three fruit classes. Number of seeds: four replications of 25 seeds per fruit class.

Table 5. Seed germination behavior per fruit class in laboratory conditions (final germination percent and mean time to complete germination, MGT) and at nursery (ambient) conditions (percentage of fully developed seedlings) of *V. dingleri*.

Seed Germination			
	In Laboratory Conditions		At the Nursery (Ambient Conditions)
Fruit Class	**Final Germination (%)**	**Mean Germination Time (MGT)**	**Fully Developed Seedlings (%)**
1st	32.0	24.7	22.0
2nd	26.7	18.8	14.5
3rd	40.0	17.8	9.5
Significance	*ns: $p > 0.05$*	*ns: $p > 0.05$*	
Mean	32.9		15.3

Approximately 30 days after planting, in the middle of spring (April 2017), in ambient conditions, *V. dingleri* germinated seeds produced fully developed seedlings; however, their growth was slow and very similar for all produced plants (statistical analysis did not reveal significant differences among fruit classes, $p > 0.05$) (Table 5). The percentage of fully developed seedlings of *V. dingleri* ranged from 2% to 30% among the fruits, regardless of the fruit diameter.

4. Discussion

4.1. Distribution and Habitat of V. dingleri

The results of this study indicate that the geographical distribution of the species *V. dingleri* lies in the Mediterranean area, at a low altitude of 100–200 m asl, on, generally, south-faced, very rocky slopes of highly degraded, overgrazed areas, in low hill sites. The climate of its habitat belongs to the Csa type, with a mean annual precipitation of 602 mm and a mean annual temperature of 14.6 °C. The species habitat is characterized by very shallow, alkaline soils, rich in organic matter, nitrogen, phosphorus, and potassium. The area consists of a grazing area, close to villages and century-old agricultural land. This means that the species habitat is not an isolated area, but the species co-exists with traditional anthropogenic activities.

From a floristic point of view, the species appears in a specific degraded shrub community where the dominant floristic elements, such as the species *P. spina-christi*, *O. europea ssp. europaea*, and *Q. coccifera*, demonstrate the presence of grazing for a long time. A quite rich herb stratum also appears, consisting of the species *Eu. dendroides*, *V. dingleri*, *P. bellardii*, and many species common in open grazing areas, such as *A. barbata*, *A. sphaerocephalon*, *C. bursa-pastoris*, and *B. tectorum*, which indicates species preference for open grazing, degraded slope areas. According to the inventory data, the species appears locally in a very limited area. Few individuals were recorded in the two years of this study, less than the limit of 500 that is currently considered the minimum number (effective population size) of individuals necessary to secure a species population genetic variability [28,29]. This creates strong uncertainty for the species future perspectives. Such low-sized populations are extremely vulnerable to extinction; however, the available data show that the species has persisted in the area for almost a century (since 1926) and grows between the cities of Kavala and Xanthi, northern Greece, just above the plate agricultural land at the foothills of the mountains, on low-altitude, rocky slopes and very shallow soils. Any specific explanation for this very restricted distribution of the species is still unknown. Probably, this may depend on species regeneration ecology and specific requirements for its propagation in the field. This species' restricted occurrence in a single well-defined area within a small part of the Mediterranean region is a characteristic element of the Mediterranean endemism [30]. Compared with *Verbascum pseudonobile*, another range-restricted *Verbascum* species, which, according to the GBIF, has been recorded in 14 localities in northern Greece, around 41.1° N, 23.624.8° E, it seems that *V. dingleri* is specialized to grow in drier habitats with mild winters, close to the sea (Aegean Sea), while *V. pseudonobile* thrives in colder habitats with a more continental climate. Thus, the species distributions are not overlapping.

4.2. Species Regeneration Ecology

The species fruit contained a quite high number (58.2 ± 3.37) of minute normal seeds. According to the germination experiments, no indication of seed dormancy was observed. Seeds quite highly germinated (up to 80.0%), depending on the fruit, at 20 °C, like many other species of the genus *Verbascum* [21,22]. In addition, the seed germination rate of *V. dingleri* in the open-air nursery, with climatic conditions similar to the natural ones, depended on the fruit from which the seeds were extracted. The seed of some fruits showed a germination of 30%, while the seeds of other fruits did not germinate. The seeds germinated and produced seedlings after approximately 30 days from planting in the middle of spring (April 2017), while their growth was slow and very similar for all produced plants.

4.3. Species Conservation

Both laboratory and nursery results analysis revealed that the seeds of *V. dingleri* do not present dormancy and germinate under favorable environmental conditions (for the species). The seeds of the species showed an average percentage of 30–40% germination at 20 °C, a temperature common in the species habitat area, especially during spring or autumn, when many species regenerate in the fields in temperate zones and with climate type Csa. Thus, we conclude that the species regeneration in the field is theoretically feasible at any time after dispersal. However, in natural conditions, a combination of ecological factors such as temperature, light, and water availability play a key role in regulating plant species germination and seedling emergence. Seeds behavior in response to these crucial environmental factors greatly differs among species, depending on each species eco-physiological attributes [7,8]. Many studies have proven that the temperature greatly influences seed germination, and almost in all species, high temperatures slow down the germination [3,4,31]. Direct sunlight also plays an important role in seed germination behavior [32] as well as in early-stage survival of many non-drought-resistant plant species. Probably, during summer, a combination of high temperatures and high intensity of direct sunlight may be a crucial factor affecting a species regeneration success in the field.

On the other hand, the low amount of precipitation, not only during summer (30–40 mm in the area of the species occurrence, according to the data from the nearest meteorological station of Kavala city), but also during the period of seed dispersion in nature, may be a crucial determining factor for species regeneration. Soil water stress commonly reduces seed germination, since this physiological process is sensitive to water availability, especially during the first stage of germination (swelling of seeds through water adsorption). However, seeds of *V. dingleri* show a tolerance to desiccation, being able to germinate in the presence of a moisture content of approximately 10% (data not shown). Considering that the seeds can survive desiccation, we conclude that *V. dingleri* seeds are orthodox, like the seeds of other species of the genus *Verbascum* [21,22,33]. Thus, in field conditions, it is expected that the tolerance of *V. dingleri* seeds to desiccation could contribute to keep them viable until the time of autumn rains. The findings of the current studies on the seed germination behavior of this species seem not to be able to explain the restricted species occurrence and its narrow endemism. Perhaps, it could be assumed that *V. dingleri* effective regeneration and habitat expansion is determined mainly by seedling propagation ability rather than seed germination in the field. It is worth pointing out that, for the soils of the general area of Kavala city [34,35], some extreme values are recorded for As, Pb, and Zn (which are enriched 7.6, 3.3, and 2.7 times, respectively, in comparison to the values of normal USA soils), even though the majority of the elements in the soils have concentrations within normal ranges. Furthermore, these elements are found at their highest concentrations in the vicinity of the industrial zone of Kavala.

The knowledge of the favorable conditions for early plant growth (germination and seedling emergence) of a rare plant species is definitely necessary for taking the appropriate measures for species conservation and the establishment of population restoration programs [10,36,37]. In the case of endangered species whose habitats are subjected to intensive anthropogenic disturbances, in situ and ex situ species conservation strategies can be suggested, and information about the reintroduction of the species by seedlings produced from seeds collected and effectively treated for germination can be provided [38]. Our study demonstrates that in situ and ex situ conservation and reintroduction of *V. dingleri* using seedlings produced from the seeds collected from a natural population is theoretically feasible.

Specifically, our findings demonstrate that ex situ propagation of *V. dingleri* is feasible from seeds, resulting in the conservation of the species diversity. Thus, these findings can contribute to the advancement of artificial seedlings' production that can be used either for ex situ species conservation or for species reintroduction when the natural population is seriously endangered. However, further research is needed to determine the key factors leading to a satisfactory in situ seedling establishment of this species. More knowledge for effective seed germination and production of high-quality seedlings is crucial for the conservation of this extremely threatened species [9].

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