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Echium vulgare and *Echium plantagineum*: A Comparative Study to Evaluate Their Inclusion in Mediterranean Urban Green Roofs

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Abstract: Green roofs (GRs) are proposed to offset against numerous environmental and socio-economic concerns associated with climate change and urban sprawl. In Mediterranean urban areas, to protect and conserve biodiversity through GRs, the use of native plant species from arid environments and with shallow roots is generally recommended. In north Europe, *Echium vulgare* L. is widely used on GRs for its tolerance to abiotic stresses and its attractiveness for bees; unfortunately, since this species requires cold winters to induce flowering and warm wet summers for vegetative growths, its adaptability to Mediterranean GRs has been questioned. The current study is based on the hypothesis that *Echium plantagineum* L. can adapt better to the Mediterranean environment than *E. vulgare* and offer blooms to pollinators, thus providing the important urban ecosystem service (UES) of protecting entomofauna biodiversity. To compare the adaptability of *E. plantagineum* vs. *E. vulgare*, both *Echium* species were grown and studied on the extensive GR installed at ENEA Casaccia Research Center, in the north of Rome, Lazio, Italy. The comparative analysis of the GR performance of the two species was based on several plant-related traits, including seed morphology, rosette stage, inflorescence, flower and root-related traits, and their biological life cycle, most of them showing significant differences (for example, rosette area was 1.42-fold major in *E. plantagineum* than in *E. vulgare*). The information provided in this manuscript will be useful to update the herbarium records for conservation biology. A dramatic water stress was purposely applied in the GR before the end of the hot summer season, and while *E. plantagineum* faced with success the imposed dehydration (88.4% vegetation cover), *E. vulgare* did not (7.5% vegetation cover), presumably because of its biennial life cycle which did not allow it to complete seed maturation (only 46.9% percentage mature seeds in *E. vulgare* respect to 89.5% in *E. plantagineum*). In summary, as the main result, this work shows that in Mediterranean areas, the inclusion of *E. plantagineum* in seed mixes for flower meadow GRs could represent a valuable alternative to *E. vulgare* in temperate areas, providing a safeguard for pollinators and allowing water and energy saving.

Keywords: *Echium vulgare*; *Echium plantagineum*; green roof (GR); urban ecosystem service (UES); herbarium record; water stress; annual plant; biennial plant; pollinator

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1. Introduction

Recently, one of the greatest threats to humanity has been identified as the collapse of the global ecosystem caused by the loss of pollinators [1–3], and public and scientific awareness of the connection between food security and pollination has increased considerably [4]. Different strategies have been proposed to reduce the pressures on pollinators, as a comprehensive evaluation of pesticide risk to the environment [5,6], and the assessment of effective habitat networks for different species [7]. In relation to this, Europe has already endorsed different policies focusing on the natural capital and the urban ecosystem services (UESs) it provides, seen as main elements of new urban models, and fostering Natural-Based Solutions (NBSs) for supporting societies to address a variety of environmental, social, and economic challenges in sustainable ways [8]. In this context, urban greenery should also be planned to serve as a refuge for pollinators, thus playing a crucial role for protecting insect biodiversity [9–11].

Among NBSs, green roofs (GRs) are the main green infrastructures on buildings for sustainable urbanization and the regeneration of cities [8,12]. In the last years, GR installations have seen a prosperous rise in north-west Europe and in North American countries [13]. To foster GR diffusion in other parts of the world, as in Italy and Mediterranean areas, research work is currently in progress to develop further valuable knowhow, allowing the exploitation of different UESs delivered by the plants, such as safeguarding and connecting biodiversity [14], but also energy saving [15], air pollution mitigation, carbon sequestration, stormwater management in rainy regions and water saving in arid ones, and others [16–18].

The design of green infrastructures in Mediterranean areas requires the selection of plants different from those used in the temperate climates, able to adapt to the typical climate of these locations, where summer can be very hot, also considering that environmental conditions on rooftops are generally quite harsh. Thus, it is advisable to include drought-tolerant species able to survive and thrive in the challenging environment of the GR [19,20], selecting them from the local autochthonous ones. Unfortunately, there is still a gap of knowledge on the suitability of various plant taxa to the Mediterranean climate and environmental conditions. Initially, succulent plants from the genus *Sedum* and other genera of the Crassulaceae family used to be considered among the best fitting species for the application on GRs, due to their shallow root systems, the CAM metabolism, their efficient water use and tolerance to extreme drought conditions [19,21,22]. Nevertheless, with reference to the emergency of the reduction in pollinators, different studies have shown that *Sedum* roofs attract a limited number of pollinator species during their almost short flowering period, compared to GRs planted with multiple forms of vegetation (herbaceous roofs) [10]. Thus, to safeguard the rich Mediterranean biodiversity of pollinators [23] through GRs, the inclusion of native plant species from arid environments [24] and with shallow roots [25] should be encouraged. To achieve this aim, interdisciplinary research is needed to assess and foresee how different environmental pressures affect pollinators and to provide evidence-based solutions as the establishment of effective habitat networks, optimizing the UESs that GRs may provide.

ENEA, in its Casaccia Research Center located in the north of Rome, Italy, has developed a GR prototype, including a flower meadow with native Mediterranean species, where experimental activities have been conducted in relation to climate change mitigation and the safeguarding of global biodiversity and pollinators. This manuscript reports a study aimed at comparing the resistance and resilience capacity under Mediterranean GR conditions of two nectar-rewarding species, namely *Echium vulgare* L. and *Echium plantagineum* L. [26]. Both plant herbaceous species from the *Echium* genus belong to the Boraginaceae family, which includes species hosting several pollen generalist bees and some species being the primary hosts for pollen specialist bees of the *Annosmia-Hoplitis* group [27]. Boraginaceae are highly variable regarding flower architecture and the mode of pollen presentation, and *Hoplitis* species possess a few different specialized morphological or behavioral adaptations to collect pollen from them [27,28]. Currently, while *E. vulgare* is frequently employed in GRs in Northern Europe [29], and indeed it is often found in areas with low temperatures and high rainfall, *E. plantagineum* occurs in areas with extreme temperatures and low rainfall [30,31], and its application on GRs is not widely reported (with the only exception of a fascinating study that aimed to establish an annual meadow on extensive GRs in the UK [32]).

In the present work, we tested the hypothesis that *E. plantagineum* can adapt better to the Mediterranean than *E. vulgare*, and that *E. plantagineum* flowers can attract specialized and generalist bee species in Mediterranean GRs likewise *E. vulgare* flowers in north

European GRs. This hypothesis is based on previous studies demonstrating that the choice and adaptation of bees to specific host plants have evolved due to physiological constraints related to pollen chemistry, implying the ability to metabolize pollen-specific toxic defense metabolites [27]. This means that the presence of the same alkaloids in the pollen and nectar of *E. vulgare* and *E. plantagineum* could play a key role in attracting the same bee species, in accordance with the host range evolution in bees [27,33]. Other studies reporting high similarities between the two species in relation to visual and olfactory floral cues [34] have reinforced this hypothesis.

To verify our hypothesis, a three-year experimentation was carried out on the ENEA GR, analyzing the morpho-physiological and phenological traits registered on the two *Echium* species and comparing their growth performance. Even though further studies are necessary to assess the effectiveness of *E. plantagineum* in GRs for the survival of Apoidea genera and families in the hot Mediterranean environment, as recognized for *E. vulgare* in the UK and north Europe temperate environments, our results endorse the cultivation of *E. plantagineum* in the Mediterranean climate and, at the same time, show that *E. vulgare* cannot survive the lack of self-propagation capacity under the extreme conditions of a non-irrigated or low-irrigated GR. Moreover, the outputs of this study present an added value since all collected information will also be useful to update the herbarium records for conservation biology [35,36], which is very important particularly under the current climate change conditions that are threatening biodiversity.

2. Materials and Methods

2.1. Bibliographic and Field Surveys

A preliminary bibliographic survey was conducted to identify spontaneous native plant species resistant to drought, with ornamental flowering particularly attractive for pollinating insects, to be planted on green roof (GR) meadows. The information sources of this study included national botanical books and manuscripts [37–39], the Portal of the Flora of Italy [40], and the 2013 ISPRA manual on herbaceous species for the restoration of anthropogenic environments [30]. This latter provides a list of about 250 spontaneous herbaceous species suggested for reintegration into urban areas and GRs. For each plant species, the ISPRA manual [30] also reports information on its biological form (annual, perennial, biennial) the adaptability to different types of habitats, the classification (rare, uncommon, common), and other phenological characteristics such as the duration of the blooms.

After the selection of the target species of interest in the literature, namely *Echium vulgare* L. and *Echium plantagineum* L., a field survey lasting 5 years was carried out in various green areas and roadside vegetation in the Center of Rome (IInd Municipio), including Villa Borghese, Villa Ada and along via Panama roadsides, Villa Glori, and Villa Chigi, and in the north of Rome (XVth Municipio), including some marginal areas of Parco di Vejo and the green areas (about 60 ha) around the ENEA Casaccia Research Center (Figure 1). These sites of study were visited approximately on a seasonal basis and even more frequently during the spring–summer season, to identify and monitor the presence of the target species of interest.

2.2. Experimental Site

The experimental activities developed to compare *E. vulgare* and *E. plantagineum* species, in terms of growth and performance under water stress, were carried out on the extensive GR located at the ENEA Casaccia Research Center, on the building F92, already described in [15,18,41]. The location is representative of Mediterranean areas. The GR surface (~90 m^2) was divided into different sectors; a sector of ~8 m^2 was dedicated to the experimentation on spontaneous herbaceous species. The soil substrate (8 cm depth) consisted of a mix of granules of volcanic origin (lava lapilli, pumice stone), for guaranteeing lightness, combined with soil substrate, natural compost, and peat. Automatic irrigation was provided daily with a calculated average water consumption of about 6 L/m^2

during the spring–summer season (except for the water stress imposition). Maintenance was minimal and included only one lawn mowing per year at the end of September, followed by one organic based N-fertilization at a concentration of 3 g m^{-2} per year. The experimentation on the GR lasted 3 years (Table 1).

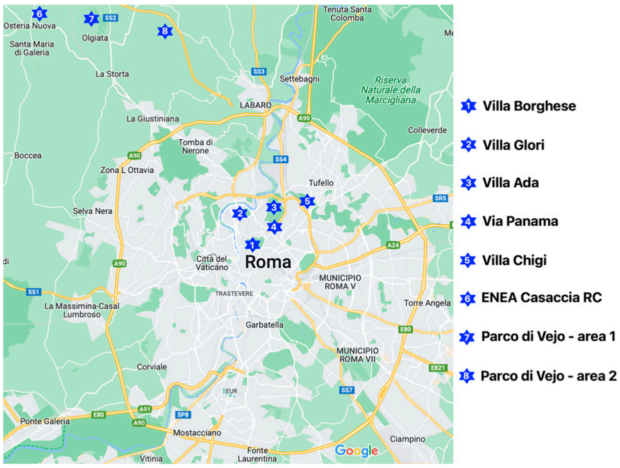

Figure 1. Map of the field survey areas monitored for 5 years and highlighted by a numbered blue star. Areas from 1 to 5 belong to the IInd Municipio, in the center of Rome; areas from 6 to 8 belong to the XVth Municipio, in the north-west of Rome.

Table 1. Activities carried out in the 3-year experimentation on *Echium vulgare* L. and *Echium plantagineum* L., planted on the green roof (GR) located at the ENEA Casaccia Research Center.

Year	Month	Activity
1st year (Dec 2018–Nov 2019)	December 2018	*E. vulgare* and *E. plantagineum* seed sowing in trays in greenhouse
	March 2019	Transfer of *E. vulgare* and *E. plantagineum* plantlets on the GR
	April 2019	First *E. plantagineum* blooms
	June 2019	First *E. vulgare* blooms
	September 2019	Water stress
	October 2019	Restart of *E. plantagineum* vegetative growth and first occasional blooms; second *E. vulgare* seed sowing
2nd year (Dec 2019–Nov 2020)	December 2019	Second transfer of *E. vulgare* plantlets on the GR
	March–June 2020	Plant rosette morphological/morphometric evaluation
	Spring 2020	Outstanding vegetative growth and blooming of *E. plantagineum*, dominant over *E. vulgare*
	July 2020	Evaluation of adult plant and inflorescence related traits
	August 2020	Evaluation of seed maturity (MS%) and root related traits
3rd year (Dec 2020–Nov 2021)	Spring 2021	Outstanding vegetative growth and blooming of *E. plantagineum*, dominant over *E. vulgare*
	Spring–Summer 2021	Not structured observations of pollinator visitations

2.3. Plant Materials

For the GR experimentation, pure seeds of *E. vulgare* were purchased from SemeNostrum [42], and a mixture of *E. plantagineum* seeds was obtained from Gardenseedsmarket [43]. Wild seeds of *E. plantagineum* collected from marginal green areas in the north-west of Rome were also planted on the GR to assess plant similarity with respect to the commercial types. Seed sowing was completed in December 2018 in OUNONA seedling starter trays under greenhouse conditions. Each pot of the tray was filled with 100 mL of a potting soil for lawns, and five seeds were placed at a depth of 1 cm. For each of the two species, 150 seeds were put to germinate. In March 2019, 50 plantlets for each of the two species were transferred in a random design on the GR. Specifically for *E. vulgare*, a second seed sowing was performed in October 2019, followed by the transfer of 50 plantlets in a random design on the GR in December 2019 (Table 1).

2.4. Morphological and Morphometric Comparison of the Two Species

Different morphological and morphometric traits were measured to compare the two *Echium* species and provide useful information for the integration of herbarium records for the scientific community. Scanning electron microscope (SEM) observations of the seeds were performed with a Zeiss EVO MA15 operated at an accelerating voltage lower than 2 kV. Indeed, low voltage conditions reduce the charging effects, due to non-conductive matter, allowing the direct observation of the seeds. Samples were prepared by depositing them on an aluminum stub covered with carbon tape. Seed length (SL) and seed basal width (SBW) were recorded by a digital millimeter caliper for 100 randomly selected seeds of each of the two species, as received from the providers.

At the leaf rosette stage, plant growth observation on the GR was carried out from March to June 2020 (Table 1). In particular, the larger rosette diameter (LRD), the smaller rosette diameter (SRD), and the largest rosette leaf length (LRLL) were recorded every ten days in 25 randomly selected plant individuals for each of the two species. Rosette area (RA) was measured at the end of April 2020 by ImageJ [44] in 5 randomly selected rosettes for each of the two species.

At the adult plant stage, the primary inflorescence length (PIL), from the insertion of the first secondary branch up to the apex, the number of secondary branches on the main inflorescence branch (NSB), and the average internode length (aIL) of the main branch, calculated as the ratio of the plant height and the number of internodes in the main branch, were registered in 25 randomly selected adult plant individuals on the GR for each of the two species. Concerning the mature flower, the calix length (CaL), the corolla length (CoL) and width (CW), the number of sepals, petals and stamens, and the pistil length (PL) were measured on graph paper with a ruler. Four randomly selected flowers per plant were measured in 25 randomly selected plant individuals for each of the two species. Both above-mentioned adult plant and inflorescence related traits were evaluated in July 2020 (Table 1).

The plant fertility was appraised by the percentage of mature seeds (MS%) calculated as a ratio between the number of fertile seeds and the total number of seeds, i.e., both fertile and sterile ones, from 5 randomly selected adult plant individuals at the beginning of August 2020 (Table 1).

The plant main root was evaluated measuring the length (RL) and width (RW) on graph paper with a ruler in 25 randomly selected plant individuals on the GR for each of the two species during three plant growth stages, namely juvenile plant (in general herbaceous plantlets in annual cycle forms and rosettes in biennial cycle forms) [45], plant at the flowering stage, and adult plant ($_{juv}$, $_{flo}$ and $_{adu}$ subscripts, respectively). Root evaluation was carried out in August 2020 (Table 1).

2.5. Evaluation of Vegetation Coverage and Water Stress Imposition on the GR

An overall estimate of plant coverage for the two *Echium* species under Mediterranean GR conditions was performed by plot-based visual evaluation [46]. A monthly estimation was carried out from March to September, at the beginning of each month, in years 2019 and 2020, evaluating the percent coverage of each of the two species in 5 plastic squares (0.25 m^2) randomly arranged on the GR herbaceous meadow under study. A severe water stress was applied at the end of August 2019 by interrupting the irrigation in the experimental GR sector for 1 month (Table 1). The regenerative capacity and the level of drought resistance of the two plant species was evaluated in terms of coverage percentages.

2.6. Entomological Biodiversity

Unstructured observations of pollinator visitation were mostly carried out on the GR and in the area of ENEA Casaccia R.C. during the sunshine hours in the spring–summer season of the 3rd year of experimentation (Table 1).

2.7. Statistical Analysis

Morphometric trait data were analyzed with IBM SPSS Statistics v 27.0.1.0. An independent samples *t*-test was used to compare the means of the data from the two groups, namely *E. vulgare* and *E. plantagineum*, after verifying the assumptions of data independence, normality, and homogeneity of variance. Linear regression analysis and R-square value estimation to define the growth trends of LRD, SRD and LRLL were performed with Excel by adding the trendline.

3. Results

3.1. Bibliographic Survey

From the bibliographic research, a group of native Mediterranean plant species was identified and selected for their potential adaptability in extensive green roofs (GRs), with a good level of drought resistance, providing shade, besides exhibiting long blooms to sustain entomophilic pollination and self-propagation ability. As a result, *Echium vulgare* L.—also known as viper's bugloss or blueweed—was selected from the ISPRA manual on herbaceous species for the restoration of anthropogenic environments (2013) [30], suggested as being able adapt to urban greening, particularly GRs [29,47], and reported to be present in the Region of Lazio by the Portal of the Flora of Italy [40]. It belongs to the Boraginaceae family. Furthermore, from the same genus, *Echium plantagineum* L. was selected as an ornamental species able to adapt to borders of flower beds and gardens [37], also found in Lazio [40], and reported as very common in many other Italian regions [39,48], and in many ruderal habitats [49]. Both herbaceous species are upright and long-lived plants initially producing a basal rosette of leaves. The main characteristics of these two plant species available from the internet and in the scientific literature are reported in Table 2.

3.2. Field Surveys

During the 5-year lasting field surveys, *E. vulgare* was never encountered in any of the monitored areas (Figure 1). In contrast, some individuals of *E. plantagineum* were identified, both annual and biennial cycle forms, and were evaluated by means of unstructured observations. They were found much more frequently in the investigated northern areas of Rome (XVth Municipio) than in the central ones (IInd Municipio). Anyway, from our field surveys, a general scarce presence of *E. plantagineum* was assessed.

3.3. Morphological and Morphometric Comparative Analysis of the Two Species Growing on the GR

3.3.1. Seed Comparison

Seeds of both species were observed under the stereomicroscope (Figure 2a,b) and the scanning electron microscope (SEM) (Figure 2c–h). In SEM, at a lower magnification, the *E. vulgare* seed surface appeared much more glabrous than *E. plantagineum*, without showy growths, while the seeds of *E. plantagineum* exhibited very evident growths on the dorsal surface (Figure 2c,d). Differently, at a higher magnification, *E. vulgare* showed a micro rough seed surface, while showing evident projecting growths and bumps in *E. plantagineum* (Figure 2e–h). In both species, the seeds showed a clearly visible septum in the dorsal surface dividing the semen into two equal parts (Figure 2c–f). Concerning the size, *E. plantagineum* seeds were 22.6% and 27.3% larger in length (SL) and basal width (SBW), respectively, than those of *E. vulgare* (Table 3). It was also noticed that in *E. vulgare* the seeds were darker and browner than those of *E. plantagineum*.

Table 2. Main distinguished features of the two boraginaceous plant species considered in this study, *Echium vulgare* L. and *Echium plantagineum* L., in Mediterranean areas.

	Echium vulgare	***Echium plantagineum***
Additional names	Preferred common name is (common) viper's bugloss; main international common name is Blueweed [50]	Preferred common name is Paterson's curse; main international common names are blue weed, purple bugloss, purple viper's bugloss [50]
Origin	Native to Europe, Western Asia, and Western China	Native to the Mediterranean region and adjacent areas of Atlantic western Europe
Plant biological cycle	Biennial to short-lived perennial. Very rarely annual	Mostly annual, sometimes biennial
Habitat	Uncultivated and arid habitats [30]	Uncultivated and arid habitats [30]
Diffusion	Not common in Mediterranean areas in rural environments [30]	Very common in Mediterranean areas, in both rural and urban environments [30,50]
Development	It produces a 20-leaf rosette during the first year of growth and a branched flowering stem during the second year [26]	It produces a 4-leaf rosette and a branched flowering stem in one season [26]
Auxiliary buds and shoots	It lacks auxiliary stems [26]	It presents auxiliary stems [26]
Inflorescence	A panicle of numerous, short helicoid cymes, each subtended by an upper foliage leaf. A stem produces as many as 50 cymes, each with about 20 flowers [51]	It has too many erect flowering branches
Blooming season	April to September [52]	March to July [52]
Additional information	- Higher levels of naphthoquinones (shikonins) involved in plant defense than *E. plantagineum* [31] - Traditionally used in green roofs in temperate Europe [29] - Increasingly used in bee-friendly gardens in temperate Europe [26] - Of interest for further application and development of medicinal products [53]	- Greater genetic diversity than *E. vulgare* [31] - Higher levels of numerous pyrrolizidine alkaloids involved in plant defense than *E. vulgare* [31] - Increasingly used in bee-friendly gardens in temperate Europe [26] - It is considered as an invasive weed in Australia [31] - Of interest for further application and development of medicinal products [53]

3.3.2. Rosette and Growing Trend Comparison

E. vulgare presented the rosette stage from February–March up to June in 2019 (when proceeding from the first seed sowing) and in 2020 (when proceeding from the second seed sowing), while only a few individuals were present in the 3rd year. Differently, the rosette stage in *E. plantagineum* was observed exclusively in the 2nd and 3rd years just for some individuals. The two species presented a different rosette morphology. As has also been described in the literature [50,51], the basal leaves in *E. vulgare* presented an oblanceolate–spatulate shape, acute at the apex and gradually narrowed at the base into a short petiole. They were covered by bristly trichomes and soft close white hairs, mostly dark green colored, and presenting clearly the primary main vein only (Figure 3a). Differently, the rosette leaf shape in *E. plantagineum* was ovate–lanceolate and petiolate, with more marked veins, including evident secondary veins, and covered with soft close hairs (Figure 3b).

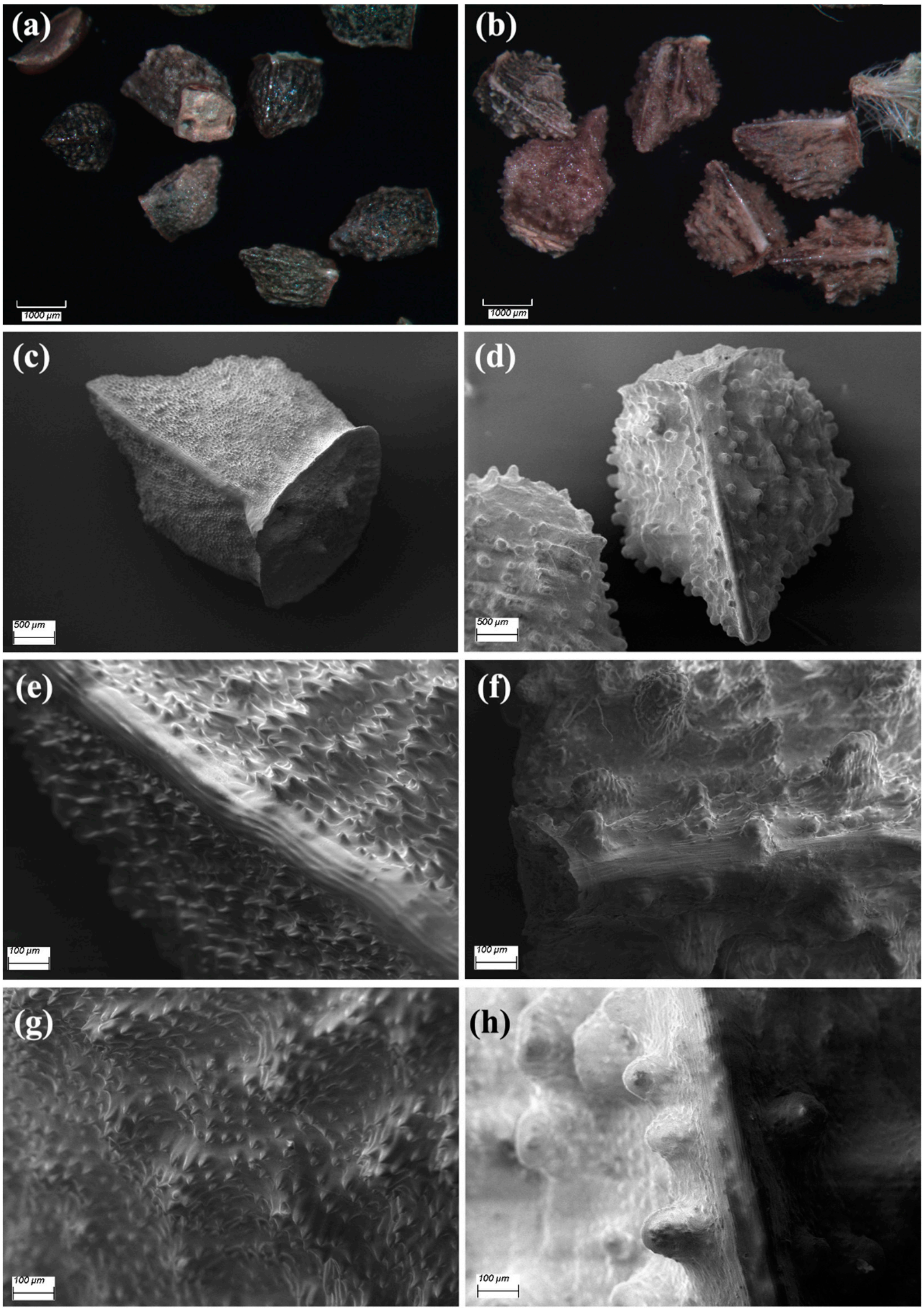

Figure 2. Seed microstructure. Stereo microscope images of a typical single seed in *E. vulgare* (**a**) and *E. plantagineum* (**b**). SEM images of a typical single seed in *E. vulgare* (**c**) and *E. plantagineum* (**d**), dorsal seed coat surface detail showing the septum in *E. vulgare* (**e**) and *E. plantagineum* (**f**); seed coat surface detail in *E. vulgare* (**g**) and *E. plantagineum* (**h**).

Table 3. Morphometric traits measured in *Echium vulgare* L. and *Echium plantagineum* L. growing in the green roof and the results for the independent samples *t*-test for a comparison of the means for each trait in the two *Echium* species.

		Morpho- and Biometric Trait Description	Acronym	Unit of Measure	N	*Echium vulgare*		*Echium plantagineum*		Independent Samples *t*-test		
						Mean	Std. Dev.	Mean	Std. Dev.	t	*df*	*p*
Seed related traits (as provided by the sellers) *		Seed length	SL	mm	100	2.095	0.211	2.706	0.219	−20.116	198	<0.001
		Seed basal width	SBW	mm	100	1.509	0.183	2.077	0.239	−18.847	198	<0.001
Rosette-related traits (at the end of April)		Larger rosette diameter	LRD	cm	25	26.90	0.48	42.00	1.30	−54.466	48	<0.001
		Smaller rosette diameter	SRD	cm	25	11.55	0.50	14.50	0.65	−17.932	48	<0.001
		Largest rosette leaf length	LRLL	cm	25	14.20	1.11	24.00	1.84	−22.770	48	<0.001
		Rosette area	RA	cm^2	10	15.52	2.31	22.07	2.23	−6.439	18	<0.001
Adult plant-related traits		Primary inflorescence length	PIL	cm	25	23.27	3.31	21.00	3.04	2.517	48	0.015
		Number of secondary branches	NSB	cm	25	33.48	3.53	14.48	1.92	23.674	48	<0.001
		Average internode length	aIL	cm	25	1.03	0.23	2.50	0.41	−15.473	48	<0.001
Inflorescence-related traits		Calix length	CaL	cm	100	0.74	0.13	1.06	0.25	−11.644	198	<0.001
		Corolla length	CoL	cm	100	1.79	0.19	1.97	0.21	−6.350	198	<0.001
		Corolla width	CW	cm	100	1.31	0.21	2.17	0.37	−20.204	198	<0.001
		Pistil length	PL	cm	100	1.99	0.43	1.59	0.34	7.279	198	<0.001
Fertility-related traits		Mature seed percentage	MS%	%	5	46.9	-	89.5	-	-	-	-
Root-related traits	Juvenile plant stage	Root length	RL_{juv}	cm	25	6.70	0.30	5.50	0.43	11.395	48	<0.001
		Root width	RW_{juv}	mm	25	1.10	0.22	1.02	0.16	1.432	48	0.159
	Flowering plant stage	Root length	RL_{flo}	cm	25	7.21	0.49	6.10	0.49	8.015	48	<0.001
		Root width	RW_{flo}	mm	25	1.73	0.52	1.40	0.42	2.484	48	0.330
	Adult plant stage	Root length	RL_{adu}	cm	25	11.5	0.65	8.20	0.65	18.035	48	<0.001
		Root width	RW_{adu}	mm	25	2.86	0.24	2.68	0.25	2.570	48	0.013

N: total number of measured samples per species; t: 2-tailed T-ratio; *df*: degrees of freedom; *P*: *p*-value; * data measured on commercial seeds.

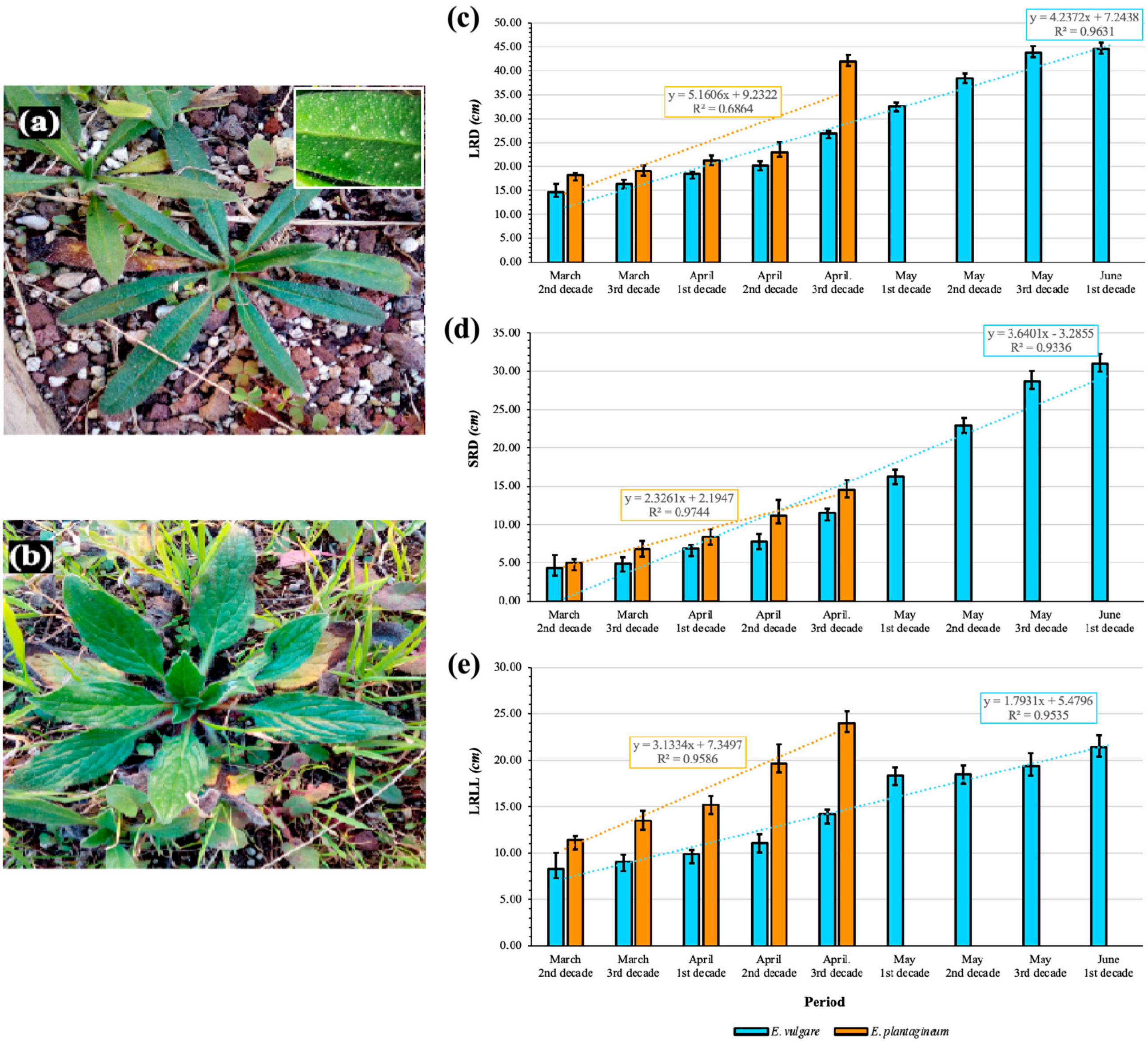

Figure 3. *Echium vulgare* L. (**a**) and *Echium plantagineum* L. (**b**) rosettes on the green roof. Graph of the basal rosette growing trend for the two species *E. vulgare* (in light blue) and *E. plantagineum* (in orange), estimated from the largest rosette diameter (LRD) (**c**), the smaller rosette diameter (SRD) (**d**), and largest rosette leaf length (LRLL) (**e**), measured on the green roof from March to June 2021. Image (**a**) includes, in the upper part on the right, a magnification of the leaf surface showing white tubercles (trichomes).

The growing trend of the rosettes evaluated from March to June of the 2nd year on the GR revealed a faster growth of *E. plantagineum* than *E. vulgare*, on the bases of the larger rosette diameter (LRD), smaller rosette diameter (SRD), and largest rosette leaf length (LRLL) measured parameters (Figure 3c–e). Except for LRD in *E. plantagineum*, all other parameters analysed in both plant species exhibited a positive linear trend ($R^2 > 0.9$), even if with some differences. Interestingly, at the end of April, most *E. plantagineum* individuals entered the flowering stage with the disappearance of the rosette. Looking at LRD, *E. plantagineum* reached more than 40 cm at the end of April, one month earlier than *E. vulgare*; SRD in *E. vulgare* at the beginning of June was double the size of *E. plantagineum* at the end of April; LRLL of *E. plantagineum* at the end of April was higher than in *E. vulgare* at the beginning of June (Figure 3c–e).

Moreover, from the values of these parameters measured at the end of April 2020, *E. plantagineum* rosettes were significantly larger than those of *E. vulgare* ($p < 0.001$). In particular, the rosette in *E. plantagineum* showed a 36.0% higher LRD, a 20.3% higher SRD, and a 40.8% higher LRLL than in *E. vulgare* (Table 3; Figure 3c–e). Based on rosette area (RA), the *E. plantagineum* rosette allowed a major (1.42-fold change) green covering of the GR than *E. vulgare* (Table 3).

3.3.3. Adult Plant and Inflorescence Comparison

Adult plant and inflorescence-related traits were evaluated in July of the 2nd year. In *E. vulgare*, spike inflorescence (PIL) was 9.8% longer and with 56.8% more branches (NSB) than in *E. plantagineum* ($p < 0.05$ and $p < 0.001$, respectively). At the same time, the average internode length (aIL) in *E. vulgare* was 75.7% lower than in *E. plantagineum*, whose branches were more spaced apart (Table 3).

Plant fertility was appraised by the percentage of mature seeds (MS%) in August of the 2nd year, and *E. plantagineum* showed a higher percentage (89.5%), close to double, of mature seeds than *E. vulgare* (46.9%) (Table 3).

Number of sepals, petals, and stamens was as known the same, five, in both *Echiums*. As reported in the literature, *E. vulgare* showed purple–blue flowers (Figure 4a), while *E. plantagineum* purple–magenta ones (Figure 4b). Significant differences ($p < 0.001$) were also recorded between the length of the calix (CaL), the length and width of the corolla (CoL and CW), and the length of the pistil (PL). In particular, CaL, CoL, and CW were, 1.4, 1.1 and 1.7 times higher, respectively, in *E. plantagineum* than in *E. vulgare*, while PL was 1.3 times longer in *E. vulgare* than in *E. plantagineum* (Table 3; Figure 4c).

3.3.4. Root Comparison

As shown in Table 3, looking at the taproot, in August of the 2nd year, *E. vulgare* exhibited a greater length (RL) than *E. plantagineum* in all the evaluated plant growth stages ($p < 0.001$; Figure 4a–c), while no significant differences were assessed regarding the width (RW) between the two species. As expected, root differences were more evident in the adult plant, when RL_{adu} was up to 1.4 times longer in *E. vulgare* than in *E. plantagineum*. Interestingly, the *E. vulgare* taproot showed an intense red color for the presence of red-colored compounds, namely shikonins, produced by the roots in some plant species in defense against a range of biotic threats (Figure 5d) [31]. Moreover, by nature, a major expansion of the root was observed as an effect of a higher soil substrate availability (Figure 5e).

3.4. Vegetation Coverage and Adaptation to Water Stress on the GR

Plant growth and vegetation coverage on the GR were monitored and evaluated during the 3-year experimentation. During the 1st year, starting from the transfer of the plantlets, the vegetation expansion allowed a GR coverage of more than 60% at the end of July 2019. Then, after the induced water stress in September 2019, the vegetation coverage showed a straight decline and all plants were completely dried and turned yellow. In the 2nd year, a GR vegetation coverage of more than 95% in June 2020 was achieved, showing only a slight decline from July to September due to the senescence of some inflorescences (Table 4; Figure 6a). Interestingly, after the imposed dehydration, only *E. plantagineum* was able to recover from the stress (Figure 6b), with first occasional blooms starting from October 2019. For this reason, the capacity of dehydration tolerance was assessed only in *E. plantagineum* and it was necessary to perform a second transfer of new *E. vulgare* plantlets on the GR to permit its further evaluation (Table 1). Under the GR experimental conditions, a major contribution to the vegetation coverage was provided by *E. plantagineum* (Table 4).

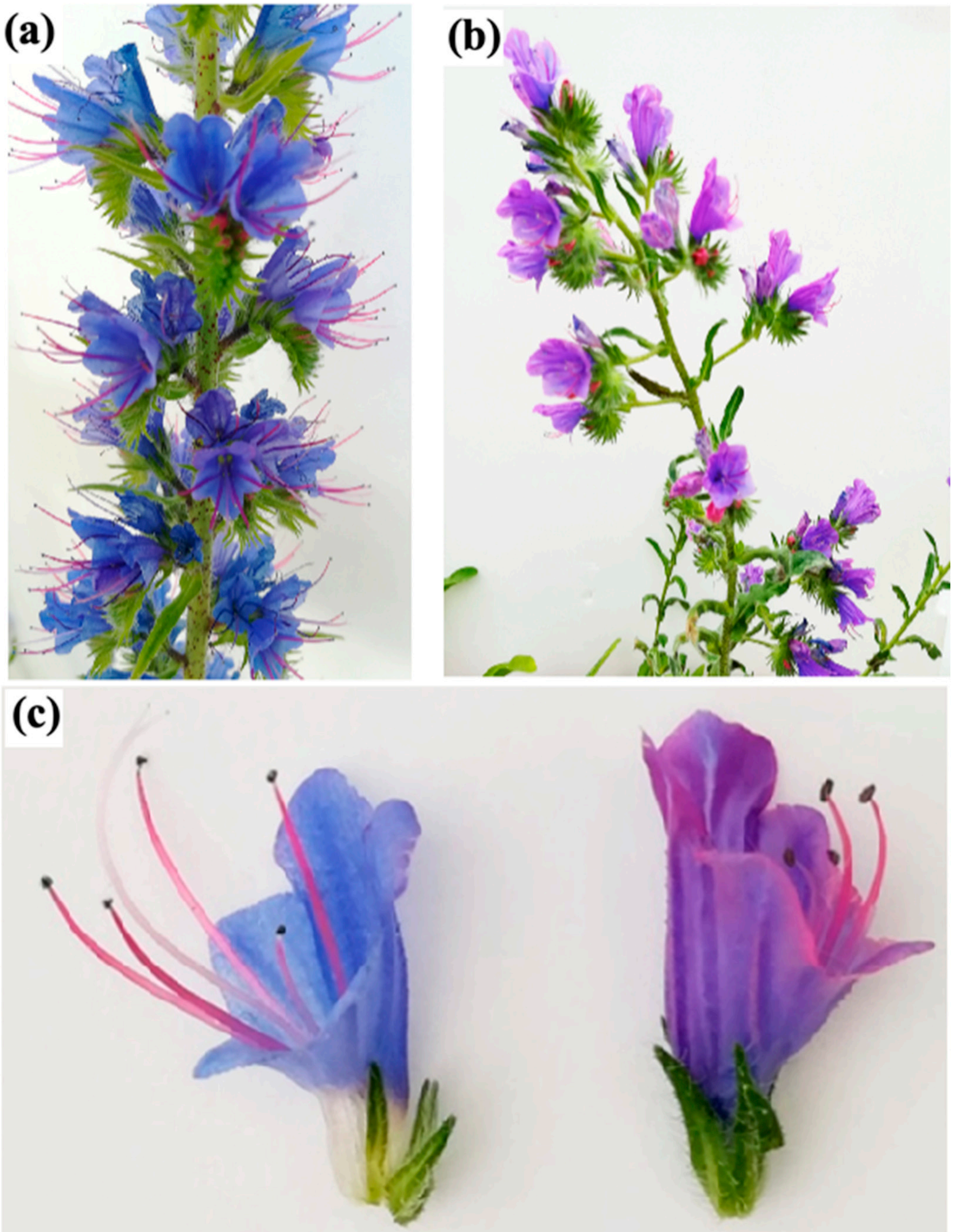

Figure 4. *E. vulgare* L. (**a**) and *E. plantagineum* L. (**b**) spike inflorescence in adult plant; *E. vulgare* (**left**) and *E. plantagineum* (**right**) flower from an adult plant (**c**).

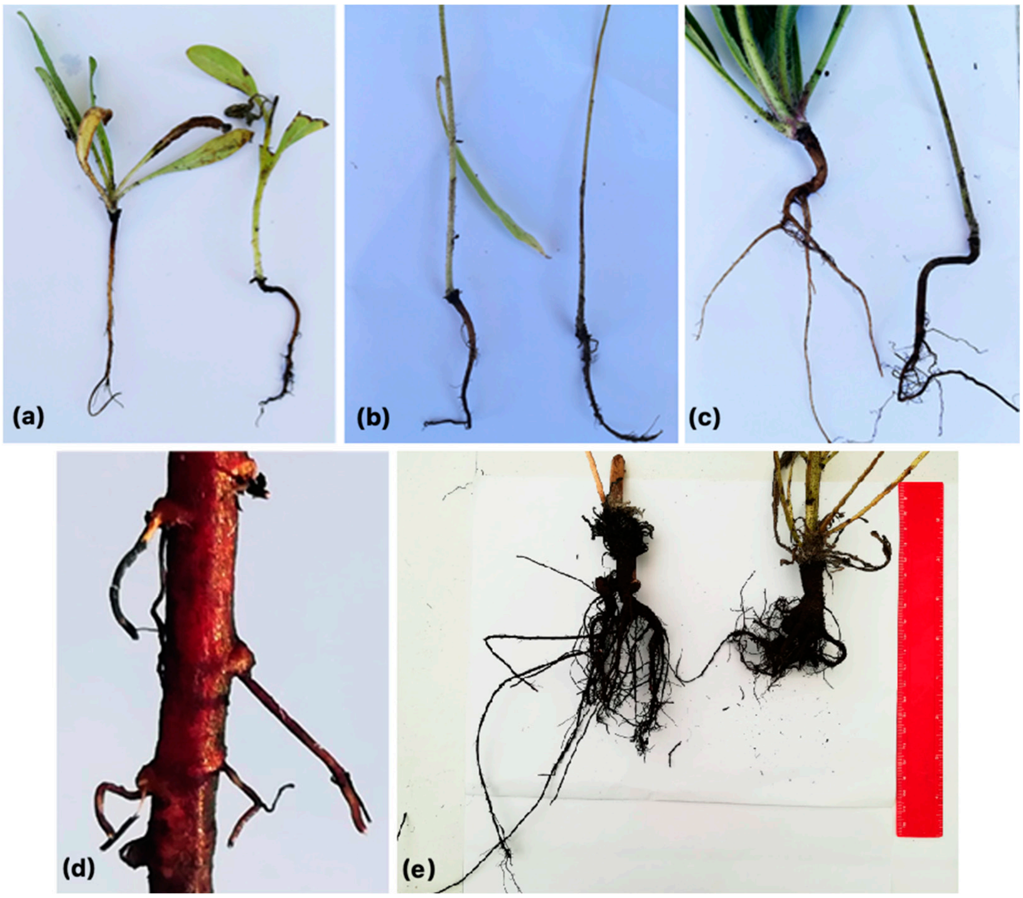

Figure 5. Taproots in *Echium vulgare* L. (on the left) and in *Echium plantagineum* L. (on the right) at the plantlet stage (**a**,**b**) and at the juvenile plant stage (**c**). Magnification of one taproot segment in adult *E. vulgare* showing the red color (**d**); *E. vulgare* root grown in a pot with a substrate depth > 20 cm (on the left) and on the green roof with a substrate depth < 8 cm (**e**).

Table 4. Vegetation coverage percent (%) of *Echium vulgare* L. and *Echium plantagineum* L. on the green roof (GR) located at the ENEA Casaccia Research Center, estimated by visual evaluation carried out from March to September in 2019 and 2020.

Year	Month	Vegetation Coverage Percent (%)		
		E. vulgare	*E. plantagineum*	*E. vulgare* + *E. plantagineum*
2019	March	0.5	12.4	12.9
	April	1.3	25.7	27.0
	May	1.9	39.5	41.4
	June	2.8	55.8	58.6
	July	2.1	59.2	61.3
	August	1.8	52.3	54.1
	September	0	0	0
2020	March	4.2	21.1	25.3
	April	7.2	42.8	48.0
	May	6.6	77.2	83.8
	June	7.5	88.4	95.9
	July	7.2	82.3	89.5
	August	3.8	72.5	76.3
	September	1.6	52.7	54.3

Each value of vegetation cover percent is from the average of five independent observations carried out on 25 × 25 cm plastic squares. Standard error of each mean was assessed as lower than 10% and it is not reported in the table.

(a) (b)

Figure 6. ENEA green roof sector with all vegetation completely dried after water stress imposition at the end of September 2019 (**a**). Vegetative blooming in April 2020 of *Echium plantagineum*, showing a high capacity for self-regeneration from the severe dehydration (**b**).

The observations of *E. plantagineum* during the experimentation on the GR in ENEA, in parallel with those conducted in the green areas around the ENEA Casaccia Research Center, provided evidence that the development of the plants growing in the GR followed almost the same phenology of the wild plants in the field. The only difference was that the blooms in field were longer, lasting up to October in the absence of lawn mowing.

3.5. Urban Ecosystem Service of Pollination

During the unstructured observations conducted on the GR and in the area of ENEA Casaccia R.C. to evaluate pollinator visitations in the 3rd year, it was assessed that both species were very appealing for pollinators, attracting a higher number of species from different orders (Hymenoptera, Lepidoptera, Coleoptera, Diptera, etc.). Several different insect species (Supplementary Materials, Figure S1), including bees (Supplementary Materials, Figure S2), were found during the natural blooming period on the plant flowers. Among the bees, several bumblebees (*Bombus* spp.), as the common *Bombus pascuorum* cf., and species from the *Anthidium* genus were observed and a few sightings in the area of ENEA Casaccia R.C. were reported at http://www.beewatching.it/#segnalazio, accessed on 29 July 2022 [54], an EU project aiming to identify and create an updated census of bee species in Italy.

4. Discussion

The experimentation conducted on the green roof (GR) at ENEA Casaccia R.C. was developed in alignment with the European "green policies" aiming at preserving flora and fauna (including entomological) biodiversity in urban environment [55]. The target plant species of this study, *Echium vulgare* L. and *Echium plantagineum* L., were selected from the bibliographic survey since they had exhibited drought-resistant behavior [51,56] and a long blooming to attract pollinators. The overall aim of comparing these two species was to assess (1) which of them could adapt better to Mediterranean GRs, and (2) if the role to attract and sustain wild bees conventionally played by *E. vulgare* in north EU and UK GRs could be played by *E. plantagineum* in Mediterranean GRs.

4.1. Comparison between E. vulgare and E. plantagineum and their Growth Performance on a Mediterranean GR

In our field survey, *E. vulgare* was not found according to ISPRA guidelines on wild Mediterranean herbaceous species which report it as non-common species (Table 2) [30]. Differently, *E. plantagineum* was only encountered with a low frequency in contrast with various sources reporting this species as very widespread (Table 2) [30,50]. The scarcity of these species in the monitored areas of the Lazio region, indicating that some adversities counteract their diffusion, represents an additional reason to recommend their inclusion into seed mixes for GRs, safeguarding them.

The morphological and morphometric comparative analyses of the two *Echium* species were oriented to provide accurate data to update the herbarium records [35,36,57] in relation to the geographical location of this study, i.e., Lazio Region (Figure 1). Indeed, since *E. vulgare* and *E. plantagineum* share numerous similar traits, misidentification between them has been often reported [31,57]. Therefore, several distinctive traits have been reported in the results to support their correct identification. Scanning electron microscopy (SEM) was used to explore seed morphological features, as it is recognized as a highly valuable technology for this purpose, and it allowed the observation that *E. plantagineum* with respect to *E. vulgare* presented larger seeds and a microstructure with much more prominent showy growths. Since the different morphology of the seed coat has been found to be associated with the protection and dispersal of seeds [58], one could hypothesize that the more protruding growths in *E. plantagineum* play a key role in the more complex and successful strategy of this species to self-regenerate and spread, becoming invasive in certain conditions [31]. The rosettes of the two species were easily distinguishable and *E. vulgare*, but not *E. plantagineum*, presented several multi-celled epidermis outgrowths (tubercles) such as trichomes (Figure 3a). Indeed, leaves of biennial and perennial species such as *E. vulgare* are known to possess multi-layered (up to five layers) tubercles, while tubercles in annual plants such as *E. plantagineum* are made up of only one cell layer [59].

Our experimentation highlighted that some key factors were significant in driving the more successful adaptation under adverse conditions of *E. plantagineum* than *E. vulgare*. As has also been found by other authors, under extreme conditions [32,60] *E. plantagineum* exhibited a considerably shorter life cycle than *E. vulgare*. It can be inferred that the short life cycle (annual cycle) facilitated a broader adaptation of *E. plantagineum* to the Mediterranean microclimate on the GR, thanks to the ability at the seed stage to face the hotter season. Besides a major fertility deduced by the high percentage of mature seeds (MS%, Table 3), *E. plantagineum* plants produced seeds continuously over time from March to December, in accordance with the high germination rate reported for this species when soil temperature ranges between 10 and 30 °C [31,60], that is from early spring to autumn in Mediterranean areas. Even after the imposition of a severe water stress in September, the seeds surveyed produced a beautiful blooming meadow on the GR in late autumn of the same year, giving proof of their good resistance against dehydration. On the other side, for *E. vulgare*, germination is typically achieved at warmer soil temperature of about 10–20 °C [31,60], i.e., during the Mediterranean summer. Under our GR conditions, after the water stress, even though the resumption of irrigation started in September when soil temperatures were favorable to seed germination, there was no development of *E. vulgare* plantlets due to the lack of fertile seed production (as for the low observed MS%, Table 3) or the low resistance to water deficit. Other factors, such as the development of the rosettes, represented a disadvantage for *E. vulgare* GR performance; indeed, they were not able to produce floral stems without vernalization at a low temperature [51].

The outputs of this study lead the authors to suggest that the annual life cycle may be advantageous for plants to resist the hot season on a GR, and to recommend *E. plantagineum* in Mediterranean extensive GRs and in dry roof gardens with a small substrate thickness (8–10 cm, as typical in *Sedum* GRs), also because of its taproot morphology, which is much shorter than in *E. vulgare* (Table 3; Figure 5). Unfortunately, due to the current knowledge, annual species such as *E. plantagineum* are rarely regarded for GR purposes, even though they represent an important part of the Mediterranean vegetation [25,32]. Concerning *E. vulgare*, its use fits better in extensive and semi-intensive GRs in temperate climates with a higher (15–20 cm) substrate thickness, endowed with summer irrigation to allow the completion of its biennial cycle and the development of its longer and robust taproot.

Up to now, *E. plantagineum* has only rarely been reported to be used in GRs [32]; presumably, its poor implementation may be due to its bad reputation as a menacing invasive plant in Australia. In their study of 2017, Zhu and colleagues [31] speculated that the high levels of genetic diversity, adaptive potential and resistance to environmental stresses, and capacity for producing defensive secondary metabolites of *E. plantagineum* could have contributed to its dramatic invasion in Australia. Anyway, there is no such risk in the native areas where this species has no invasive potential, as reported for the non-native America continent [61], and also resulting from the field survey in the center of Italy reported in this manuscript.

4.2. The Urban Ecosystem Service of Safeguarding Pollinators

Currently, urban ecosystems have been confirmed as highly suitable environments for hosting and protecting the life and activities of pollinating entomofauna, due to the lack of pesticides and seed tanning still spread in agricultural ecosystems, which have been ascertained as the main causes of the death of bees [62,63]. The genus *Echium* is notoriously appealing to pollinators [33,64]; therefore, by promoting its inclusion among the GRs vegetations, the authors aimed to stimulate the attractiveness of different pollinating insects, particularly wild bee species, and protect them at the same time. Given that at the Mediterranean latitudes the earliest bee species already appear in February–March and late species fly as long as the local weather conditions allow it, we wanted to verify the visits of pollinators on *E. plantagineum* in this period. As a result, the inclusion of *E. plantagineum* on Mediterranean GRs appeared to be tightened with the flight periods of early and late wild Apoidea species. In this context, *E. plantagineum* provides an important urban ecosystem service (UES) thanks to its long blooms, by reducing the mismatches caused by global warming between plant and their pollinators [65]. Further research is required to establish how and how much the green roof stress conditions may alter the relationships between the flowers and the pollinators, and the presence of green corridors and networks through the urban space to provide opportunities for pollinators to move along and across has to be considered at the same time [66], evaluating the regional geographic distribution of native species compared to non-native ones [67].

5. Conclusions

This manuscript reported a comparative study on two herbaceous plant species, *Echium vulgare* L. and *Echium plantagineum* L., with high potential for being included in mixes of seeds for Mediterranean green roofs (GRs) with very hot summers and water saving needs, and to prevent the biodiversity decline of pollinators. In the experimentation carried out on ENEA GR, located in a Mediterranean area, *E. plantagineum* was more suitable than *E. vulgare*, as it was able to germinate, grow, flower, and produce many seeds in a very short time period, and to self-propagate by overcoming the critical summer period. Moreover, the higher coverage attained by *E. plantagineum* plantlets and rosettes than *E. vulgare* as well as its shorter taproots grown in a shallow substrate (8 cm) make this species very suited for being incorporated in non-irrigated or poorly irrigated GRs in Mediterranean areas.

Furthermore, the morphological information provided by the comparative analysis of the two *Echium* species, including the SEM observation of seeds, will enrich the little taxonomic knowledge currently available on these non-agronomic plants, which is foreseen to augment in the next few years with the primary goal of promoting and conserving biodiversity. In conclusion, the outcomes of the current work are very relevant when planning the vegetation to be planted on a GR in urban environment, but further experimentation would be worthwhile to support the different stakeholders around GR technology adoption for climate resilience. Considering that roofs are an intensely urbanized district that may represent about 25% of the overall surface, their "greening" may have a significant positive effect on the local environment and human wellbeing; so, there is need of research to achieve more evidence related on plant species for GRs covering more geographical locations and contexts in the Mediterranean climate, in relation to the different UESs that are specifically required.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14159581/s1, Figure S1: Entomological biodiversity observed on *Echium vulgare* and *Echium plantagineum* on the green roof and in the area of ENEA Casaccia R.C. Figure S2: Bee pollinators biodiversity observed on *Echium vulgare* and *Echium plantagineum* on the green roof and in the area of ENEA Casaccia R.C.

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