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Ecology and Diversity of Solanum trisectum Dunal From Madeira Island: Implications for Its Conservation

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ABSTRACT

Solanum trisectum Dunal is an endemic Solanaceae of Madeira Island (Portugal), considered critically endangered due to its extreme rarity. Since its discovery this species has shown a narrow distributional range mainly on the northern side of the island, with scattered and small populations and large time gaps between sightings. The present work aimed to understand the underlying ecological underpinnings for this species rarity towards an effort to increase the success of conservation actions. Data on historical locations, environmental conditions field surveys, reinforcement in situ and ex situ germination trials were obtained. The species was found to currently occur only in two wild populations and two reinforcement sites, amounting to twenty-four plants in total. Statistical analysis of these data related wild populations and successful reinforcement sites, separating them from other sites where the reinforcement did not succeed. This suggests that the successful reinforcement sites showed conditions more similar to wild population sites compared to non-successful ones. The data obtained supports the hypothesis that S. trisectum distribution and rarity is determined by species specific habitat needs, i.e., abiotic conditions, which have been for the first time assessed during the present work. Further, this study also reinforces the classification of S. trisectum as a top priority species for conservation.

Keywords: Solanum trisectum, Propagation, Ecology; Population reinforcements, Conservation

INTRODUCTION

The Madeira archipelago is part of the biogeographic region of Macaronesia, located about 900 km southwest of the Iberian Peninsula and 630 km of the north-western Africa (Morocco) [1,2]. The native and naturalized vascular flora comprises around 1,186 taxa, of which 147 are endemic [2]. Madeira is the largest island of the archipelago and holds the third highest number of endemics and the second highest density of endemic taxa amongst all Macaronesian archipelagos [2]. The present-day degree of endemism in the island of Madeira is certainly related to its biological and geological history, patterns of human occupancy, subsequent loss of diversity, and the impact of non-native species [2]. The mountainous landscape hinders access to many areas, enabling the preservation of numerous habitats, such as the evergreen laurel forest, Laurisilva. This forest occupies around 20% of the total island area [3] and hosts 200 vascular taxa of which 76 are endemic [4] including some of the rarest Madeiran endemic plants [5] such as *Solanum trisectum* Dunal. Thus Laurisilva is one of the most important habitats of the archipelago in terms of its occupancy, distribution range and species richness. "Macaronesian laurel forests (Laurus, Ocotea)" are classified as a priority habitat for preservation in the European Union [6] and included in the European Union network of protected areas Natura 2000 [4].

Solanum trisectum has been reported to grow in the Madeiran Laurisilva [1,5,7-10]. This forest is characterized as a broad-leaved thermo-mesotemperate submediterranean humid to hyperhumid forest found mostly on andosols (AN)

[11]. Although occurring in a forest's environment, *S. trisectum* appears to grow more abundantly in open canopy areas with some degree of soil disturbance [5,8] *Solanum trisectum* an herbaceous annual plant, was first described in 1838 by Richard Thomas Lowe as Nycterium triphyllum (Figure 1). Later, Dunal [12] transferred the species into the genus *Solanum*. In 1872 Lowe created the genus Normania in order to incorporate the Madeiran endemic species renamed as Normania triphylla (Lowe) Lowe (Syn: *Nycterium triphyllum, Solanum trisectum*).

Figure 1: Detail of the flower of *S. trisectum*

Lowe suggested grouping it with the Canary Island endemism *Solanum* nava Webb and Berthel, a mere suffrutescent form or state of the Madeiran species. Unlike Lowe's Dunal taxonomy was followed by subsequent authors. Francisco-Ortega et al. [13] restated the generic status of Normania comprising two species *N. tryphylla* Lowe and *N. nava* (Webb and Berthel.) Franc.-Ort. and Lester. However Bohs and Olmstead [14] based on phylogenetic analysis from the chloroplast ndhF gene and the nuclear ITS region suggested that Normania and the monotypic genus Triguera from Spain and north-western Africa were sister taxa nested within a broadly defined genus *Solanum*. Subsequently, Bohs [15] supported the placement of Normania and Triguera within *Solanum* sect. Normania (Lowe) Bitter and confirmed the taxonomic treatment of Bohs and Olmstead [14].

The first known collections of *S. trisectum* date back to 1837 made by Lemann and by Lippold in two distinct localities the only ones mentioned by Lowe [7] in the original description. In 1872 Lowe considered *S.trisectum* to be a very rare species i.e. in several spots but only sparingly,a few detached plants here and there mentioning four populations and therefore adding two new locations. Several authors [1,13,16] reported a rediscovery of this species in 1991 by naturalist Priest Manuel Nóbrega. Faria et al. [10] and Garden et al. [5] later reported this species to persist in the wild with an estimated number of mature plants below 50. However shortly after Delmail and Autret [17] and Delmail et al. [16] did not confirm such findings considered instead that *S.trisectum* had become extinct in the wild after its rediscovery in 1991. Due to its recognized rarity *S.trisectum* was evaluated under International Union for Conservation of Nature (IUCN) criteria and classified as Critically Endangered (CR) in 2006 by Garden et al. [5]. It was later listed in the top 100 taxa prioritized for conservation and management in the archipelago of Madeira based on its narrow distribution and small number of populations and individuals [10]. However, despite matching the criteria required for a CR status, *S. trisectum* was not included in the most recent European IUCN Red List of vascular plants [18].

The most current information mentions the rarity of *S. trisectum* and its vulnerability to pressures and threats [5,10]. The pressures are related to its low populational growth and density potentially leading to inbreeding and genetic impoverishment processes and reduced habitat area either due to habitat degradation fragmentation by anthropogenic or/and natural catastrophic events [10,19]. The threats could be habitat competition by invasive species [5,10]. Garden et al. [5] considered that its long-term survival depends of the development of a conservation strategy based on ecological and biological information. Rare species conservation requires a detailed knowledge of habitat preferences [20]. *Solanum trisectum* is the only endemic species of this genus present in the Laurisilva, and could be an additional source of genetic interest since is a close relative of Solanaceae crops. The aim of the present work is to bring up new information on the distribution, habitat, ecological conditions and population dynamics of *S. trisectum*, to identify the causes of species rarity and design an efficient conservation strategy.

MATERIALS AND METHODS

Species distribution surveys

Thirty-one field surveys were carried out to 14 sites between April 2011 and July 2012. Information regarding species sites prospection was gathered from literature herbarium observations and from personal communications. Also information of reinforcements made by Madeira Botanical Garden-Eng. Rui Vieira Funchal (MADJ) between 1996 and 2010 were used for prospection (Table 1; Figure 2). The sites were: Ginjas (GI), Levada dos Lamaceiros (currently designated as Levada do Furado), Portela (POR), Vereda das Voltas (VV), Vereda do Galhano (GA), Trancuada (TR), Vereda da Cavaca (VC), Levada dos Cedros (LC), Chão da Ribeira (CR), Encumeada (EN), Portas da Vila do Porto Moniz (PV) or Forestry Services House (Porto Moniz), Ribeiro Frio (RF). Additional sites were selected with the aim of expanding the search for *S. trisectum* occurrence to other areas: Vereda da Terra Chã and Portal das Fontaínhas (PF) (Table 1, Figure 2).Some sites were not visited due to logistical difficulties, such as vague description unspecified place and difficult access. From the 14 sites visited 8 were selected to carried out the present work through the monitorization of the encountered populations and reinforcements actions. A numbered code was assigned to each studied site and a letter code was attributed to identify each plant spot when sites showed fragmented populations or were subdivided for assessment purposes: Encumeada (1a, 1b, 1c, 1d), Portas da Vila do Porto Moniz (2a, 2b), Trancuada (3a, 3b), Portal das Fontaínhas (4), Levada dos Cedros (5), Ribeiro Frio (6a, 6b), Vereda da Cavaca (7) and Chão da Ribeira (8a, 8b) (Table 2).

Table 1: Sites for prospection were selected based on literature, herbarium records, from personal communications and additional sites were selected with the aim of expanding the search for *S. trisectum* occurrence to other areas

Code	Sites	Information source	References	Origin	Presence in 2011	Sites studied (2011-2012)
GI	Ginjas	Historical	Lowe [7,8]]	Wild	No	No
VV	Vereda das Voltas	Historical	Lowe [8] ; Menezes [3] MNHN-P-P00490050 [1]; BM000072291a [2]; BM000072291b [2]	Wild	No	No
POR	Portela	Historical	K000414058 3; K000414059 4	Wild	No	No
LF	Levada dos Lamaceiros (currently designated Levada do Furado)	Historical	Lowe [7,8]	Wild	No	No
EN	Encumeada	F. Fernandes, pers. comm.	F. Fernandes [43], pers. comm.	Reinforcement	Yes	Yes
PV	Portas da Vila do Porto Moniz	F. Fernandes, pers. comm.	F. Fernandes [43], pers. comm.	Reinforcement	No	Yes
TR	Trancuada	Historical	MADM4741; MADJ05372;MADJ05373; MADJ05374	Wild and Reinforcement	No (The exact site is unknown)	Yes
PF	Portal das Fontaínhas	Selected place	-	Prospection	No	Yes
LC	Levada dos Cedros	Historical	MADJ11474	Wild	Yes	Yes
RF	Ribeiro Frio	F. Fernandes, pers. comm.	F. Fernandes [43], pers. comm.	Reinforcement	Yes	Yes
VC	Vereda da Cavaca	Historical	MADJ06439	Wild	Yes	Yes
CR	Chão da Ribeira	F. Fernandes, pers. comm.	F. Fernandes [43], pers. comm.	Reinforcement	No (The exact site is unknown)	Yes
VG	Vereda do Galhano	Historical	P. Gouveia [43], pers. comm.	Wild	No	No
VTC	Vereda da Terra Chã	Selected place	-	Prospection	No	No

[1]National Museum of Natural History https://science.mnhn.fr/institution/mnhn/collection/p/item/p00490050?listIndex=34&listCount=86 , 2011
[2]Natural History Museum, BM http://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb?q=Solanum+trisectum&view_id=6b611d29-1dcf-4c60-b6b5-4cbb69fdf4fe ,2011
[3] Royal Botanic Gardens http://specimens.kew.org/herbarium/K000414058 , 2011
[4]Royal Botanic Gardens http://specimens.kew.org/herbarium/K000414059 , 2011

In the references: The code MADM means Herbarium in the Funchal Natural History Museum; The code MADJ means Herbarium in the Botanical Garden of Madeira.

Population assessment

Populations of *S. trisectum* were assessed to identify pressures and threats, climate and soil requirements, topography plant community and population structure. In average three visits were carried out to each site with the measurement of populations and ecological parameters. Geographical coordinates and altitudes (Alt) were recorded using a GPS (Garmin Oregon 550t). The habitat, vegetation, e.g. floristic covers and possible threats around the populations of *S.trisectum* were assessed applying an ad-hoc sampling strategy to a perimeter stroke around that population [21] (Table 3). A total of 27 variables, including 4 biotic, 8 physical and 15 describing soil composition were monitored to characterize the main biotic and abiotic conditions.

Ecological characterization was performed by recording climatic and soil parameters. The soil was randomly sampled at each site (20 cm depth) and soil parameters were measured and its temperature (Tsoil) and soil moisture (SM) recorded using a Soil Sensor Reader probe (Spectrum Technologies Inc.). The soil pH was analyzed by standard water (H_2O) and potassium chloride (KCl) methods according to Pinheiro de Carvalho et al. [22] and organic matter (OrgM) content measured using the modified wet oxidation Walkley-Black method [23]. Mineral elements were quantified by flame atomic absorption spectroscopy method (Perkin Elmer Instruments, AAnalyst 800) according to Temminghoff and Houba [24], namely macronutrients (potassium (K), magnesium (Mg) and calcium (Ca)) and micronutrients (sodium (Na), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B)). Textural classification was determined by pipette method [25, 26], and soil fractions classified according to particle size and sand, silt or clay percentages. Other parameters measured included percentage of soil saturation (Sat), cationic exchange capability (CExC), assimilable potassium (Kass) and phosphorus (Pass) in the soil.

The plant community, population structure and topography were performed *in situ*. In each site the identification of species that existed around the populations (Sp) was recorded, as native species (SpN), introduced species (Spin) and endemic species (SpEn). The topography (Tp) was based on a set of categories, (i.e., (1) on a slope; (2) on undisturbed soil; (3) close to an irrigation canal; (4) close to a watercourse; (5) on a landslide). Population inventories were conducted to record the number of specimens. Whenever available, fruits and the number of seeds per fruit were counted. Some of the fruits in the plant were collected and the seeds were stored at the ISO Plexis Genebank and in the MADJ seed bank.

Propagation and reinforcement actions

In this work the reinforcement actions in the selected sites were carried out with seeds collected in Levada dos Cedros (LC) and plants germinated at University of Madeira. In ex situ germination trials seeds (40) collected in LC were sown in the same day of collection, and no growth hormones or other treatment were added. Rich organic soil collected from wild populations was used for the ex situ germination trials performed under open-air environmental conditions (in outdoor temperature, with natural daylight and rainfall) at the University of Madeira. Ex situ germination rate was recorded at the end of a 5-month period. The plants obtained were later used in population reinforcements that were carried out in Trancuada (TR), Chão da Ribeira (CR), Encumeada (EN), Ribeiro Frio (RF) and in Portal das Fontaínhas (PF). One plant provided by the MADJ was placed in the Portas da Vila do Porto Moniz (PV). Plant survival (or establishment) in each site was recorded 2 months after the plantation.The *in situ* germination trials were performed using seeds collected in LC, these seeds were stored at the ISOPlexis Genebank. The trials were carried out in TR, CR, EN, RF, PF and PV. The seeds used have not suffered any treatment process or hormones.Germination trials both *in situ* and *ex situ*, were conducted after soil disturbance and the seeds were scattered directly in the soil. Germination success was calculated at the end of trial. The seeds were recorded as germinated whenever they showed an emerged radicle [27].

Data treatment

Statistical analyses were performed using SPSS (Statistical Package for the Social Science) version 22.0 for Windows and MVSP (Multivariate Statistical Package) version 3.22 for Windows. The Kolmogorov-Smirnov non-parametric test was applied to test data normal distribution. Multivariate Analysis of the studied sites based on 27 variables were performed. The Principal Component Analysis (PCA) was run using standardize data, loge transformed, and axes were extracted using Kaiser's rule. The F test of One-Way ANOVA and Tukey HSD post-hoc test was applied to evaluate variance and robustness of sites classification. The differences were considered statistically significant when $p \leq 0.05$.

RESULTS

Species distribution surveys

Solanum trisectum was seen in 13 natural occurring sites between 1837 and 2008 and in 12 reinforcements sites made by Madeira Botanical Garden Funchal (MADJ) between 1996 and 2010 (Figure 2) of the 13 natural occurring sites 12 lied along the northern side of the island and only one Ribeira de Santa Luzia (Funchal) was in the south (Figure 2). The field surveys undertaken in the present work have revealed that the populations of Levada dos Cedros (LC), Vereda da Cavaca (VC), Encumeada (EN) and Ribeiro Frio (RF) is persisting (Table 2 and Figure 2).

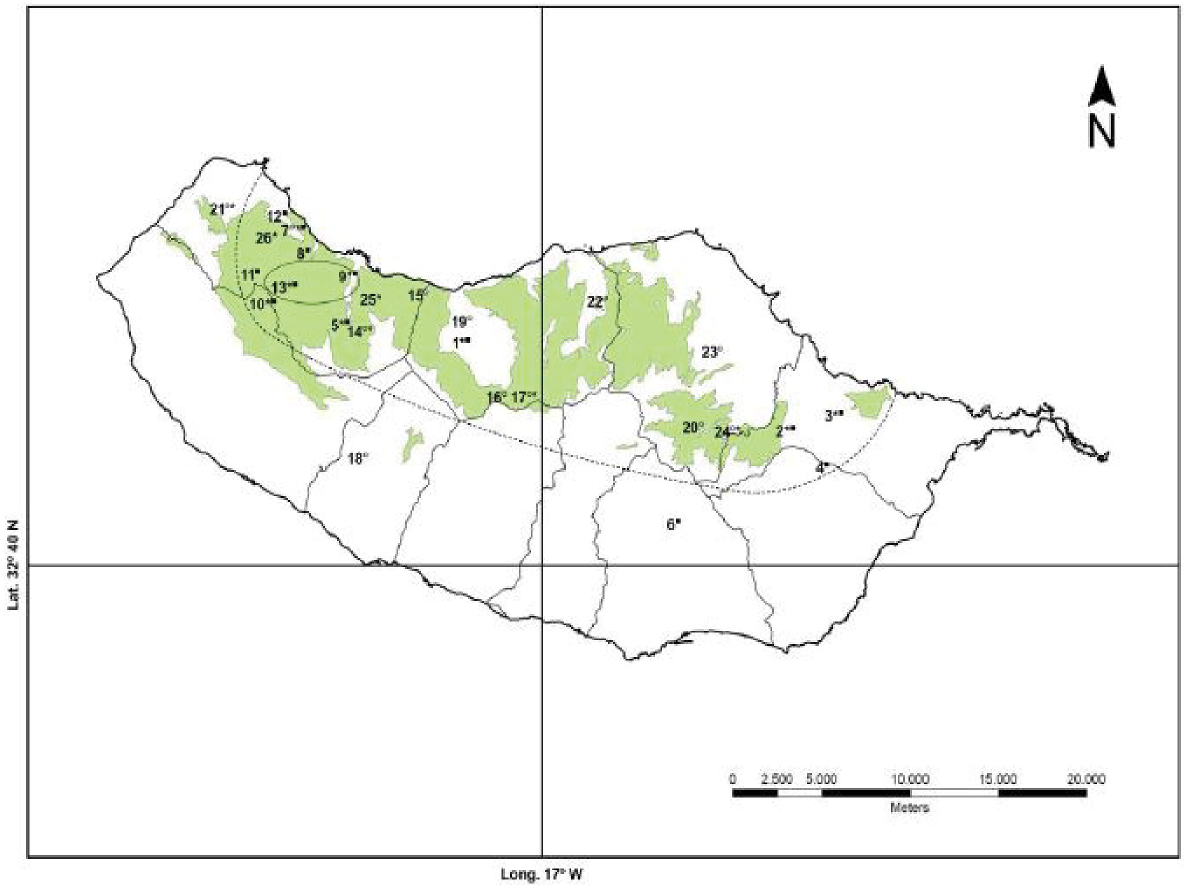

Figure 2: Map of Madeira Island and areas of prospection and occurrence of *S.trisectum*.

1: Ginjas; 2: Levada dos Lamaceiros (currently designated Levada do Furado); 3: Portela; 4: Santo António da Serra; 5: Vereda das Voltas; 6: Ribeira de Santa Luzia; 7: Trancuada; 8: Ribeira Funda - Fanal; 9: Vereda da Cavaca; 10: Vereda do Galhano; 11: Levada da Central da Ribeira da Janela; 12: Ribeira da Janela; 13: Levada dos Cedros; 14: Chão da Ribeira; 15: Ribeira do Inferno (São Vicente); 16: FSH at Encumeada (São Vicente); 17: Encumeada (São Vicente); 18: FSH at Malhadinha (Ponta do Sol); 19: FSH do Passo (São Vicente); 20: FSH at Fajã da Nogueira (Santana); 21: FSH (Porto Moniz); 22: FSH at Fajã do Penedo (São Vicente); 23: FH at Pico das Pedras (Santana); 24: FSH at Ribeiro Frio (Santana); 25: Vereda da Terra Chã; 26: Portal das Fontaínhas.

The symbols: – means the sites described in literature and herbarium; º – means the sites were the population reinforcement took place in 1996-1998 and 2009-2010; * – means the sites were the surveys were made in this work; (----) Borders of the hypothetical species distribution; (–) Borders of wild populations; ( ) Area of occupancy of laurel forest – Laurisilva.

FSH: Forestry Services House; FH: Forestry House

Table 2: Results on *S. trisectum* censuses and results on population reinforcements actions carried out in the present work.

Numerical codes are according to those used for the PCA analysis. SdSw – Nº. of seed sown; SdGerm - % of seed germination; PlEm – Nº. of plants emerged; EstPl – Nº. of established plants; EstRt - Establishment rate, i.e. nº. of established seedlings or plants/ total nº. of seeds sown or small plants used in reinforcement.

Code		Sites	Altitude (m a.s.l)	Type of site	Initial census (2011)	*In situ* and ex situ germination trials and reinforcements				Reinforcements with plants			Final census
					Plants (nº)	SdSw (nº)	SdGerm (%)	PlEm [1] (nº)	EstRt (%)	Plants (nº)	EstPl (nº)	EstRt (%)	Plants (nº)
EN	1	Encumeada	920	R	4	35	0	-	-	4[b]	4	100	8
PV	2	Portas da Vila do Porto Moniz	709	R	-	20	5	1	5	1[a]	-	-	-
TR	3	Trancuada	538	H	-	10	0	-	-	4[b]	3	75	-
PF	4	Portal das Fontaínhas	735	P	-	-	0	-	-	2[b]	2	100	2
LC	5	Levada dos Cedros	869	H	10	-	0	-	-	-	-	-	10
RF	6	Ribeiro Frio	900	R	2	30	0	-	-	2[b]	-	-	-
VC	7	Vereda da Cavaca	670	H	4	-	0	-	-	-	-	-	4
CR	8	Chão da Ribeira	540	R	-	20	25	5	25	2[b]	2	100	-
-	-	University of Madeira	n/a	n/a	n/a	40	47.5	15	35	n/a	n/a	n/a	n/a
			Total (nº of plants)		20	155	25	21	13	15	10	73.3	24

H - Historical reported sites (See Figure 2)
R - Reinforcement sites according to *F. Fernandes* (pers. comm. and Figure 2)
P - Prospection of new sites
[1]Plants reaching the 5-leaved developmental stage
[a]Plants obtained from cuttings at the Madeira Botanical Garden – Eng. Rui Vieira, Funchal (MADJ)
[b]Plants obtained from germination trials at the University of Madeira facilities
n/a: Not applicable.

Population size and structure

The surveys of 14 assessed sites in 2011 (Table 1) allowed recording a total of 20 plants 14 plants in the LC and VC populations and 6 plants in the reinforcement sites of Encumeada (EN) (4 plants) and Ribeiro Frio (RF) (2 plants) (Table 2). Some plants of LC (6) and RF (1) produced a total of 34 fruits and 660 seeds, an average of 19 seeds (5 to 35 seeds) per fruit. During the population survey of the adult plants of LC in wild conditions, were collected 32 fruits (average 6.4 fruits collected per plant), 620 seeds (average 19.4 seeds collected per fruit) in 2012 only the LC population showed plants at different developmental stages from seedlings (7 specimens) to adult fructifying plants (3 specimens). The VC population held only 4 immature plants and in the reinforcement sites, EN showed 8 plants and Portal das Fontaínhas (PF) 2 plants.

Propagation and reinforcement trials

The success of germination *in situ* was considered when the seeds showed an emerged radicle. Was verified a low germination success (25%), with all the plants failing to reach the 5-leaved developmental stage (Table 2). However when reinforcements were performed with plants from a nursery at the 5-leaved development stage the establishment rate reached 73.3% of planted specimens (Table 2). The *ex situ* germination trials showed a higher germination rate reaching 47.5%, with a plant survival rate of 35.0% (Table 2).

Habitat

Existing wild populations grow at 869 m a.s.l. in Levada dos Cedros (LC) and at 670 m a.s.l. in Vereda da Cavaca (VC)

(Table 2) near human-built water channels (levadas) or rocky slopes mostly in openings or clearings of Laurisilva. The tree stratum in both sites (LC and VC) was dominated by Ocotea foetens (Aiton) Baill, Laurus novocanariensis Rivas Mart et al., Clethra arborea Aiton and Myrica faya (Ait.) Wilbur (Table 3), indicating that *S.trisectum* can be found growing in a Clethro arboreae-Ocoteetum foetentis JC Costa, Lousã, Fontinha, Jardim, Sequeira and Rivas-Martinez (temperate til laurisilva) environment according to the latest syntaxonomical classification of Madeiran vegetation [11] (Table 3).

Table 3: List of species found in each site. Nomenclature follows Borges et al (2008)

		EN	PV	TR	PF	LC	RF	VC	CR
Acer pseudoplatanus L.	i						x		
Ageratina adenophora (Spreng.) R.M. King & H. Rob.	i	x	x			x	x		
Apollonias barbujana (Cav.) Bornm	MAC					x			
Asplenium sp.	n					x			
Blechnum spicant (L.) Roth subsp. *spicant*	n	x			x			x	
Carex sp.	n							x	x
Cedronella canariensis (L.) Webb & Berthel.	MAC	x				x		x	x
Clethra arborea Aiton	END	x	x	x	x	x	x	x	x
Cryptomeria japonica (L.f.) D.Don.	i		x				-		
Cytisus scoparius (L.) Link subsp. *scoparius*	i						x	x	
Diplazium caudatum (Cav.) Jermy	n			x	x			x	x
Duchesnea indica (Jacks.) Focke	i	x							x
Erica platycodon Webb & Berthel. subsp. *maderincola* D.C. McClint.	END	x	x	x	x	x	x	x	x
Erigeron karvinskianus DC	i	x	x					x	
Eucalyptus globulus Labill.	i		x	x					
Euphorbia mellifera Aiton	MAC							x	x
Festuca donax Lowe	END	x		x	x		x	x	x
Geranium palmatum Cav	END								x
Hymenophyllum tunbrigense (L.) Sm.	n	x							
Hypericum grandifolium Choisy	MAC				x	x		x	
Laurus novocanariensis Rivas Mart., Lousã, Fern. Prieto, E. Dias, J.C. Costa & C. Aguiar	MAC	x	x	x	x	x	x	x	x
Myrica faya Aiton	n	x		x	x	x	x	x	x
Myrtus communis L.	n					x			
Ocotea foetens (Aiton) Baill.	MAC	x	x	x	x	x	x	x	x
Persea indica (L.) Spreng.	MAC							x	x
Phyllis nobla L.	MAC							x	x
Picconia excelsa (Aiton) DC.	MAC						x	x	x
Pinus pinaster Aiton	i			x					
Plagiomnium undulatum (Hedw.) T.J.Kop.									x
Porella canariensis (F.Weber) Underw.	n								x
Pseudoscleropodium purum (Hedw.) M.Fleisch.			x						
Rubia agostinhoi Dans. & P. Silva	n	x		x		x	x	x	x
Rubus sp.	n	x		x		x	x	x	x
Selaginella denticulata (L.) Spring	n							x	x
Sibthorpia peregrina L.	END	x	x	x	x	x	x	x	x
Sideritis candicans Aiton	END							x	
Solanum nigrum L.	np					x		x	
Ulex europaeus L. subsp. *latebracteatus (*Mariz) Rothm.	i		x	x			x		
Vaccinium padifolium Sm.	END	x		x	x	x	x	x	
Viola odorata L.	n					x			
Woodwardia radicans (L.) Sm.	n				x			x	

The x indicates the presence of species. EN: Encumeada; PV: Portas da Vila do Porto Moniz; TR: Trancuada; PF: Portal das Fontaínhas; LC: Levada dos Cedros; RF: Ribeiro Frio; VC: Vereda da Cavaca; CR: Chão da Ribeira. END: Endemic to the island of Madeira; MAC: Endemic to Macaronesia; n: Native; np: Possible native; i: Introduced.

Abiotic conditions

Major soil features of the assessed sites are shown in Table 4. The majority sites assessed showed soil classification of dystic rugged terrain (EN, PV, PF, LC, VC and CR) and andosols (TR and RF) (Table 4). Overall soil pH varies between 4.1 in TR (3a) and 5.7 in EN (1c). Sites holding plants showed pH between 4.4 and 5.7. Soil moisture ranged between 7.2% and 37%, in RF (6b) and EN (1c), respectively. In sites with plants these values varied between 12.7% (RF 6a) and 37% (EN 1c). Soils were moderately rich in organic matter being higher in reintroduction sites reaching 21.7% (Table 4). Overall the surveyed soils showed higher contents of macronutrient K than P except in PF, where P was higher than K (Table 4). The analysis of other soil parameters showed significant differences ($p \leq 0.05$) in soil macronutrients (e.g., Mg and Ca) and micronutrients (e.g., Fe and Zn). They revealed a trend in the soils of wild populations, with narrow variation of these nutrients when compared with the other soil samples (data not shown).

Table 4: Soil ecological characterization of wild population and population reinforcement sites of *S.trisectum.*

Sites	Codes		Soil texture type	Moisture (%)	pH[a]	Organic Matter (%)	P (mg. Kg^{-1})	K (mg. Kg^{-1})	Soil classification (Ricardo et al., 1992)
Encumeada	EN	1[a]	1	31.7	4.6	21.74	46	360	TAd
		1b	2	29.7	4.8	21.14	46	360	TAd
		1c	3	37	5.7	7.73	46	180	TAd
		1d	2	36.1	4.9	21.16	46	300	TAd
Portas da Vila do Porto Moniz	PV	2a	1	31	4.5	15.06	46	120	TAd
		2b	1	12.2	5.2	13.02	46	132	TAd
Trancuada	TR	3a	4	33.6	4.1	21.72	46	264	AN
		3b	1	31.8	4.7	10.34	64	156	AN
Portal das Fontaínhas	PF	4	1	36.6	5.3	10.34	779	336	TAd
Levada dos Cedros	LC	5	5	16.1	4.4	10.34	82	240	TAd
Ribeiro Frio	RF	6a	1	12.7	4.5	10.34	96	372	AN
		6b	5	7.2	4.5	10.34	114	612	AN
Vereda da Cavaca	VC	7	1	21.4	5.4	6.94	55	528	TAd
Chão da Ribeira	CR	8a	5	30	4.6	10.34	243	444	TAd
		8b	5	34	4.5	10.34	114	516	TAd

Soil texture type 1: silty clay loam; 2: clay; 3: sandy loam; 4: silt loam; 5: clay loam. Soil classification: Tad: dystric rugged terrain; AN: andosols

[a]water standard method

Statistical analyses

The abiotic and biotic characterization of the assessed sites was statistically analyzed using 27 variables (Table 5). These variables showed a normal distribution according to the Kolmogorov-Smirnov non-parametric test. The variables, organic matter, macronutrients (Ca and Mg), micronutrients (Zn, Fe, Mn and B) and soil physical features (Sat, silt and clay), according to ANOVA, were significant in the variation ($p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$). In the PCA analysis, group 2 was composed by two microsites of EN (1b and 1d) holding specimens, and group 3 joined the sites and microsites of wild populations LC (5) and VC (7) with the reinforcement sites of EN (1c), TR (3b) and RF (6a) (Figure 3), where some plants persevered. Statistically, the most relevant variable was Fe (Table 5), influencing only the separation of the group 2. According to the Tukey HSD post-hoc test, 100% of sites were correctly classified into these groups. This analysis showed that compositional (OrgM, Ca, Mg, B, Zn, Mn and Fe) and physical (Sat, Silt, Clay) variables exhibited significant differences between the groups ($p \leq 0.05$) (Table 5).

Table 5: Tukey HSD multiple comparisons between PCA groups, showing variables allowing separation the groups: Tukey HSD multiple comparisons between PCA groups, showing variables allowing separation the groups: Species (Sp); Native species (SpN); Introduced species (Spin); Endemic species (Spen); Topography (Tp); Altitudes (Alt); Soil temperature (soil (ºC)); Soil moisture (SM (%)); soil pH analyzed by standard water (pHH2O); soil pH analyzed by standard potassium chloride (pHKCl); Organic matter (OrgM); Assimilable phosphorus in the soil (Pass); Assimilable potassium in the soil (Kass); Calcium (Ca); Magnesium (Mg); Potassium (K); Sodium (Na); Cationic exchange capability (CExC); Percentage of soil saturation (Sat.); Boron (B); Copper (Cu); Zinc (Zn); Manganese (Mn); Iron (Fe)

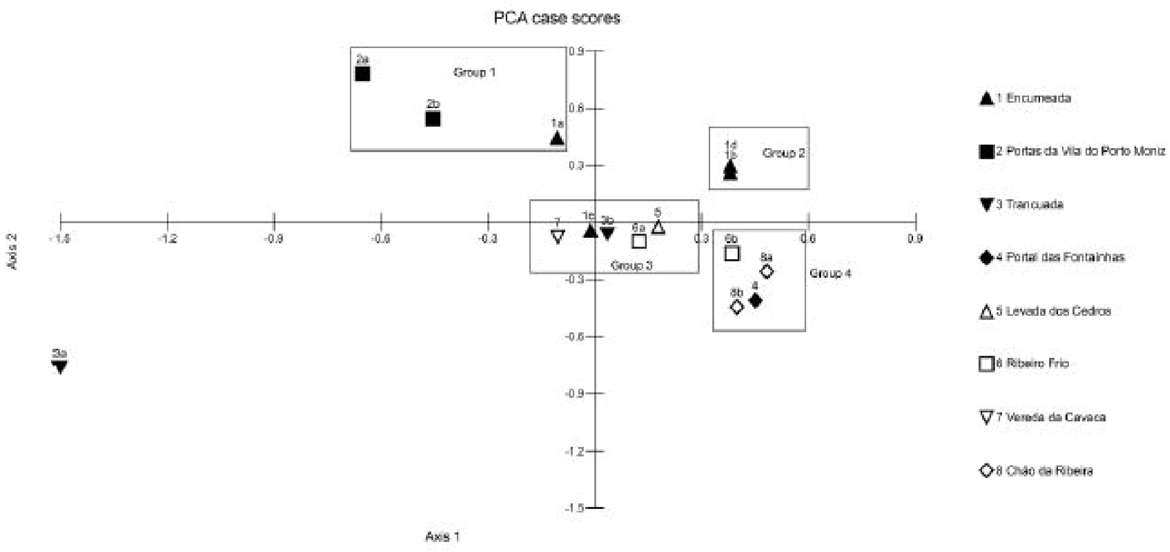

Figure 3: Representation of the Euclidean biplot by principal component analysis (PCA) with of all the parameters (variables) in analysis. However, only the variables Fe, Ca, Mg, Zn, Sat and Silt showed a significant variation between groups and contributed to their separation in the PCA analysis.

Variables	Groups			
	1	2	3	4
Sp	12.67 ± 2.89	16.00 ± 0.00	17.40 ± 4.39	17.25 ± 4.50
SpN	7.67 ± 4.62^{a,b}	13.00 ± 0.00^{a}	14.40 ± 4.56^{b}	15.25 ± 4.35
Spin	4.33 ± 1.16	3.00 ± 0.00	2.60 ± 1.14	1.50 ± 1.73
Spen	6.00 ± 1.73	8.00 ± 0.00	9.20 ± 2.78	10.00 ± 2.31
Tp	1.00 ± 0.00	1.00 ± 0.00	2.20 ± 1.30	3.00 ± 2.31
Alt	779.33 ± 121.41	919.50 ± 0.71	775.40 ± 161.94	680.25 ± 173.60
soil (ºC)	11.07 ± 1.62	8.35 ± 0.21	9.38 ± 1.30	9.88 ± 0.63
SM (%)	24.97 ± 11.06	32.90 ± 4.53	23.80 ± 10.33	26.95 ± 13.44
pHH2O	4.77 ± 0.38	4.85 ± 0.07	4.94 ± 0.58	4.73 ± 0.39
pHKCl	4.03 ± 0.31	4.10 ± 0.14	4.46 ± 0.36	4.53 ± 0.32
OrgM	16.61 ± 4.56^{b,c}	21.15 ± 0.01^{d,e}	9.14 ± 1.67^{b,d}	10.34 ± 0.00^{c,e}
Pass	46.00 ± 0.00	46.00 ± 0.00	68.60 ± 20.29	312.50 ± 18.34
Kass	204.00 ± 135.23	330.00 ± 42.43	295.20 ± 154.75	477.00 ± 116.50
Ca	3.00 ± 2.17^{c}	21.40 ± 5.09^{e}	16.48 ± 7.27	36.98 ± 8.28^{c,e,}
Mg	4.67 ± 2.78^{c}	10.50 ± 1.56^{e}	8.30 ± 1.25^{f}	17.68 ± 3.81^{c,e,f}
K	0.50 ± 0.35	0.90 ± 0.14	0.84 ± 0.52	1.45 ± 0.48
Na	0.20 ± 0.00	0.30 ± 0.14	0.28 ± 0.13	0.40 ± 0.08
CExC	75.03 ± 19.75	98.70 ± 0.71	67.74 ± 20.58	91.58 ± 11.33
Sat.	10.00 ± 6.08^{b,c}	33.50 ± 6.36^{e}	39.00 ± 9.98^{f}	62.50 ± 13.87^{c,e,f}
B	1.20 ± 0.17^{c}	1.15 ± 0.21	0.66 ± 0.36	0.50 ± 0.00^{c}
Cu	0.90 ± 0.69	1.25 ± 1.06	1.82 ± 0.73	2.68 ± 1.46
Zn	1.67 ± 0.58^{b,c}	2.00 ± 1.41	5.84 ± 2.75^{b}	6.30 ± 0.62^{c}
Mn	23.33 ± 4.16^{a}	135.00 ± 35.36^{a,d}	61.00 ± 18.00^{d}	73.75 ± 34.37
Fe	576.67 ± 280.06	740.00 ± 127,28^{d}	294.00 ± 110.36^{d}	487.50 ± 108.13
Sand	18.00 ± 5.20^{a}	21.00 ± 0.00	43.80 ± 22.19	41.50 ± 6.28

Silt	46.00 ± 1.73[a,b,c]	2.00 ± 0.00[a,d,e]	27.20 ± 11.86[b,d]	24.75 ± 4.57[c,e]
Clay	36.00 ± 3.46[a]	77.00 ± 0.00[a,e]	29.00 ± 12.37[d]	33.75 ± 6.29[e]

Different letters in the same column represent statistically different results (p ≤ 0,05) by Tukey HSD test; a - between 1 and 2; b - 1 and 3; c - 1 and 4; d - 2 and 3; e - 2 and 4; f - 3 and 4.

DISCUSSION

Species distribution surveys

The comparison of the literature herbarium observations personal communications and reinforcements with our surveys reveal a significant reduction of the species former geographical distribution and number of populations (Table 1; Figure 2). Moreover the earlier reports showed that populations located in areas continuously exposed to anthropogenic pressures, such as competition by invasive species, land use for agriculture, forestry, pasture and forest fires [7, 8]. It might have impacted strongly the species distribution from the early days of human settlement up to the present day, but it is difficult to postulate on the relative weight that they might had on the species abundance and distribution.

Habitat

Historical data [8] Nóbrega pers. comm. 2013; herbarium specimens held in MADJ and in MADM] and present-day data on geographical distribution. Figure 2 showed that *S. trisectum* occurs in association with the plant community of the temperate til laurisilva [5, 9]. However, *S. trisectum* was not found in the understory of this habitat, but instead, in forest openings or clearings and edges, along footpaths or near water channels, and in recently disturbed sites, as stated by Jardim et al. [5], Lowe [8] and Nóbrega (pers. comm. 2013). In the present work, this was demonstrated in the LC population where the species was found in a forest clearing, holding small sized, but age-structured population. However, the ephemeral character of such habitats due to natural plant succession might lead to the population disappearance within a short time period.

The presence of *S. trisectum* in some types of habitats that might share a set of features could be determinant for the species establishment, such as specific abiotic features. The assessment of *S. trisectum* occurrence reinforcement and potential sites using 27 biotic and abiotic variables aimed to analyze such hypothesis. Madeira Island has an altitudinal gradient and orography that can impose significant variation in the biotic and abiotic conditions, which may affect species adaptation [23]. Therefore abiotic conditions including soil composition could determine the rarity of *S.trisectum* as occurs with other plant species [20]. The *S. trisectum* soil types fall under the classification of andosols according with Driessen et al. [28] and ISRIC [29] haplic umbric andosols and dystric rugged terrain according with Ricardo et al. [30]. The biotic and abiotic variables analysis using multivariate analysis showed that the major differences were found between group 3 and groups 1 and 4, and involve organic matter macronutrients (Ca, Mg, K, P) and micronutrients (Zn, Fe, B, Mn), with species sites showing more narrow variation of soil features (Table 5). In fact it has been recognized that the bioavailability of phosphorus and magnesium decreases in extremely acidic environments with high aluminum concentration [23]. Moreover the field observations found plants of *S.trisectum* in PF, EN and LC with chlorosis of old leaves which progress to the young leaves. These symptoms are consistent with magnesium deficiency [31], a detrimental plant disorder that occurs most often in strongly acidic, light and sandy soils [32].

The occurrence of populations of *S. trisectum* in disturbed habitats could also be related with the species requirements for specific conditions such as an open space and sunlight. This kind of habitat could be favored by stochastic major disturbances such as landslides. Thus undisturbed forests, with dense canopy and without open spaces reduces the availability of suitable niches for *S. trisectum*. The influence of elements like rainfall, air temperature and insolation on *S. trisectum* needs to be monitored. These observations can be substantiated by the fact that Solanaceae family or members are known to play a role as light gap colonizers in the ecosystem and as pioneer species of disturbed areas such as clearings, roadsides and forest edges [33,34]. Therefore *S. trisectum* could be fulfilling its ecological role as habitats colonizer. In fact seeds of pioneer species usually display some form of dormancy so that they can remain viable in the soil for long periods [34]. Thus the long-term soil seed bank viability could also explain the large time gap seen in some sites described in literature but not confirmed in this work. The results have shown a single site (LC) with a mature and well-structured population that appears to indicate that this species is dependent on very specific habitat conditions. Moreover is still necessary to pursue intensive surveys to locate new wild populations that are fundamental for building up a more accurate characterization of species optimal habitat.

Population dynamics

S. trisectum survival might require a life strategy to ensure its reproductive success. The results of ex situ germination trials showed higher germination rates (47.5%) than *in situ* (5.2%) (Table 2). This could suggest that seeds had greater ability to germinate under suitable soil and climatic conditions e.g., longer exposure to sunlight higher water input and average temperatures. However no specific experimental trials were conducted to establish the importance and effectiveness of each parameter in determining germination rate.

Annual plants, such as *S. trisectum* showing large populations fluctuations would be highly dependent on large seed production and availability, long-term seed viability soil seed bank and dormancy mechanisms. This behavior of seed dormancy evolved as a life strategy allowing a species to ensure minimal sizes viable population, waiting for proper niche or adequate ecological conditions for reestablishment [35,36]. A *S. trisectum* adult plant in optimal conditions produces an average amount of fruits and seeds per plants that is consistent with this strategy. This survival strategy is highly dependent on a seed bank which in the case of *S. trisectum* is supplied by a reduced number of adult plants in the population 10 in LC population during both monitoring seasons. Low germination rates *in situ* are consistent with a survival strategy depending of soil seed bank. These low numbers contrast with other *Solanum* species which release a higher number of seeds [37-39]. Therefore survival strategy of *S. trisectum* seems not to rely on a high seed production. Furthermore, the comparison between the number of seeds produced and the number of individual plants in the populations indicates a low rate of plant recruitment, which can be related either with seed germination success, plant survival due to predation detected in the field surveys or even other ecological factors. Several factors can be responsible for species rarity in nature, being either due to intrinsic aspects, dispersal capacity, abiotic conditions, or habitat specificity [20, 40] or yet a combination of these factors.

The success of open areas colonization such as appears in *S. trisectum* appears to thrive could be favored by pollination and seed dispersal [33] so the role of both factors should be analyzed. On the other hand seed dispersal by zoochory as seen in Solanaceae could enable the spread of this species along paths and forest edges through feeding routes, enrichening seed banks and facilitating regeneration [33]. These observations suggest a role of pollination and seed dispersal in the conservation of *S.trisectum* but their precise contribution is still unknown.

Pressures and threats

Habitat competition due to the spread of invasive species was considered by Jardim et al. [5] as the only serious pressure and threat posed to the survival of this rare species. The present work points out to a broader set of pressures, namely: inbreeding depression and genetic drift; habitat intrinsic constrains and natural pressures by herbivores (observed at VC and PV); disruption of the ecosystem (e.g., landslides); climatic factors as predicted by Santos and Aguiar [41]. This work points out another pressures as direct and indirect anthropogenic actions: vegetation cutting and cleaning; specimens destruction and collection; application of herbicides for weed control observed at LC and attack by pests. The potential impact of insect plagues on wild populations should also be considered. In fact whiteflies (Aleyrodidae) and the cosmopolitan black cutworm Agrotis ipsilon Hufnagel 1766 were observed in ex situ germination trials, resulting in plant damage. Also, larvae of an unidentified species of Chrysodeixis Hübner 1821 (Noctuidae) was found *in situ* at VC site. The species of this genus is known to feed on various plant species and can be considered a plants pest. One of threats detected in the present work was competition by exotic plants. The above-mentioned types of pressures and threats fit, in general terms, within those considered to be major threats to rare species on islands [19,42].

Conservation efforts

The campaigns led by the Madeira Botanical Garden, Funchal (MADJ), between 1996 and 2010 aiming *in situ* species reinforcement are shown in Figure 2. The first conservation efforts were led by Nóbrega in 1994, involving collecting seeds at TR that were later sent to J. Y. Lesouëf at the Conservatoire Botanique National de Brest (CBNB) [43; Nóbrega pers. comm.]. Plants and seeds obtained at the CBNB were subsequently used in population reinforcements (Figure 2) carried out in 1996 and 1998 [F. Fernandes and S. Fontinha pers. comm], and again in 2009 and 2010 [F. Fernandes, pers. comm]. Overall, 12 reinforcement trials, using 3,500 seeds and 17 plants were conducted (data not shown). Seed reinforcements showed very low success, with a germination rate of 0.6% and plant establishment rate of 12%. The census conducted in 2011 showed the persistence of plants only in two of those reinforcement sites, EN and RF (Table 2). In comparison, the use of nursery plants sown in the wild gave higher success rates 12% in the work made by MADJ and 73.3% in this work (2011-2012) in plant establishment (Table 2). After 14 years of reintroductions only 6 plants were found to persist at reinforcements sites, making clear that these efforts had minimal improvement in species conservation status. It was not possible to draw up any definitive conclusions on the outcomes of the *S.trisectum* reinforcement trials. However, van Wieren [44] refers to the number of individuals of the founding population as crucial for achieving the desired levels of success. Thus, the number of plants and the randomly site selection used in *S. trisectum* reintroduction efforts possibly has not been the most suitable, making them more susceptible to increased extinction risks as small populations facing environmental fluctuations, demographic stochasticity and inbreeding depression [44]. Therefore it would be preferable to release a high number of individuals in sites with higher quality habitat [44] implying a bigger chance of success. For *S.trisectum* this means the release of a higher number of individuals per site and in fewer sites selected based on a robust knowledge of species habitat requirements such as the ones gathered in this work.

CONCLUSIONS

In this work, the assessment and description of *S. trisectum* habitat features was performed for the first time. The germination reported is the most precise estimations of this species reproductive success available albeit they represent very rough estimations. *Solanum trisectum* appears to have a survival strategy that copes with ephemerality of its populations showing endogenous physiological dormancy and low germination rates under non-optimal conditions. The low outcome of the *S. trisectum* conservation efforts showed that reinforcements trials should release a higher number of individuals and to target high quality habitat that suits its ecogeographic specific requirements. The habitat assessment performed in this study allows determining the species ecogeographic baseline, which allows monitoring and establishing a clearer strategy for site selection, as well as an urgency for reinforcement actions that needs to be better designed. Moreover, the present study unraveled some of the potential key habitat factors (soil and vegetation) responsible for the *S.trisectum* occurrence and rarity in nature.

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COMPETING AND CONFLICTING INTERESTS

The authors declare no conflict of interest.

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