Short Communication

Conservation status of the narrow endemic gypsophile *Ononis tridentata* subsp. *crassifolia* in southern Spain: effects of habitat disturbance

Miguel Ballesteros, Ana Foronda, Eva María Cañadas, Julio Peñas and Juan Lorite

Abstract *Ononis tridentata* L. subsp. *crassifolia* (Leguminosae) is a narrow endemic plant restricted to gypsum outcrops in south-east Spain. Its habitat and populations are currently threatened by anthropogenic disturbance. Because of the paucity of information concerning its distribution, abundance and response to disturbance, we assessed its status and evaluated the impacts of quarrying, ploughing, grazing and afforestation. Distribution and population size were estimated by field surveys, censuses and mapping. We measured cover, plant volume, fruit and seed production, seed predation and seedling recruitment to asses any effects of disturbance. The species' area of occupancy is 1.6 km^2 and its extent of occurrence 337 km^2, in 29 habitat patches and 16 populations between 705 and 1,125 m altitude, and its population size was estimated to be 531,605. Quarrying, ploughing, overgrazing and afforestation negatively affected the species, in this order. We recommend this subspecies be categorized as Vulnerable on the IUCN Red List. A species recovery plan is required, and the ecological restoration of altered areas would mitigate negative effects on the species and improve the overall conservation of gypsum habitats.

Keywords Conservation status, grazing, gypsophile, gypsum quarrying, habitat disturbance, narrow endemic, *Ononis tridentata*, ploughing

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Gypsum outcrops harbour rare and endemic flora restricted to this substrate (Mota et al., 2004, 2011), which is often negatively affected by human disturbances, especially quarrying (Mota et al., 2003, 2004). *Ononis tridentata* subsp. *crassifolia* (Dufour ex Boiss.) Nyman (Leguminosae) is a rare subspecies with a narrow distribution restricted to gypsum outcrops in south-east Spain (centre-west Granada province), where its habitat quality is declining because of anthropogenic disturbance (Ballesteros et al., 2011). The *Ononis tridentata* complex is endemic to gypsum outcrops in Spain and Morocco (Supplementary Fig. S1). Other than taxonomic studies (Devesa & López, 1997) there is little information on the biology, distribution and conservation status of *O. tridentata* subsp. *crassifolia*. It is categorized on the Red List of the vascular flora of Andalusia as Data Deficient (Cabezudo et al., 2005). Assessment of the conservation status of Data Deficient species is vital, as it can reveal the existence of threats (e.g. Good et al., 2006). Following a preliminary assessment as Near Threatened (Ballesteros et al., 2011), the aims of this study were to (1) assess thoroughly the subspecies' conservation status, (2) quantify the impact of human disturbance, and (3) recommend conservation measures.

The distribution of *O. tridentata* subsp. *crassifolia* was first determined using biodiversity databases, including the Global Biodiversity Information Facility (GBIF, 2008) and Anthos (Castroviejo et al., 2008), herbarium records (GDA and GDAC), and available literature (Devesa & López, 1997; Marchal et al., 2008). Neighbouring areas potentially suitable for the subspecies were identified based on the ecology of known populations and aerial photographs. Field surveys were conducted during 2008–2011. Presence records were mapped, using *Quantum GIS v. 1.7* (Quantum GIS Development Team, 2011), to establish the subspecies' known extent of occurrence (EOO) and area of occupancy (AOO; IUCN, 2001). We estimated population size by counting all reproductive individuals in a total of 142 linear transects of 25 × 2 m (50 m^2) throughout the subspecies' known range, and extrapolating mean density to AOO. All the information available was then used to assess the status of *O. tridentata* subsp. *crassifolia* using the IUCN criteria (2001, 2011).

The impact of habitat disturbance was determined using qualitative data (IUCN/SSC, 2001), available literature (Cabezudo et al., 2005; Escudero, 2009), information on land use (REDIAM, 2008) and earlier observations (Ballesteros et al., 2011). Areas with four types of disturbance

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were considered: afforestation (with *Pinus halepensis* c. 15 years ago), gypsophile shrubland ploughed for cultivation but abandoned c. 15 years ago, topsoil removal (area quarried, with topsoil removed 2 years ago), and overgrazing by free-ranging goats and sheep. These were compared with apparently undisturbed areas (i.e. five 'treatments' in total). Three uniform plots were selected per treatment, giving 15 plots in total, with plots at least 100 m apart.

Cover was estimated using five intercept point transects of 25×2 m (50 m^2), with three contact points per m (i.e. 75 per transect), in each plot. All reproductive individuals were recorded and recruitment was estimated by counting all seedlings along five transects of 10×2 m (20 m^2) per plot. To determine size structure we randomly selected 30 reproductive individuals per plot (a total of 450 individuals) and estimated their volume as the volume of a semi-spheroid (Lorite et al., 2010). To examine the effect of disturbance on the fitness of these selected individuals we counted the number of fruits produced at the end of July. To examine any effects of habitat disturbance on seeds, 10 ripe fruits were collected from each of 10 individuals per plot (i.e. a total of 1,500 fruits), dissected, and the number of well-formed, aborted and predated seeds counted. Well-formed seeds, by plot, were separated in 5 subsets of 5 seeds and weighed to a precision of ± 0.1 mg, and the mean weight per plot calculated.

We estimated the total number of individuals, with confidence interval, by bootstrapping the average density from censuses ($n = 10{,}000$, $P < 0.05$) and calculating it for the AOO. To evaluate the effect of habitat disturbance on the plant parameters examined, we fitted Generalized Linear Models, with a normal function for continuous variables, Poisson error distribution and log as a link function for count data, and binomial error distribution and logit as the link function for proportions. For some analyses we performed a multiple-comparison test with the Bonferroni correction. For all statistical analyses we used *R v. 2.13* (R Development Core Team, 2011).

Using c. 20,000 presence records of the species we estimated AOO to be 1.6 km^2 and EOO to be 337 km^2 (Ballesteros et al., 2011). The average density, from censuses ($n = 142$) and computed by bootstrapping ($n = 10{,}000$), was 0.33 m^{-2} (0.29–0.37, at $P < 0.05$). The estimate of the population is at least 531,605 (467,831–584,564, $P < 0.05$). We located the species in 29 habitat patches, forming 16 populations (defined as a patch or cluster of patches separated by at least 500 m; i.e. a pollinator's foraging distance, Gathmann & Tscharntke, 2002; Fig. 1). Projected habitat depletion from quarrying, assuming a 39.9% (63.9 ha) of habitat lost in the next 60 years (unpubl. environmental impact assessment), would involve the loss of 212,310 individuals (186,840–233,460, $P < 0.05$).

Our findings suggest that *O. tridentata* subsp. *crassifolia*, should be categorized as Vulnerable, with criteria A3cd, D2 (IUCN, 2001, 2011): a projected population reduction (A3), with a decline in AOO, EOO and quality of the habitat (c), and actual or potential exploitation levels (d), and an AOO of < 20 km^2 (D2).

The results show that all the disturbances examined have negative consequences for the species (Table 1): topsoil removal, ploughing, overgrazing and afforestation, in descending order. The number of seeds per fruit was similar between areas, although the predation rate differed significantly between areas and was greatest in undisturbed sites. This could be related to vegetation cover (Meiss et al., 2010), which was highest in undisturbed sites. There were no significant differences in seed weight between areas.

The major threat to *O. tridentata* subsp. *crassifolia* is habitat destruction and fragmentation because of human activities, as for most narrow endemic species (e.g. Fenu et al., 2011; Peñas et al., 2011). In general all of the disturbances we examined have negative consequences for the species, except afforestation (where the pre-existing individuals were not removed during plantation). In studies of other species afforestation has been found to be detrimental (Maestre et al., 2004). As expected, quarrying and ploughing create the greatest disturbance. Mining has been shown to have significant impacts on gypsicolous flora (e.g. Mota et al., 2003, 2004). Livestock may enhance seed germination by digestive tract effects on hard-coated seeds (Baskin & Baskin, 1998) and by eliminating interspecific competition or creating micro-niches for seedling colonization, but may injure seedlings by trampling and browsing (e.g. Hobbs, 2001), as we observed in this study.

Our recommendations for the conservation of *O. tridentata* subsp. *crassifolia* are similar to those generally made for plants of gypsum habitats (Escudero, 2009): minimize the risk of direct destruction and fragmentation by controlling human activities and land use. Impacts could be reduced by identifying areas with good quality habitat for the species and then designing reserves composed of patches of habitat (Mota et al., 2011). The negative impacts of quarrying necessitate a recovery programme; we have found that the species responds to restoration, either as planting (authors, unpubl. data) or sowing with the appropriate soil preparation (Ballesteros et al., 2012). The habitat of *O. tridentata* subsp. *crassifolia* is included in the EU Habitat Directive (92/43/EEC) as a priority for conservation, and therefore no additional measures are needed in this respect. Our results will be put at the disposal of the regional government and taken into account in the development of measures to restore the vegetation of gypsum habitats in this area.

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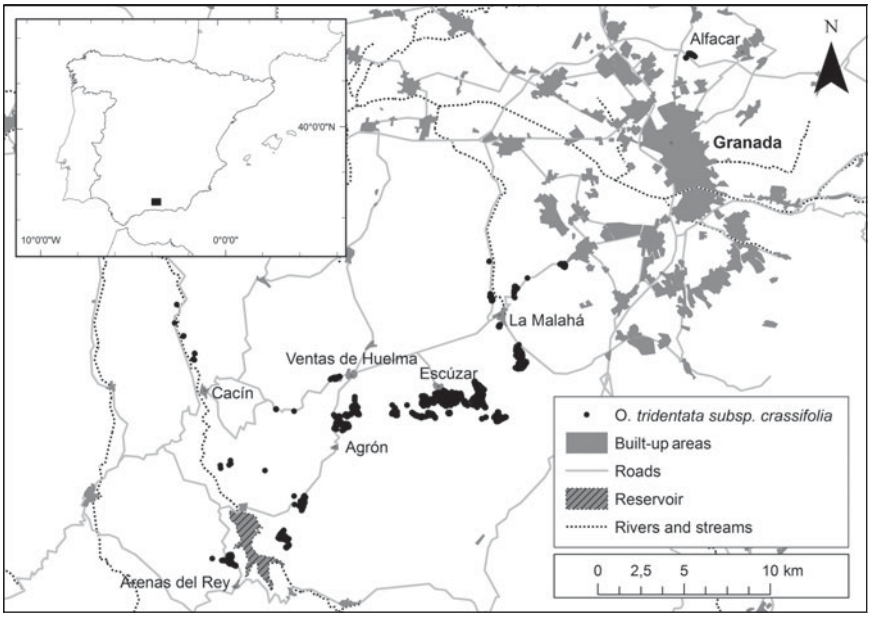

FIG. 1 Presence records of *Ononis tridentata* subsp. *crassifolia* recorded in our surveys. The rectangle in the inset indicates the location of the main figure in southern Spain.

TABLE 1 Characteristics (mean values ± SE) of *Ononis tridentata* subsp. *crassifolia* in undisturbed areas and in areas subjected to four types of disturbance in southern Spain (Fig. 1), and results for Generalized Linear Models used to examine the effect of habitat disturbance on these characteristics (see text for details). Different superscripted letters indicate significant differences in the post hoc test after Bonferroni correction at $P < 0.05$.

	Undisturbed	Afforestation	Overgrazing	Ploughed land	Topsoil removal	df	χ^2	P
Cover (%)	15.56 ± 2.71^{a}	9.15 ± 1.85^{a}	9.66 ± 1.44^{a}	2.82 ± 0.52^{b}	2.31 ± 0.85^{b}	4	242.61	<0.001
Plant volume (dm^3)	135.16 ± 12.92^{b}	234.98 ± 32.33^{a}	38.56 ± 4.20^{c}	95.81 ± 13.52^{bc}	29.74 ± 4.67^{c}	4	23.25	<0.001
Seedling density (m^{-2})	0.07 ± 0.03^{ab}	0.32 ± 0.15^{a}	0.02 ± 0.01^{b}	0.04 ± 0.02^{ab}	0.08 ± 0.03^{ab}	4	139.09	<0.001
Fruit production (%)	63.05 ± 3.22^{a}	48.16 ± 3.43^{b}	38.89 ± 3.72^{b}	41.84 ± 3.92^{b}	73.45 ± 4.06^{a}	4	1,436.74	<0.001
No. of seeds per individual	103 ± 21^{ab}	158 ± 32^{a}	57 ± 9^{b}	68 ± 12^{b}	105 ± 16^{ab}	4	1,488.99	<0.001
No. of seeds per fruit	0.94 ± 0.07	1.03 ± 0.08	1.09 ± 0.09	1.05 ± 0.07	1.35 ± 0.08	4	2.23	0.6928
Predated seeds (%)	67.42 ± 3.20^{a}	63.69 ± 3.45^{ab}	54.31 ± 3.86^{ab}	50.31 ± 3.75^{b}	36.45 ± 3.02^{c}	4	2,362.10	<0.001
Empty seeds (%)	24.96 ± 3.00^{b}	24.02 ± 3.20^{b}	29.59 ± 3.64^{b}	33.39 ± 3.57^{b}	47.57 ± 3.15^{a}	4	2,119,423	<0.001
Well-formed seeds (%)	7.62 ± 1.69^{b}	12.29 ± 2.30^{ab}	16.10 ± 2.74^{ab}	16.31 ± 2.72^{ab}	15.98 ± 2.19^{a}	4	877,594	<0.001
Mean seed weight (mg)	6.3 ± 0.7	7.2 ± 0.5	6.6 ± 0.5	6.6 ± 0.6	7.3 ± 0.3	4	0.00	1

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Biographical sketches

The research of the authors is devoted to the conservation of threatened and endemic flora and the ecological restoration of disturbed areas in Mediterranean environments. Miguel Ballesteros focuses on developing measures to recover gypsum habitats affected by quarrying in south-east Spain. Ana Foronda's research interests include biodiversity conservation and ecological restoration in areas affected by quarrying activities. Eva María Cañadas is interested in plant–environment relationships and their importance in plant conservation and habitat restoration. Julio Peñas is interested in biogeographical patterns and the conservation of endemic vascular plants in the Western Mediterranean region. Juan Lorite has worked on conservation biology and restoration ecology, with a special focus on montane areas. He is leading several projects on the conservation and restoration of gypsicolous habitats affected by quarrying.