Unpaved roads disrupt the effect of herbivores and pollinators on the reproduction of a dominant shrub.

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Abstract

Anthropogenic linear developments, such as trails and firebreaks, also called soft linear developments (SLD), can influence animal behavior, altering the ecological interactions in which animals are involved. For example, SLD can affect the behavior of pollinators and herbivores, but little is known about the combined effect of these three elements on plant reproduction.

We evaluated the combined effect of SLD, insect pollinators and herbivores (ungulates) on three reproductive output variables (fruit set, seed set, and seed mass) of a Mediterranean shrub (*Halimium halimifolium*). We considered two different habitats (SLD verges vs. adjacent scrublands), two scenarios of herbivory (with and without ungulates), and three scenarios of pollinator activity (without pollinators, with manual pollination and with natural pollination).

SLD had contrasting effects on *H. halimifolium* reproduction. In the absence of herbivores, overall fruit set was lower in the verges of SLD than in adjacent scrublands, probably due to lower flower pollination rates. Where herbivores were present, overall fruit set was similar between habitats, because ungulate browsing was lower in SLD verges than in adjacent scrublands. The quantity and weight of seeds per fruit was similar in both habitats, probably because all fertilized flowers received similar amounts of pollen.

SLD can alter the interaction among pollinators, herbivores and plants, leading to changes in the reproductive performance of the latter. These changes can have strong negative impacts on endangered plants that rely on fruit and seed production to persist. However, SLD verges could be safe places for plants particularly sensitive to herbivory by ungulates.

Zusammenfassung

Anthropogene lineare Landschaftselemente wie Wanderwege oder Feuerschneisen können das Verhalten von Tieren beeinflussen und damit die ökologischen Interaktionen, an denen die Arten beteiligt sind, verändern. Diese grünen linearen Strukturen (GLS) können beispielsweise das Verhalten von Bestäubern und Herbivoren beeinflussen, aber wenig ist über den gemeinsamen Effekt dieser drei Elemente auf die Reproduktion von Pflanzen bekannt.
Wir untersuchten den gemeinsamen Effekt von GLS, bestäubenden Insekten und Herbivoren (freilaufende Nutz- und Wildtiere) auf drei die reproduktive Leistung beschreibenden Variablen (Fruchtansatz, Samenansatz und Samengewicht) bei einem mediterranen Strauch (*Halimium halimifolium*). Wir untersuchten zwei Habitattypen (Ränder von GLS und benachbartes Buschland), zwei Herbivorievarianten (mit und ohne Huftiere) und drei Bestäubungsszenarien (ohne Bestäuber, manuelle Bestäubung und natürliche Bestäubung).


GLS können die Interaktionen zwischen Bestäubern, Herbivoren und Pflanzen verändern, was zu veränderter reproductiver Leistung bei den Pflanzen führt. Diese Veränderungen können stark negative Auswirkungen auf gefährdete Pflanzenarten haben, die auf Frucht- und Samenproduktion angewiesen sind, um zu persistieren. Indessen können GLS-Ränder sichere Orte für Pflanzen sein, die gegen Pflanzenfraß durch Huftiere empfindlich sind.

**Keywords:** ecosystem services, fruit set, *Halimium halimifolium*, herbivory, pollination, trails.
Introduction

Human-induced changes in plant and/or pollinator populations often lead to the disruption of pollination mutualisms and consequently to pollen limitation (Ashman, Knight, Steets, Amarasekare, Burd et al. 2004; Gómez, Abdelaziz, Lorite, Muñoz-Pajares & Perfectti 2010; González-Varo, Arroyo & Aparicio 2009). Pollen limitation leads to decreased fruit and seed production in countless crops and wild plants, which negatively affects agricultural yields (Garibaldi, Aizen, Klein, Cunningham & Harder 2011) as well as the colonization ability and the persistence of wild plants (Biesmeijer, Roberts, Reemer, Ohlemüller, Edwards et al. 2006; Bond 1994; Burd 1994).

Understanding how and to what extent human activities affect plant-pollinator interactions and, ultimately, plant reproductive performance, is therefore a priority for conserving the supply of goods and services that plants provide (Biesmeijer et al. 2006; Bond 1994; Isbell, Calcagno, Hector, Connolly, Harpole et al. 2011). At a global scale, human activities have led to the so-called “pollination crisis” (Kearns, Inouye & Waser 1998). More specifically, pollinators are declining due to habitat loss and fragmentation (i.e. reduced availability of feeding and nesting resources), agriculture intensification (which usually involves pesticide and herbicide application), and climate change, in addition to the spread of pests, pathogens and alien species (Potts, Biesmeijer, Kremen, Neumann, Schweiger et al. 2010). At smaller scales, pollinator abundance and behavior can be negatively influenced by local anthropogenic habitat transformation (Elliott, Lindenmayer, Cunningham & Young 2012; Kennedy, Lonsdorf, Neel, Williams, Ricketts et al. 2013; Winfree, Aguilar, Vázquez, LeBuhn & Aizen 2009).

One of the most pervasive human-mediated habitat transformations is the construction of paved roads (e.g. highways), as well as similar structures that involve...
minor habitat transformation such as trails and firebreaks (also known as “Soft Linear Developments”, SLD; Suárez-Esteban, Delibes & Fedriani 2013a). Paved roads can have contrasting effects on plant pollination. For example, they can reduce the frequency of pollinating birds visiting flowers (Geerts & Pauw 2011; but see Francis, Kleist, Ortega & Cruz 2012; Magrach, Guitián & Larrinaga 2011). On the other hand, roadsides often host flourishing grasslands that provide suitable habitats for diverse and abundant populations of pollinators (Hopwood 2008; Noordijk, Delille, Schaffers & Sýkora 2009).

SLD are as pervasive as paved roads (e.g. Pasher, Seed & Duffe 2013). However, very few studies have assessed their effects on plant reproduction. For example, SLD can result in increased habitat fragmentation and edge effects, such as dust deposition on flowers, extreme climate conditions and pollinator mortality (Cunningham 2000; Huang, Sun, Yu, Luo, Hutchings et al. 2009; Jules & Rathcke 1999; Kolb 2008; but see Magrach, Santamaria & Larrinaga 2013), that can reduce the abundance of plants and pollinators, and also affect the behavior of the latter. On the other hand, shrub hedgerows may exist in the verges of SLD, either because they were planted (Karim & Mallik 2008) or because they established naturally (Suárez-Esteban, Delibes & Fedriani 2013b). In such cases, SLD might assist pollination, as hedgerows are known to provide food resources and shelter for pollinators (Morandin & Kremen 2013; Schmucki & De Blois 2009). Interestingly, SLD can also modify the behavior of large herbivores such as ungulates. Wild ungulates usually avoid SLD and other similar habitat edges (Cadenasso & Pickett 2000; Suárez-Esteban et al. 2013a). Therefore, SLD could buffer herbivore damage on plant reproduction (e.g. predation of leaves, flowers, unripe fruits or whole plants), limiting the loss of plant attractiveness for pollinators (Gómez 2003).
Although both pollinators and ungulates can be affected by SLD (Huang et al. 2009; James & Stuart-Smith 2000), and all these three elements pervasively co-occur in the wild, no prior study has assessed their combined effect on plant reproduction. A better understanding on the potential of SLD to disrupt key plant-animal interactions (e.g. pollination, herbivory) will allow us to better predict the effects of SLD and increase our efficiency when planning SLD networks. We specifically chose to evaluate the combined effect of SLD, ungulate herbivores (e.g. deer, cattle) and pollinators on the reproductive output of the common shrub *Halimium halimifolium* L. (Cistaceae) in several patches of Mediterranean scrubland. We compare three measures of reproductive output (fruit set, seed set, and seed mass) in naturally-pollinated flowers between two habitats (SLD verges and scrubland) under two scenarios of herbivory (with and without ungulates). In order to link reproductive output and pollination (Cunningham 2000), we compared flowers exposed to different pollination scenarios (without pollinators, with manual pollination and with natural pollination).

With this experimental design, we aimed to answer the following questions: does herbivory by ungulates limit the reproductive output of *H. halimifolium*? Are the effects of ungulates conditioned by the presence of SLD? Is the reproductive output of *H. halimifolium* dependent upon pollination? Is this species’ reproductive output affected by SLD? Based on the literature, the net effect of SLD on *H. halimifolium* predispersal reproductive performance is difficult to anticipate.

**Materials and methods**

**Study system**

This study was carried out during April-August of 2010 and 2011 in Doñana National Park (SW Spain; 37º 9’ N, 6º 26’ W; 510 km²; elevation 0-80 m). The climate
is Mediterranean sub-humid, characterized by dry, warm summers (June–September) and mild, wet winters (December–March). Annual rainfall is irregular, averaging 577 mm ± 39 SE, with 88.4% of rain falling between October and April (data from Natural Processes Monitoring Group, Doñana Biological Station, http://www-rbd.ebd.csic.es/Seguimiento/seguimiento.htm).

The Doñana area contains several habitats (e.g. marshland, scrubland, dunes) and a vast SLD system (over 2,000 km) composed mainly of dirt trails (62.5%) and firebreaks (35.5%). The scrubland patches harbor a diverse community of native Mediterranean shrubs. This community of shrubs includes our model species, *Halimium halimifolium* L., an abundant Cistaceae shrub that grows in a wide range of environmental conditions in the Western Mediterranean. At Doñana, it dominates extensive scrubland patches from the edges of marshlands to mobile dunes (Díaz Barradas, Zunzunegui & García Novo 1999). Throughout the study area, it reaches similarly high densities in both SLD verges (10.14 ± 1.33 mean number of individuals/m² ± SE) and in adjacent scrublands (9.79 ± 1.13; Suárez-Esteban et al. 2013b).

*H. halimifolium* has large (up to 62 mm in diameter) hermaphrodite yellow flowers that bloom mainly in late-spring and early-summer (May-June; Herrera 1988). Flowers emerge as apical inflorescences and are pollinated mainly by pollen-eating beetles, such as *Heliotaurus ruficollis* Fabricius (Tenebrionidae), and less frequently by some bees (Halictidae; (Herrera 1986). Both beetles and bees are usually observed feeding on *H. halimifolium* flowers both near and far from SLD in our study area (Authors personal observation). Fertilized flowers lead to small dry fruits, containing 25 seeds on average (Herrera 1987a). After the fruiting period, fruits dry up and usually drop their seeds on the ground. Although no evident specialized dispersal mechanism is
observed, this species seems to have a great colonization ability, as documented in other Cistaceae species (Bastida & Talavera 2002).

Within our study area, browsing ungulates such as red deer (*Cervus elaphus* L.), fallow deer (*Dama dama* L.), and livestock (e.g. cattle, horses) reach high densities, because of the absence of both hunting pressure and natural predators. These ungulates can severely damage *H. halimifolium* (Silva, Barradas & Zunzunegui 1996).

**Experimental design**

Because reproductive output can vary in time and space, we surveyed three different study sites separated from each other by 2.5 – 14 km that are called “Reserva”, “Rocina” and “Matasgordas” (described in detail in Suárez-Esteban et al. 2013a), during two consecutive years (2010 and 2011). At each site, we set up two independent experimental blocks separated by a minimum of 1 km (Fig. 1). Within each experimental block we set up four plots (~ 36 m² each). Two of the plots were located along SLD verges and the other two were located in the scrubland, 60 meters away from SLD (since most edge effects cease to be significant within 50 m; Murcia 1995; Fig. 1). Of the two plots established in each habitat, one was fenced to exclude large herbivores (i.e. ungulates such as deer and cattle), while another was left completely open (Fig. 1). Open and fenced plots within each habitat were adjacent, ensuring that they were subjected to the same environmental conditions. In total, we established 24 plots (12 in SLD verges, 12 in the scrubland), all of them containing at least ten reproductive *H. halimifolium* individuals.

To evaluate whether herbivory by ungulates affected the reproductive output of *H. halimifolium* both near and far from SLD verges, we compared fruit set, seed set, and seed mass between open and fenced plots in SLD verges and in the scrubland. To assess whether SLD alone affected the reproductive output of *H. halimifolium*, we also
compared the same three reproductive measures between fenced plots (i.e. controlling
for the effect of herbivory) in SLD verges and in the scrubland.

Linking habitat differences in reproductive output with differential pollination
requires confirmation that pollen reception limits the reproductive output (Cunningham
2000). To do this, in each plot we simulated three scenarios of pollinator activity:

a) Without pollinators:

After counting the flower-buds, we tagged and bagged a number of
inflorescences (five in 2010 and two in 2011) of each of five randomly selected *H.
halimifolium* plants within each plot to preclude pollinator access (Parker 1997).

Overall, we tagged and bagged 779 inflorescences with 53152 flowers. After the
flowering period (July-August), we counted the resulting fruits and randomly sampled
three of them from each bagged inflorescence. All collected fruits were stored in paper
bags, then dissected in the lab to count the number of seeds produced per fruit and to
measure their mass (using a precision scale).

b) Manually supplemented cross-pollination:

Using the same five *H. halimifolium* plants mentioned above, we randomly
selected five open inflorescences that were different from those that had been bagged to
exclude pollinators. For each of these open inflorescences, we added supplemental
pollen to one randomly selected flower per inflorescence (hereafter “supplemented
flower”), by rubbing its stigma with collected anthers from distant (20-30 m)
conspecifics until it was completely covered with pollen.

Pollen addition may overestimate the magnitude of pollen limitation if plants
reallocate resources from non-manipulated flowers to supplemented flowers (Haig &
Westoby 1988; Knight, Steets & Ashman 2006). To assess whether such resource
reallocation occurs (thereby potentially affecting our results), for each supplemented
flower we labeled three control flowers: one within the same inflorescence as the supplemented flower (hereafter “intra-inflorescence control”), one within another inflorescence on the same plant (hereafter “inter-inflorescence control”), and the last one on another adjacent individual, outside the plot (hereafter “external control”). All control flowers were exposed to natural pollination (no pollen was added). For this, we tagged and monitored 1034 flowers for each of the four treatments (i.e. supplemented cross-pollination and three controls). After the flowering period, we counted all resulting fruits set by supplemented and control flowers, harvesting and processing all of them as above.

c) Natural pollination

To estimate *H. halimifolium* natural fruit set, we tagged five inflorescences (when possible) of each of five additional *H. halimifolium* plants (different from the five plants used to simulate the absence of pollinators and the effect of supplemented cross-pollination) within each plot and counted their flowers, which were exposed to natural pollination (hereafter “naturally-pollinated flowers”). Overall, we tagged 1149 inflorescences with 80038 flowers. As above, we counted the number of fruits set by all tagged inflorescences and randomly sampled three fruits from each. In the 2011 surveys, we selected the same individuals used in 2010 when possible.

**Statistical analyses**

Fruit set of bagged flowers (i.e. those that were not exposed to pollinators) was ~2% in both habitats, confirming that *H. halimifolium* reproductive output relies on animal pollination. Because of its small sample size, we excluded this treatment from the analyses.

Using data on the supplemented flowers, we evaluated whether habitat, herbivory or their interaction altered the importance of pollination for *H. halimifolium*
reproductive output. To address the effect of habitat, we compared plots in SLD verges vs. plots in the scrubland. To address the effect of herbivory by ungulates, we compared open vs. fenced plots. Finally, to quantify a potential interaction between habitat and herbivory, we compared SLD verges with the scrubland in terms of any differences between open and fenced plots.

We fitted three generalized linear mixed models (GLMMs) with three different response variables: (1) the proportion of flowers that set fruits, i.e. fruit set, per plant (using a binomial error-distribution and logit-link function), (2) the number of seeds produced per fruit, i.e. seed set (using a negative binomial distribution and log-link function), and (3) the overall seed mass per fruit (using a Gaussian distribution and identity-link function). In these models, we also included data on control flowers to assess the potential occurrence of resource reallocation.

Using data on natural pollination, we evaluated the effect of habitat, herbivory and their interaction on the reproductive output of naturally-pollinated flowers. To do so, we fitted three additional GLMMs with the same response variables as above (i.e. fruit set per plant, seed set per fruit and seed mass per fruit).

All GLMMs were implemented using the SAS 9.2 GLIMMIX procedure (Littell, Milliken, Stroup, Wolfinger & Schabenberger 2006) and adjusted using maximum likelihood (Bolker, Brooks, Clark, Geange, Poulsen et al. 2009). In all models we considered the habitat (SLD verges vs. scrubland), herbivory by ungulates (open vs. fenced plots), and their interaction as fixed factors. When analyzing whether pollen reception and resource reallocation varied among factor combinations, we also included the pollination treatment (i.e. supplemented vs. control flowers; referred to as “Supplement” in Table 1) and its interactions with habitat and herbivory as fixed factors. When any interaction was significant, we performed tests for the effect of a
factor at the different levels of the other factor (“tests of simple main effects”) using the SLICE option in the LSMEANS statement (Littell et al. 2006).

The use of GLMMs allowed us to model non-normal variables as well as to introduce random factors in order to account for temporal and spatial heterogeneity, in addition to potential individual effects that might influence our results. Specifically, we included the year, the study site, the plot (nested within site), and the plant (nested within plot) as random factors. For every model concerning seed set and seed mass, we removed from the dataset all fruits that presented some fault (e.g. with depredated, unripe or rotten seeds), which happened rarely.

**Results**

**Supplemented cross-pollination**

Pollen supplementation increased fruit set by 114.6%, seed set by 35.0% and seed mass by 29.0%, as compared with control (naturally-pollinated) flowers (see “Supplement” in Table 1; Fig. 3). This suggests that the reproductive output of *H. halimifolium* (all three variables) is generally limited under natural conditions. As found for naturally-pollinated flowers, fruit set of both supplemented and control flowers was slightly higher in fenced plots than in open plots in the scrubland, although these differences were only marginally significant (Table 1).

With respect to the possibility of resource reallocation, control flowers showed similar (*P > 0.05*) fruit set (Fig. 2), seed set (Fig. 3) and seed mass. This indicates the lack of resource reallocation in *H. halimifolium* at any scale, reinforcing the strength of our inference on the link between pollen reception and reproductive output in our study system.

The effect of pollen supplementation on fruit set was consistent in both habitats, and in both fenced and open plots (i.e. habitat, herbivory and their interactions with the
pollination treatment were not statistically different between either habitats or plots; Table 1). However, when analyzing seed set and seed mass, we found marginally significant differences between habitats (see “Habitat” in Table 1). These marginal differences were the result of a higher seed set and seed mass of pollen-supplemented flowers in the scrubland than in SLD verges (see Fig. 3). We did not find any other significant effect of habitat or herbivory on seed set or seed mass (Table 1).

**Natural pollination**

Naturally-pollinated flowers set 27689 fruits (overall fruit set 34.6%; n = 80038). Habitat and herbivory did not significantly affect fruit set (Table 1). However, the interaction between habitat and herbivory was significant (Table 1), suggesting that the effect of ungulates differed between habitats. Indeed, the exclusion of ungulates had a significant effect in the scrubland, but not in SLD verges. In SLD verges we found no differences in the fruit set between open and fenced plots. In the scrubland, however, fruit set was 33.0% higher in fenced than in open plots (Fig. 4). Considering exclusively the differences between fenced plots in both habitats (i.e. controlling for the effect of herbivory), the fruit set per plant was a 33.1% higher in the scrubland than in SLD verges (test of slices: $F_{1,113} = 6.90 ; P < 0.01$; Fig. 4). Thus, SLD negatively affected *H. halimifolium* fruit set in absence of ungulates.

We collected 2612 fruits (9.43% of the counted fruits) to estimate the number of seeds and their overall mass per fruit of naturally-pollinated flowers. The number of seeds produced per fruit ranged between 1 and 72. The mean number of seeds produced per fruit was similar between SLD verges (20.12 ± 1.81; mean ± SE) and adjacent scrublands (21.87 ± 1.96; Table 1). We did not find a significant effect of herbivory by ungulates on seed set (Table 1). This lack of ungulate effect was consistent in both habitats (the interaction Habitat*Herbivory was not significant; Table 1).
The overall seed mass per fruit ranged between 0.1 and 31.4 mg. As was true for the average seed set, seed mass was similar between SLD verges (8.97 ± 0.59 mg; mean ± SE) and adjacent scrublands (9.80 ± 0.59 mg). We did not detect any significant effect of habitat, herbivory, or their interaction on seed mass (Table 1). These results for both seed set and seed mass suggest that SLD did not affect seed production.

**Discussion**

**Effects of SLD on pollination**

As found by Herrera (1987a), bagged *H. halimifolium* barely produced fruits in either habitat. Furthermore, Herrera (1987b) found that self-pollinated flowers of a closely related species (*H. calycinum*) did not set any fruit. This evidence suggests that *H. halimifolium* is highly dependent on cross-pollination mediated by insects regardless of the presence of SLD. *H. halimifolium* did not reallocate resources in response to pollen supplementation. This confirms that the more pollen a plant receives, the more fruits and seeds it produces, i.e. *H. halimifolium* is pollen-limited, as found for other species (Burd 1994; Parker 1997). This pollen-reception dependence was consistent in both habitats.

We found that, in the absence of herbivores, plants in SLD verges produced relatively fewer fruits despite having a similar seed yield (i.e. seed number and mass) per fruit than plants in the scrubland. Given that pollen-reception is positively correlated with both fruit and seed production, our results suggest that the proportion of pollinated flowers was lower in SLD verges than in the scrubland (which resulted in a lower fruit set), but all pollinated flowers received similar amounts of pollen, regardless of the habitat (which explains the consistency of seed set and seed mass between habitats).

Lower flower pollination rates in SLD verges relative to adjacent scrubland in the absence of herbivores can be related to the reception of fewer or poorer quality
pollen grains (Aizen & Harder 2007). Lower pollen arrival is usually associated with either lower visitation rates by pollinators (Kolb 2008; Parker 1997) or with lower pollinator efficiency when removing and transporting pollen, e.g. due to briefer visits (Aizen et al. 2007; Wilson & Thomson 1991). As our results suggest that all pollinated flowers received a similar amount of pollen, we assume that pollinator efficiency did not vary between habitats. Thus, we propose that this pattern is largely explained by lower visitation rates.

Flower visitation rates by pollinators can be affected by abiotic conditions (Jules et al. 1999). As a matter of fact, we observed that *H. halimifolium* flowers tended to close with windy weather. Wind turbulence and exposure is probably much higher at SLD verges, given the lack of surrounding, protective vegetation. This probably decreased the period of time during which *H. halimifolium* flowers along SLD were available relative to those within the scrubland. Reduced availability of open flowers could have reduced the likelihood of pollen reception and fecundation in SLD due to both a reduced time of exposure for each flower, as well as an overall reduction in attractiveness for pollinators. Furthermore, wind intensity and turbulence can hinder pollinator activity (Sayre, Kelty, Simmons, Clayton, Kassam et al. 2013), which could act in synergy with the reduced availability of flowers.

Considering that in the absence of herbivores, naturally-pollinated flowers set more fruits in the scrubland than along SLD verges, the overall production of seeds in the scrubland was higher than in SLD verges. Although the number of seeds per fruit set by naturally-pollinated flowers was similar in both habitats, we found that pollen-supplemented flowers set a significantly higher number of seeds per fruit in the scrubland than along SLD verges (see “Suppl” in Fig. 3). This suggests the existence of
other effects of SLD (e.g. dust deposition and interference with pollen, Lewis, Schupp
& Monaco 2012) that would affect negatively the seed set when pollen is unlimited.

**Combined effect of SLD and ungulates on plant reproduction**

Large ungulates such as red deer and caribou (*Rangifer tarandus*) usually avoid linear structures such as SLD (James et al. 2000; Suárez-Esteban et al. 2013a). Accordingly, we only found a negative effect of ungulates on *H. halimifolium* fruit set in the scrubland (Fig. 4). Thus, SLD had a partly beneficial effect on *H. halimifolium* reproductive output. We did not detect any significant effect of herbivory by ungulates on the number of seeds produced per fruit. This suggests that ungulates mainly disrupt fruit set, probably by removing flowers and entire inflorescences (Vázquez & Simberloff 2004). By reducing the fruit set in the scrubland, ungulates diminished in turn the overall production of seeds in that habitat, but not at SLD verges. However, this potentially positive effect of SLD was counterbalanced by other negative effects on pollination (see above). We expect this reduction in herbivory mediated by SLD will be rather advantageous in other plant ontogenetic stages, such as the seedling and the sapling stage (Cadenasso et al. 2000).

**Conclusions and implications**

Effective management of plant populations in anthropic ecosystems requires an understanding of the ecological drivers of plant reproduction and how they interact with pervasive human features. Our results suggest that SLD can have negative effects on local pollination and thereby on the reproductive output of *H. halimifolium*, a dominant Mediterranean shrub. However, limited fruit or seed production do not necessarily mean a negative effect on plant populations (Herrera, Medrano, Rey, Sánchez-Lafuente, García et al. 2002). Negative effects of SLD on pollination might be overcome by positive effects at other stages such as seed dispersal (Suárez-Esteban et al. 2013a),
while the resulting net effect will ultimately determine plant fitness (Magrach et al. 2013). Despite the importance of considering all ontogenetic stages as a whole, very little is known about whether SLD affect seed predation, germination, and seedling establishment (Ogden, Heynen, Oslender, West, Kassam et al. 2013; but see Suárez-Esteban 2013). Thus, further research is clearly needed.

Given that *H. halimifolium* readily colonizes SLD after one year without perturbations (*Authors personal observation*), fruit and seed set do not seem to be the most limiting factors for population size. Rather, the availability of empty sites without competitors is more likely to influence *H. halimifolium* dynamics. However, the fact that we detected a negative effect of SLD on the reproductive output of such locally widespread and abundant species suggests that SLD disturbances could have a deeper impact on other taxa (Cunningham 2000). For instance, those plants with small populations, depending upon specialist pollinators sensitive to SLD derived effects (e.g. wind, dust), and those whose fitness relies mainly on sexual reproduction, will likely be significantly deterred by SLD. On the other hand, SLD could have a positive effect on plant populations whose viability is negatively affected by ungulates. Therefore, careful SLD development in conjunction with further research is desirable to effectively manage sensitive plant populations.

SLD effects are likely heterogeneous among different species (i.e. SLD effects are context-dependent; Suárez-Esteban et al. 2013b). This highlights the importance of local scale studies that can be translated into management practices. In order to reduce the negative effects of SLD on pollination, we propose the conservation of roadside native vegetation. SLD verges have the potential to host shrubby hedgerows (Suárez-Esteban et al. 2013b) that can act as wind and dust screens, as well as provide pollinators with habitat for both foraging or nesting (Morandin et al. 2013; Mwangi,
Kasina, Nderitu, Hagen, Gikungu et al. 2012). Natural hedgerows along roadsides can be achieved by conserving endozoochorous seed dispersal vectors that positively select SLD verges for defecation (Suárez-Esteban et al. 2013a) and by promoting shrub establishment along SLD verges (Karim et al. 2008; Suárez-Esteban et al. 2013b).
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References


Table 1. Results of the GLMMs fitted for testing the effects of habitat, herbivory, pollen-supplementation (Supplement) and their interactions on the fruit set, seed set and seed mass. *P*-values are represented by asterisks (m.s. *P* < 0.07; *P* < 0.05; *** *P* < 0.0001).

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<td>127.89***</td>
<td>3,1369</td>
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<td>Hab*Suppl</td>
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<td>1,763</td>
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**Figure captions**

**Fig. 1.** Spatial distribution of our three study sites inside Doñana National Park (SW Spain). In each of the three sites, we set up two experimental blocks, with open and fenced plots both along SLD (dark grey stripe) verges and in the scrubland, as shown by the diagram in the top-right.

**Fig. 2.** Model-adjusted means (± SE) of fruit set by pollen-supplemented (“Suppl”) and control flowers (from left to right: intra-inflorescence, inter-inflorescence and external controls) along SLD verges (black bars) and in the scrubland (white bars). We only found significant differences between supplemented (a) and all types of control flowers (b). No significant differences were found between habitats (n.s. = non significant, $P > 0.05$).

**Fig. 3.** Model-adjusted means (± SE) of seed set by pollen-supplemented (“Suppl”) and control flowers (from left to right: intra-inflorescence, inter-inflorescence and external controls) along SLD verges (black bars) and in the scrubland (white bars). We only found significant differences between supplemented and control flowers. No significant variations were found between habitats, though the overall seed set was slightly higher in the scrubland as compared with SLD verges (** $P < 0.01$; m.s. $P = 0.054$; n.s. = non significant, $P > 0.054$).

**Fig. 4.** Model-adjusted means (± SE) of fruit set by naturally-pollinated flowers along SLD verges (black bars) and in the scrubland (white bars) between open and fenced plots (i.e. with and without ungulates, respectively). Letters show whether differences between least-square means were significant (n.s. = non significant).
FIGURE 2

Proportion of flowers that set fruits

<table>
<thead>
<tr>
<th></th>
<th>Scrubland</th>
<th>SLD verges</th>
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<td>Intra C</td>
<td>n.s.</td>
<td>b</td>
</tr>
<tr>
<td>Inter C</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>External C</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>n.s.</td>
<td></td>
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</tbody>
</table>
FIGURE 3

#### Number of seeds per fruit

<table>
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<tr>
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<th>Scrubland</th>
<th>SLD verges</th>
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<td>30</td>
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<td>n.s.</td>
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<tr>
<td><strong>Inter C</strong></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>External C</strong></td>
<td>n.s.</td>
<td>m.s.</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>n.s.</td>
<td>m.s.</td>
</tr>
</tbody>
</table>
FIGURE 4

![Graph showing proportion of flowers that set fruits in different conditions.](image-url)