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Facilitation by herbivore-mediated nurse plants in a threatened tree, *Taxus baccata*: local effects and landscape level consistency

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We investigated the regeneration of a threatened tree, the yew *Taxus baccata*, in relation to the presence of fleshy-fruited woody plants acting as seed dispersal foci as well as protecting yew recruits against ungulate herbivores. We seek to determine if local facilitative effects are consistent across landscape in the Cantabrian range (NW Spain). Yew seed rain by birds mostly concentrated under yew trees and beneath hollies *Ilex aquifolium*. Seedling emergence distributed similarly to seed rain, but first-year seedling survival was higher beneath hollies. In one site where woody vegetation was structured as nucleation centres (multispecific patches of fleshy-fruited plants acting as foci for seed rain) yew recruits mostly occurred in yew-dominated centres, suggesting dispersers-mediated facilitation. However, holly was the main nurse plant for most of these recruits, considering the nurse as the species whose canopy covered directly the yew recruit. Living beneath nurse plants reduced herbivore damage on saplings and enhanced seedling survival. A planting experiment with yew rooted-cuttings beneath different spiny shrubs corroborated this effect. Additional evidence on yew recruitment limitation by herbivory emerged from one population where ungulates were fence-excluded. Our results suggest that nurse plants mitigate the negative effect of herbivores on yew regeneration, by providing defence against browsing and trampling. Shelter ability related to nurse structure, cone-shaped shrubs with branches at their bases acting better as a barrier. Paradoxically, this structure resulted from heavy browsing on nurse plants. The study of yew regeneration and habitat structure in seven sites provided evidence for the consistency of facilitation by holly at the landscape level, since local values of yew recruitment positively related to nurse ground cover. Range-scale yew management must consider the local functioning of the interaction among avian seed-dispersers, nurse fleshy-fruited plants and ungulate herbivores, in combination with regional measures, targeting the habitats where facilitation emerges.

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Facilitation among plants happens when one species enhances the survival, growth or fitness of another, resulting in the spatial association among them (Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997, Holmgren et al. 1997). A benefactor plant (termed nurse, Franco and Nobel 1989, Tewksbury and Lloyd 2001) facilitates another by making the physical environment under its canopy more suitable for the benefitiary, by modifying light (Greenlee and Callaway 1996, Weltzin and McPherson 1999), temperature (Franco and Nobel 1989, Valiente-Banuet and Ezcurra 1991), and soil moisture (Joffre and Rambal 1993, Verdú and García-Fayós 1996) or nutrient regimes (Callaway et al. 1991, Belsky 1994). Besides these direct mechanisms, facilitation also emerges indirectly, when mediated by a third organism (Callaway 1995). For example, some plants attract frugivores, seed dispersers of other species, by acting as a perch or by being themselves endo-zoochorous, and the final result is an accumulation of seeds (dispersal focus) promoting dif-

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ferential recruitment of the facilitated species under the nurse plant (Debussche and Issenmann 1994, Verdú and García-Fayós 1996, 2003). In other cases, nurse plants protect others from damage by herbivores, by having mechanical defences such as an imbricate branching or a prickly foliage (McAuliffe 1984, 1986, Callaway 1992, García et al. 2000). Moreover, both disperser-mediated and herbivore-mediated effects might co-occur, as it happens when a plant firstly acts as dispersal focus for another and after protects it against herbivores (García et al. 2000, see also Suzán et al. 1996, Verdú and García-Fayós 2003). Then, the final outcome of facilitation will depend on the behaviour of the multispecific system composed of seed dispersers, fleshy-fruited nurse plants and herbivores.

Facilitation among plants has been focused from the conservation standpoint due to its role in structuring plant communities and promoting biodiversity (Callaway and Walker 1997, Hacker and Gaines 1997, Tewksbury and Lloyd 2001). In this sense, the identification of facilitation mechanisms has proven useful for local management of threatened plants, since their population viability is often determined by the ability of nurse plants to provide biogenic suitable habitats (e.g. Suzán et al. 1994, Tewksbury et al. 1999, García et al. 2000). However, the outcome of facilitation frequently depends on how stressful are the local environmental conditions. In fact, the interaction between two species might result positive in stressful environments, but the same benefactor and beneficiary species turn to competitors under more benign conditions (Callaway et al. 1991, Bertness and Callaway 1994, Greenlee and Callaway 1996). The spatial variation of facilitation outcome has thus important consequences since nurse plants, by modifying habitat suitability for other species in many different localities, are able to determine not only the local viability of benefitiary species but also their persistence throughout large portions of the range (Callaway and Davis 1998, Tewksbury and Lloyd 2001). Therefore, when considering facilitation for conservation purposes, a major challenge is to determine if local facilitative effects are consistent at larger spatial scales, or, if conversely, facilitation emerges only at particular spatial scales due to environmental stress gradients.

An excellent system to investigate facilitation at different spatial scales and its implications for conservation is the case of yew *Taxus baccata*, a relictic tree showing severe decline all over its range in Europe (Tittensor 1980, Mitchell 1990, Hulme 1996, Svenning and Magard 1999, García et al. 2000). Several works have shown a positive spatial association between yew and shrubs, suggesting that regeneration might depend on facilitative effects. For example, Watt (1926) concluded that yew could establish better in the shelter of prickly shrubs, such as juniper and hawthorn, after finding dead bushes beneath yew canopies. Tittensor (1980), Mitchell (1990) and Hulme (1996) also considered the scrublands of fleshy-fruited plants (juniper, hawthorn, holly) as seral stages previous to yew woods development. Finally, García et al. (2000), working in a Mediterranean environment, suggested that facilitation was herbivore-mediated, since saplings escaped herbivory when protected by shrubs, but also infered an initial effect of shared seed dispersal, because most of these shrubs were fleshy-fruited. In this paper, we report on the ecology and conservation of yew in the Cantabrian mountain range (NW Spain), providing observational and experimental evidences about the effect of facilitation on yew regeneration. More specifically, we addressed the following points: 1) determine the regeneration status of yew populations over a range of localities; 2) examine the effect of herbivore ungulates on yew regeneration; 3) evaluate differentially the effects of bird-mediated seed rain and herbivore-mediated seedling survival in the facilitative role of fleshy-fruited plants; and 4) examine the consistency of local facilitative effects at the landscape level, in order to provide yew management tools at local and regional scales.

Methods

Species

Yew *Taxus baccata* (Taxaceae) is a dioecious, slow-growing, evergreen tree that is scattered throughout western Europe and limited to low altitudes in the north and to high altitudes in the south (Tutin et al. 1964). It flowers from February to April, is wind-pollinated and the arillated seeds ripen sequentially from late July to October. The seed is immersed in a fleshy cup-like red aril and is both dispersed by birds (thrushes *Turdus* sp., Snow and Snow 1988) and mammals (Tittensor 1980, Bermejo and Guitián 2000). After dispersal, seeds are consumed by rodents (*Apodemus flavicollis*, *A. sylvaticus* and *Clethrionomys glareolus*, Hulme 1996). Germination occurs from late spring to early summer, most of seedlings emerging in June. Seedlings are tolerant to deep shade (Hättenschwiler 2001). The shoots and needles are readily consumed by large herbivores despite their toxicity, apparently because browsers are able to build an immunity to yew if regularly fed small quantities (Haeggström 1990).

In the Cantabrian range (northern Spain), yew is widespread as isolated individuals within the beech forest (Peñalba 1994), but there are also true populations within open beech forests, ash *Fraxinus excelsior* dominated forests and second growth forests dominated by fleshy-fruited woody plants, mainly holly *Ilex aquifolium* and hawthorn *Crataegus monogyna*. The regeneration of this species faces serious problems and it is legally protected in the region (Anon. 2002).

Study sites

The study was conducted at seven main localities (Sueve, Río Color, Peña Mayor, Teixeu, Aramo, Agüeria, and Sobia) in the Cantabrian Range, chosen to be representative of medium-to-large populations of yew in the Asturias province (Table 1). All these yew populations occurred on northern-oriented slopes at mid-altitude areas, with calcareous shallow soils and karstic physiognomy. The climate of the region is Atlantic, with precipitation distributed throughout the year. Recruitment of yew shrubs and trees from seedlings is rare at these sites, populations being mostly composed by adult individuals. Main yew seed-dispersers at these sites are the song thrush *Turdus philomelos* and the mistle thrush *T. viscivorus*. Browsing ungulates (cattle *Box taurus*, goats *Capra hircus*, red deer *Cervus elaphus*, fallow deer *Dama dama*, and wild boar *Sus scrofa*) are very abundant (1–2 ha^{-1}) and damage to seedlings and saplings is frequent (35–50%).

Spatial distribution of dispersed seeds and first-year seedlings

The spatial distribution of yew seeds after dispersal (seed rain) was studied at Sueve and Aramo sites. Ten permanent quadrats of 50 × 50 cm were established in September 2001 at each one of four microhabitats: beneath *Taxus*, *Ilex*, and *Crataegus*, and in open sites (forest gaps at Sueve, pastures at Aramo). Quadrats were monthly monitored until January 2002, collecting all seeds deposited by thrushes in droppings. Undispersed seeds falling from trees were easily distinguishable from avian-handled seeds by the presence of remains of aril. They were discarded from sampling since they constitude a negligible fraction and are most unviable since fallen after fruit abscission. We considered as seed rain both intact seeds and seeds attacked by rodents after dispersal (*Apodemus* sp., *Clethrionomys glareolus*), easily identifiable by teeth marks. Seed removal from quadrats due to predators was considered negligible since rodents eat seeds in situ (see García 2001, for a similar procedure). No sign of losses due to ungulate trampling or abiotic factors was detected.

The abundance of first year seedlings and their survival from summer to winter was examined at Sueve and Aramo sites. First year seedlings are easily distinguishable because they present two cotyledons, three to four opposite pairs of leaves produced above the cotyledons and a greenish stem which turn to brown at the second year (see also Thomas and Polwart 2003). These seedlings were counted in June 2001 and mapped beneath the canopies of different tree species and counted again in January 2002. At both sites seedling density was estimated at different microhabitats: beneath *Taxus*, *Ilex*, and other trees (*Crataegus* and hazel *Corylus avellana* at Aramo and *Fraxinus* at Sueve) and in open sites (forest gaps at Sueve, pastures at Aramo). Ten replicates were obtained by microhabitat and site by searching seedlings in circular plots of 3-m radius around the tree trunk. In open areas, the seedlings were sampled in 75 1-m^2 plots, distributed along ten linear transects.

Distribution of recruits in relation to nucleation centres and nurse plants

The distribution of different yew regeneration stages in relation to microhabitat was examined in more detail at Aramo site, where they showed relatively higher abundance. There, vegetation structure is characterised by patches of woody plants surrounded by a matrix of pastures and open stony areas. Woody patches are nucleation centres (sensu Verdú and García-Fayós 1996) containing different species, mainly fleshy-fruited plants which may act as foci for bird-mediated seed rain (yew, holly, hawthorn, but also blackthorn *Rhamnus alpinus* and rowans *Sorbus* sp.). Nucleation centres may be differentiated by the major species, dominant in terms of canopy cover and height. Then, we studied the

Table 1. Characteristics of the study sites and yew populations (SGF = Second growth forest; DBH = Mean diameter at breast height of individuals with > 5 cm of diameter). Means are given ± 1SD (N). Sapling (plants 0.5–5.0 cm trunk diameter) and tree densities were measured in ten to fifteen 20 × 20 plots per site. Lillo site was sampled for additional data on herbivore exclusion effect on yew regeneration.

Locality	Altitude m a.s.l.	Geographical coordinates	Vegetation type	DBH	Ratio saplings to trees
Sueve	680	43°26′25″N/5°15′19″W	Mixed forest	55.51 ± 25.52 (42)	0.69 ± 1.88
Río Color	850	43°16′56″N/5°15′38″W	SGF, pastures	48.63 ± 24.16 (30)	1.26 ± 2.33
Teixeu	1000	43°17′43″N/5°30′17″W	Open beech forest, SGF, pastures	54.22 ± 37.24 (38)	0.22 ± 0.36
Peña Mayor	1100	43°18′40″N/5°30′35″W	Open beech forest, SGF, pastures	50.12 ± 27.62 (42)	0.02 ± 0.09
Aramo	1400	43°14′26″N/5°56′22″W	SGF, pastures, rock	46.92 ± 29.37 (41)	2.79 ± 1.99
Sierra Sobia	1450	43°08′29″N/6°01′35″W	SGF, rock, pastures	35.62 ± 33.32 (49)	1.02 ± 1.18
Agüeria	1500	43°03′38″N/5°56′11″W	SGF, pastures	23.23 ± 23.03 (44)	12.04 ± 10.29
Lillo	1320	43°02′12″N/5°20′30″W	Dense beech forest	_	_

distribution of yew recruits considering six microhabitat categories, representing nucleation centres respectively dominated by yew, holly, hawthorn, hazel, and other fleshy-fruited species (blackthorn, rowan, etc), as well as open sites (pastures). Ten replicates of variable surface (ten transects of 1×10 m in open sites) were sampled per microhabitat during June–July 2001, accounting for the following total surfaces (in m^2): yew, 280; holly, 135; hawthorn, 234; hazel, 146; other fleshy-fruited species, 40. All yew recruits, including first year, second year and older than second year seedlings and saplings (0.5–5.0 cm of trunk diameter, see also Hulme 1996), occurring in the sampled areas were counted and individually marked. The recruits were examined for the presence of damage by herbivores, and were counted again in January 2002 to determine their probability of survival. Each recruit was assigned to the corresponding nucleation centre type but also to the nurse plant type, that is, the species whose canopy covered the recruit. A recruit might be nursed by the dominant species in the nucleation centre but also by a different species, inhabiting also the same nucleation centre (Fig. 1). Under heavy browsing, some nurse trees or large shrubs show a conical shape and cover the ground level, their canopy contacting directly with yew recruits and potentially providing shelter against herbivores. The percentage of cover at ground level provided from nurses was estimated for each nucleation centre, and varied from zero, in yew centres, to 90.0%, in holly centres. Some species like holly and hawthorn also develop prickly foliage or spiny branching under browsing (Obeso 1997). Finally, for each individual yew recruit we also estimated visually the percentage of the recruit surface that was directly covered by the canopy of nurse trees or shrubs, as a measure of shelter (Fig. 1, for a similar procedure see García et al. 2000).

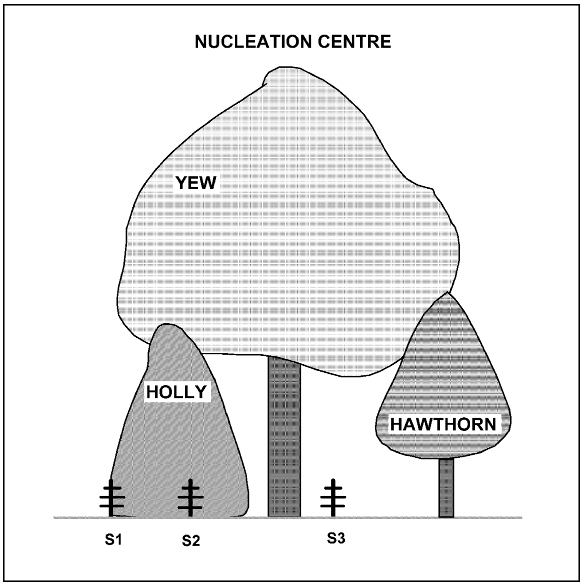

Fig. 1. Representation of yew recruits it relation to nucleation centre (the patch of woody vegetation differentiated by the dominant species, where the recruit occurs) and nurse plant (the species directly growing above the recruit, either the nucleation species or other species co-occurring in the nucleation centre). Each recruit is also characterised by a percentage of sheltering, the percentage of the recruit surface contacting directly with the nurse canopy. S1, S2 and S3 exemplify three recruits in a yew-dominated nucleation centre, with different nursing and sheltering conditions: S1 shows 50% sheltering, nursed by holly; S2 shows 100% sheltering, nursed by holly; S3 shows 0% sheltering, nursed by yew.

Yew regeneration and habitat structure at the regional scale

Densities of yew saplings (0.5–5.0 cm trunk diameter), juveniles (> 5.0 cm DBH) and adult (reproductive) trees were recorded in ten to fifteen 20×20-m plots per population (Table 1). The ratio sapling/adult tree density was used as an estimation of regeneration for each population (see also Hulme 1996). The diameter at breast height (DBH) was determined for pre-reproductive and adult trees in the plots and in the surroundings. Although the relationship among tree girth and age might perform differently in different areas, we considered DBH to be an effective parameter for representing coarse differences in age structure among populations, since no strong variations in soil type or productivity seemed to occur among sites (see also Thomas and Polwart 2003).

To examine across populations the relationship between yew regeneration and habitat structure, we recorded vegetation cover data for each site. The percentage of cover of the shrubs and trees of all species was determined visually at both canopy and ground level, in the same 20×20-m plots used for sampling yew population structure.

Experimental procedures

Considering that conditions other than herbivory might also account for the association between *Taxus* recruits and spiny shrubs, a transplantation program was performed at Aramo site, in order to evaluate experimentally nurse effect against herbivores. For that, more than two hundred yew terminal shoots were collected at Aramo population in December 2001 and translated to individual plastic pots (0.25 l) containing a 2:1 mixture of potting soil (based on 100% blond peat) and siliceous sand after application of rooting promoter (IBA 2%, see Eccher 1988, for a similar procedure). The pots were kept in a temperature-controlled (12–25°C) glasshouse at the Univ. of Oviedo. Some of the explants died during the winter but most of them produced callus and rooted during spring 2002. These rooted cuttings were transplanted to field at 21 June 2002, following an experimental design that considered three spatial blocks (different altitudes within Aramo site) and tree nurse habitats: beneath *Ilex* spiny shrubs, beneath *Crataegus* spiny shrubs, and control (open pastures). Seven nurse plants per species and block were selected and three rooted cuttings were transplanted beneath each of these nurse plants. All shrubs used as nurse were selected to show a relatively uniform and high cover at ground level ($>75\%$ of individual canopy). Rooted cuttings at open sites were close to the spiny shrubs (30–50 cm apart from the shrub) and consisted of 22 per block to obtain a balanced design. The transplanted cuttings were examined for herbivore damage (bitten and/or trampled by cattle) at 10 and 27 July 2002. Taking into account that nurse plants provide shade and reduce water evaporation, the original experimental design included watering of the cuttings, However, this was not necessary because they got rain or fog most of the days during that period.

Additional experimental evidence of the effect of browsing ungulates on yew regeneration was obtained from the locality of Lillo (León province), where a small group of adult trees grows in a dense beech forest, many of them inside a 1-ha exclosure built in 1996 as a practical conservation measure to promote forest regeneration. In June 2001, we estimated the density of yew recruits (1st yr, 2nd yr and >2 yr seedlings and saplings) inside and outside the exclosure, by means of ten transects including ten 50×50 cm quadrats at each side of the fence. To limit errors from pseudo-replication derived from the existence of just one large exclosure, samples from inside the exclosure were located far from each other and selected to span all the range of variability in tree cover. In addition, sampling was performed seeking to reduce possible biases in yew recruitment related to seed rain differences inside and outside the fence. For that, the position of transects relative to adult yews was similar at both sides of the exclosure, since many fenced trees were, in fact, adjacent to the fence, and at least five adult trees were located outside the exclosure. On the other hand, due to the relatively recent built-up of the exclosure, there is no reason to suspect that transects differed in their characteristics before exclosure (i.e. there were no differences in shading).

Statistical analyses

Selective seed dispersal among microhabitats was analysed by using a χ^2 test to compare the observed distribution of sampled seeds with an expected distribution representing a random distribution where seed occurrence was proportional to microhabitat cover at each site. The surface of the microhabitats was derived from canopy and ground cover values obtained in the habitat structure sampling. Microhabitat cover was calculated as the ratio of each microhabitat surface to the total surface of sampled microhabitats (see Tewksbury et al. 1999, for a similar procedure). Selection for individual microhabitats was checked by partial χ^2 tests whose levels of significance were corrected by Bonferroni adjustment at $p < 0.05$ (Rice 1989).

First year seedling survival was compared among microhabitats and localities, by using repeated measures ANOVA with log-transformed data, considering the density in summer and winter as the repeated factor and using the season × microhabitat interaction as a test for survival differences.

The percentage of yew recruit covered by the shelter shrub was used as a predictor variable for herbivore damage (damaged vs undamaged, for all recruitment stages) and survival (live vs dead, for first and second year seedlings) in respective logistic regression models. The effects of the microhabitat acting as nucleation centre or nurse plant on herbivore damage and seedling survival were examined performing χ^2-tests.

To examine the effect of the microhabitat on rooted cutting damage, we used a Generalized Linear Model with binomial error. The effect of ungulate herbivore exclosure on yew recruitment abundance was tested separately for each recruitment stage with a nested ANOVA with the factor transect nested within treatment.

Data used in the ANOVAs were tested for normality and homoscedasticity and transformed if necessary. Diameter at breast height was log-transformed. Means are given ± 1 SD.

Results

Spatial distribution of dispersed seeds and first-year seedlings

Seed rain among microhabitats differed significantly from random at both sites (Aramo: $\chi^2_{(3)} = 814.4$, $p < 0.001$; Sueve: $\chi^2_{(3)} = 729.9$, $p < 0.001$). Dispersed seeds occurred more than expected under adult yews, very few seeds being placed at open areas, at both Sueve and Aramo (Table 2). Hollies were positively selected at Aramo, but counterselected at Sueve. Hawthorns received seeds in a proportion similar to their cover at both sites.

There were between sites differences in the number of seedlings that emerged during the spring; the first year seedlings showed higher densities and higher mortality at Sueve than at Aramo (Fig. 2, Table 3). There were also differences between microhabitats, first year seedlings exhibited higher density beneath yews and hollies than beneath other trees or shrubs, and survived better beneath holly canopies at Aramo site (significant interactions season by microhabitat, and season by locality by microhabitat). We found no seedlings in open sites ($N = 75$ 1-m^2 plots at each site).

Table 2. Percentages of recorded bird-dispersed seeds in different microhabitats at Aramo and Sueve sites ($N = 575$ seeds at Aramo and 1877 seeds at Sueve) and the coverage of sampled microhabitats (the microhabitats covered 80.5 and 68.0% of the total area at Aramo and Sueve, respectively). The values and significance levels of partial χ^2 tests, indicating microhabitat selection, are also shown ($DF = 1$; *, $p < 0.05$; n.s., $p > 0.05$; after Bonferroni correction).

	Aramo			Sueve		
Microhabitat	% seeds	% cover	χ^2	% seeds	% cover	χ^2
Taxus	68.00	8.04	485.8 *	73.42	40.00	434.4 *
Ilex	26.61	18.81	9.6 *	15.45	25.73	61.8 *
Crataegus	4.00	2.65	1.8 n.s.	10.70	12.79	3.5 n.s.
Open	1.39	70.50	719.2 *	0.43	21.47	537.7 *

Distribution of recruits in relation to nucleation centres and nurse plants

At Aramo site, the probability that a yew recruit (all stages) suffered herbivore damage decreased with increasing percentage of yew sheltered by nurse plants ($\chi^2_{(1)} = 20.90$, $p < 0.001$; Fig. 3). In the same way, the probability of seedling survival (first to > 2 yr-old seedlings) increased with shelter by nurse plants ($\chi^2_{(2)} = 15.51$, $p < 0.001$; Fig. 3). Most of yew recruits (92.0%) where associated to fleshy-fruited trees and shrubs but no recruit was found in open areas, despite being the dominant microhabitat in the site (56.7% cover). Yew trees were the main nucleation centres for yew recruits, followed by holly-dominated patches (Table 4). However, holly was the main nurse species, since most of the recruits in holly nucleation centres were directly nursed by holly. Additionally, 20% of recruits under yew nucleation-centres were nursed by holly shrubs growing beneath adult yews. Holly was the nurse species providing better shelter, as shown by the comparative percentage of shelter of yew recruits among different nurse species (Table 4). The probability to escape damage by herbivores significantly increased for recruits beneath holly and yew nucleation centres and, specially, for recruits nursed by holly (Table 4; nucleation centre: $\chi^2_{(3)} = 18.72$, $p < 0.001$, $N = 225$; nurse plant: $\chi^2_{(3)} = 14.03$, $p < 0.003$, $N = 205$). The probability of seedling survival was also affected by both the identity of the nucleation centre and the nurse plant (considering yew, holly and hawthorn; nucleation centre: $\chi^2_{(2)} = 23.41$, $p < 0.001$, $N = 106$; nurse plant: $\chi^2_{(2)} = 16.82$, $p < 0.001$, $N = 98$).

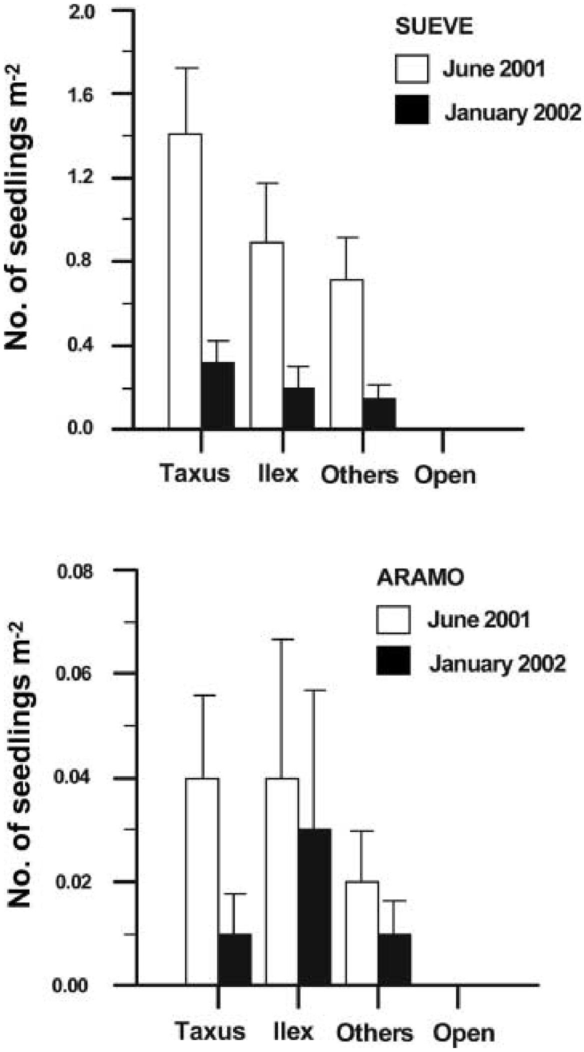

Fig. 2. Density of first year seedlings (number m^{-2}) at two study sites (Sueve and Aramo) during summer (June, 2001) and winter (January, 2002). Note that y-axes show different scales. Means (+1SD) correspond to 10 replicates per microhabitat and site.

Yew regeneration and habitat structure at the regional scale

Mean tree size significantly differed between sites ($F_{(6,\ 279)} = 16.20$, $p < 0.001$; Table 1). The density of saplings ha^{-1} differed among sites ($F_{(6,\ 73)} = 19.23$, $p < 0.001$) and ranges from 1.7 ± 6.3 (15) at Peña Mayor to 707.5 ± 669.5 (10) at Agüeria site. The presence of juvenile (non-reproductive) trees only was important at this later site reducing significantly the average DBH value. The sites differ markedly in patterns of recent regeneration, estimated as the ratio saplings to trees (Table 1). At the population level there was a significant negative relationship between log-transformed

Table 3. ANOVAR examining the density of first year seedlings m^{-2} between localities (Sueve, Aramo), microhabitats (beneath *Taxus*, *Ilex* and other species) and seasons (repeated measure, summer vs winter). The term "subjects" refers to plots where seedlings were counted.

Source of variation	DF	SS	F	p
Within subjects				
Season	1	3.52	33.19	<0.001
Season × locality	1	3.25	30.64	<0.001
Season × microhabitat	3	1.60	5.03	0.003
Season × locality × microhabitat	3	1.44	4.53	0.006
Error	77	8.17		
Between subjects				
Locality	1	7.67	24.03	<0.001
Microhabitat	3	4.11	4.29	0.008
Locality × microhabitat	3	3.66	3.82	0.013
Error	77	24.59		

mean density of saplings and log-transformed mean DBH ($R^2 = 0.732$, $F_{(1,\ 5)} = 13.68$, $p < 0.014$).

At the regional scale, the abundance of yew saplings depended on the availability of nurse plants at population level, as shown by the positive and highly significant relationship between the average density of saplings and ground level cover by woody plants (Fig. 4). Holly cover at ground level provided the best fit (92% of the variance explained) and the ground level cover by other species did no provide additional information (the cover of holly and other species at ground level is correlated). When differences between sites were examined using holly cover at ground level as a covariable there was a significant interaction site by covariable ($F_{(6,\ 57)} = 4.76$, $p = 0.001$), which means that the slope of the relationship between sapling density and holly cover at ground level differed between sites. The ratio saplings to adult trees was significantly correlated to holly ground cover (Pearson product-moment correlation with log-transformed variables: $r = 0.945$, $p < 0.002$, $N = 7$), indicating a positive relationship between nurse plant availability and yew regeneration irrespective of adult yew density.

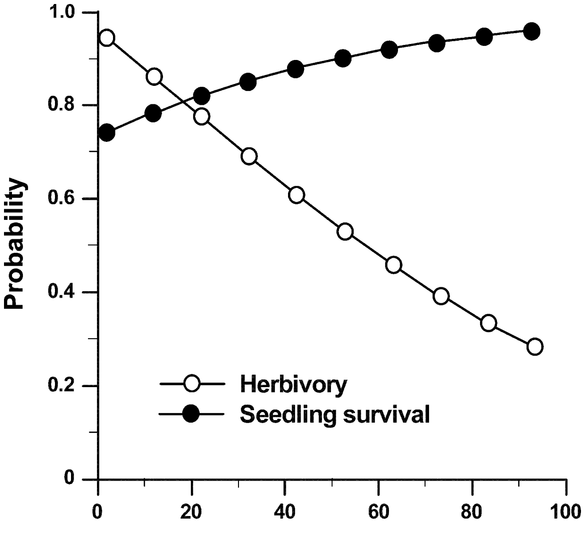

Fig. 3. Probability of herbivore damage (all regeneration stages combined) and probability of seedling survival (first yr to more than two-yr old seedlings) in relation to the percentage of the yew recruit sheltered by nurse bushes. Probability values were obtained from the respective logistic regression models.

Experimental procedures

The rooted cuttings experiment at Aramo site confirmed the above observational results. The number of rooted cuttings damaged by large herbivores (mainly cattle) significantly differed between habitats (Partial $\chi^2_{(2)} = 27.25$, $p < 0.0001$, GLM), since most of cuttings under holly and hawthorn escaped browsing or trampling, whereas a half of cuttings in open suffered damage (Fig. 5).

The density of yew recruits inside the herbivore exclosure at Lillo site was significantly higher than outside (Table 5). These differences were more evident for late regeneration stages such as seedlings older than 2 yr and saplings.

Discussion

Yew regeneration limitation in the Cantabrian range

Yew regeneration was severely constrained in many of the examined populations in the Cantabrian range. Despite that the sites were selected to represent populations with large adult abundances, the presence of recruits was extremely low in Sueve, Peña Mayor and Teixeu, as shown by the sapling-to-adult ratios. These sites also showed older adult age, as depicted by mean tree diameter, indicating a longer regenerative collapse (see also Hulme 1996, García et al. 1999). Conversely, populations like Agüeria and Aramo were characterised by younger age structures, juvenile presence or higher

Table 4. Number of yew recruits (all stages combined) sheltered by different nurse species in different nucleation centres (defined by the dominant species within the patch, FFS indicates other fleshy-fruited trees or shrubs, mainly *Rhamnus alpina* and *Sorbus* sp.). The percentage of recruits occurring under different nurse species and in different nucleation centres, the percentage of sheltering (mean ± 1SD) provided by different nurse species, and the percentage of recruits damaged by ungulate herbivores under different nurse species and in different nucleation centres, are also shown.

	Nurse species					% recruits	% damage
Nucleation centre	*Taxus*	*Ilex*	*Crataegus*	*Corylus*	Other FFS		
Taxus	51	16	0	9	4	37.56	28.40
Ilex	0	59	2	1	1	29.57	16.41
Crataegus	5	1	37	0	2	21.13	48.93
Corylus	0	0	0	18	0	8.45	52.17
Other FFS	0	5	0	0	2	3.29	43.85
% recruits	26.29	38.03	18.31	13.15	4.22		
% of sheltering (mean ± SD)	5.2 ± 17.3	78.2 ± 35.5	32.4 ± 39.5	46.2 ± 36.7	67.2 ± 31.9		
% damage	32.14	18.52	51.28	37.93	33.33		

sapling densities, suggesting active long-term recruitment. Thus, the main feature of yew regeneration status in the Cantabrian range was the variability in the recruitment ability across the landscape. Differences among sites in the succession development from second ground forest to mature beech forest could account for this variability in recruitment, as suggested by Svenning and Magard (1999) for Denmark yew populations, where the shade of tall-statured deciduous trees affects negatively adult reproductive output. However, our data did not support this hypothesis for current Cantabrian populations, since most of studied populations, even those suffering regenerative collapse, occurred in open beech forest and open holly-dominated second growth forest. In addition, the Lillo population inhabited a closed beech forest and, nevertheless, it showed active regeneration. In this site, given the methodological limitations imposed by the use of a single exclosure, the differences in regeneration at both sides of the fence must be interpreted cautiously, since we cannot exclude some confounding effects in the recruitment patterns, specially from seed rain differences, among fenced and unfenced areas. However, most of differences in regeneration were accounted by more than two-year old seedlings and saplings, fenced area weakly differing from outside in the densities of first- and two-year seedlings, suggesting similar seed input at both sides of the exclosure. Thus, enhanced long-term recruitment in this site seems to be related to the absence of browsers imposed by the exclosure. On the other hand, the absence of regeneration in most of other sites cannot be attributed neither to seed limitation, since viable seeds are beared in all sites (data not shown), nor to seed predation (see Hulme 1996) or microsite limitation for seedling establishment, since first-year seedlings appeared in all populations at early summer. Although we did not quantify specific causes for seedling losses at our surveys, evidence of severe browsing or trampling was found in many cases, indicating a significant role of ungulates as a mortality factor. This was also experimentally suggested by the rooted cutting experiment, where a half of recruits were lost from open areas after ungulate damage. Then, both observational and experimental data suggest that the lack of yew regeneration in the Cantabrian Mountains may be attributed to a population bottleneck between seedling emergence and sapling establishment, caused by herbivore-mediated mortality (see also Hulme 1996, García et al. 2000).

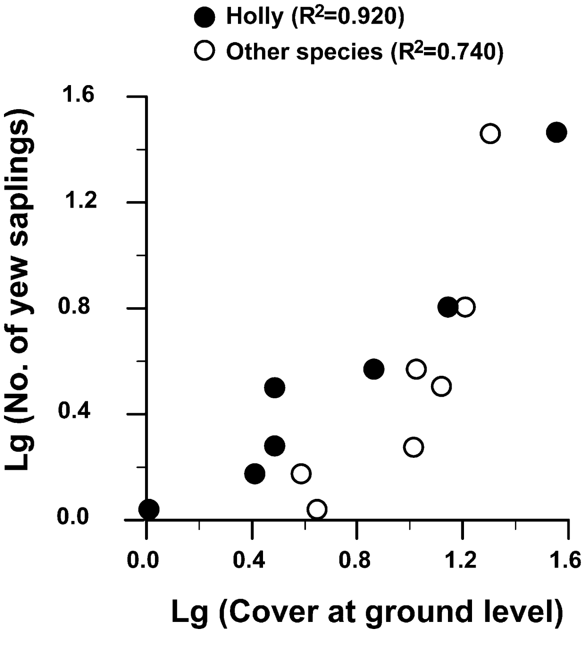

Fig. 4. Mean number of yew saplings from the seven localities included in Table 1 against mean cover of holly (filled circles: $R^2 = 0.920$, $F_{(1,\ 5)} = 57.306$, $p < 0.001$) or other bushes (open circles: $R^2 = 0.704$, $F_{(1,\ 5)} = 11.914$, $p < 0.018$) at ground level. Mean values correspond to 10–15 plots per study site.

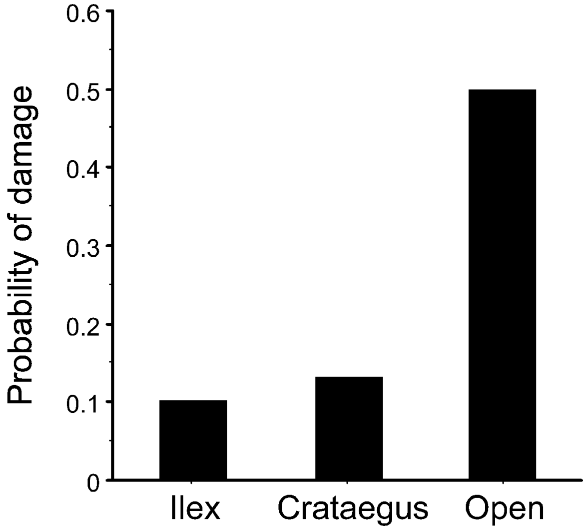

Fig. 5. Probability of herbivore damage of yew rooted cuttings transplanted to different nurse woody species and open areas.

Association among yew recruitment and woody plants

A clear spatial association between yew recruits and woody plants emerged from our results at Sueve and Aramo sites. Most of yew seed rain appeared under adult trees and under other fleshy-fruited shrubs like holly, probably as a result of positive selection of these microhabitat by seed dispersing birds in their post-foraging movements (Jordano and Shupp 2000). This initial seed distribution probably accounted for the differences among microhabitats in the abundance of first-year seedlings at both sites (Fig. 2). In addition, yew trees were the nucleation centres for most recruits at Aramo site (Table 4). Thus, the association between yew regeneration and woody plants might be firstly explained by the formation of nucleation centres dominated by fleshy-fruited plants, as a result of seed dispersal foci in those plants which attract seed dispersing birds with fruit resources (Debussche and Issenmann 1994, Verdú and García-Fayós 1996, 2003) and protection from predators (as is the case of yew and holly, the only species exhibiting evergreen canopies during autumn and winter in the study sites, see also Obeso and Fernández-Calvo 2003). However, when considering the distribution of recruits in relation to the nurse species (Table 4), recruits occurred more under holly than under the species acting as main dispersal foci (yew). This happened because some recruits in yew nucleation centres were actually nursed by hollies, which occurred themselves under yews, probably also as a result of facilitation by dispersal. The fact that the main nurse species (holly) is also providing better shelter suggests that bird-mediated seed rain is not the only mechanism underlying the spatial regeneration pattern of yew at the local scale. In fact, living beneath nurse plants decreased herbivore damage and enhanced seedling survival, as shown by surveys at Aramo. In addition, experimental rooted cuttings planted at Aramo beneath prickly hollies and hawthorns suffered less damage by herbivores than those planted in open areas. We cannot blame drought for damage of cuttings planted in open because the experimental period was particularly rainy. All these observational and experimental data strongly suggest a facilitative effect of woody plants on yew recruitment by providing defence against herbivores. Additional benefits from living under nurse plants, in comparison to open sites, might be related to shelter from frost, improvement of soils and litter layer conditions, and a space free from herbaceous competitors (Franco and Nobel 1989, Belsky 1994, Callaway 1995, Weltzin and McPherson 1999, Holl 2002). To sum up, nurse plants provided not only the best micro-environmental conditions but also shelter from herbivory.

Nurse plant structure and herbivore defence

The defence against herbivores in the studied populations seems to depend on nurse structure, trees and shrubs with branches at their bases acting better as an effective barrier against herbivores. In this sense, the effect of nurse plants on both seedling survival and herbivore damage at Aramo site depended on the proportion of yew recruit sheltered by nurse plants, which were cone-shaped hollies in a high proportion. In addition, the differences in seedling survival beneath holly

Table 5. Density of yew recruits (number m^{-2}) of different regeneration stages inside and outside a large herbivore exclosure (treatment) at Lillo Site. Means (± 1SD) correspond to 10 transects per treatment. The results of the ANOVA examining the differences in the abundance for each different stage are also shown (transect factor is nested within treatment, DF: treatment = 1; transect = 9).

	Fenced	Unfenced	Treatment		Transect	
			F	p	F	p
First yr seedlings	0.39 ± 0.44	0.13 ± 0.28	3.50	0.094	2.59	0.008
Second yr seedlings	0.24 ± 0.17	0.18 ± 0.19	0.74	0.411	1.05	0.400
>2 yr old seedlings	0.51 ± 0.23	0.18 ± 0.19	12.75	0.006	0.96	0.471
Saplings	0.57 ± 0.27	0.13 ± 0.11	23.55	0.001	0.97	0.468

between Sueve and Aramo sites (Fig. 2) may be explained because hollies were cone-shaped at Aramo (branches at soil level) but tree-shaped at Sueve (without low branches). This effect is better reflected when considering yew regeneration across all sites, since high levels of holly ground cover, a direct consequence of the occurrence of cone-shaped trees, apparently determined the abundance of yew saplings. On the other hand, holly could exert the nurse function better than the remaining species thanks to its prickly leaves. Both cone-shaped branching and prickly foliage are the result of ungulate browsing on holly saplings (Obeso 1997, see also Cuartas and García-González 1992), the occurrence of cone-shaped shrubs being dependent on sporadic events of moderate herbivore pressure, permitting establishment, followed by periods of heavy browsing moulding shrub canopy. Thus, paradoxically, herbivory by ungulates is the same factor determining yew regenerative collapse in the Cantabrian range but promoting the occurrence of functionally nurse plants that allow yew regeneration in some populations. The differences in susceptibility to herbivory among yew and its potential nurse species would make possible this situation. That is, current herbivory levels at Cantabrian range preclude yew regeneration, but, in the case of holly, the same levels of herbivory allow sporadic establishment and the formation of cone-shaped shrubs, promoting the occurrence of nurse plants (see also Mitchell 1990). Both structural characteristics and its role as seed dispersal focus in some populations would make the holly the main yew facilitator, suggesting a species-specific facilitation (Callaway 1998).

Consistency of local facilitative effects at the landscape scale

This work evidences that the mechanism underlying yew regeneration in the Cantabrian range is the facilitation by nurse woody plants that provide a regeneration niche (Grubb 1977) by acting as dispersal foci and by protecting yew recruits against herbivores. At the local scale, the potential for yew regeneration would depend on vegetation composition (the occurrence of potential nurse species such as holly) but also on the herbivory management history, moulding vegetation structure towards biogenic safe sites. Moreover, if this process is examined at the regional scale, the role of nurse plants generating suitable habitat for yew recruitment is strongly evident, since the magnitude of yew regeneration at site level (estimated by the abundance of yew recruits as well as by the ratio saplings to trees) depended on the holly ground cover (Fig. 4). This clear landscape pattern emerges from the consistency of local facilitative effects across different sites, since a positive relationship among nurse cover and yew regeneration was found in most of localities. However, the slope of this relationship varied among sites. This was probably related to the differences in vegetation structure among sites, rather than to variation in herbivory pressure. That is, in sites like Teixeu and Sueve, the values of holly ground cover were rather low and homogeneous for all plots within the site, generating smaller gradients of sheltering potential and bearing flatter slopes. Conversely, in sites like Aramo and Agüeria, higher values and stronger differences among plots in holly ground cover would promote stepper slopes. On the other hand, the landscape pattern found here is indirectly suggesting that the high levels of environmental stress (herbivore pressure) promoting facilitation are widespread, since this pattern probably would be masked by the occurrence of low stress (low herbivory) sites where yew recruitment is independent of nurse presence (see also Tewksbury and Lloyd 2001). In conclusion, our study demonstrates that habitat structure, characterised by the presence of functional nurse plants, is accounting for most of variation in yew recruitment across the Cantabrian range, evidencing, therefore, a direct link between facilitation and regeneration at the landscape scale.

Ecological implications for yew management

This work exemplifies how positive interactions among plants must be considered for conservation of threatened plants at both local and regional scale. Its first applied conclusion is that the management of yew must consider simultaneously all the components of the ecological web determining yew regeneration, that is, avian seed dispersers, herbivores and the potential nurse plants (see also Tewksbury et al. 1999). Among the guidelines to improve the current regeneration status of yew in the Cantabrian range we firstly suggest the use of exclosures for ungulate in those populations lacking of nurse plants. When fences are not possible, cattle rotation or selective hunting of wild ungulates would promote low herbivore pressure, allowing initial recruitment of yew or, at least, the establishment of potential nurse plants (holly) whose canopy could be shaped towards cone-like patterns by means of further moderate herbivory. In addition, yew population restoration should consider the use of cone-shaped holly trees as transplanting microsites (see also Castro et al. 2002).

The local measures must be combined with regional-scale management, considering holly woodlands as a target for protection, in order to preserve yew suitable habitat at the landscape level. Moreover, management might be also accomplished at the scale of the biogeographical region, since facilitative effects have been also suggested for yew populations in the Mediterranean mountains of southern Spain, where fleshy-fruited shrubs promote regeneration under drought and herbivory stress (García et al. 2000). The maintenance of the functional interactive triad composed of yew, avian dispersers, and nurse woody plants, irrespective of the identity of species dispersing and nursing yew in each different region, is, therefore, a recommendation for range-scale conservation of this widely threatened species.

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