

Reproductive ecology of *Ochetophila trinervis* in Northwest Patagonia

M. Fernanda Reyes^{A,B}, Miriam E. Gobbi^{A,C} and Eugenia E. Chaia^A

^ADepartamento de Biología General, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue-INIBIOMA, Quintral 1250, 8400 San Carlos de Bariloche, Argentina.

^BCátedra de Ecología, IFEVA, Facultad de Agronomía, Universidad de Buenos Aires-CONICET, Av. San Martín 4453, 1417 Buenos Aires, Argentina.

^CCorresponding author. Email: miriam.gobbi@crub.uncoma.edu.ar

This paper originates from a presentation at the 16th International Meeting on Frankia and Actinorhizal Plants, Oporto, Portugal, 5–8 September 2010.

Abstract. Native actinorhizal species *Ochetophila trinervis* (Gillies ex Hook. & Arn.) Poepp. ex Miers, also known as *Discaria trinervis* (Kellermann *et al.* 2005), grows along watercourses and distant from them, along a rainfall gradient in north-west Patagonia. We studied the reproductive ecology of this species under different macro- and micro-environmental conditions, in three zones in a rainfall gradient (western, intermediate and eastern) with two sites for each one, near and distant to a watercourse (riparian and dry-land). We performed field studies and germination trials. Plant size, reproductive effort (seed abundance per branch) and seed bank size of *O. trinervis*, were favoured by the proximity of streams in the drier environments of the gradient. The abundance of seedlings and saplings in the field was very low, which was in agreement with a lack of germination in the field, despite the good germination capacity of seeds. Sexual reproduction of *O. trinervis* was affected by the low abundance of seedlings. Lower rainfall, higher temperatures and a longer growing season along the gradient favour the potential regeneration of the species. Because of its nitrogen-fixing capacity and other features, *O. trinervis* has potential for reclamation of eroded lands in Patagonia. Patterns of seed biology and regeneration presented in this study will aid in the use of *O. trinervis* in the reclamation of disturbed lands.

Additional keywords: environmental gradient, forest–steppe ecotone, reproductive effort, seed bank, sexual regeneration, Rhamnaceae, shrublands.

Introduction

Ochetophila trinervis (Gillies ex Hook. & Arn.) Poepp. ex Miers, also known as *Discaria trinervis* (Kellermann *et al.* 2005) (Family Rhamnaceae), is a native actinorhizal plant from South America, found from 31° to 48°S (Tortosa 1983a). In north-west Patagonia, it occurs along an eastward rainfall gradient from the ecotone between mesic forest and steppe, to the shrubby steppe, either as a riparian plant along rivers and streams or distant from watercourses. *O. trinervis* also grows in degraded sites such as roadsides and may survive long drought periods in poor soils (Silvester *et al.* 1985). Like other species in the same family, it is a deciduous tree or shrub, with spines, proliferating synflorescences, tri-carpel hermaphrodite flowers, dehiscent fruits (Tortosa 1983a) and a high sprouting capacity.

O. trinervis plants are nodulated by the nitrogen-fixing bacteria *Frankia* under different ecological conditions (Chaia *et al.* 2006) and obtain a significant amount of N from N₂ fixation (Chaia and Myrold 2010). Their foliar N concentration was about twice that of non-actinorhizal shrubs and the senescent leaves had a relatively high N proportion as compared with other deciduous plants of the region (Mazzarino

et al. 1998; Chaia and Vobis 2000; Chaia and Myrold 2010). The highest content of N in its litter could contribute to improve soil conditions (Huss-Danell 1997). Therefore, *O. trinervis* probably plays an important role in the N economy of north-west Patagonian ecosystems (Chaia and Myrold 2010). This species is a good food resource for herbivores in the region because it has high forage value due to the presence of young leaves with higher N concentration, plenty of green shoots, and high palatability for cattle, sheep, deer and hares (Kitzberger *et al.* 2005; Ortiz and Bonino 2007). Moreover, *O. trinervis* could facilitate the entry of new individuals to the system as a nurse plant, as was reported for *Discaria articulata* (Phil.) Miers (Raffaele and Veblen 1998, 2001; Kitzberger *et al.* 2000).

In north-western Patagonia, regeneration of native plants is, in many cases, restricted to their ability to sprout; as a result of severe problems associated with desertification by heavy grazing and fire (Raffaele and Veblen 1998). For this reason, the presence of species with the characteristics of *O. trinervis* can lead to natural regeneration (Dawson 1990). Sexual reproduction includes a sequence of demographic processes: production of flowers, fruits and seeds, seed dispersal, seed conservation in the

soil (seed bank), germination and development of seedlings and saplings. These processes are highly susceptible to macro- and micro-environmental conditions that affect the generation of new reproductive individuals to complete the cycle. If any of them has a low probability of success, the feedback is limited or fails (Houle 1995). The loss of propagules in each demographic stage determines a possible recruitment 'bottleneck' (Jordano *et al.* 2002).

The study of demographic stages of sexual regeneration of *O. trinervis* will help in developing guidelines for the conservation and management of this native species, as well as for possible use in the restoration of degraded environments (Ferrari and Wall 2004; Turner *et al.* 2005).

The aim of this work was to determine: (1) the state of regeneration, (2) macro- and micro-environmental conditions associated with regeneration and (3) the importance of the *O. trinervis* seed bank in communities located in different zones of the environmental gradient in north-western Patagonia.

Materials and methods

Study area

Study area was located in an environmental gradient along the transitional region between the xeric forest and the steppe (ecotone) of north-west Patagonia, in the provinces of Neuquén and Río Negro, Argentina. We determined three zones, designated as western and intermediate (placed inside the Nahuel Huapi National Park) and eastern (Fig. 1; Table 1). The zones included a sequence of mesic forest vegetation, forests and xeric shrublands, mixed thickets of tall shrubs in post-disturbed areas and patches of *Austrocedrus chilensis* (D. Don) Pic.Serm. and Bizzari or *Maytenus boaria* Mol. forests, in the steppe. The climate is temperate cool, with a steep precipitation gradient decreasing eastward as a consequence of moisture discharge of winds coming from the Pacific Ocean; the temperature increases as the air masses become drier (Paruelo *et al.* 1998). These combined factors enlarge the extent of the growing season along the gradient (Jobbágy *et al.* 2002).

Study of the natural regeneration

Natural regeneration of *Ochetophila trinervis* (Gillies ex Hook. & Arn.) Poepp. ex Miers was studied in different shrublands of *O. trinervis* located in the western, intermediate and eastern

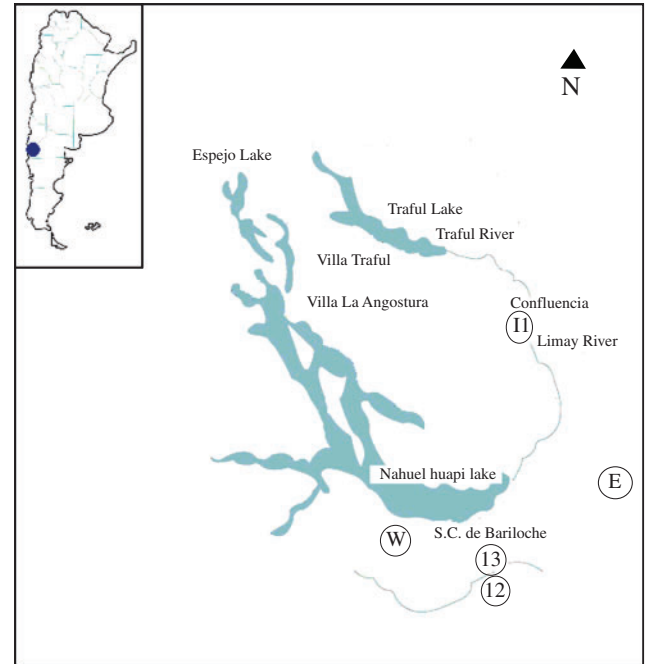


Fig. 1. Study area of the reproductive ecology of *Ochetophila trinervis*. Circles indicate zones of the gradient: western, W ($41^{\circ}08'19''$;S, $71^{\circ}24'30''$;W); intermediate, I1 ($41^{\circ}03'45''$;S, $71^{\circ}03'02''$;W), I2 ($41^{\circ}10'00''$;S, $71^{\circ}18'00''$;W) and I3 ($41^{\circ}08'45''$;S, $71^{\circ}18'45''$;W); eastern, e ($41^{\circ}03'46''$;S, $70^{\circ}59'44''$;W).

zones (Fig. 1, W, I1 and E). Two sites were selected in each zone: a riparian site (associated to a watercourse, stream or river) and a dry-land site, (distant to the watercourse, Table 1). There were no presumptive hybrid individuals between *O. trinervis* and *Discaria chacaye* (G. Don) Tortosa. with morphologically intermediate features (Tortosa 1983b) in the sites.

The first sampling was conducted in early autumn when fruits of *O. trinervis* were at maturity stage. At each site, we recorded the species diversity. To study the reproductive effort and characterise *O. trinervis* mature plants we randomly selected five individuals. In each plant, height, stem basal diameter and crown projection were measured. Then the last 40 cm of three branches, located at the four cardinal positions, were cut and placed into plastic bags. We counted the number of fruits per

Table 1. Description of the study area which included three zones (western, intermediate and eastern) and the study sites (riparian and dry-land) of the reproductive ecology of *Ochetophila trinervis*, in the environmental gradient of north-west Patagonia (see Fig. 1 for the sites localisation)

Zone	Western		Intermediate		Eastern	
Locality	Virgen de las Nieves		Villa Llanquín		Estancia San Ramón	
Average annual precipitation (mm)	1350		750		450	
Average annual temperature (°C)	8.0		8.2		8.4	
Sites	Riparian	Dry-land	Riparian	Dry-land	Riparian	Dry-land
Altitude (m)	815	818	650	660	870	890
Distance to stream (m)	9	150	2	150	2	150

branch and measured the dry weight of leaves, stems and fruits. Reproductive effort was estimated by calculating the mean number of seeds per branch (mean number of seeds in 20 fruits times the total number of fruits per branch). We also measured the mean seed weight.

To study the microsite effect, a set of four 40 × 40 cm plots were laid out below and outside the crown of *O. trinervis* trees. In each plot we estimated the vegetation cover as a percentage and the abundance of seedlings and saplings of *O. trinervis*.

To study the size of the *O. trinervis* seed bank, we performed a second sampling after the period of fruit dehiscence, in late fall. Four soil samples were collected under the edge of the canopy of each plant with a soil corer (10 cm diameter) to a depth of 5 cm. These soil samples were kept at 4°C for a week to stratify seeds contained in the sample. To determine the seed bank size we used the seedling emergence technique (Van Der Valk and Davies 1978), as we considered that only the seeds with the capacity to germinate contribute to the seed bank size. The individual soil samples were placed in trays in a growth chamber with 16 h photoperiod provided by halogen lamps (PAR 100 μM m⁻² s⁻¹), and watered three times a week. Average night and day temperatures were 10 and 20°C, respectively.

Field test germination

To analyse the *O. trinervis* seed germination under natural conditions we conducted two field experiments in areas devoid of individuals of the species, located in the intermediate zone (see Fig. 1, I2 and I3 for trials 1 and 2, respectively). The seeds were collected in March 2005 in a neighbouring area (Pampa de Huenuelo). The first trial was established to determine the effect of sowing time, shade and the presence of litter and, the second to study the effect of shade and moisture. Seeds used for the field assays were stratified at 4°C for 15 days. To prevent fungal infection during the stratification, the seeds were first surface sterilised with sodium hypochlorite 5% m/v for 10 min, and thoroughly rinsed with sterile water. We estimated the average maximum germination capacity of the lot by placing seeds in sterile Petri dishes containing a mixture of sand and vermiculite. We considered that seeds had attained the maximum germination capacity when there was no further germination after 4 days. The dishes were placed in a growth chamber with 16 h photoperiod (PAR 318 μM m⁻² s⁻¹), provided by halogen lamps (Philips HPI-T and Philips 400W SON-T Plus 400; Royal Philips Electronic Holland, Belgium), and watered twice a week. Average maximum and minimum temperatures were 26 and 21°C, respectively, and RH was 24%.

The first trial was conducted in Challhuaco streambank (Fig. 1, I2), near a shrubland of this species growing downstream. The site vegetation was dominated by shrubs of *Diostea juncea* (Gillies & Hook.) Miers, *Schinus patagonicus* Raddi and *Berberis microphylla* G. Forst. and the herbaceous cover was very low. The proportion of bare soil was 50%. We considered two sowing seasons (spring and autumn, November 2005 and March 2006, respectively), two shade levels (below bushes cover and in shrubland gaps) and two levels of soil cover with litter (presence and absence). Sowing was performed in

0.30 m line sowing (experimental units). Trial was done with 15 replicates for each combination of treatment levels (120 experimental units), with 50 seeds each.

The second trial was conducted in November 2006 in the field of the Centro Regional Universitario Bariloche (Fig. 1, I3). The trial design included two shade levels (same as trial 1) and three moisture levels (low, medium and high). Humidity levels were established according to the water holding capacity (WHC) of the soil. Soil characteristics in this site were: pH 6.3, electrical conductivity 9.2 μS cm⁻¹, C content 2.4%, N content 0.17% and field capacity 43%.

Moisture levels, measured by gravimetry, were established as: low (half of the moisture level recorded at the moment of the soil collection to perform the trial), medium (soil moisture level at the moment of soil collection) and high (corresponding to WHC 80% of the soil).

We used plastic pots with holes on the base to allow water drainage. Each pot was filled with 1 kg of soil previously collected in I2 (Fig. 1) and 50 seeds of *O. trinervis*. Pots were watered with the amount of water corresponding to the different moisture treatments (low, medium and high). Then, 18 pots were buried below the shrub cover and the other 18 in the shrubland gap. The pots were unearthed and weighted three times a week. The pots were refilled with water according to the corresponding weight for each treatment level and then reburied. Trial was done with six repetitions for each combination of treatment levels.

Data analysis

The similarity of species composition among sites in each zone was calculated with the Jaccard coefficient of community (Matteucci and Colma 1982). The association between gradient zones and sites, for reproductive effort and the seed bank size, was established by using Chi-square (χ^2) of observed versus expected values. Expected values were calculated as the average of the observed ones. We used Spearman correlation to assess the relationship between reproductive effort and seed bank size with height, canopy diameter and stem basal diameter of the plants ($n=30$). We considered the probability of 0.05 as the level of rejection.

Results

Zones and sites differed in vegetation composition and physiognomy (Table 2). The shrublands occurring in the Intermediate and the eastern zones were dominated by *O. trinervis*. The western zone had mixed shrublands with the highest species richness and with the smallest *O. trinervis* shrubs in the gradient.

The total vegetation cover, both under *O. trinervis* shrubs and in gaps between them, was higher in the eastern and western zones, whereas the intermediate zone had the higher proportion of bare soil (sandy and stony). Other tree species under the canopy of *O. trinervis* shrubs were absent. The highest similarity value of species composition between riparian and dry-land environments corresponded to the western zone (Jaccard coefficient of community (CC_{R,T})=0.71, 0.44 and 0.53 for western, intermediate and eastern zones, respectively).

Table 2. Vegetation description, vegetative cover and morphological characteristics of *Ochetophila trinervis* in the study sites of the reproductive ecology of the species, in the environmental gradient of north-west PatagoniaThe morphological data are mean \pm s.d. ($n=30$)

Zone	Western		Intermediate		Eastern	
	Riparian	Dry-land	Riparian	Dry-land	Riparian	Dry-land
<i>Acaena splendens</i> H. & A.				X	X	X
<i>Austrocedrus chilensis</i> (D. Don) Pic.Serm. and Bizzarri	X	X				
<i>Berberis microphylla</i> G. Forst.	X	X	X	X	X	X
<i>Chusquea culeou</i> E. Desv.	X	X				
<i>Conium maculatum</i> L.		X			X	
<i>Dactylis glomerata</i> L.		X		X		X
<i>Diostea juncea</i> (Gillies & Hook.) Miers	X	X				
<i>Discaria chacaye</i> (G. Don) Tortosa	X	X		X		
<i>Equisetum bogotense</i> Kunth	X				X	
<i>Fabiana imbricata</i> Ruiz & Pav.			X	X		
<i>Festuca</i> sp.	X	X	X	X	X	X
<i>Fragaria chiloensis</i> (L.) Mill.	X	X				
<i>Geranium sessiliflorum</i> L.					X	
<i>Juncus</i> sp.					X	
<i>Maytenus boaria</i> Mol.	X	X		X		
<i>Mentha</i> sp.		X			X	
<i>Mulinum spinosum</i> (Cav.) Pers.		X		X		X
<i>Mutisia spinosa</i> Ruiz and Pav.	X		X	X		
<i>Nothofagus antarctica</i> (G.Forst.) Oerst.	X	X				
<i>Nothofagus dombeyi</i> (Mirb.) Oerst.	X	X				
<i>Ochetophila trinervis</i> (Gillies ex Hook. & Arn) Poepp. ex Miers	X	X	X	X	X	X
<i>Plantago lanceolata</i> L.	X	X		X		
<i>Rosa rubiginosa</i> L.	X	X	X	X	X	X
<i>Rumex acetosella</i> L.					X	X
<i>Salix humboldtiana</i> Willd.					X	
<i>Schinus patagonicus</i> Raddi	X	X	X			
<i>Senecio filaginoides</i> De Candolle	X	X	X		X	X
<i>Solidago chilensis</i> Meyen				X	X	X
<i>Stipa speciosa</i> Trin. & Rupr.			X	X	X	X
<i>Species richness</i>	17	19	9	14	15	11
<i>Vegetal cover (%)</i>						
Under the canopy of <i>O. trinervis</i>	100	80	16	52	100	92
In gaps of vegetation	89	43	1	37	100	82
In gaps of vegetation	11	55	30	17	0	10
<i>Morphometry of O. trinervis</i>						
Height (m)	1.9 \pm 0.5	1.8 \pm 0.2	2.8 \pm 1.0	4.1 \pm 1.1	3.6 \pm 0.6	1.7 \pm 0.2
Mid-canopy (m ²)	2.1 \pm 0.2	1.8 \pm 0.9	3.6 \pm 1.5	5.4 \pm 2.8	7.0 \pm 4.0	3.1 \pm 0.6
Stem basal diameter (cm)	5.7 \pm 2.8	3.8 \pm 1.9	11.8 \pm 6.0	21.3 \pm 11.5	28.6 \pm 17.9	10.3 \pm 5.1

Reproductive effort

The reproductive effort of *O. trinervis* showed variations between the gradient zones and sites (Fig. 2). Plants of the western zone had lower seed abundance per branch ($\chi^2 = 379.25$, $P < 0.0001$, $df = 2$) than plants of the other two zones, which were similar ($\chi^2 = 0.07$, $P = 0.779$, $df = 1$). Five percent of the total registered seeds corresponded to the western zone and 48 and 47% to the intermediate and eastern zones, respectively. In riparian sites there was a marked increase in the number of seeds per branch from the western to the eastern zones ($\chi^2 = 577.21$, $P < 0.0001$, $df = 2$), but in the dry-land sites, the largest quantity of seeds was recorded in the intermediate zone.

In the eastern zone, recorded values were intermediate as compared with the other two zones ($\chi^2 = 572.81$, $P < 0.0001$, $df = 2$). The relationship between seed abundance and branch dry weight followed the same pattern of reproductive effort for zones and sites under study (data not shown).

The average number of seeds per fruit was lower than the potential maximum of three seeds per fruit. This average was higher in riparian environments at the extremes of the gradient (Fig. 3). The average seed weight was 2.2 mg (± 0.27) and similar in all zones and sites. We found seed-eating weevils (Family Curculionidae) within a very small number of fruit (less than 1% of fruits).

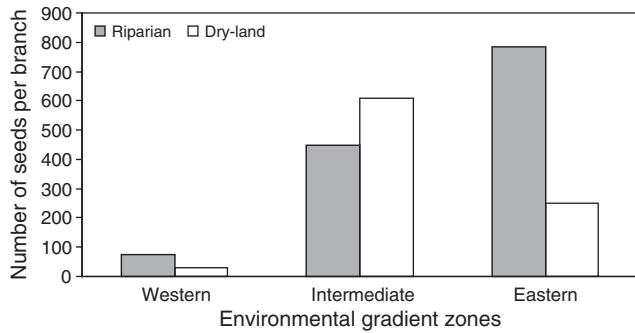


Fig. 2. Mean reproductive effort of *Ochetophila trinervis* in the sites across the environmental gradient of north-west Patagonia.

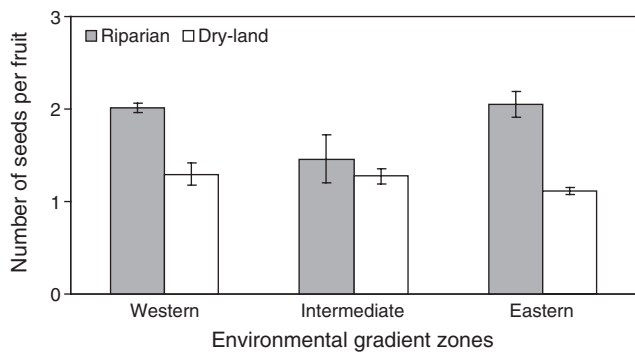


Fig. 3. Mean number of seeds per fruit of *Ochetophila trinervis* in the sites of the environmental gradient of north-west Patagonia ($n=30$).

Seed bank size

Seed bank size of *O. trinervis* (Fig. 4) from the eastern and intermediate zones was similar ($\chi^2 = 1.05$, $P < 0.303$, $df = 1$), and higher than those from the western zone ($\chi^2 = 47.29$, $P < 0.0001$, $df = 2$). The seed bank of riparian and dry-land sites showed different trends in the gradient. It was absent in the riparian site from the western zone and had ~ 45 seedlings m^{-2} in the intermediate and eastern zones ($\chi^2 = 0.05$, $P < 0.821$, $df = 1$). The dry-land sites of the intermediate and eastern zones had a bigger seed bank than that in the western zone, which produced only 2% of the total seedlings ($\chi^2 = 5.06$, $P < 0.024$, $df = 1$). Within each zone, sites were similar to each other ($\chi^2 = 2.37$, $P < 0.123$, $df = 1$ and $\chi^2 = 0.94$, $P < 0.332$, $df = 1$, for the intermediate and eastern zones, respectively).

The relationship between seed bank size and reproductive effort was very low (Fig. 5). Up to 16% of the seeds produced a seedling in the bank. In dry-land environments this ratio increased eastward, and in riparian sites it followed the opposite trend. In the intermediate zone, this relationship was similar in both environments. The seed bank size and the reproductive effort of *O. trinervis* were positively correlated with height, canopy cover and stem basal diameter of *O. trinervis* plants (Table 3).

Regeneration of *O. trinervis* and microsite conditions

The abundance of seedlings and saplings of *O. trinervis* recorded in the field was always very low. *O. trinervis* seedlings were only

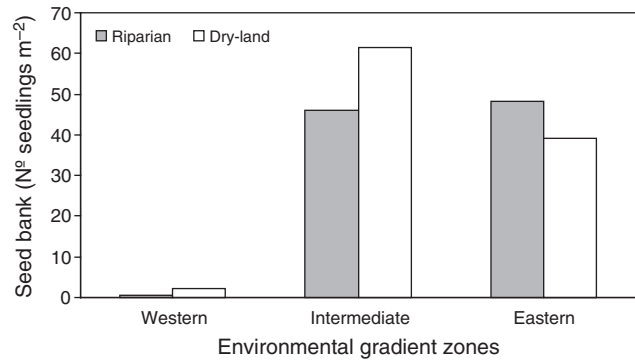


Fig. 4. Mean size of the seed bank of *Ochetophila trinervis* in the sites of the environmental gradient of north-west Patagonia ($n=30$).

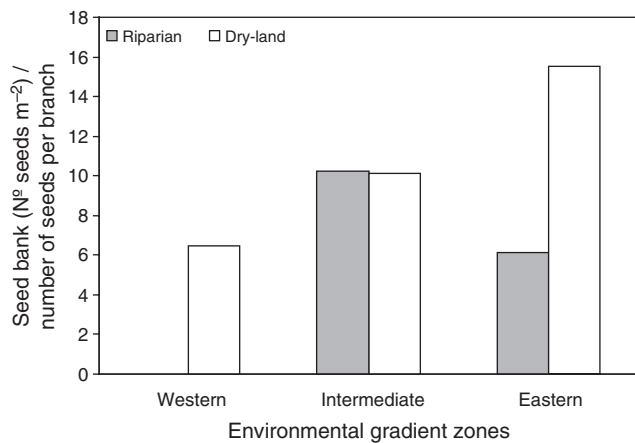


Fig. 5. Relationship between the mean size of the seed bank of *Ochetophila trinervis* and reproductive effort in the sites of the environmental gradient of north-west Patagonia ($n=30$).

Table 3. Correlation matrix between reproductive effort and seed bank size, with height, canopy and stem basal diameter of *Ochetophila trinervis* plants

Spearman's correlation coefficient (r) and probability value ($n=30$)

	Reproductive effort (no. of seeds per branch)		Seed bank size (no. of germinated seedlings m^{-2})	
	r	P -value	r	P -value
Plant height (m)	0.63	0.0001	0.45	0.0120
Mid-canopy (m^2)	0.53	0.0026	0.57	0.0010
Stem basal diameter (cm)	0.50	0.0044	0.68	<0.0001

found in the dry-land site of the eastern zone (2 ± 4.5 seedlings m^{-2}). The low regeneration density did not allow relationships with microsite conditions to be found.

Germination of *O. trinervis*

Although the germination rate of *O. trinervis* seeds under laboratory conditions was high (65% within 7 weeks after sowing), no germination was recorded in the field trials. In

October 2006, after the wet season, we found evidence of river flooding on 33% of the sowing line.

Discussion

The reproductive ecology of *O. trinervis* varied along the environmental gradient in north-western Patagonia, showing a significant reduction in the number of reproductive structures from one phenological stage to the next. This finding indicates the existence of a recruitment bottleneck in the species.

The performance of individuals, in terms of reproductive effort, seed bank size and plant size, was lowest in the western zone where the mean annual rainfall is the highest, but increased eastward in riparian environments where moisture is not a limiting factor. This suggests that in the absence of water limitations, other factors such as the conditions of the semiarid steppe (longer growing season, lower vegetal cover and a higher proportion of bare soil) could have favoured a higher plant size, which was found to be a good determinant of seed production (Zammit and Zedler 1993).

As in other shrubby species (Ramírez 1992), *O. trinervis* had a reduced number of seeds per fruit (indicated by one or more empty locules) with an increased number of eggs, flowers and aborted fruits. Developing fruits may abort one or more seeds per plant in response to intrinsic factors, like competition between seeds for limited resources (Ramírez 1992) or environmental conditions as seed predation (Aizen 1992), although the latter was negligible for *O. trinervis* in this study. For other species of Rhamnaceae, reduced seed production has been attributed to a limitation in insect-mediated pollination or to the loss of eggs due to summer droughts (Hampe 2005).

Seed mean weight was the only factor not affected either by the gradient or by the environment (riparian or dry-land). In other woody species, the range of variability of seed weight is considerably higher and generally decreases with the number of seeds, which is also related to flower and fruit production (Ramírez 1992).

The results of the present study support previous findings that the sexual regeneration in native shrubs and trees is low under field conditions (Heinemann *et al.* 2000; Rovere *et al.* 2004; Heinemann and Kitzberger 2006). The absence of *O. trinervis* seedlings in the field, despite of the existence of seed banks, could be explained by two non-exclusive causes: reduced seed viability and germination under field conditions and/or unfavourable conditions for germination (Squeo *et al.* 2006). In riparian sites, the absence of seedlings in the field could be related to increases in stream levels due to thaw or rains, resulting in dragged seed banks, which was observed in the sowing trials. Flooding of rivers and streams after the rainy season can increase seed mortality due to friction between seeds and riverbed sediments. Further, the seed bank may be moved, and seeds deposited in unsuitable microsites for germination (Hampe and Arroyo 2002). This could also explain the absence of seedlings and saplings of other tree species after the rainy season at these sites.

The moist storage in the soil could be a severe limiting factor for seed survival if germination does not occur in the spring following its dispersal. Fluctuations in soil moisture as well as pathogens and predators attack could result in reduced

germination rate and survival of newly germinated seedlings (Louda 1989). Under growth-chamber conditions, we found high germination rates for seeds contained in the seed bank and for seeds directly collected from plants, which were surface sterilised. Treating the seeds with oxidising agents and large volumes of water can lead to a loss of compounds being positive or negative for microbial infection, which, in some cases, favour plant growth (Chaia *et al.* 2010). Nevertheless, since an objective of the trial was to evaluate germination capacity, we did not take in account an eventual effect due of seed disinfection. Although the results of both germination trials expresses only potential regeneration, which seldom occurs in natural environments, they were used as a reference for a comparison with results under field conditions. Field seed germination was not enhanced either by moisture or by the protection provided by litter or the shade of bushes.

Germination failure was in agreement with the absence of seedlings and saplings in natural conditions at most of the sites. The site with increased recruitment of the species was the eastern dry-land site, where the seed bank was not exposed to river floods. Keogh and Bannister (1992) suggested that the lack of germination under natural conditions of *Discaria toumatou* seeds could be related to a double dormancy (a watertight seed coat and embryo dormancy) that reversed with stratification and scarification with gibberellic acid. This does not seem to be the case for *O. trinervis* seeds, which do not need scarification to achieve a high germination rate. A similar pattern was found for *D. pubescens*, an Australian Rhamnaceae, whose seeds germinated only under greenhouse conditions, therefore, restraining species regeneration under natural conditions (Humphries 1993). In several species of *Rhamnus*, which have seeds with high germination capacity, seedling recruitment is strongly limited by water, especially during the first summer after germination when roots of seedlings are not well-developed. These plants typically germinate under the canopy of shrubs where shade reduces water stress in seedlings, thus, increasing their survival in the first year compared with seedlings that grow in clear areas (Pratt *et al.* 2007).

O. trinervis and other species belonging to Rhamnaceae are part of the diet of herbivores in the region (Farji-Brener and Ghermandi 2004; Kitzberger *et al.* 2005; Ortiz and Bonino 2007); therefore, it is possible that the abundance of seeds or seedlings is reduced by the herbivory pressure. We suggest that there is a relationship between the recruitment, the lack of germination in the field and the environmental conditions during the years in which we conducted sowing trials. Both grazing and summer drought may affect the mortality of seedlings and saplings of native woody species (Veblen *et al.* 1989; Gobbi and Schlichter 1998; Relva and Veblen 1998; Kitzberger *et al.* 2000; Relva *et al.* 2009).

The absence of seedlings and saplings of other tree species under the canopy of *O. trinervis*, calls into question its potential as a nurse plant.

In conclusion, macro- rather than micro-environmental conditions have a negative effect in the recruitment of *O. trinervis* in the region. The actual regeneration of *O. trinervis* in north-western Patagonia is strongly limited by recruitment under natural conditions. Despite the great potential

for regeneration of the species, environmental conditions in the region are probably not usually suitable for these plants, preventing them from becoming more dominant in these communities.

Ecological features of *O. trinervis*, such as its ability to grow in poor soils, to fix N, to form riparian scrubs, to sprout (Kitzberger et al. 2000; Raffaele and Veblen 2001) and to form part of the herbivores diet, means that *O. trinervis* represents a potentially useful option for the recovery of vegetation in degraded environments in the study region. Further research on the influence of other aspects like temperature on the recruitment of *O. trinervis* and the effect of different types of herbivores on its saplings could contribute to a better understanding of the regeneration process of the species and to promote its sustainable management.

Acknowledgements

We thank the Universidad Nacional del Comahue and the Nahuel Huapi National Park for the award of a fellowship to MF Reyes, and permission to conduct field studies and seed collection. We also thank Cecilia Brion, Cecilia Ezcurra, Facundo Cabezas and Luciana D'Acunto for comments and suggestions, and Beatriz Santos and Claudia Cártes for language corrections.

References

- Aizen MA (1992) Predación de semillas de *Acacia aroma* por el *Pseudopachymeria grata* en función de la posición de las semillas y el número de semillas por vaina. *Ecología Austral* **1**, 17–23.
- Chaia EE, Myrold DD (2010) Variation of ^{15}N natural abundance in leaves and nodules of actinorhizal shrubs in north-west Patagonia. *Symbiosis* **50**, 97–105. doi:10.1007/s13199-009-0040-z
- Chaia EE, Vobis G (2000) Seasonal change of the actinorhizal nodules and the movement of N in *Discaria trinervis*. In 'Nitrogen fixation: from molecules to crop productivity'. (Eds FO Pedrosa, M Hungria, M Geoffrey Yates, WE Newton) p. 469. (Kluwer Academic Publishers: Dordrecht, The Netherlands)
- Chaia EE, Fontenla S, Vobis G, Wall LG (2006) Infectivity of soilborne *Frankia* and mycorrhizae in *Discaria trinervis* along a vegetation gradient in Patagonian soil. *Journal of Basic Microbiology* **46**, 263–274. doi:10.1002/jobm.200510095
- Chaia EE, Wall LG, Huss-Danell K (2010) Life in soil by the actinorhizal root nodule endophyte *Frankia*. A review. *Symbiosis* **51**, 201–226. doi:10.1007/s13199-010-0086-y
- Dawson JO (1990) Interactions among actinorhizal and associated plant species. In 'The biology of *Frankia* and actinorhizal plants'. (Eds CR Schwintzer, JD Tjepkema) pp. 299–317. (Academic Press: New York)
- Farji-Brener AG, Ghermandi L (2004) Seedling recruitment in a semi-arid Patagonian steppe: facilitative effects of refuse dumps of leaf-cutting ants. *Journal of Vegetation Science* **15**, 823–830.
- Ferrari A, Wall LG (2004) Utilización de árboles fijadores de nitrógeno para la revegetación de suelos degradados. *Revista de la Facultad de Agronomía, La Plata* **105**, 63–87.
- Gobbi M, Schlichter T (1998) Survival of *Austrocedrus chilensis* seedlings in relation to microsite conditions and forest thinning. *Forest Ecology and Management* **111**, 137–146. doi:10.1016/S0378-1127(98)00314-4
- Hampe A (2005) Fecundity limits in *Frangula alnus* (Rhamnaceae) relict populations at the species' southern range margin. *Oecologia* **143**, 377–386. doi:10.1007/s00442-004-1811-0
- Hampe A, Arroyo J (2002) Recruitment and regeneration in populations of an endangered South Iberian Tertiary relict tree. *Biological Conservation* **107**, 263–271. doi:10.1016/S0006-3207(02)00061-7
- Heinemann K, Kitzberger T (2006) Effects of position, understorey vegetation and coarse woody debris tree regeneration in two environment contrasting forests of north-western Patagonia: a manipulative approach. *Journal of Biogeography* **33**, 1357–1367. doi:10.1111/j.1365-2699.2006.01511.x
- Heinemann K, Kitzberger T, Veblen TT (2000) Influences of gap microheterogeneity on the regeneration of *Nothofagus pumilio* in a xeric old-growth forest of northwestern Patagonia, Argentina. *Canadian Journal of Forest Research* **30**, 25–31.
- Houle G (1995) Seed dispersal and seedling recruitment: the missing link(s). *Ecoscience* **2**, 238–244.
- Humphries RK (1993) Hairy anchor plant *Discaria pubescens*. Flora y Fauna Guarantee Action Statement 47. Available at Department of Conservation and Natural Resources Victoria, Melbourne.
- Huss-Danell K (1997) Transley Review No. 93. Actinorhizal symbioses and their N_2 fixation. *New Phytologist* **136**, 375–405. doi:10.1046/j.1469-8137.1997.00755.x
- Jobbágy EG, Sala OE, Paruelo JM (2002) Patterns and controls of primary production in the Patagonian steppe: a remote sensing approach. *Ecology* **83**, 307–319.
- Jordano P, Zamora R, Marañón T, Arroyo J (2002) Claves ecológicas para la restauración del bosque mediterráneo. Aspectos demográficos, ecofisiológicos y genéticos. *Ecosistemas* **11**(1). Available at <http://www.revistaecosistemas.net/articulo.asp?Id=312>.
- Kellermann J, Medan D, Aagesen L, Hilger H (2005) Rehabilitation of the South American genus *Ochetophila* Poepp. ex Endl. (Rhamnaceae: Colletieae). *New Zealand Journal of Botany* **43**, 865–869. doi:10.1080/0028825X.2005.9512996
- Keogh JA, Bannister P (1992) A method for inducing rapid germination in seed of *Discaria toumatou* Raoul. *New Zealand Journal of Botany* **30**, 113–116.
- Kitzberger T, Steinaker DF, Veblen TT (2000) Effects of climatic variability on facilitation of tree establishment in northern Patagonia. *Ecology* **81**, 1914–1924. doi:10.1890/0012-9658(2000)081[1914:EOCVOF]2.0.CO;2
- Kitzberger T, Raffaele E, Veblen T (2005) Variable community responses to herbivory in fire-altered landscapes of northern Patagonia, Argentina. *African Journal of Range & Forage Science* **22**, 85–91. doi:10.2989/10220110509485865
- Louda SM (1989) Predation in the dynamics of seed regeneration. In 'Ecology of soil seed banks'. (Eds MA Leck, VT Parker, RL Simpson) pp. 65–89. (Academic Press: San Diego)
- Matteucci SD, Colma A (1982) Metodología para el estudio de la vegetación. Monografía Científica No. 22, Serie de Biología, Secretaría General de la Organización de los Estados Americanos. (Washington, DC)
- Mazzarino MJ, Bertiller M, Schlichter T, Gobbi M (1998) Nutrient cycling in Patagonia ecosystems. *Ecología Austral* **8**, 167–181.
- Ortiz C, Bonino NA (2007) Dieta estacional del ciervo colorado (*Cervus elaphus*) en el Parque Nacional Nahuel Huapi, Argentina. *Ecología Austral* **17**, 281–286.
- Paruelo JM, Beltrán A, Jobbágy E, Sala OE, Golluscio RA (1998) The climate of Patagonia: general patterns and control on biotic processes. *Ecología Austral* **8**, 85–101.
- Pratt RB, Jacobsen AL, Golgotiu KA, Sperry JS, Ewers FW, Davis SD (2007) Life history type and water stress tolerance in nine Californian chaparral species (Rhamnaceae). *Ecological Monographs* **77**, 239–253. doi:10.1890/06-0780
- Raffaele E, Veblen TT (1998) Facilitation by nurse shrubs or resprouting behavior in a post-fire shrubland in northern Patagonia, Argentina. *Journal of Vegetation Science* **9**, 693–698. doi:10.2307/3237287

- Raffaële E, Veblen TT (2001) Effects of cattle grazing on early post-fire regeneration of matorral in northwest Patagonia, Argentina. *Natural Areas Journal* **21**, 243–249.
- Ramírez N (1992) Las características de las estructuras reproductivas, niveles de aborto y semillas producidas. *Acta Científica Venezolana* **43**, 167–177.
- Relva MA, Veblen TT (1998) Impacts of introduced large herbivores on *Austrocedrus chilensis* forests in northern Patagonia, Argentina. *Forest Ecology and Management* **108**, 27–40. doi:10.1016/S0378-1127(97)00313-7
- Relva MA, López Westerholm C, Kitzberger T (2009) Effects of introduced ungulates on forest understory communities in northern Patagonia are modified by timing and severity of stand mortality. *Plant Ecology* **201**, 11–22. doi:10.1007/s11258-008-9528-5
- Rovere A, Gobbi ME, Relva MA (2004) Regeneración de *Austrocedrus chilensis*. In 'Ecología y Manejo de los bosques de Argentina. EDULP'. (Eds MF Arturi, JL Frangi, JF Goya) (CD-ROM) (La Plata, Bs. As.: Argentina)
- Silvester WB, Balboa O, Martínez JA (1985) Nodulation and nitrogen fixation in members of the Rhamnaceae (*Colletia*, *Retanilla*, *Talguenea* and *Trevoa*) growing in the Chilean matorral. *Symbiosis* **1**, 29–38.
- Squeo FA, Arroyo MT, Jara PA, León M, Olivares NA (2006) Banco de semillas en los Andes desérticos de Chile. In 'Geoecología de los Andes Desérticos: La Alta Montaña del Valle del Elqui'. (Ed. J Cepeda) pp. 107–119. (Ediciones Universidad de La Serena: La Serena, Chile)
- Tortosa RD (1983a) El género *Discaria* (Rhamnaceae). *Boletín de la Sociedad Argentina de Botánica* **22**, 301–335.
- Tortosa RD (1983b) Una especie polimorfa de *Discaria*: *D. chacaya* (G. Don) comb. nov. (Rhamnaceae) y sus híbridos presuntivos. *Parodiana* **2**, 79–98.
- Turner SR, Merritt DJ, Baskin CC, Dixon KW, Baskin JM (2005) Physical dormancy in seeds of six genera of Australian Rhamnaceae. *Seed Science Research* **15**, 51–58. doi:10.1079/SSR2004197
- Van Der Valk AG, Davies CB (1978) Role of seed banks in the vegetation dynamics of prairie glacial marshes. *Ecology* **59**, 322–355. doi:10.2307/1936377
- Veblen TT, Mermóz M, Martín C, Ramilo E (1989) Effects of exotic deer on forest regeneration and composition in northern Patagonia. *Journal of Applied Ecology* **26**, 711–724. doi:10.2307/2404095
- Zammit CA, Zedler PH (1993) Size structure and seed production in even-aged populations of *Ceanothus greggii* in mixed chaparral. *Journal of Ecology* **81**, 499–511. doi:10.2307/2261528

Manuscript received 13 January 2011, accepted 23 June 2011