

RESEARCH PAPER

***Juniperus communis*: victim of the combined action of climate warming and nitrogen deposition?**K. Verheyen¹, S. Adriaenssens¹, R. Gruwez¹, I. M. Michalczyk², L. K. Ward³, Y. Rosseel⁴, A. Van den Broeck⁵ & D. García⁶

1 Laboratory of Forestry, Ghent University, Melle-Gontrode, Belgium

2 Philipps-University of Marburg, Faculty of Biology, Conservation Biology, Marburg, Germany

3 Centre for Ecology and Hydrology, Crowmarsh Gifford, Wallingford, Oxon, UK

4 Department of Data Analysis, Ghent University, Gent, Belgium

5 Research Institute for Nature and Forest, Geraardsbergen, Belgium

6 Depto. de Biología de Organismos y Sistemas, Universidad de Oviedo, and ICAB (Instituto Cantábrico de Biodiversidad, CSIC-Universidad de Oviedo-Principado de Asturias), Oviedo, Spain

Keywords

Drought; Europe; growing degree-days; *Megastigmus bipunctatus*; nitrogen deposition; seed limitation; structural equation modelling.

Correspondence

K. Verheyen, Laboratory of Forestry, Ghent University, Geraardsbergsesteenweg 264, B-9090 Melle-Gontrode, Belgium.
E-mail: kris.verheyen@ugent.be

Editor

M. Riederer

Received: 12 January 2009; Accepted: 24 March 2009

doi:10.1111/j.1438-8677.2009.00214.x

ABSTRACT

Research on the combined effects of climate change and nitrogen deposition on reproductive traits, and especially on the production of viable seeds, is still scarce despite their importance for population persistence and expansion. Hence, in this study we set out to investigate the direct and indirect effects of the above-mentioned global change drivers on seed viability in the coniferous shrub *Juniperus communis* L. In many parts of its European range, juniper is increasingly threatened, partly because of a lack of sexual reproduction. We hypothesised that this regeneration failure is partly due to poor seed viability. Using data from 39 populations throughout Europe, we were able to demonstrate that a strong, triangular-shaped relationship exists between the percentage of viable seeds produced and the percentage of juniper seedlings occurring in a population, which indicates that the species is indeed partly seed limited. Furthermore, based on an extended dataset of 42 populations, we found that seed viability was negatively affected by temperature, measured as mean annual growing degree-days, and nitrogen deposition (but not by drought). Suggestions are made about the processes behind the observed patterns, but more research is required. Nevertheless, our results do raise serious concerns for the conservation of juniper in light of the predicted rise in temperature and global nitrogen emissions. Furthermore, it is likely that similar patterns can also be observed for other species.

INTRODUCTION

During the last decades, an increasing number of studies have been performed on the impacts of the global change (GC) factors, climate and nitrogen deposition, on vegetative growth of plant species (e.g. Saxe *et al.* 2001; Högberg *et al.* 2006; Magnani *et al.* 2007; De Vries *et al.* 2008). However, studies of such impacts on reproductive traits, and especially on the production of viable seeds, are less numerous, despite their importance for population persistence and expansion (cf. Silvertown *et al.* 1996). The

impacts of temperature on viable seed production have been considered in several studies (e.g. Pigott & Huntley 1981; Meunier *et al.* 2007; Hovenden *et al.* 2008; Graae *et al.* 2009; Hedhly *et al.* 2009), but there is little information on the potential effects of nitrogen deposition (but see Callahan *et al.* 2008). Furthermore, increased nitrogen deposition and higher temperatures are also likely to have indirect effects on seed viability, for instance, *via* effects on the population dynamics of seed predators and pathogens (e.g. Roux *et al.* 1997; Callahan *et al.* 2008). Hence, it is difficult to predict whether the

joint effects of multiple GC factors on viable seed production will be antagonistic, additive or synergistic (cf. Brook *et al.* 2008; Darling & Côté 2008).

Unravelling all these interacting effects of GC requires either well-designed, but inevitably complex, experiments or large-scale observational studies covering broad environmental gradients (cf. Sagarin *et al.* 2006). In this study, we adopt the latter approach to investigate the direct and indirect effects of GC drivers on seed viability in the coniferous shrub *Juniperus communis* L. A good understanding of the factors explaining the variation in seed viability in juniper is of particular interest, as poor seed viability may be linked with strong population declines that are actually occurring in large parts of its range, including northwest European lowlands and Mediterranean mountain regions. In Flanders (*i.e.* the northern part of Belgium), the number and sizes of juniper populations have declined dramatically in the past two decades, and at present only three populations remain that contain more than 100 individuals (Adriaenssens S., Baeten L., Crabbe S., Verheyen K., Ghent University, Ghent, unpublished results). Also, in surrounding regions, including Wallonia (southern Belgium; Frankard 2004), the Netherlands (Oostermeijer & de Knegt 2004), northern and western Germany (Hüppe 1995) and England (Clifton *et al.* 1997), the species is increasingly threatened and is a target for conservation. A need for conservation measures also exists in the Mediterranean mountains of Spain (García *et al.* 1999). It is therefore not surprising that *J. communis* communities are listed in Annex I of the EU Habitat Directive (code 5130). Strikingly, in other parts of Europe (*e.g.* the Alps, Scandinavia, Poland) expanding populations encroaching into abandoned open spaces are frequently encountered (Falinski 1980; Rosén 1995; Rosén & Bakker 2005).

The main reasons for the juniper decline probably include habitat destruction, habitat degradation and very limited recruitment in remaining populations (*e.g.* Verheyen *et al.* 2005). Due to its protected status, the destruction of juniper habitat is at present less common, and for the same reason, actions are being undertaken to prevent further habitat degradation in extant populations. Degradation of juniper habitat is mostly related to progressive forest succession and subsequent out-shading. Nevertheless, despite habitat protection and management, populations continue to decline because of the very limited spontaneous regeneration (*e.g.* García *et al.* 1999; Verheyen *et al.* 2005). The absence of microsites suitable for germination and establishment, notably bare ground, and high grazing pressures have been identified as possible causes for the poor regeneration success of the species (Ward 1973, 1982; Fitter & Jennings 1975; Gilbert 1980). In Mediterranean mountains, seedling mortality due to summer droughts can be added to these factors (*e.g.* García 2001). However, there is plenty of field evidence that the removal of regeneration bottlenecks alone is not sufficient to promote regeneration (*e.g.* Verheyen *et al.* 2005). Therefore, another factor hampering regeneration is likely to be seed limitation caused by the poor seed viability

(cf. García 2001). The production of sufficient viable seeds is obviously a prerequisite for sexual reproduction, and it has already been shown that viable seed production is highly variable among juniper populations throughout Europe (García *et al.* 2000).

Indications exist that the GC factors climate and nitrogen deposition can have an impact on phenotypic variation in seed viability. García *et al.* (2000) suggested a climatic impact on seed viability, and Ortiz *et al.* (2002) found the percentage of filled seeds was significantly larger at higher elevations. The latter authors explained their results by the more favourable conditions for wind pollination in high-elevation stands. In contrast, Houle & Babeux (1994) suggested that pollen viability might be lower in colder climates, giving rise to lower fertilization and a lower proportion of filled seeds. Barkman (1989) hypothesised a negative impact of acidifying and eutrophic depositions on seed viability, but to our knowledge this has never been explicitly tested. Krupa (2003) reviewed the multitude of primary and secondary effects of nitrogen deposition, but in the context of this study, the possibly deleterious effects on mycorrhizal associations (mostly endomycorrhizae in the case of *J. communis*; Thomas *et al.* 2007) might be the most important, as Bakker (1988) noted a possible relationship between juniper vitality and the presence of mycorrhizae. The possibility of indirect effects of climate and nitrogen deposition mediated *via* predispersal seed predation also requires more investigation (*e.g.* García 1998; Falke 2004). It should be noted, however, that no unequivocal relationships between seed viability, predation rates and recruitment success have been found until now.

The specific goals of this study were: (i) to evaluate if juniper is indeed seed-limited by relating seed viability (measured as the percentage of filled seeds per shrub) to the frequency of seedling establishment in European populations with variable regeneration success; (ii) to determine variations in juniper seed viability across its distribution range in Europe and assess the ability of climate, nitrogen deposition and predispersal seed predation to explain this variation; and (iii) using a subset of the data, to determine the extent to which these environmental drivers may affect seed viability *via* their impact on vegetative shrub characteristics.

METHODS

Study species

Juniper has one of the widest distribution ranges of all plant species, and its European range spans from the Iberian Peninsula up to northern Scandinavia and from Ireland to Russia (Jalas & Suominen 1973). Juniper is a dioecious and wind-pollinated coniferous shrub. In spring, female individuals bear axillary cones that take up to 2 years to develop into fleshy spherical structures, called galbulae (Thomas *et al.* 2007; Ward L.K., unpublished results). Cones ripen fully in the autumn of the second or

third year of development, becoming blue-grey, approximately 6.5 mm in diameter and containing 1–3 or rarely four seeds per cone (García *et al.* 2000). The percentage of viable seeds is generally low, but exhibits considerable variation across Europe (see below). Ripe cones are available to dispersers from September through winter and spring. Birds, especially thrushes (*Turdus* spp.), are the main dispersal vectors, and juniper does not produce a persistent seed bank (Kollmann 1994). García (2001) estimated first-year seedling mortality to be as high as 75–80%, and the same author estimated that only six out of 10,000 seeds are able to produce a seedling that survives its first year. In the UK, Grubb *et al.* (1996) found lower seedling mortality rates for the species in unshaded conditions. According to Ward (2007), young females (mean annual shoot growth 6.7 cm) grow less than males (8.1 cm), presumably because of greater costs associated with female reproductive effort. Age at first reproduction is *ca.* 12 years (Ward 2007), and the lifespan of *J. communis* is about 100 years in southern England on chalk. In northern England, exceptional individuals reach over 200 years (Ward 1982), and in northern Finland even *ca.* 1000 years (Kallio *et al.*

1971). Ward (1982) found longevity was related to growth rate: slower-growing individuals lived longer and the life-span was correlated with growth rate in the early years.

Data collection

Cone, seed and recruitment variables

Data on seed viability were collected in 42 populations from across the species' distribution range in Europe (Fig. 1). Twenty-seven populations were sampled by García *et al.* (2000) during the autumn of 1994–1996, encompassing sites on the Iberian Peninsula (17 populations), in the Alps (four populations), Great Britain (two populations) and northern Scandinavia (four populations). This dataset was complemented by 15 populations located in the lowlands of northwestern Europe that were sampled in the autumn of 2005 by the second author of this paper. These populations included sites in northern France (three populations), Belgium (three populations), the Netherlands (four populations) and northwestern Germany (five populations). All selected populations were in unshaded locations and were sufficiently large to

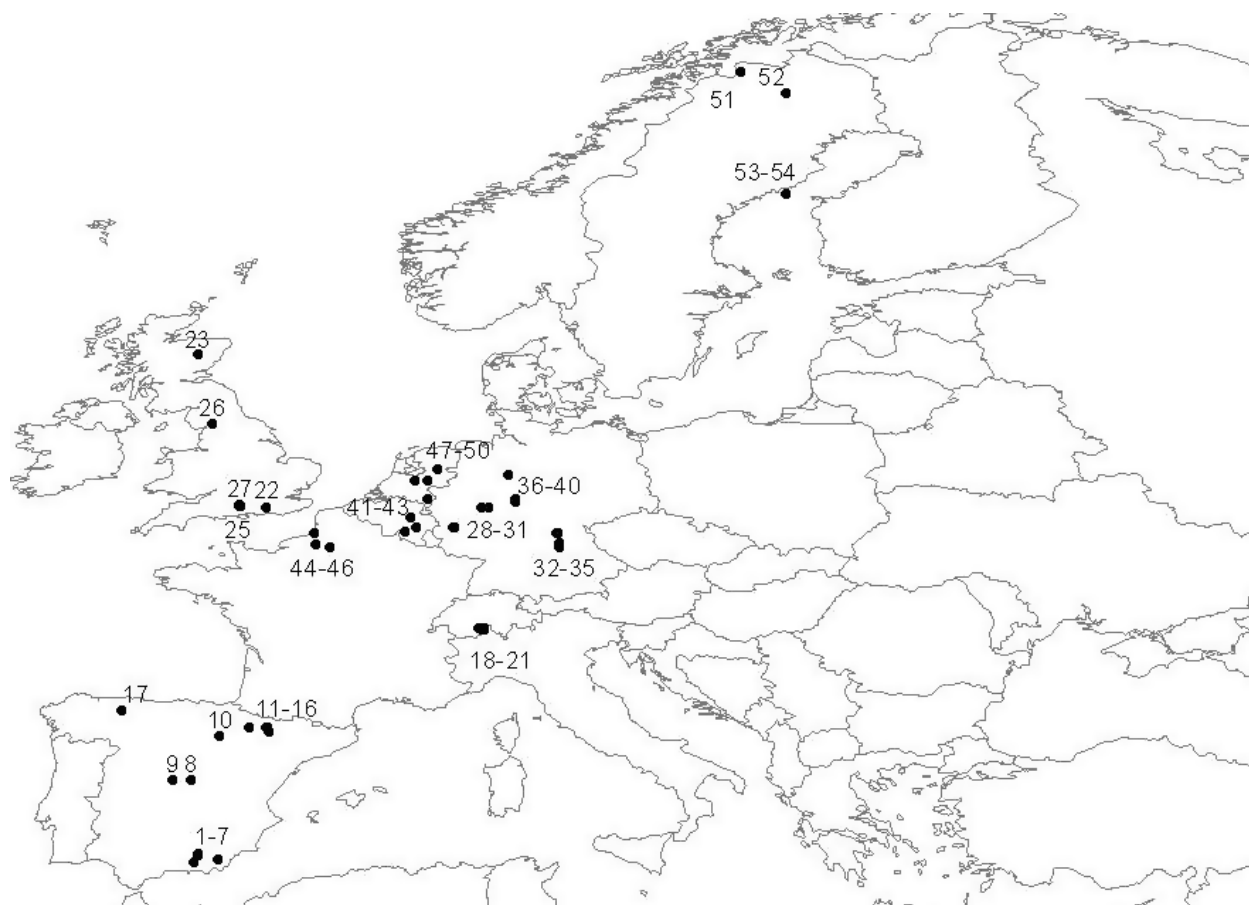


Fig. 1. Map of Europe (scale 1:25,000,000) showing the locations of the juniper populations used in this study. More information on the populations can be found in Appendix S1.

minimize Allee effects [see Fig. 1, Appendix S1 and García *et al.* (2000) for more details].

The seed viability sampling protocols in both datasets were similar. In all populations, female shrubs were selected in a stratified random way, assuring that the full size range of seed-bearing plants was sampled (520 plants in the 1994–1996 dataset, with a range of 5–75 plants per population, and 198 plants in the 2005 dataset, with a range of 6–27 plants per population). Per plant, random samples of 30–70 ripe cones (mean: 36 cones) were collected in 1994–1996 and of 1–166 cones (mean: 57 cones) in 2005. In the laboratory, all collected cones were inspected for signs of predation by the chalcid *Megastigmus bipunctatus* (Hymenoptera, Torymidae; see Roques & Skrzypczynska (2003) for a review of the seed-infesting chalcids of the genus), as indicated by the presence of a small circular hole in the cone. Although many other seed predators occur (see Thomas *et al.* 2007 for an overview), *M. bipunctatus* is considered the main predispersal seed predator (García *et al.* 2000). Furthermore, seed predation by *M. bipunctatus* can easily and unequivocally be assessed. Next, the cones were opened, the number of seed counted and each seed was assigned to one of two categories: *empty*, due to seed abortion, poor seed maturation or predation, or *filled*, having an apparently undamaged, well developed embryo. For the 2005 data only, additional viability testing on the filled seeds was performed by exposing seeds to a 1% 2,3,5 trifenylyl tetrazolium chloride solution (see *e.g.* Miller 2004 for more details on the method).

Recruitment, expressed as the percentage of individuals in a population less than 25 cm in height, was found for all 15 populations in the 2005 dataset, but only for 12 populations of the 1994–1996 dataset. However, similarly collected recruitment and viability data on eight populations in central Germany (four in the Fanconian Alb and four in the Rhenish Uplands; unpublished results collected by Michalczyk in 2006 and 2005, respectively) and on four populations in England (Ward L.K., unpublished results) resulted in 39 populations for which both recruitment and viability data were available (see Fig. 1 and Appendix S1 for more details).

Environmental and plant variables

Climatic data (average 1960–1991) for all populations were obtained through the Local Climate Estimator (LCE; <http://www.fao.org/sd/locclim>) based on the population's coordinates (latitude–longitude and altitude in m a.s.l.). The average monthly temperatures obtained through the LCE were used to derive average yearly growing degree-days >0 °C ($GDD_{>0}$; cf. Hall *et al.* 2002). GDD is a widely used heuristic tool in phenology that has many applications and correlates well with plant development in general and seed development in particular (see Meunier *et al.* 2007 for a recent example). The $GDD_{>0}$ values in our dataset ranged between 904 and 4542, with a mean of 3007. Average monthly precipitation (P) and potential evapotranspiration (PET) were also obtained through the

LCE and were used to derive an ordinal variable 'drought' with five classes ranging from 'very dry' (value 2) to 'very wet' (value –2). An ordinal variable was created because PET values were not available in the LCE for five populations (Glen Gairn, Kiruna, Nydala 1, Nydala 2 and Abisko), and these populations could therefore only be classified into a wetness class using a combination of the known P, $GDD_{>0}$ and expert knowledge. To attribute a drought class to the 37 populations with known monthly P and PET values, first the following six variables, with specific attention to quantification of summer drought (cf. García 2001), were derived: annual precipitation (aP), annual evapotranspiration (aPET), ratio aP/aPET, number of months with aPET $>$ aP, difference aP – aPET and summed difference between P and PET for the summer months only (June–September). Next, a principal component analysis was performed on these variables, resulting in one component that explained 86% of the variation and that was strongly correlated with all six variables (weakest correlation is with aPET: $r = 0.77$). The factor scores on the component ranged between 1.71 and –2.18, and these values were used to attribute the 37 populations to the wetness classes: very dry (factor score ≥ 1.5), dry (≥ 0.5), neither dry nor wet (< 0.5 and > -0.5), wet (≤ -0.5) and very wet (≤ -1.5).

The nitrogen deposition data were obtained from the European Monitoring and Evaluation Programme database (<http://www.emep.int>). EMEP is the 'Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air pollutants in Europe' and provides scientific information on the emission, transport and deposition of air pollutants. In this paper, we used the estimates for 2000 (*i.e.* in the middle of the period in which our data were collected) of total inorganic nitrogen deposition (wet and dry) expressed as $\text{kg N ha}^{-1}\text{-year}^{-1}$ in 50×50 km grid cells covering Europe. The values for the 42 populations under study ranged from $1 \text{ kg N ha}^{-1}\text{-year}^{-1}$ to $31 \text{ kg N ha}^{-1}\text{-year}^{-1}$, with a mean of $11 \text{ kg N ha}^{-1}\text{-year}^{-1}$.

Finally, the following additional plant characteristics were recorded for the 15 populations in the 2005 dataset only: size of plant, vitality of plant and number of blue cones produced. Given the variable morphology of *J. communis* shrubs, the crown surface area (m^2) was used as a measure of plant size. Crown surface area was calculated using four measures, plant height, height of the crown base and two perpendicular measures of crown diameter taken at the height of the maximum crown width. Plant vitality was assessed in three ordered classes based on the degree of defoliation, measured as the percentage of needles lost (1, $>60\%$ loss, 2, 30–60% loss; 3, $<30\%$ loss; cf. Verheyen *et al.* 2005). Blue cone production was estimated as crown surface (m^2) \times blue cone density (number of cones m^{-2}). The latter variable was determined in a maximum of ten 20×20 cm squares randomly distributed over the shrub's crown surface. The mean and maximum blue cone number was 2591 and 50,255, respectively; only seven out of 198 juniper bushes

had over 10,000 cones. These values are comparable with Falinski (1980), who found a mean value of 1112 cones and a maximum of 22,625 cones per shrub in eastern Poland.

Data analysis

The data were analysed in the same order as the three research questions.

1 The relation between the mean percentage of filled seeds per shrub and the percentage of young (<25 cm) juniper shrubs per population was assessed by means of a Spearman rank correlation (r_s).

2 To determine the degree of variation in seed viability between regions and populations across Europe, a general linear model analysis was performed, with region and population (nested within region) as random factors. The factor 'region' had four levels representing geographically distinct regions in Europe (Iberian Peninsula, Alps, northwestern Europe including Great Britain, and northern Scandinavia). The ln-transformed percentage of filled seeds was the response variable for this analysis. Next, structural equation modelling (SEM; Grace 2006) was used to determine the direct impacts of climate, nitrogen deposition and latitude on the percentage of viable juniper seeds per cone, as well as the indirect impacts of these environmental variables *via* their influence on the degree of predispersal seed predation by *M. bipunctatus* and the average number of seeds per cone. Therefore, we developed an *a priori* model to represent hypothesized dependencies between the predictor and response variables based on the expected causal relationships (Fig. 2) out-

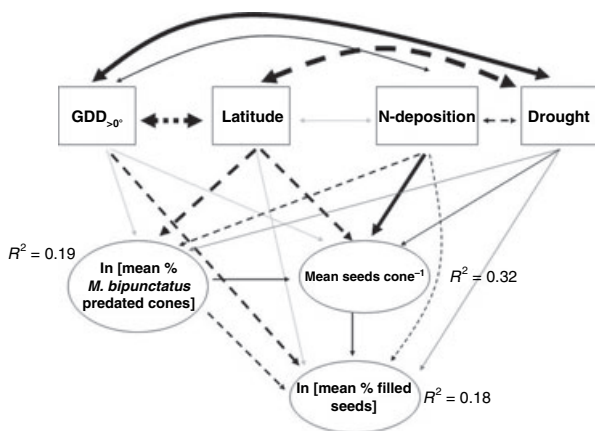


Fig. 2. Path diagram displaying the relationships between environmental variables and selected juniper variables. Double-headed arrows represent correlations between independent variables, while single-headed arrows describe assumed causal relationships. Significant ($P < 0.05$) relationships are indicated in black, with full and dotted lines indicating positive and negative relationships, respectively. The width of the lines indicates the strength of the relationships. Non-significant relationships are indicated in grey and were not included in the final model.

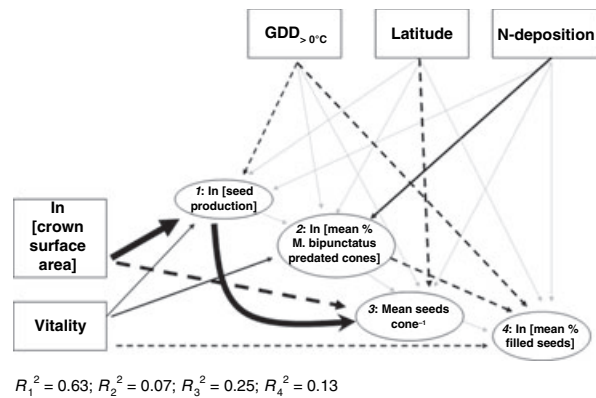


Fig. 3. Path diagram displaying the relationships between environmental variables and selected juniper variables (including vegetative shrub traits) for a subset of 15 populations in northwest Europe. The single-headed arrows describe assumed causal relationships. Significant ($P < 0.05$) relationships are indicated in black, with full and dotted lines indicating positive and negative relationships, respectively. The width of the lines indicates the strength of the relationships. Non-significant relationships are indicated in grey and were not included in the final model.

lined in the introduction. SEM provides a framework for handling correlations between predictors that are related to a common response. It is based on a simultaneous solution procedure, where the residual effects of predictors are estimated (partial regressions) once common causes from intercorrelations have been statistically controlled. To evaluate our model, standard SEM procedures using *Mplus* (version 4.1) were used. If necessary, variables were ln-transformed to linearize relationships. As our initial model was saturated (*i.e.* the model included as many free parameters as the number of available correlations among the variables), non-significant relationships (based on z -values) were eliminated from the model to allow assessment of its overall fit using a chi-square test and the determination of the root mean square error of approximation (RMSEA). All the above-mentioned analyses were performed at the shrub level, and $n = 718$.

3 To assess the importance of the direct and indirect impacts of vegetative shrub characteristics on viable seed production, an extended model was constructed (Fig. 3) and tested for the subset of shrubs for which characteristics were available ($n = 196$). The drought variable was, however, not included in this analysis as all but two populations fell into the 'neither dry nor wet' class.

RESULTS

The mean percentage of filled seeds per population was significantly positively correlated ($r_s = 0.47$, $P = 0.003$, $n = 39$) with the percentage of seedlings in the population (Fig. 4). The relationship was more or less triangular in shape, in the sense that consistently low seedling

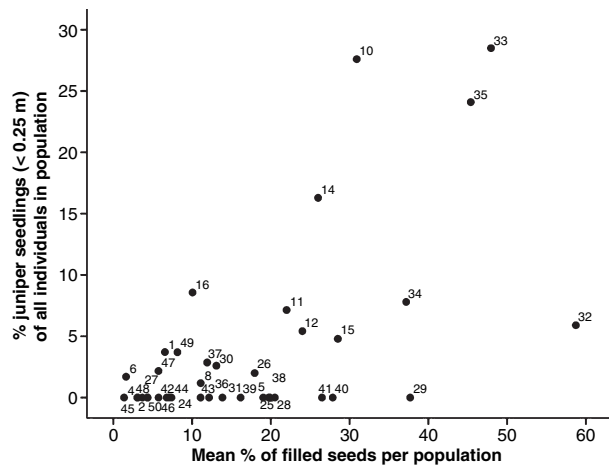


Fig. 4. Relationship ($r_s = 0.47$, $P = 0.003$) between the mean percentage of filled seeds per shrub and the percentage of juniper seedlings (*i.e.* shrubs < 25 cm) in 39 populations distributed throughout Europe (numbers refer to the locations given in Fig. 1).

numbers were found in populations with low percentages of filled seeds, whereas both high and low seedling numbers were found in populations with a higher percentage of filled seeds. The percentage of filled seeds exhibited large variability (Fig. 5), but most variation occurred between populations in a single region ($F_{38, 676} = 12.207$, $P < 0.001$), and only to a lesser extent between regions ($F_{3, 39} = 3.627$, $P = 0.023$). The overall average of filled seeds was 17%, and the mean values (min–max) in the Iberian Peninsula, Alps, northwest Europe and northern

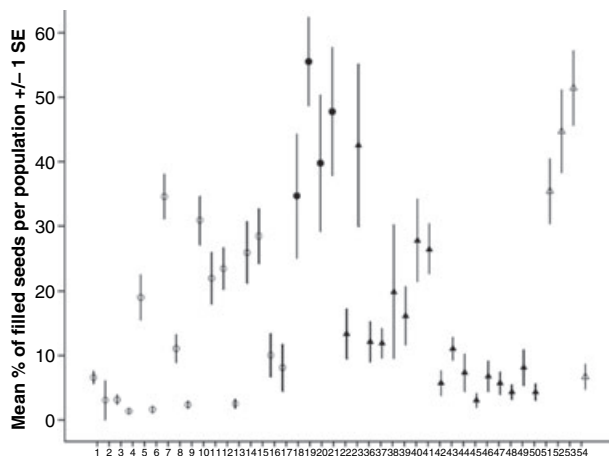


Fig. 5. Variation in mean percentages of filled seeds per shrub (\pm standard error, SE) in 42 populations from four regions across Europe: Iberian Peninsula (empty circles), the Alps (filled circles), northwest Europe (filled triangles) and northern Scandinavia (empty triangles). The numbers on the x-axis refer to the locations given in Fig. 1.

Scandinavia were: 12% (0–71%), 47% (2–86%), 13% (0–100%) and 36% (0–100%), respectively. There was a relatively strong correlation between the percentage of filled seeds and percentage of tetrazolium-treated seeds that turned red (and were assumed truly viable) ($r_s = 0.74$, $P < 0.001$, $n = 198$), indicating that the percentage of filled seeds is a good indicator of seed viability. Nevertheless, the mean percentage of viable seeds (3%) was four times smaller than the percentage of filled seeds (12%). On average, 2.41 seeds per cone were found, with mean values (min–max) in the four regions of: 2.44 (1.00–3.15), 2.14 (1.03–2.91), 2.68 (1.00–3.30) and 1.78 (1.00–3.00), respectively. There was no correlation between the number of seeds per cone and the percentage of filled seeds ($r_s = -0.01$, $P = 0.882$, $n = 718$). A clear triangular relationship between the percentage of filled seeds and predation rate was found (Fig. 6). The percentage of *M. bipunctatus* predated cones per shrub was, on average, 15% (range: 0–100%). Highest predation rates were found on the Iberian Peninsula and in one of the two Nydala populations in northern Scandinavia. In populations with the lowest $GDD_{>0^\circ}$ [~ 1000 ; *i.e.* the most northern Scandinavian (Kiruna, Abisko) and highest alpine (Grimsensee and Eigerletscher) populations], *M. bipunctatus* was completely absent.

The model in Fig. 2 explained 18% of the variation in the percentage of filled seeds and a good fit between the data and model was obtained ($\chi^2 = 9.97$, $df = 5$, $P = 0.08$; RMSEA = 0.037). As expected, the environmental predictor variables are more or less correlated. It should be noted that, despite the weak correlation between latitude and nitrogen deposition, a non-linear relationship does exist between these variables: nitrogen

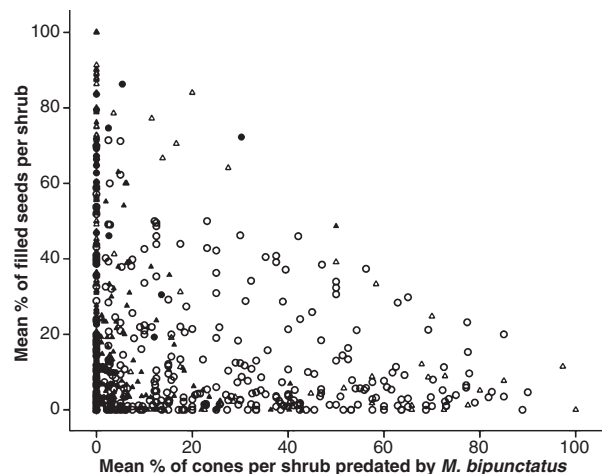


Fig. 6. Relationship ($r_s = -0.24$, $P < 0.001$) between the mean percentage of *M. bipunctatus* predated cones per shrub and the mean percentage of filled seeds per shrub in 42 populations in four different regions across Europe: Iberian Peninsula (empty circles), the Alps (filled circles), northwest Europe (filled triangles) and northern Scandinavia (empty triangles).

deposition was low at both the lower and upper end of the latitudinal gradient, whereas the highest nitrogen deposition was at intermediate latitudes ($\pm 50^\circ$). However, these intercorrelations did not result in high variance inflation factors (VIFs) in any of the regressions (VIFs always ≤ 2). The model results indicate positive effects of nitrogen deposition (strong) and drought (weak) on the average number of seeds per cone and a negative effect of latitude (Fig. 2). The percentage of cones predated by *M. bipunctatus* is lower at higher latitudes and in regions with high nitrogen deposition. The percentage of filled seeds is negatively affected by the degree of *M. bipunctatus* predation and, especially, by the number of $GDD_{>0^\circ}$ (Fig. 7). The latter variable only has a direct effect on the percentage of filled seeds. Nitrogen deposition also has a direct negative effect on the percentage of filled seeds, but this effect was counterbalanced by positive indirect effects *via* the number of seeds per cone and the degree of *M. bipunctatus* predation (Fig. 2).

The model for the northwestern European subset (Fig. 3) explains 13% of the variation in filled seed and also displays a good fit with the data ($\chi^2 = 16.47$, $df = 14$, $P = 0.29$; RMSEA = 0.030). Compared to results for the entire dataset, more or less similar relationships with $GDD_{>0^\circ}$, latitude and nitrogen deposition were found, although the strength of the relationships varied in some cases (Fig. 3). Inclusion of the three extra shrub traits helped to explain an additional part of the variation, especially for the number of seeds per cone, which was positively related to the number of cones produced and negatively to the size of the shrub (Fig. 3). By contrast, the tested shrub characteristics do not explain much additional variation in the percentage of filled seeds and,

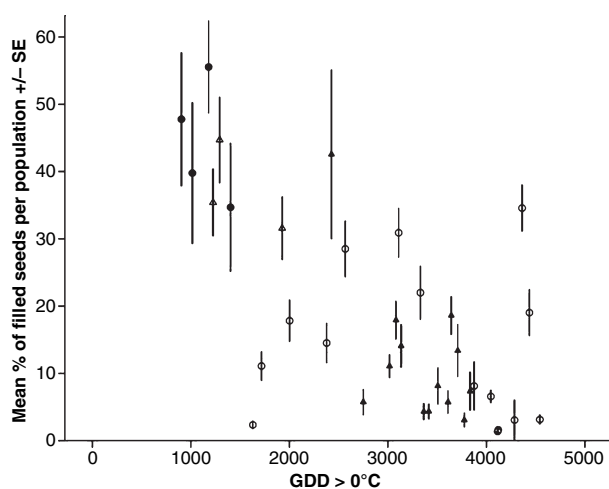


Fig. 7. Relationship ($r_s = -0.38$, $P < 0.001$) between the mean annual growing degree-days above 0°C (1960–1991) and the mean (\pm standard error, SE) percentage of filled seeds per population across four different regions of Europe: Iberian Peninsula (empty circles), the Alps (filled circles), northwest Europe (filled triangles) and northern Scandinavia (empty triangles).

rather surprisingly, a negative relationship was found with shrub vitality (Fig. 3).

DISCUSSION

In this paper, we demonstrated that the limited availability of viable seeds is indeed an important factor explaining the poor regeneration success of juniper in parts of Europe. Furthermore, we found that the variation in seed viability is mainly determined by temperature, but also by latitude and nitrogen deposition *via* their impact on predispersal *Megastigmus* predation and seed number per cone.

The triangular relationship between seed viability and percentage of seedlings in a population is consistent with earlier observations that juniper regeneration is hampered by the absence of microsites suitable for germination, and by overgrazing and/or drought stress (cf. above). These factors are probably responsible for the large variation in seedling recruitment even when sufficient viable seeds are available (cf. Fig. 4). The results also suggest that when seed viability is low, the chances of successful regeneration are very low (cf. García 2001). Although based on observational data, the positive response of local recruitment to the availability of viable seeds is indicative of seed limitation at a large scale for the plant's range in Europe (Eriksson & Ehrlén 1992; Münzbergová & Herben 2005). More importantly, seed viability may therefore be used as a proxy for juniper recruitment potential in a large-scale scenario of global change in Europe.

Variation in seed viability is large both between regions and between populations within regions. This is in accordance with Johnsen & Alexander (1974), Ward (1982) and Forbes & Proctor (1986), who also found large differences between populations. No evidence was found for correlated seed viability patterns among neighbouring regions (Fig. 5), which may suggest the absence of any important genetic control on seed viability at a regional scale. Studies using neutral genetic markers found a lack of spatial clustering between and within regions and high degrees of genetic variation within populations (cf. Van der Merwe *et al.* 2000; Oostermeijer & de Knecht 2004; Michalczyk *et al.* 2006; Van den Broeck *et al.*, unpublished results). Hence, the likelihood of a strong genetic imprint on the observed seed viability patterns seems rather low. As well as the large spatial variation, significant interannual variability in viable seed percentages also occurs. Ward (unpublished results) monitored seed quality in two juniper populations at Porton (UK) between 1997 and 2006 and found seed viability varied between 1% and 15% and between 7% and 33%, respectively, in these 2 years. Also, García *et al.* (2002) found a 65–90% range in seed abortion during a 6-year monitoring campaign in a juniper population in the Mediterranean mountains of southern Spain. The variability appeared to be strongly related to rainfall during the pollen delivery month. This interannual variability may partly account for the significant percentage of unexplained variation in our models.

However, the question remains, what are the mechanisms behind the large variation in seed viability and how can the observed relationships with the GC drivers be explained? As, on average, only 15% of the cones per shrub were predated by *M. bipunctatus*, predispersal seed predation by this species seems to be somewhat less important to explain the low viability of juniper seeds. However, *M. bipunctatus* does have an important impact on seed viability in some, more southerly populations (cf. Figs 2 and 6). Furthermore, the higher impact in southern Europe is more likely to be explained by the presence of other host plants for *M. bipunctatus* (*J. sabina*, *J. oxycedrus*) than by temperature limitation, as the threshold GDD level for *M. bipunctatus* presence is low (~ 1000 GDD $_{>0}$; cf. Fig. 2). The high percentage of non-filled seeds (83%) is therefore either primarily due to (i) poor ovule fertilization or (ii) processes other than *M. bipunctatus* predation that cause seed development failure during maturation (cf. Hedhly *et al.* 2009).

Fertilization failure (i) may be the result of low pollen quality and quantity or malfunctioning of female reproductive organs. Recently, Mugnaini *et al.* (2007) found that retraction of the juniper pollination drop, *i.e.* the liquid drop secreted by the ovule and exposed outside of the micropyle, can be triggered by dead juniper pollen, by heterospecific pollen of *e.g.* *Pinus*, and even by non-biological particles of an appropriate size. Hence, the low pollination selectivity of juniper could reduce pollination success in regions where high aerial concentrations of pollen-sized particles are present. Furthermore, Gottardini *et al.* (2004) found a specific negative effect of nitrogen oxides on pollen viability of *Pinus nigra* in Italy. This finding may also hold for juniper and could help to explain the observed negative impact of nitrogen deposition on the percentage of viable seeds (Fig. 2). It should also be stressed that, apart from pollen quality, pollen quantity could also be a limiting factor. However, since mostly larger populations were sampled in open areas (*i.e.* facilitating wind pollination, cf. Ortiz *et al.* 2002), pollen quantity is less likely to explain seed viability patterns in this study. Whether higher temperatures have a negative impact on juniper pollen quality and quantity is not yet known (cf. Hedhly *et al.* 2009).

Poor post-fertilization seed maturation (ii) may be another cause of the low seed viability. This is corroborated by Hopster & Greeve (1999) who found seed development failure mainly between the second and third year of seed development. Since there are currently no indications that higher temperatures could have a negative impact on seed maturation (but see Hedhly *et al.* 2009), the more plausible explanation for the strong negative impact of the number of GDDs on seed viability may be an indirect effect of temperature on the frequency and distribution of seed predators or pathogens, other than *M. bipunctatus*. An important seed predator that may be more strongly limited by temperature is the mite *Trisetacus*

quadrissetus (Thom; Acarina, Eriophyiidae). Ward (1973) found >80% of seeds were affected on some sites in southern England, whereas this mite appears to be absent from northern Britain. The mite is also scarce in southern Finland (Raatikainen & Tanska 1993), although García (1998) found *T. quadrissetus* was not an important predator in the Sierra Nevada (Campos de Otero, GDD $_{>0}$ = 4045), which conflicts with our hypothesis. Thomas *et al.* (2007) provided an overview of insects, mites and fungi associated with juniper, among which several are reported to have an impact on pollen and seeds.

Juniper is mainly colonized by arbuscular endomycorrhizae (Thomas *et al.* 2007). As mycorrhizae are sensitive to nitrogen deposition, it is possible that deficient uptake of nutrients might be a cause of poor seed maturation, thus explaining the negative relationship between nitrogen deposition and seed viability (Fig. 2). Nitrogen deposition seems to have a positive impact on the number of seeds produced per cone. This is in line with results of Callahan *et al.* (2008), who recently found that oak species increase reproductive output but not offspring quality when exposed to ecosystem manipulations that enhance vegetative growth. However, as the variation in seed number per cone is small compared to the variation in the percentage of viable seeds per cone, the impact of nitrogen deposition on viable seed output per cone is rather small.

Reproductive failure due to increased plant–plant competition in warmer regions could also serve as an explanation for the negative correlations between seed viability, temperature and nitrogen. Junipers have low competitive ability and are therefore confined to marginal sites. It is possible that when growing conditions improve, resource uptake by juniper may be hindered by species that are more competitive under these circumstances.

It is clear that further research is required to identify the exact mechanisms behind the observed impacts of GC drivers on juniper seed viability. In this respect, data to assess more long-term trends in seed viability would be very instructive. Such data would allow us to check whether changing environmental conditions (*e.g.* increasing temperatures and nitrogen deposition) over time result in similar impacts on seed viability to those observed in this study. In a report of the ‘Nederlandse Boschbouw Vereniging’ (Anonymous 1946), germination of 60–90% was reported, but no details on test protocols are provided. In 1975, Breek (1978) collected seeds near Dwingeloo in the province of Drenthe (the Netherlands) and found 24% of the seeds were filled. In the nearby population at Mantingerzand sampled in this study, Hopster & Greeve (1999) found $\sim 10\%$ filled seeds, and we only found 4% filled seeds. These limited, rather anecdotal, data could suggest a slightly decreasing trend in seed viability, but whether this is due to aging of shrubs or changing environmental conditions cannot be confirmed; although, based on our results, the latter seems more realistic than the former. Whereas the negative impact of shrub ageing has been suggested

several times (e.g. Diotte & Bergeron 1989), in this study only a weak negative correlation between shrub size and percentage of filled seeds ($r_s = -0.18$, $P = 0.014$, $n = 196$) was found. Furthermore, this relationship disappeared after controlling for other variables (Fig. 3). The weak relationship may be due to the fact that shrub size is not a 100% reliable proxy for age (Breek 1978; Forbes & Proctor 1986), but it could also be a genuine pattern.

CONCLUSIONS

In this study, we showed that low seed viability is an important factor explaining recruitment failure of juniper in Europe. Furthermore, clear indications were found for both direct and indirect effects of temperature and nitrogen deposition on juniper seed viability, whereas drought and shrub traits, such as size and vitality, only had minor effects on seed viability. Although our understanding of the mechanisms behind the negative effects of nitrogen deposition and temperature on seed viability is still limited, it does raise serious concerns, especially in light of predicted rises in temperature (IPCC 2007) and in global nitrogen emissions (Galloway *et al.* 2004) during the next century. If adult juniper persistence is not negatively affected by these GC factors, the species may survive as remnant populations (*sensu* Eriksson 1996) for several more decades, but it is clear that there is a limit to the long-term survival of juniper in central and southern Europe because it is likely that seed viability will further decrease in the future. In addition, seedling success will also be weaker under more stressful conditions caused by competition from more strongly growing vegetation and/or increased summer droughts.

Finally, it is not yet clear to what extent the reproductive output of other species will be similarly negatively affected by rising temperatures and/or nitrogen deposition, but results from both observational (Graae *et al.* 2009) and experimental studies (Hovenden *et al.* 2008) at least suggest that juniper may not be unique in this respect.

ACKNOWLEDGEMENTS

KV and SA thank Guillaume Decocq, Leo Goudzwaard, Martin Diekmann and various local managers for locating and providing access to the sampled populations. DG thanks Regino Zamora, José M. Gómez, José A. Hódar and Pedro Jordano for collaboration in data collection and previous analyses of geographical patterns of seed production. During the writing of this paper, DG was supported by research grant MEC-CGL2006-279872E from the Spanish Government.

SUPPORTING INFORMATION

Additional Supporting information may be found in the online version of this article:

Appendix S1. Characteristics of the sampled *Juniperus communis* populations

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

REFERENCES

- Anonymous (1946) *Boomzaden*. Report of the Nederlandse Boschbouw Vereniging. Nederlandse Boschbouw Vereniging, Arnhem, pp. 47–48.
- Bakker A. (1988) *Gymnosporangium bij Juniperus communis L. en het verband tussen taksterfte in de toppen van Juniperus communis en het VAM-percentage*. PhD thesis, Wageningen University, Wageningen.
- Barkman J.J. (1989) *Syllabus caput selectum: Nederlandse boomsoorten II: Juniperus communis L. Course Material*, Wageningen University, Wageningen.
- Breek J. (1978) *De kiemingsecologie van Juniperus communis L.* MSc thesis, Utrecht University, Utrecht.
- Brook B.W., Sodhi N.S., Bradshaw C.J.A. (2008) Synergies among extinction drivers under global change. *Trends in Ecology and Evolution*, **23**, 453–460.
- Callahan H.S., Del Fierro K., Patterson A.E., Zafar H., (2008) Impacts of elevated nitrogen inputs on oak reproductive and seed ecology. *Global Change Biology*, **14**, 285–293.
- Clifton S.J., Ward L.K., Ranner D.S. (1997) The status of juniper *Juniperus communis* L. in northeast England. *Biological Conservation*, **79**, 67–77.
- Darling E.S., Côté I.M. (2008) Quantifying the evidence for ecological synergies. *Ecology Letters*, **11**, 1278–1286.
- De Vries W., Soldberg S., Dobbertin M., Sterba H., Laubhahn D., Reinds G., Nabuurs G.-J., Gundersen P., Sutton M.A. (2008) Ecologically implausible carbon response? *Nature*, **451**, 1–2.
- Diotte M., Bergeron Y. (1989) Fire and the distribution of *Juniperus communis* L. in the boreal forests of Quebec, Canada. *Journal of Biogeography*, **16**, 91–96.
- Eriksson O. (1996) Regional dynamics of plants: a review of evidence for remnant, source–sink and metapopulations. *Oikos*, **77**, 248–258.
- Eriksson O., Ehrlén J. (1992) Seed and microsite limitation of recruitment in plant populations. *Oecologia*, **91**, 360–364.
- Falinski J.B. (1980) Vegetation dynamics and sex structure of the populations of pioneer dioecious plants. *Vegetatio*, **43**, 23–38.
- Falke B. (2004) Die Verjüngungsproblematik des Wacholders in Nordwestdeutschland: Welchen einfluss haben spezialisierte Samenprädatoren? *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie*, **14**, 49–52.
- Fitter A.H., Jennings R.D. (1975) The effects of sheep grazing on the growth and survival of seedling junipers (*Juniperus communis* L.). *Journal of Applied Ecology*, **12**, 637–642.

- Forbes A.R.D., Proctor J. (1986) The Glen Artney juniper wood. *Transactions of the Botanical Society of Edinburgh*, **45**, 63–72.
- Frankard P. (2004) Evolution de la population de *Juniperus communis* L. dans la réserve naturelle domaniale de la genévrière de Cour pendant ces vingt dernières et impact des mesures de gestion appliquées. *Parcs et Réserves*, **59**, 32–37.
- Galloway J.N., Dentener F.J., Capone D.G. et al. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.
- García D. (1998) Interaction between juniper *Juniperus communis* L. and its fruit insect pests: pest abundance, fruit characteristics and seed viability. *Acta Oecologica*, **19**, 517–525.
- García D. (2001) Effects of seed dispersal on *Juniperus communis* recruitment on a Mediterranean mountain. *Journal of Vegetation Science*, **12**, 839–848.
- García D., Zamora R., Hódar J.A., Gómez J.M. (1999) Age structure of *Juniperus communis* L. in the Iberian Peninsula: conservation of remnant populations in Mediterranean mountains. *Biological Conservation*, **87**, 215–220.
- García D., Zamora R., Gómez J.M., Jordano P., Hódar J.A. (2000) Geographical variation in seed production, predation and abortion in *Juniperus communis* throughout its range in Europe. *Journal of Ecology*, **88**, 436–446.
- García D., Zamora R., Gómez J.M., Hódar J.A. (2002) Annual variability in reproduction of *Juniperus communis* L. in a Mediterranean mountain: relationship to seed predation and weather. *Ecoscience*, **9**, 251–255.
- Gilbert O.L. (1980) Juniper in Upper Teesdale. *Journal of Ecology*, **68**, 1013–1024.
- Gottardini E., Cristofolini F., Paoletti E., Lazzeri P., Pepponi G. (2004) Pollen viability for air pollution bio-monitoring. *Journal of Atmospheric Chemistry*, **49**, 149–159.
- Graae B.J., Verheyen K., Kolb A., Van der Veken S., Heinken T., Chabrierie O., Diekmann M., Valtinat K., Zindel R., Karlsson E., Ström L., Decocq G., Hermy M., Baskin C.C. (2009) Germination requirements and seed mass of slow- and fast-colonizing temperate forest herbs along a latitudinal gradient. *Ecoscience*, in press.
- Grace J.B. (2006) *Structural Equation Modeling and Natural Systems*. Cambridge University Press, Cambridge.
- Grubb P.J., Lee W.G., Kollmann J., Wilson J.B. (1996) Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*. *Journal of Ecology*, **84**, 827–840.
- Hall B., Motzkin G., Foster D.R., Syfert M., Burk J. (2002) Three hundred years of forest and land-use change in Massachusetts, USA. *Journal of Biogeography*, **29**, 1319–1335.
- Hedhly A., Hormaza J.I., Herrero M. (2009) Global warming and sexual plant reproduction. *Trends in Plant Science*, **14**, 30–36.
- Högberg P., Fan H.B., Quist M., Binkley D., Tamm C.O. (2006) Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology*, **12**, 489–499.
- Hopster G., Greeve R. (1999) *De achteruitgang van de jeneverbes in Nederland: een gecombineerd actuo- en paleoecologisch onderzoek*. Thesis report, University of Amsterdam, Amsterdam.
- Houle G., Babeux P. (1994) Variation in rooting ability of cuttings and in seed characteristics of five populations of *Juniperus communis* var. *depressa* from subarctic Quebec. *Canadian Journal of Botany*, **72**, 493–498.
- Hovenden M.J., Wills K.E., Chaplin R.E., Vander Schoor J.K., Williams A.L., Osonai Y., Newton P.C.D. (2008) Warming and elevated CO₂ affect the relationship between seed mass, germinability and seedling growth in *Austrodanthonia caespitosa*, a dominant Australian grass. *Global Change Biology*, **14**, 1–9.
- Hüppe J. (1995) Zur problematik der Verjüngung des Wacholders (*Juniperus communis*) unter dem Einfluß von Wildkaninchen in Hudegebieten pleistozäner Sandlandschaften. *Zeitschrift für Ökologie und Naturschutz*, **4**, 1–8.
- IPCC (2007) *Climate Change: The Physical Science Basis*. Cambridge University Press, Cambridge.
- Jalas J., Suominen J. (Eds) (1973) *Atlas Florae Europaeae. Distribution of Vascular Plants in Europe. 2. Gymnospermae (Pinaceae to Ephedraceae)*. The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki.
- Johnsen T.N., Jr, Alexander R.A. (1974) *Juniperus* L. *Juniper*. In: Schomeyer C.S. (Ed). *Seeds of Woody Plants in the United States*. USDA Forest Service, Washington: 460–469.
- Kallio P., Laine U., Mäkinen Y. (1971) Flora of Inari Lapland. 2. Pinaceae and Cupressaceae. *Reports of the Kevo Subarctic Research Station*, **8**, 73–100.
- Kollmann J. (1994) Ausbreitungsökologie endozochorer Gehölzarten. *Veröffentlichen Projekt 'Angewandte Ökologie'*, **9**, 1–212.
- Krupa S.V. (2003) Effects of atmospheric ammonia (NH₃) on terrestrial vegetation: a review. *Environmental Pollution*, **124**, 179–221.
- Magnani F., Mencuccini M., Borghetti M., Berbigier P., Berninger F., Delzon S., Grelle G., Hari P., Jarvis P.G., Kolari P., Kowalski A.S., Lankreijer H., Law B.E., Lindroth A., Loustau D., Manca G., Moncrieff J.B., Rayment M., Tedeschi V., Valentini R., Grace J. (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, **447**, 848–852.
- Meunier C., Sirois L., Bégin Y. (2007) Climate and *Picea mariana* seed maturation relationships: a multi-scale perspective. *Ecological Monographs*, **77**, 361–376.
- Michalczyk I.M., Sebastiani F., Buonamici A., Cremer E., Mengel C., Ziegenhagen B., Vendramin G.G. (2006) Characterization of highly polymorphic nuclear microsatellite loci in *Juniperus communis* L. *Molecular Ecology Notes*, **6**, 346–348.

- Miller A.L. (2004) Tetrazolium testing for flower seeds. In: McDonald M.B., Kwong F.Y. (Eds). *Flower Seeds: Biology and Technology*. CABI Publishing, Wallingford: 229–310.
- Mugnaini S., Nepi M., Guarnieri M., Piotto B., Pacini E. (2007) Pollination drop in *Juniperus communis*: response to deposited material. *Annals of Botany*, **100**, 1475–1481.
- Münzbergová Z., Herben T. (2005) Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. *Oecologia*, **145**, 1–8.
- Oostermeijer J.G.B., de Knecht B. (2004) Genetic population structure of the wind-pollinated, dioecious shrub *Juniperus communis* in fragmented Dutch heathlands. *Plant Species Biology*, **19**, 175–184.
- Ortiz P.L., Arista M., Talavera S. (2002) Sex ratio and reproductive effort in the dioecious *Juniperus communis* ssp. *alpina* (Suter) Celak. (Cupressaceae) along an altitudinal gradient. *Annals of Botany*, **89**, 205–211.
- Pigott C.D., Huntley J.P. (1981) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. 3: nature and causes of seed sterility. *New Phytologist*, **87**, 817–839.
- Raatikainen M., Tanska T. (1993) Cone and seed yields of the juniper (*Juniperus communis*) in southern and central Finland. *Acta Botanica Fennica*, **149**, 27–39.
- Roques A., Skrzypczynska M. (2003) Seed-infesting chalcids of the genus *Megastigmus* Dalman, 1820 (Hymenoptera: Torymidae) native and introduced to the West Palearctic region: taxonomy, host specificity and distribution. *Journal of Natural History*, **37**, 127–238.
- Rosén E. (1995) Periodic droughts and long-term dynamics of Alvar grassland vegetation on Öland, Sweden. *Folia Geobotanica Phytotaxonomica*, **30**, 131–140.
- Rosén E., Bakker J.P. (2005) Effects of agri-environment schemes on scrub clearance, livestock grazing and plant diversity in a low-intensity farming system on Öland, Sweden. *Basic and Applied Ecology*, **6**, 195–204.
- Roux G., Roques A., Menu F. (1997) Effect of temperature and photoperiod on diapause development in a Douglas fir seed chalcid, *Megastigmus spermotrophus*. *Oecologia*, **111**, 172–177.
- Sagarin R.D., Gaines S.D., Gaylord B. (2006) Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends in Ecology and Evolution*, **21**, 524–530.
- Saxe H., Cannell M.G.R., Johnsen B., Ryan M.G., Vourlitis G. (2001) Tree and forest functioning in response to global warming. *New Phytologist*, **149**, 369–399.
- Silvertown J., Franco M., Menges E. (1996) Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. *Conservation Biology*, **10**, 591–597.
- Thomas P.A., El-Barghathi M., Polwart A. (2007) Biological Flora of the British Isles: *Juniperus communis* L. *Journal of Ecology*, **95**, 1404–1440.
- Van der Merwe M., Winfield M.O., Arnold G.M., Parker J.S. (2000) Spatial and temporal aspects of the genetic structure of *Juniperus communis* populations. *Molecular Ecology*, **9**, 379–386.
- Verheyen K., Schreurs K., Vanhollen B., Hermy M. (2005) Intensive management fails to promote recruitment in the last large population of *Juniperus communis* (L.) in Flanders (Belgium). *Biological Conservation*, **124**, 113–121.
- Ward L.K. (1973) The conservation of juniper. I. Present status of juniper in southern England. *Journal of Applied Ecology*, **10**, 165–188.
- Ward L.K. (1982) The conservation of juniper: longevity and old age. *Journal of Applied Ecology*, **19**, 917–928.
- Ward L.K. (2007) Lifetime sexual dimorphism in *Juniperus communis* L. var. *communis*. *Plant Species Biology*, **22**, 11–21.