

Biomass allocation and growth responses of Scots pine saplings to simulated herbivory depend on plant age and light availability

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Received: 22 March 2007 / Accepted: 18 October 2007 / Published online: 2 November 2007
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Abstract This study experimentally analyses the response to simulated herbivory of juvenile Scots pine of two different ages in contrasting abiotic scenarios, focusing on the potential dual role of browsing ungulates: negative, by removing aerial biomass, and positive, by stimulating compensation capacity and providing nutrients by depositing their excrement. Compensation against herbivory was investigated by experimentally clipping a set of Scots pine (*Pinus sylvestris* L. *nevadensis*) juveniles, grown under different levels of light and nutrient availability. The responses analysed were survival, trunk-diameter growth, leader-shoot growth, increment in number of meristems, RGR, biomass of needles, shoots, root and whole plant, and root-to-shoot ratio. Clipping consistently resulted in a worse survival and performance of pines with respect to unclipped ones. From the factors analysed, light availability was responsible mainly for the variations in plant performance, while the addition of nutrients was much less important. Age was also important, with older pines

showing in general better performance after clipping. Overall, clipping invariably had a negative effect on Scots pine, since none of the combinations of abiotic factors used resulted in overcompensation. However, the intensity on this negative effect proved quite variable, from almost an exact compensation in clipped older pines under full sunlight availability to very poor performance and high death probability in younger pines in shade. Scots pine cannot overcompensate after clipping, but, depending of the environmental conditions, the negative result of clipping varies from severe undercompensation to almost exact compensation. Also, small differences in sapling age can promote significant differences in sapling response to clipping and light environment.

Keywords Biomass allocation · Compensation · Experimental clipping · Light · Plant age · Plant growth · RGR · *Pinus sylvestris* · Survival

Introduction

Woody plants often undergo mammalian herbivory that severely limits plant growth and survival, especially at early life stages (Danell and Bergström 2002). However, herbivores can also have positive effects on plants, adding nutrients to the soil by dunging (McNaughton 1985; Ruess 1987; Pastor et al. 1993) or stimulating compensation in damaged plants for a positive net balance (Rosenthal and

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Kotanen 1994; Aarssen 1995). Herbivore attack and experimental clipping frequently lead to compensation (Edenius et al. 1993; Hjaltnén 1999; Vanderklein and Reich 1999; Lehtilä et al. 2000), and even to overcompensation when the intensity of damage is low (Rosenthal and Kotanen 1994; Chamberlin and Aarsen 1996; Hawkes and Sullivan 2001). In fact, the responses to herbivory depend on the timing, type, and extent of damage (McNaughton 1985; Rosenthal and Kotanen 1994; Whitham et al. 1991), as well as the environmental conditions in which browsing takes place (Danell et al. 1991b, 1997; Canham et al. 1994; Harmer 1999; Saunders and Puettmann 1999; Hawkes and Sullivan 2001; Baraza et al. 2004; see Wise and Abrahamson 2005 for a review). Several plant–herbivore theories try to explain how plants respond to herbivory under different resource conditions, sometimes producing contradictory predictions. Thus, the continuum of responses model (Maschinski and Whitham 1989) predicts that plants are able to compensate better after herbivory when growing in resource-rich environments, because the lost tissues can be replaced more easily when resources are abundant, while the growth rate model (Hilbert et al. 1981) concludes the opposite, that is, plants will recover better in low than in high resources, because plants growing at low rate can increase more easily their growth rhythm.

Furthermore, plant intrinsic characteristics previous to herbivory are also important (Obeso 1993; Haukioja and Koricheva 2000). For example, plant age has been proved to affect the individual response to herbivory (Donovan and Ehleringer 1991; Karban and Thaler 1999; Horvitz and Schemske 2002; see Boege and Marquis 2005 for a review). The plant ontogeny conditions meristem availability, photosynthetic rates, storage capacity, growth rate, and many other important plant processes (del-Val and Crawley 2005; Boege 2005), because the plant allocation priorities to growth, storage, defences or reproduction are changing (Boege 2005; Barton 2007). According to these changes in allocation priorities, the plant age hypothesis (Bryant et al. 1992) states that plant compensatory ability should increase as a plant develops, mainly due to their large reserves, with which it can manage the damage inflicted by herbivores. Thus, young woody plants would be less able to recover after herbivory. Although smaller plants are usually less prone to herbivory, as they are

less apparent to ungulate herbivores (Gómez et al. 2001; Baraza et al. 2006), they can also suffer herbivory when grazing pressure is high. This is the case of woody plants of the Mediterranean woodlands, where plants are frequently subjected to high herbivory pressure both by domestic livestock and by wild ungulates (Le Houérou 1981; Papanastasis 1998; Zamora et al. 1999).

In this study, our aim was to test the response to clipping of a woody species, the Scots pine (*Pinus sylvestris* L.) when both intrinsic (age) and extrinsic (light and nutrient availability) factors are changing. Our general hypothesis was that pines would compensate better the biomass lost by clipping when older than younger, and with higher levels of light and nutrients, than with lower levels. Thus, the presumed best compensation conditions would be for old pines with light and nutrients, and the worst ones for young pines without light and nutrients, giving a continuum of responses (sensu Maschinsky and Whitham 1989). The effect of the abiotic conditions on the Scots pine response to browsing has been documented mostly with observational data (Danell et al. 1991a, b, 1994; Edenius et al. 1993, 1995; but see Millard et al. 2001). Our focus is the potential dual role of browsing ungulates: negative, by removing aerial biomass, and positive, by stimulating compensation capacity (defined as compensatory regrowth to recover the biomass lost by herbivory) and adding nutrients with their excrement. Furthermore, we used two different juvenile ages in order to examine the effect of different developmental stages of the plants (Karbon and Thaler 1999). Even if overcompensation is not likely in any combination of abiotic factors, it is a matter of interest how Scots pines can compensate, partially or totally, for the herbivore damage that they routinely undergo (Zamora et al. 2001), and what abiotic conditions promote a better tolerance to herbivory.

Specifically, the following questions were addressed:

- (1) What are the effects of herbivory in growth and survival of juvenile Scots pine?
- (2) Can juvenile age or size influence the effects of clipping in growth and survival?
- (3) Can light and nutrient availability improve the compensating capacity of the juvenile Scots pines after clipping?

Materials and methods

Study site

The study area was located at 1,650 m a.s.l. at the Trevenque area, in the National Park of Sierra Nevada (SE Spain, 37°5′N, 3°28′W). The climate is Mediterranean continental, with hot, dry summers, and cold winters. The mean minimum temperature in the coldest month (January) is -1.2°C , and the mean maximum of the hottest month (July) is 28.5°C . Annual rainfall, concentrated mainly in autumn and spring, is 879 mm (average 1990–2003). The experiment was conducted in a 12.4 ha fenced area, the Jardín Botánico de la Cortijuela (Junta de Andalucía 2001). The fenced area was excluded from mammal herbivores. Vegetation at the zone is dominated by Scots and black pine (*Pinus nigra* ssp. *salzmannii*), both natural and afforested, while the understorey is composed of a variety of deciduous spiny shrubs (see Junta de Andalucía 2001 for further description). In southern Spain, relict Scots pine populations belonging to the endangered subspecies *Pinus sylvestris nevadensis* Christ (Boratynski 1991) survive in Sierra Nevada and Sierra de Baza mountains, between 1,600 and 2,100 m in altitude (Hódar et al. 2003).

Experimental design

Juveniles of *Pinus sylvestris nevadensis* were provided from a nursery located in the National Park of Sierra Nevada. Seedlings were germinated in beds, and root-pruned when 1-year old. In March 1996, the day before planting, pines were lifted and bare-root transported to the study site.

The pines were of two different ages: 2 and 3 years old at the start of the experiment (hereafter 2- and 3-year-old pines, respectively). Pines were potted in plastic pots of 2.5 l for 2-year-old pines and 6.2 l for 3-year-old pines, containing topsoil (9.4 kg dry weight in large pots, 3.8 kg in small pots) without litter from a place where the conspecific trees were growing. Pot size and spatial arrangement was chosen in order to allow a good growth of the trees according with their size, allowing the root expansion and avoiding that the proximity of the aerial part produced shadow at the neighbouring potted plants. We planted 256 pines of each age, according

to a full-crossed factorial design with four main sources of variation: light, fertilization, age and clipping, two levels per factor (eight treatments), and 32 plants per treatment. The factors and levels used in the experiment were the following.

Light

We defined two levels of irradiance: natural sunlight radiation (hereafter sun), and reduced sunlight radiation provided by a horticultural shade netting (hereafter shade). Owing to practical field limitations, we used just one shading tent for the entire shade treatment, thus the lack of replicates could represent as a case of pseudoreplication. However, since plants were potted (avoiding root competition) and pines were separate enough within the tent to avoid that the shadow of one plant interfered the growth of the neighbours (see above), we assume that every potted plant can be considered as an independent experimental unit. In mid-summer, the full light photosynthetic photon flux density in a clear, cloudless mid-day was $2033.6 \pm 3.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, while under the shade was 96.2 ± 8.1 (mean \pm SE, both $n = 20$, measured using a Li-Cor LI-200 sz pyranometer sensor connected to a LI-1000 data logger, Li-Cor Inc., Lincoln, Nebraska, USA). This level of light is similar to that found under the canopy of the adult pine trees in natural forests ($81.4 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$, $n = 20$).

Fertilization

Half of the pines received no additional nutrients (unfertilized), while the other half were fertilized (fertilized), receiving 58 g (small pots) or 112 g (large pots) of dry goat dung collected nearby (N total 1.79%, P total 0.92%, K total 1.38%). The nutrient concentration in the dry dung represents a considerable increase in comparison with the low nutrient availability of the natural soil (0.28% N total, 12 ppm P assimilable, 22 ppm K assimilable), especially for P and K. We chose this kind of natural fertilizer because it corresponds with the fertilizer that plants can receive in the field, since goats (both domestic goat and wild Spanish ibex) are the main ungulates present in the natural forests of Scots pine.

Furthermore, pines have ectomycorrhizae (mostly Basomycetes), which play an important role in the plant growth and development (Allen 1991; Smith and Read 1997), and the natural mycorrhization process is favoured in the presence of organic residues, while high levels of mineral nutrients inhibit mycorrhization (e.g. Read 1999).

Clipping

Half of the pines were subjected to experimental clipping (clipped), in which 50% (or 50% +1 in the case of odd numbers) of the apical shoots of each seedling was removed with scissors. The other half left untouched (unclipped). This kind of clipping resembles the type and amount of damage that ungulates cause to natural seedlings in the area (Gómez et al. 2001; Zamora et al. 2001; Baraza et al. 2004), and forces plants to grow through the remaining undamaged meristems.

Initial height, trunk diameter and initial number of meristems of the pines were recorded once they were planted. Plants were randomly assigned to treatments, and their position was also randomly assigned. Once per month, the pot positions were interchanged in order to homogenise the conditions in which every pine grew. Once per week, pines were watered with 0.75 l for small pots and 1.5 l for large pots, a quantity enough to reach the field capacity after the watering. The watering schedule was kept from May to September of 1996 and 1997. Fertilization was applied at the beginning of the first growing season (May 1996), and clipping at the end of the first growing season under the experimental conditions (September 1996). Pots were regularly weeded to avoid the competitive effect of grass and forbs. The level of natural herbivory undergone by the plants was also monitored during the experiment. Since insect herbivory attack was very low, restricted to a few aphids in very few plants, no insecticide was applied, and their effects were considered irrelevant to the experimental treatments.

Target variables

In February 1998, all surviving pines were harvested, gently uprooted and washed with clean water. Once

cleaned and tagged, seedlings were individually bagged and preserved in a freezer at -20°C until analysed. In the laboratory, seedlings were thawed, and several morphological variables were recorded.

The variables for analysis were:

- (a) *Plant survival*, number of plants of each treatment still alive, recorded during the uprooting process.
- (b) *Growth of the leader shoot*, growth of the leader shoot during the 1997-growing season (1 mm precision, measured with a stop-zero rule).
- (c) *Increment in trunk diameter*, calculated as the difference between final and initial trunk diameter (0.01 mm, measured with a digital calliper at the root collar).
- (d) *Increment in number of meristems*, calculated as the difference between final and initial number of meristems. In clipped plants, the number of initial meristems was the remaining after clipping.
- (e) *Relative growth rate RGR*, calculated as $\ln(\text{He}) - \ln(\text{Hb})$, being Hb and He the total height of the sapling at the beginning and at the end of the experiment, respectively (Zamora et al. 2001).
- (f) *Needle mass of the current year*, mass of the needles borne during the 1997 growing season.
- (g) *Shoot mass of the current year*, mass of the shoots borne during the 1997 growing season.
- (h) *Final root mass*, total mass of the root at the end of the experiment.
- (i) *Total plant mass*, total mass of the plant at the end of the experiment.
- (j) *Root to shoot ratio*, calculated as the ratio between the total mass of the root and the total mass of the aerial part (needles plus shoots) at the end of the experiment.

Dry mass was obtained by drying the samples at 45°C in a stove until constant weight, and then weighed by using an electronic balance (± 0.1 mg).

Statistical analyses

All data were analysed using JMP 5.0 statistical package (SAS 2002). Survival was analysed with a logit analysis, in which the factors were introduced as independent variables and pine survival as the response variable. For metric variables, four-way ANOVAs were used, except for the increment in trunk diameter,

in which a four-way ANCOVA was used, with the initial trunk diameter of the plant as a covariate. That is, for analysis, each of the response variables was introduced as a dependent variable, the factors as fixed factors and the pine as the experimental unit. Previous to the ANOVAs, the impact of treatments on metric variables was analysed using MANOVA, in order to consider the correlation among all traits and reduce type I errors (Scheiner 2001). Since the analysis was significant (data not shown) we consider the univariate analysis the most appropriate way to analyse the effect of treatments. We used type-III Sum of Squares, due to the unbalanced nature of data, after removing from the analyses those seedlings that died before the end of the experiment (Shaw and Mitchell-Olds 1993). Analyses were performed with full models. However, when interactions were not significant ($P > 0.05$) we used a pooling procedure (Zar 1996), to increase the degrees of freedom. To improve homocedasticity, arcsin and logarithmic transformations were performed on the variables (Zar 1996).

Results

At the beginning of the experiment, older pines were taller, trunk diameter was bigger and bore more meristems than younger ones ($P < 0.0001$ in all, t -test). Initial height, trunk diameter and initial number of meristems of the pines were 36.3 ± 0.8 mm (mean \pm SE, range 2–74), 2.44 ± 0.05 mm (0.5–6.8) and 2.2 ± 0.1 meristems (1–9) for 2-year old pines, and 72.8 ± 1.6 (10–151), 3.59 ± 0.06 (1.5–7.8) and 6.4 ± 0.2 (1–21) for 3-year-old pines, respectively. Three plants failed to root during the first month after planting and died, leaving the initial sample size per treatment between 30 (minimum) and 32 (maximum), for a total of 509 plants. After that, all plant deaths were considered due to experimental treatments.

Survival

Survival was affected mainly by Light ($\chi^2 = 36.49$, $P < 0.0001$), age ($\chi^2 = 34.80$, $P < 0.0001$) and Clipping ($\chi^2 = 17.04$, $P < 0.0001$), while nutrients did not reach significance ($\chi^2 = 3.79$, $P = 0.0515$; overall $r^2 = 0.14$; Wald χ^2 , d.f. = 1 in all cases). The highest percentage of survival (96.8%) was for

3-year-old pines in treatment sun fertilized unclipped, while the minimum survival (23.3%) was for 2-year-old pines, treatment shade unfertilized clipped (Fig. 1a). On average, twice as many 3-year-old pines than 2-year old ones survived to the end of the experiment.

Growth parameters

For growth parameters, all variables analysed showed similar results. There was a significant effect of Clipping, Age and, above all, Light. By contrast, the significance of fertilization as factor that explained most of the variation in growth of the shoots, as well as the interactions, was scant. Interestingly,

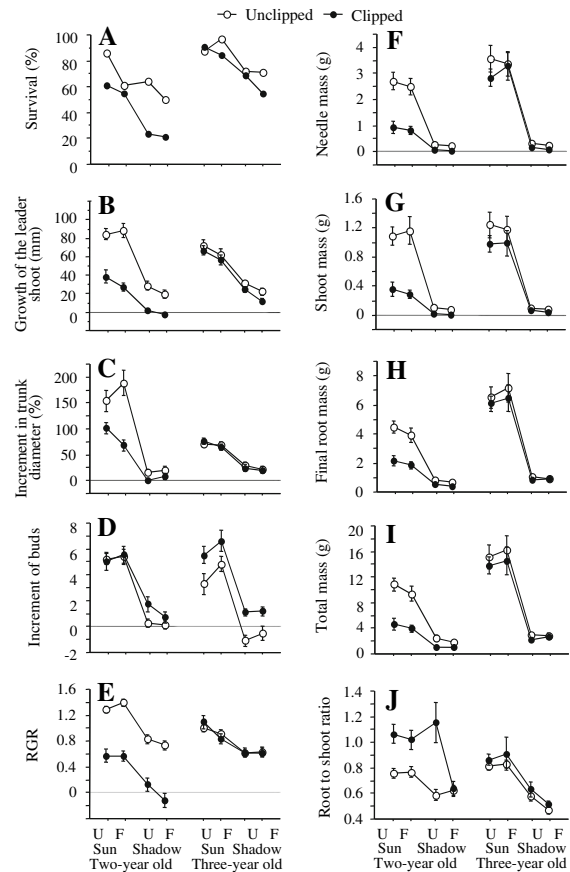


Fig. 1 Percentage of survival (a), growth parameters (b–e), allocation parameters (f–h), total biomass (i), and root-to-shoot ratio (j) of the Scots pine seedlings from different treatments. Two- and Three-year-old are the two pine ages used, U and F are the treatments of fertilization (unfertilized and fertilized, respectively), and Unclipped and Clipped are the treatments of experimental clipping. Values are mean \pm SE

interactions were in general nonsignificant, with the exception of Age \times Clipping, that resulted significant for all the variables analysed.

Growth of the leader shoot growth was affected by Age, Light, Nutrients and Clipping, as well as some of the interactions including these factors (Table 1). The main factor was Light, although Clipping was also very important: the growth of the leader shoot was greater for pines growing with full sunlight and for unclipped ones. Growth in 2-year-old, unclipped pines was similar to or greater than that of 3-year ones under the same treatment, implying a strong growth investment in the leader shoot of younger pines. Growth was very weak in clipped, fertilized pines in shade, and the positive effect of growing in sun was smaller when plants were fertilized and/or clipped (Fig. 1b). By contrast, the increment in trunk diameter showed a sharply different response for 2- and 3-year-old pines. The main factors were Age, Light, Clipping and the initial trunk diameter as covariate for both pine ages, as well as the Age \times Clipping interaction (Table 1), because in 3-year-old pines there was no apparent effect of clipping or nutrients in trunk diameter, while in 2-year-old ones, clipping consistently reduced the increment in trunk diameter (Fig. 1c).

The number of new meristems borne by pines depended on Light and Clipping, while this time, Age did result significant only in the interaction Age \times Clipping (Table 1). Clipped pines produced, on average, more meristems than did unclipped ones, and pines growing in sun more than in shadow. This difference was especially noteworthy for 3-year-old pines; in fact, unclipped pines at shadow reduced the number of meristems, while clipped ones slightly increased (Fig. 1d). Finally, RGR again showed Age, Light and Clipping to be significant factors, with Age \times Light, Age \times Clipping and Age \times Light \times Nutrients being significant interactions (Table 1). There were two important features of the significance for the RGR. First, Age sharply decreased in significance, despite remaining significant as a single factor and participating in all the significant interactions. Second, while clipping did not affect the RGR at all in 3-year-old pines, the differences in 2-year-old ones were noteworthy, unclipped pines being able to grow significantly more than clipped pines (Fig. 1e). Similarly, pines growing in sun consistently showed greater RGR than did their counterparts growing in shade (Fig. 1e).

Table 1 *F* values and significance from ANOVAs for the response variables measured for the experimental Scots pine seedlings

| Variable | Growth leader shoot | Increment trunk diameter | Increment number of meristems | RGR | Needle mass | Stem mass | Root mass | Total mass | Root/shoot ratio |
|---------------------------------------|---------------------|--------------------------|-------------------------------|------------|-------------|------------|------------|------------|------------------|
| Model (r^2): | 0.629 | 0.581 | 0.451 | 0.462 | 0.657 | 0.660 | 0.731 | 0.684 | 0.348 |
| Age | 78.08**** | 17.80**** | 2.28 n.s. | 4.54* | 44.45**** | 38.59**** | 128.61**** | 147.18**** | 28.27**** |
| Light | 441.43**** | 263.76**** | 240.99**** | 143.30**** | 579.48**** | 601.71**** | 823.68**** | 604.79**** | 72.79**** |
| Nutrients | 23.99**** | 0.11 n.s. | 3.50 n.s. | 2.16 n.s. | 1.19 n.s. | 2.47 n.s. | 1.58 n.s. | 0.42 n.s. | 9.66** |
| Clipping | 142.62**** | 9.08** | 12.93**** | 115.86**** | 42.16**** | 46.14**** | 34.47**** | 51.64**** | 37.72**** |
| Age \times Light | 44.02**** | 7.04** | | 10.48** | | | | | 5.97** |
| Age \times Clipping | 76.30**** | 9.29** | 7.08** | 103.62**** | 19.02**** | 36.51**** | 21.58**** | 30.83**** | 27.32**** |
| Light \times Nutrients | 10.16** | | | | | | | | 4.21* |
| Nutrients \times Clipping | | | | | | | | | 5.86* |
| Age \times Light \times Nutrients | | | | | | | | | |
| Age \times Light \times Clipping | 6.30* | | | 5.44* | | | | | |
| Initial trunk diameter | | 76.48**** | | | | | | | |

Only significant interactions are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Biomass allocation

The allocation of biomass to needles and shoots, the total weight of the root and the total weight of the plant followed a similar pattern. Light and Age were the determining factors, followed by Clipping, as well as the Age \times Clipping interaction (Table 1). Pines in sun produced far more needles and shoots, and grew greater roots, than did pines in shade (Fig. 1f–j). However, among 3-year-old pines in sun, clipped ones produced almost the same quantity of needles, shoots and roots, while among 2-year-old pines in sun, the clipped ones did not reach the production levels of their unclipped counterparts (Fig. 1f–h). A slightly different picture is given by the root-to-shoot ratio, which shows the same significance factors and interactions; however, Nutrients, alone or in interaction, proved significant also (Table 1). That is, when we consider the relationship between aerial and belowground parts of the plants, nutrients acquire a relevance that did not appear previously. The root-to-shoot ratio was greater in plants growing in sun than in shade, and as a direct consequence of clipping, the ratio was also greater in clipped plants than in unclipped ones, although the difference was not significant in 3-year-old pines or in shaded, fertilized 2-year-old pines (Fig. 1j).

Discussion

Three main conclusions can be drawn from the experimental results. First, as expected, none of the experimental treatments set prompted overcompensation, although there was a wide variety in the degree of undercompensation, depending on the treatment conditions, from the death of the plant to almost exact compensation in older plants. Clipping consistently decreased plant performance, with respect to unclipped plants, resulting in lower survival and less growth in leader shoot, trunk diameter, lower needle and shoot production and lower RGR; however, the differences between clipped and unclipped plants varied from nonsignificant for 3-year-old pines in sun to highly significant for almost all the response variables analysed for 2-year-old-pine treatments (Table 1, Fig. 1b–j; Puettman and Saunders 2001).

Secondly, age greatly influenced pine performance and the capacity of response to herbivory. Most studies

dealing with tree responses to clipping have been performed with just one age or life phase (see Karban and Thaler 1999). When considering plant age as a factor, some studies have differentiated between adult and juvenile phases (del-Val and Dirzo 2003; Boege 2005) or between plants of different stages of development in herbaceous species (del-Val and Crawley 2005; Barton 2007). The strong effect of the plant age in determining the effects of the treatments highlights the importance of studying the different developmental stages of the plant—even those differing by just 1 year of age, as in this case. Probably the greater size of the older pines (thus having more reserves) can account for most part of this result, although other mechanisms, as photosynthetic rate or nutrient acquisition could be implicated. Our study suggests that even the same species with the same treatments can provide different results when working with different developmental stages of the plants, and, therefore, the results cannot be extrapolated to other life phases (Donovan and Ehleringer 1991; Karban and Thaler 1999; Boege and Marquis 2005). This is especially evident from the results found with the RGR, given that this factor is considered for the effect of plant size, and allowing to assess differences in growth between ages. RGR shows that, when clipped, older pines can grow at the same rate as unclipped ones, while younger pines, which in fact have greater RGR than older ones when unclipped, show a lower response when clipped. This suggests an ontogenetic change in herbivory susceptibility of Scots pine saplings.

Thirdly, Fertilization had only a minor influence on the experimental pines, and only root-to-shoot ratio was slightly affected. Dung addition did not benefit pine development, despite that the soil used in the experiment was notably oligotrophic, especially in P and K (see “Methods”). The amount of dung added to each plant in experimental pots, although realistic, was larger than usually found under natural conditions near a seedling, thus the benefit of herbivores via dung deposition is unlikely.

Fourthly, Light is the factor that provided the most dissimilar results. Pines in the shade survived and grew less than did pines in the sun, and the differences were accentuated in the younger pines (Fig. 1a). Thus, light availability is a main requisite for an adequate recovery after clipping (Heikkilä and Mikkonen 1992; Bast and Reader 2003, and references therein). However, the lack of a significant

Light \times Clipping interaction indicates that, although plants consistently perform worse in shadow, the shadow does not worsen the response to clipping in respect to the response found at sun. This is especially noteworthy in our study site, since shady microhabitats are the best sites for seedling establishment (Castro et al. 2004) but the worst for sapling recovery after herbivory.

Studies on tolerance of woody plants to herbivory have traditionally assumed a low compensatory potential, although this vision has recently been debated (Haukioja and Koricheva 2000, and references therein). Our experiment shows that none of the treatments promoted overcompensation, but at least in older pines growing in sun resulted in nearly exact compensation, while younger pines are more negatively affected. Other studies have shown overcompensation in woody plants (Hjältén 1999; Vanderklein and Reich 1999; Lehtilä et al. 2000) and for Scots pine, previous studies have reported a positive effect of herbivory on growth (Honkanen and Haukioja 1994, 1998; Honkanen et al. 1994). However, these studies focus on defoliation or debudding, which corresponds to the usual kind of damage inflicted by insects. This kind of damage only removes photosynthetic tissues and some reserves, but allows the production of new leaves, the budburst of the next growing season. By contrast, the kind of ungulate herbivory undergone by Scots pine is much more severe, removing terminal buds, needles and shoots of the current-year shoots (Gómez et al. 2001; Zamora et al. 2001). Under clipping treatments, simulating ungulate browsing instead of insect defoliation or debudding, Scots pine saplings did not recover the biomass lost in terms of needle mass or number of needles (Millard et al. 2001), although needles were larger after clipping, and other authors found compensation, at least under some circumstances (Edenius et al. 1993).

For Scots pine in Mediterranean mountains, the results found in this work evidence additional sources of regenerative bottleneck. Seeds of Scots pine are massively preyed upon (Castro et al. 1999), seedlings suffer high rates of mortality due to summer drought (Castro et al. 2004, 2005), and, once established, saplings and juveniles undergo severe browsing by ungulates (Gómez et al. 2001; Zamora et al. 2001). This work shows that the best places for seedling establishment and survival, the shady microhabitats

(Castro et al. 2004), are the worst for plant growth and recovery after herbivory, as proved by our experimental results. By contrast, sunny places are better for plant growth after herbivory, but seedlings and saplings are conspicuously scant there (Castro et al. 2004). That is, Scots pine in Mediterranean mountains shows uncoupling between its requirements for establishment (shade and soil humidity) and for growth and recover after herbivory (light).

In conclusion, our experiment clearly shows the negative effects of herbivory for juvenile Scots pines, the damage being greater the younger the tree, by reducing the tree-growth capacity, as shown by the marked reduction of RGR in younger, clipped pines. The clipped plants showed worse survival and performance than unclipped ones, but there was a wide variety of responses, depending on the light environment. Furthermore, there was no positive effect of dunging. In short, herbivory in Mediterranean Scots pine results in a negative net balance, because the detrimental effects of herbivory cannot be alleviated by their potential benefits, but the age of the sapling and the abiotic context in which the sapling is living can greatly condition the final outcome. This study clearly shows, for the first time, that even small differences of age can be very important when considering the sapling response to herbivory and light environment. Long-term studies that capture ontogenetic changes are needed in order to understand sapling growth potential of long-lived woody plants in contrasting scenarios.

Acknowledgements We thank the Consejería de Medio Ambiente, Junta de Andalucía, for permitting our field work in the Sierra Nevada Protected Area. Ángel and Joaquín took good care of the plants during the experiment, and helped us in the collection of data. Three anonymous referees provide useful and constructive criticisms. David Nesbitt looked over the English version of the manuscript. Financial support was provided by PFPU-MEC grants to Jorge Castro and Daniel García, and projects AGF98-0984 and REN2002-04041-CO2-01/GLO (HETEROMED) from MCYT.

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