

## Short Delay in Timing of Emergence Determines Establishment Success in *Pinus sylvestris* across Microhabitats

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• **Background and Aims** The date of emergence may have far-reaching implications for seedling performance. Seedlings emerging early in the growing season often have a greater rate of survival or grow better if early emergence provides advantages with respect to an environmental cue. As a result, the benefits of early emergence may be lost if the environmental stress creating the differences among cohorts disappears. The experimental manipulation under field conditions of the factors that constitute the main sources of stress for seedling establishment is thus a straightforward method to evaluate the impact of date of emergence on seedling establishment under realistic conditions.

• **Methods** Two field experiments were performed to analyse the effect of emergence date on survival and first-year growth of Scots pine seedlings in natural mountain forests in south-east Spain. Two main environmental factors that determine seedling success in these mountains were considered: (1) microhabitat type (monitoring the effect of date of emergence in the three most common microhabitats where seedlings recruit); (2) summer drought (monitored by an irrigation treatment with control and watered sampling points).

• **Key Results** Overall, early emergence resulted in a higher probability of survival and better growth in the two experiments and across microhabitats. However, the reduction in summer drought did not diminish the differences observed among cohorts: all cohorts increased their survival and growth, but early cohorts still had a clear advantage.

• **Conclusions** Date of emergence determines establishment success of *Pinus sylvestris* seedlings, even if cohorts are separated by only a few days, irrespective of the intensity of summer drought. The experimental design, covering a gradient of light intensity and soil moisture that simulates conditions of the regeneration niche of Scots pine across its geographical range, allows the results to be extrapolated to other areas of the species. Date of emergence is thus likely to have a large impact on the demography of Scots pine across its geographical range.

**Key words:** Cohort effects, cohort of emergence, date of emergence, delayed emergence, irrigation experiments, *Pinus sylvestris*, seedling establishment, Sierra Nevada National Park, summer drought.

### INTRODUCTION

It is common for seedlings emerging early in the season to do better than those emerging later, as they have a higher rate of survival, improved growth or even fitness (Miller, 1987; Weisner and Ekstam, 1993; Jones *et al.*, 1994; Kelly and Levin, 1997; Teasdale *et al.*, 2004; Verdú and Traveset, 2005). There may be several reasons for this, such as the more vigorous growth of the root system reaching greater soil moisture for early cohorts (Lonsdale and Abrecht, 1989), competitive hierarchies (Ross and Harper, 1972; Abul-Fatih and Bazzaz, 1979; Stanton, 1985), or better access to light (Seiwa, 1998; Pommel *et al.*, 2002; Stibbe and Märlander, 2002). A common point in all cases is the advantage conferred by having more days to grow during the growing season. Even so, early emergence does not always guarantee advantages and may even have disadvantages. Usually such cases may be explained by peculiarities of the study system, such as unexpected germination in the dry season after rainfall (Fowler, 1988), time of flooding (Jones and Sharitz, 1989), climatic hazards such as storms and early frost events (Fernández-Quintanilla *et al.*, 1986; Quintana *et al.*, 2004), or due to developmental constraints of the species studied (e.g. species with determinate growth form, for which initial

growth is determined mostly by seed size; Seiwa, 2000). Thus, the effect of date of emergence depends heavily on the stochastic nature of environmental factors (e.g. Miller, 1987). However, if environmental hazards are reduced so that differences among cohorts are related mostly to differences in number of days for growing (as for instance under greenhouse conditions, in a growth chamber or in agricultural systems), a positive effect of early emergence either on survival or growth can be expected (e.g. Sorensen, 1978; Jones and Sharitz, 1989; Wang and Lechowicz, 1998; Espigares *et al.*, 2004; Teasdale *et al.*, 2004), which may even be predicted in terms of growing degree-days (Teasdale *et al.*, 2004).

The relative importance that the timing of emergence exerts in determining seedling success may, however, be mediated by the environmental conditions encountered by the seedlings (Fowler, 1988; Miller *et al.*, 1994; Seiwa, 1998; Angadi *et al.*, 2004; Quintana *et al.*, 2004). Provided that the effect of date of emergence is related to the advantage gained by early cohorts with respect to some environmental cue, any situation reducing the differences in stress undergone by cohorts could reduce the relative importance of emergence date. For instance, Battaglia (1996) found that the positive effect of early emergence in two *Eucalyptus* species was more pronounced in a harsh site than in a mild one. Seiwa (1998) found that early

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cohorts of *Acer mono*—a species with its establishment constrained by low light levels in the forest understorey—had higher survival rates than later ones due to higher light interception before canopy closure, but this difference was greater inside the forest than on the sunnier forest edge. Similarly, Espigares *et al.* (2004) found that the positive effect of early emergence in *Retama sphaerocarpa* under competition with grasses disappeared with higher water availability under some circumstances. Thus, an experimental manipulation under field conditions of factors presumed to determine seedling performance will help to elucidate the implications that time of emergence has for establishment success.

The present study explores the effect of date of emergence on seedling survival and first-year growth for Scots pine on a Mediterranean mountain, considering the two factors that exert the greatest impact on seedling establishment in these ecosystems, i.e. microhabitat type and summer drought (Castro *et al.*, 2004, 2005a). Microhabitats where seeds arrive after dispersal differ in abiotic conditions, with a strong influence on recruitment such as soil moisture and radiation intensity (Castro *et al.*, 2004). On Mediterranean mountains, Scots pine seeds germinate between April and early May, and emergence starts in May (Castro *et al.*, 2004, 2005b), during a typically rainy period and, hence, without drought stress. However, soil moisture declines over the growing season (summer), and summer drought actually becomes the main cause of mortality (Castro *et al.*, 2004, 2005a). In consideration of these two main constraints, four specific questions were addressed in these experiments. (1) How do short time lags in emergence date affect seedling establishment in Scots pine? (2) How does microhabitat influence the survival and growth patterns established by differences in emergence time? (3) How does water stress affect the survival and growth patterns established by differences in emergence time? (4) What are the consequences for forest regeneration?

## MATERIALS AND METHODS

### *Study site and species*

The study was conducted in the surroundings of La Cortijuela Botanical Garden (37°05'N, 3°28'W; Sierra Nevada National Park, south-east Spain), in natural Scots pine forests that, in these southern populations, grow between 1600 and 2200 m a.s.l., forming the treeline. The climate is continental Mediterranean, with dry summers, cold winters, and a mean annual rainfall of 879 mm (for further details on climate conditions, see Castro *et al.*, 2004, 2005a). Scots pine forests form a canopy of approx. 25% cover in a typical woodland stand. The understorey is composed of areas of bare soil with intermingled shrubby species, mostly junipers (*Juniperus communis* and *J. sabina*) and tall spiny shrubs [sloe (*Prunus ramburii*) and barberry (*Berberis hispanica*) being particularly abundant]. This allows the recognition of three main microhabitats where seedlings may be recruited, i.e. areas of bare soil, areas under the canopy of adult pines, and

areas under the canopy of shrubs (Castro *et al.*, 2004). These microhabitats represent >75% of the available microsites for seedling establishment (Castro *et al.* 2005b).

Abiotic conditions differ markedly among microhabitats. Areas of bare soil receive full sunlight, and have the highest soil temperature as well as the lowest soil moisture during the growing season. Areas under adult pine canopies receive approx. 5–10% of the radiation received by bare soil, have the lowest soil temperatures, and maintain the highest soil moisture during the growing season. Areas under the canopy of shrubs have intermediate values of the above abiotic variables. See Castro *et al.* (2004, 2005a, b) for details on these abiotic variables in different years. Overall, soil moisture declines steadily in all the microhabitats with the advance of the summer (e.g. Castro *et al.*, 2004, 2005b), as expected under a Mediterranean-type climate.

Seed dispersal spans January to March (Castro *et al.*, 1999). Seeds are not dormant and germinate quickly with sufficient warmth and moisture (Castro *et al.*, 2005b), emergence starting under field conditions in May (Castro *et al.*, 2004). By that time, leaf flushing has occurred in the deciduous shrubs, and thus seedlings becoming established under shrubs are subjected to relatively homogeneous radiation throughout the growing season (June–September). Seedling mortality during the first growing season is very high ( $\geq 90\%$ ; Castro *et al.*, 2004, 2005a). Summer drought is the main mortality factor in all the microhabitats (Castro *et al.*, 2004, 2005a).

### *Experimental design*

*Experiment 1.* This experiment was set up in 1996 to analyse the effect of date of emergence and microhabitat on seedling survival and growth. The four microhabitats most representative of the forest were chosen according to coverage: (1) open—interspaces of bare soil; (2) spiny—under the canopy of tall (approx. 1.5 m in height), spiny—deciduous shrubs, either *Berberis hispanica* or *Prunus ramburii*; (3) juniper—under the canopy of *Juniperus communis*; (4) pine—under the canopy of adult Scots pines. For each microhabitat, 60 sampling stations were haphazardly assigned, in which 25 seeds were sown at 1 cm depth, forming a quadrat of 5 × 5 seeds, with a distance of 4 cm between seeds (6000 sown seeds: 4 microhabitats × 60 sampling stations × 25 seeds). The sowing date was 12–13 April. Wire cages of 1.3 cm mesh protected sampling stations against predators and trampling. Sampling stations were censused regularly, noting emergence, survival and cause of mortality (see Table 1 for identification of cohorts of emergence). Seedlings were considered to have emerged when any evidence of cotyledon emergence was detected.

A total of 2685 seedlings emerged, of which 2664 were used for the analysis of the effect of time of emergence (the rest were eliminated because they belonged to cohorts of emergence not included in the analysis of emergence; see Table 1). A total of 261 seedlings survived the first growing season, of which 214 could be used for the growth analysis (the rest were eliminated either because they belonged to cohorts of emergence not included in the analysis of

TABLE 1. Cohorts distinguished for seedling emergence of Scots pine in (A) expt 1 and (B) expt 2

Cohort no.	Interval of emergence*	No. of emerged seedlings per microhabitat				No. of seedlings surviving per microhabitat							
		Open	Spiny	Juniper	Pine	Open	Spiny	Juniper	Pine				
(A) Experiment 1													
1	23/05–30/05	<b>673</b>	<b>442</b>	<b>231</b>	<b>103</b>	43	<b>56</b>	<b>45</b>	<b>6</b>				
2	31/05–05/06	<b>63</b>	<b>195</b>	<b>240</b>	<b>112</b>	1	<b>24</b>	<b>46</b>	<b>3</b>				
3	06/06–21/06	2	<b>126</b>	<b>246</b>	<b>146</b>	0	<b>10</b>	<b>24</b>	2				
4	22/06–04/07	0	5	<b>45</b>	<b>42</b>	–	0	1	0				
5	05/07–23/07	0	0	1	7	–	–	0	0				
6	24/07–06/08	0	0	0	6	–	–	–	0				
Cohort no.	Interval of emergence*	No. of emerged seedlings per microhabitat						No. of seedlings surviving per microhabitat					
		Unwatered			Watered			Unwatered			Watered		
		Open	Spiny	Pine	Open	Spiny	Pine	Open	Spiny	Pine	Open	Spiny	Pine
(B) Experiment 2													
1	01/05–07/05	<b>97</b>	<b>167</b>	4	<b>101</b>	<b>179</b>	7	37	<b>33</b>	0	<b>53</b>	<b>115</b>	0
2	08/05–19/05	<b>22</b>	<b>107</b>	77	<b>16</b>	<b>131</b>	<b>102</b>	2	<b>14</b>	<b>18</b>	<b>4</b>	<b>39</b>	<b>62</b>
3	20/05–28/05	1	<b>19</b>	<b>70</b>	1	<b>27</b>	<b>87</b>	0	2	<b>7</b>	0	<b>6</b>	<b>28</b>
4	29/05–12/06	1	10	<b>81</b>	0	2	<b>88</b>	0	0	1	–	0	<b>16</b>
5	13/06–20/06	0	0	10	0	0	6	–	–	0	–	–	0
6	21/06–30/06	0	0	1	0	0	1	–	–	0	–	–	0

The interval of emergence is marked by the date of sampling and the date of the following sampling (except for cohort number 1, for which the starting day is the date when the first seedlings were detected).

In bold type, cohorts used for analyses in each microhabitat; the rest of seedlings were excluded due to low sample size for analyses.

\*Day/month.

emergence or because they suffered damage by trampling; Table 1). There was an average of  $11.0 \pm 0.4$  s.e. emerged seedlings per sowing point, ranging from  $7.0 \pm 0.7$  in the pine microhabitat to  $12.7 \pm 0.8$  in the juniper microhabitat. The average number of live seedlings per sowing point after the growing season (thus seedlings used for growth analyses) was  $1.1 \pm 0.2$ . See Castro et al. (2004) for further details related to experimental set-up, seedling demography, and abiotic parameters in each microhabitat.

*Experiment 2.* This experiment, set up in 1997, was designed to include the effect of water supplementation, a factor reducing the seedling stress and putatively modulating the effect of date of emergence on survival and growth. The open, spiny and pine microhabitats defined above were chosen; juniper was not included due to the absence of this microhabitat near the area around the spring used as a water source for the experiment. In each of the microhabitats, 20 sampling stations were randomly located, with two sampling points established in each roughly 75 cm apart. One sampling point was subjected to irrigation and the other kept as a control. At each sampling point, 25 seeds were sown on 19 March 1997 using the same procedure described above (3000 sown seeds in total: 3 microhabitats  $\times$  2 irrigation levels  $\times$  20 sample stations  $\times$  25 seeds), and similarly protecting the seeds with a wire cage. Plots assigned to irrigation were sprinkler irrigated 12 times at around 10-d intervals during 1997 from the onset of emergence (first watering on 12 May) to the end of summer drought (15 September, when the first major rainfall was recorded). At each irrigation time, 2 L of water were added, equivalent (considering the surface area irrigated) to 32 mm of precipitation. This simulates the periodic strong

summer storms on a Mediterranean mountain, and fits with the overall summer precipitation in more mesic, northern areas within the distribution of the species, where summer drought is mild compared with Mediterranean-type ecosystems (Castro *et al.*, 2005a). Watered plots of the open microhabitat registered a modest increase in herb coverage, and were carefully weeded, when herbs were still small, to levels comparable to the control plots (nearly bare ground). Sampling stations were checked regularly, recording emergence, survival and cause of mortality (see Table 1 for identification of cohorts of emergence).

A total of 1414 seedlings emerged, of which 1371 were used for the analysis of the effect of date of emergence on seedling survival (Table 1; the other seedlings were eliminated for the same reasons explained for expt 1). A total of 437 seedlings survived the first growing season, of which 434 were used for the analysis of growth parameters (the rest eliminated for the same reasons explained for expt 1; Table 1). There was an average of  $12.0 \pm 0.6$  s.e. emerged seedlings per sowing point, ranging from  $6.4 \pm 1.1$  in the non-watered plots of the open microhabitat to  $16.9 \pm 1.0$  in the watered plots of the spiny microhabitat. The average number of live seedlings after the growing season per sowing point (thus seedlings used for growth analyses) was  $3.8 \pm 0.4$ . See Castro *et al.* (2005a) for further details related to experimental set-up, seedling demography and abiotic parameters in each microhabitat.

#### Performance estimates

In the two experiments, the survival and growth of seedlings from different cohorts was compared at the end

of the first growing season (October). Growth was estimated *in situ* with non-destructive methods using several parameters: (a) length of the shoot (considered from cotyledon insertion level up because it was not possible to identify the root collar); (b) number of leaves; and (c) length of the largest leaf. Causes of mortality were assigned to: (a) 'drought', seedlings turned brown and dried out without any visible damage; (b) 'pathogens', damping-off of seedlings, generally with a region of necrosis at root-neck level; and (c) 'invertebrate herbivory', seedlings severed by insects. Other minor causes of mortality (e.g. vole tunnels, trampling) were not considered in this study due to their low relevance in the data set (for further details on causes of mortality, see Castro *et al.*, 2004, 2005a).

#### Data analysis

In the absence of any negative correlations between the number of emerged seedlings per sowing point and survival or growth after the first growing season in either of the two experiments, it was assumed that the pattern of survival and growth were not mediated by density-dependent effects. Thus each seedling was considered to be a replicate. The relationship between cohort of emergence and probability of survival was analysed by using a contingency table. When more than one factor was included (expt 2, cohort and irrigation), a multivariate contingency test was used, with simultaneous consideration of the two factors and their interaction. The relationship between cohort of emergence and seedling growth was analysed with ANOVAs. Type-III sum of squares were used, and data were log-transformed to improve homocedasticity (Zar, 1996). Analyses were performed with JMP 5.0 software (SAS Institute, Cary, NC, USA).

Independent analyses were performed for each microhabitat, given that cohorts of different microhabitats may be subjected to different environmental conditions and, in addition, the consideration of all microhabitats simultaneously implies differences in age among seedlings (see Table 1). The analyses were restricted to cohorts having sample sizes sufficient to allow statistical treatment without violating the assumption of the models (Zar, 1996; for sample size, see Table 1).

## RESULTS

### Experiment 1

Seedlings that emerged earlier in the season showed higher survival rates (Fig. 1). The relationship was not significant in the spiny microhabitat ( $\chi^2 = 2.18$ , d.f. = 2,  $P = 0.34$ ), but was close to marginal significance for the open ( $\chi^2 = 2.36$ , d.f. = 1,  $P = 0.12$ ) and pine microhabitats ( $\chi^2 = 5.91$ , d.f. = 3,  $P = 0.12$ ) and highly significant for the juniper microhabitat ( $\chi^2 = 22.68$ , d.f. = 3,  $P < 0.0001$ ; Fig. 1). However, the trend was clear, i.e. higher survival for early (first or second) cohorts even in microhabitats where differences were not statistically significant (Fig. 1). Lack of strong statistical differences is likely to be due to the small number of seedlings from later cohorts (Table 1).

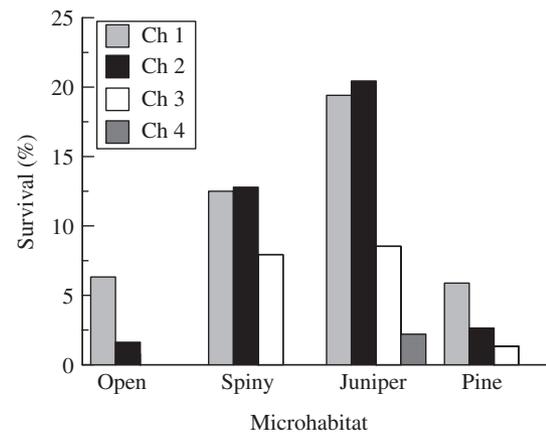


FIG. 1. Percentage survival of Scots pine seedlings from different cohorts of emergence (Ch) in four microhabitats in the 1996 experiment. Differences were analysed for each microhabitat with a contingency test. Chi-square  $P$ -values for each microhabitat are 0.12 for open, 0.33 for spiny,  $<0.0001$  for juniper, and 0.11 for pine. See Table 1 for time of emergence and sample size of each cohort.

The cause of mortality could be identified for 96.0% of the dead seedlings during the first growing season, with a total of 2285 seedlings that died due to summer drought, pathogens or insect herbivory. The cause of mortality differed among cohorts of emergence ( $\chi^2 = 42.96$ , d.f. = 4,  $P < 0.0001$ ; all microhabitats pooled; seedlings from cohort 4 excluded from analysis due to low sample size). Overall, the relative importance of drought as a mortality factor increased for late-emerged seedlings. Thus, for cohort 1, drought caused 73.4% of deaths compared with 74.4%, 85.0% and 95.7% for cohorts 2, 3 and 4, respectively. This was associated with a decrease in death due to pathogens from 22.9% (cohort 1) to 19.9% (cohort 2), 9.6% (cohort 3) and 0% (cohort 4).

Growth parameters were generally higher for early- than for late-emerging seedlings in the spiny and juniper microhabitats, with the exception of the length of the largest leaf in the juniper microhabitat ( $P = 0.20$ ) (Fig. 2; data from the open microhabitat was not analysed due to lack of sample). Shoot length showed the largest differences, with seedlings from the first cohort nearly doubling the length reached by seedlings that emerged in the second or third cohorts (Fig. 2). By contrast, the pine microhabitat showed no differences among cohorts for any of the growth parameters measured (Fig. 2).

### Experiment 2

Seedlings that emerged earlier in the season showed higher survival rates, with a clear stairway pattern among cohorts of emergence in all the microhabitats (Table 2 and Fig. 3). Irrigation also determined survival (Table 2), which rose for all the cohorts in all the microhabitats (Fig. 3).

The cause of mortality was identified for 96.4% of dead seedlings, with a total of 862 seedlings dying from summer drought, pathogens or insect herbivory. The cause of mortality differed among irrigation treatments ( $\chi^2 = 37.89$ , d.f. = 1,  $P < 0.0001$ ; all microhabitats and cohorts pooled;

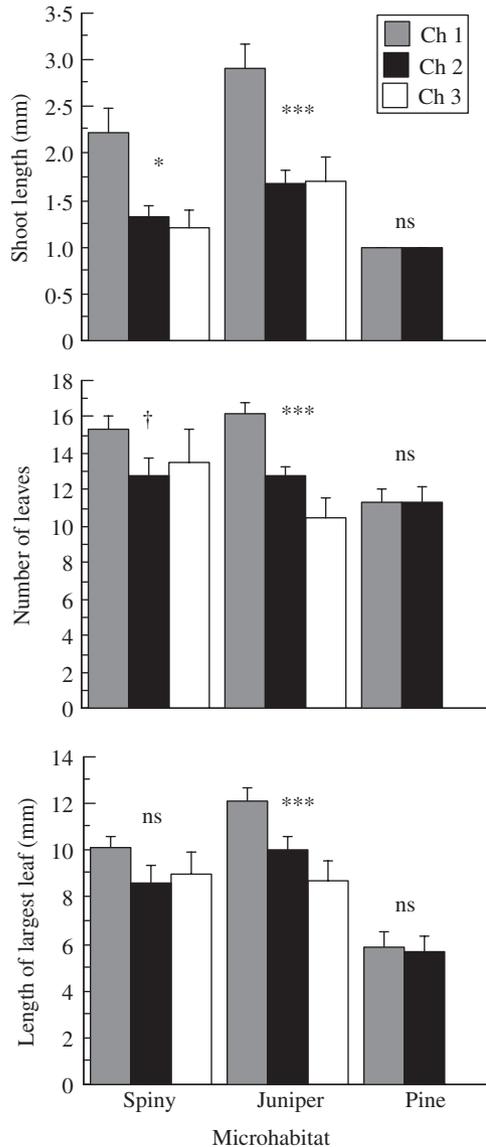


FIG. 2. Growth parameters after the first growing seasons for Scots pine seedlings from different cohorts of emergence (Ch) in the 1996 experiment. Differences within microhabitats were analysed with a one-way ANOVA: †,  $P < 0.1$ ; \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ ; ns, not significant. See Table 1 for sample size of each cohort.

seedlings death by insect herbivory excluded from analysis due to low sample size). Overall, irrigation reduced the mortality by drought and increased the mortality by pathogens. This trend was consistent among cohorts, with mortality by pathogens roughly doubling in the watering treatment in all cases except cohort 4 (Table 3).

## DISCUSSION

### Effect of date of emergence on seedling performance

The results show a positive effect of early emergence on seedling survival, as expected from the larger time span for seedling development before the onset of summer drought.

TABLE 2. Summary of the multivariate contingency analysis for survival of seedlings from different microhabitats in Experiment 2, considering watering treatment and cohort of emergence as factors

	Source	d.f.	Wald $\chi^2$	P
Open	Watering (A)	1	3.30	0.0692
	Cohort emergence (B)	1	9.41	0.0022
	A $\times$ B	1	0.40	0.5274
	Model	3	18.78	0.0003
Spiny	Watering (A)	1	15.91	0.0001
	Cohort emergence (B)	2	26.50	0.0000
	A $\times$ B	2	6.40	0.0408
	Model	5	120.17	<0.0001
Pine	Watering (A)	1	25.08	0.0000
	Cohort emergence (B)	2	31.23	0.0000
	A $\times$ B	2	1.57	0.4548
	Model	5	110.83	<0.0001

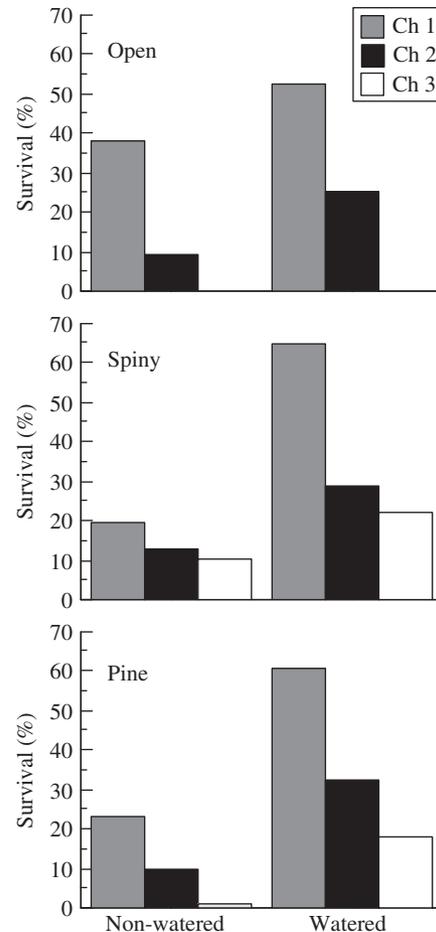


FIG. 3. Percentage survival of Scots pine seedlings from different cohorts of emergence (Ch) in three microhabitats in expt 2 under two levels of irrigation (watered and unwatered). See Table 1 for time of emergence and sample size of each cohort; see Table 2 for results of the multivariate contingency analysis.

In fact, summer drought had a higher impact for later cohorts, suggesting that early emergence has a positive effect on survival via more vigorous root or whole-seedling development. These differences in survival appeared even

TABLE 3. Percentage of causes of mortality per cohort of emergence for non-watered and watered seedlings in expt 2

Cohort	Mortality factor	Irrigation treatment	
		Control	Watered
1	Drought	81.5	65.7
	Pathogens	3.3	8.8
	Insect herbivory	15.2	25.5
2	Drought	70.1	43.9
	Pathogens	27.3	51.1
	Insect herbivory	2.6	5.0
3	Drought	64.9	41.1
	Pathogens	29.9	54.8
	Insect herbivory	5.2	4.1
4	Drought	86.7	82.8
	Pathogens	12.0	13.8
	Insect herbivory	1.3	3.4

Data of all microhabitats are pooled.

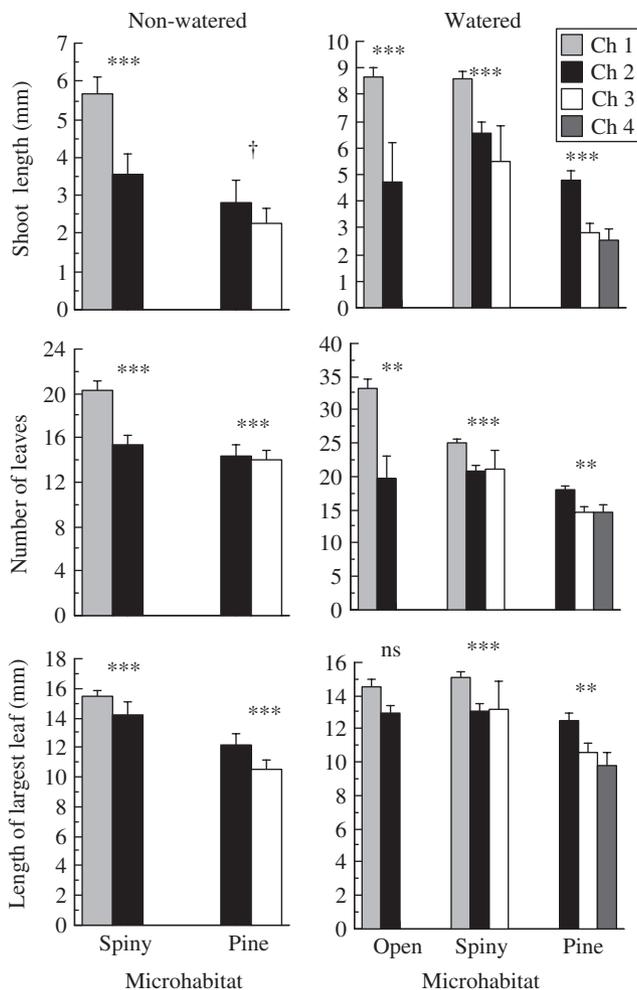


FIG. 4. Growth parameters after the first growing seasons for watered and unwatered Scots pine seedlings from different cohorts of emergence (Ch) in the 1997 experiment. Differences within microhabitats were analysed with one-way ANOVA: †,  $P < 0.1$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns, non-significant. See Table 1 for sample size of each cohort. Comparisons between cohorts in the unwatered treatment of the open microhabitat were not possible due to lack of seedlings.

though the time lag among cohorts of emergence was in the range of days (for similar results with other species, see Miller, 1987; Streng *et al.*, 1989; Jones *et al.*, 1994; Wang and Lechowicz 1998; Seiwa, 1998). The differences in the survival pattern for different years (Figs 1 and 3) may be related to yearly variation in experimental design (emergence dates and cohort intervals differed among years and microhabitats) and environmental conditions (e.g. rainfall) which may slightly alter the survival patterns, but in any case the results are clear cut: overall, early emergence resulted in higher seedling survival.

Growth parameters also differed among cohorts of emergence, early-emerging seedlings having higher performance. As in the case of survival, there were peculiarities for each year and microhabitat, although the overall trend was clear. Thus, seedling growth increased with early emergence in almost all the microhabitats in the two experiments, but there were no differences among cohorts in the pine microhabitat in expt 1 (Fig. 2). The lack of differences under the pine canopy may in fact be a predictable result, as radiation intensity in this microhabitat is likely to be the limiting factor for seedling growth (Castro *et al.*, 2004, 2005a). Seedling growth may depend almost exclusively on seed reserves in this microhabitat, which would reduce differences among cohorts (e.g. Seiwa, 2000). Thus, the relationship between date of emergence and seedling performance may be microhabitat-dependent, and factors such as light intensity under field conditions may cancel the advantage conferred by early emergence.

Seedling growth differed among cohorts even after watering. This is logical given that, even when the main source of stress is reduced, earlier-emerging seedlings still have more days to grow before the end of the growing season. However, alleviation of drought stress did not diminish the differences in survival among cohorts of emergence, but boosted seedling survival in all cohorts (e.g. Fig. 3). The reduction of differences on stress undergone by seedlings has been shown to decrease the advantage of early germination in other species (e.g. Battaglia, 1996; Seiwa, 1998; Espigares *et al.*, 2004). The lack of a reduction in the survival differences among cohorts in this study cannot be ascribed to a putative low intensity of the irrigation treatment, as in fact the amount of water added proved to be effective, considerably reducing the number of deaths by drought and boosting survival, which doubled (Castro *et al.*, 2005a; this study). One possible explanation is the strong impact of summer drought for Scots pine seedlings in these southernmost areas of its distribution, which causes mortality rates of around 75% at the end of the first growing season. This implies that irrigation is also extremely beneficial for early cohorts, masking the detection of possible differences among cohorts in the effect of watering.

*Possible mechanisms underlying differences in date of emergence among cohorts*

Given the differences in survival and growth among cohorts, the results support the hypothesis that date of emergence is a key point for seedling survival. Hence, an

important question for understanding the survival probability of seedlings is to know the factors that determine differences in date of emergence. Differences among emergence dates may be based either on phenotypic and genetic effects related to seeds, or to environmental conditions experienced by the germinating seed (Jones *et al.*, 1997). Genetic and phenotypic effects were not controlled in the present study, but they are unlikely to be the main factor determining emergence rate for several reasons (see also Jones *et al.*, 1997). Scots pine seeds germinate quickly under appropriate conditions, reaching values of approx. 90 % in 1 week (Nyman, 1963; Castro *et al.*, 2005b). This time is much shorter than the temporal range of emergence found in the present study, supporting the contention that environmental factors (e.g. soil moisture, soil temperature, soil texture, microtopography, etc.) rather than genetic constitution, control the germination rate, which in turn translates as differences in survival. Phenotypic traits such as seed mass (which may also be partially determined by a genetic component) may also determine the emergence date and thus establish a size hierarchy (Castro *et al.*, 2006). However, this situation is again unlikely in the present study system; in fact, under nursery conditions, neither date of emergence nor early seedling survival correlate with seed mass in Scots pine (Castro, 1999; J. Castro, unpubl. res.). Thus, it is highly probable that differences in emergence dates found here are due primarily to differences in environmental conditions surrounding the germinating seed (e.g. microtopography, soil temperature, etc.). If this were the case, seedling emergence might act as a stochastic process that affects the probability of survival, and hence might counteract the selection of particular genotypes.

#### Consequences for regeneration

The consequences that date of emergence has for seedling establishment in these southern Scots pine forests are clear: early-emerging seedlings have higher chances of establishment, and this pattern is consistent across all the microhabitats where seedlings recruit. Only in the case of the Pine microhabitat there was a clear lack of cohort effect upon growth for one of the years (Fig. 2). This had no implications for seedling recruitment, as survival was still higher for early cohorts under pine canopies. In addition, recruitment is hampered in this microhabitat due to low light availability, which results in very low values of seedling growth (Castro *et al.*, 2004, 2005a) and led to seedling death in the mid-term (Castro *et al.*, 2004). Areas of bare soil and areas underneath the canopies of shrubs represent the microsites available for recruitment in these forests (Castro *et al.*, 2004), and in the two cases the emergence date determined chances of survival and growth. This has both ecological and management implications. First, seedling mortality may be enhanced by a cohort effect in case that germination is delayed for some reason (e.g. a cold spring that might result in an overall delayed emergence). Second, the impact of date of emergence should be considered when planning regeneration programmes that involve seeding.

The experimental design in this study may be extrapolated to other areas of the species' distribution range. The irrigation treatment is based on the rainfall registered in other mesic areas of the Scots pine distribution (Castro *et al.*, 2005a) and creates a situation of lower summer drought (typical of the main distribution area of the species). On the other hand, the gradient of radiation used, from full sunlight to approx. 5 % of incident radiation, covers the gradient of light intensity that the species may encounter across its geographical range. All this supports the contention that date of emergence may be important for Scots pine recruitment across the entire geographical range of the species.

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