

CONSTRAINTS ON TREE SEEDLING ESTABLISHMENT IN MONTANE GRASSLANDS OF THE VALLES CALDERA, NEW MEXICO

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Abstract. Montane and subalpine grasslands are prominent, but poorly understood, features of the Rocky Mountains. These communities frequently occur below reversed tree lines on valley floors, where nightly cold air accumulation is spatially coupled with fine soil texture. We used field experiments to assess the roles of minimum temperature, soil texture, grass competition, and ungulate browsing on the growth, photosynthetic performance, and survival of transplanted ponderosa pine (*Pinus ponderosa*) seedlings at 32 sites straddling such reversed tree lines in the Valles Caldera National Preserve (VCNP) of the Jemez Mountains, New Mexico (USA). Seedling growth increased most strongly with increasing nighttime minimum temperatures away from the valley bottoms; seedlings experiencing the coldest temperatures on the caldera floor exhibited stunted needles and often no measurable height growth. Based on the chlorophyll fluorescence ratios Φ_{PSII} and F_v/F_m , we found that low minimum temperatures, low soil moisture, and fine soil texture all contributed to photoinhibition. Neighboring herbs had only minor negative effects on seedlings. We found no effect of ungulates, but golden-mantled ground squirrels (*Spermophilus lateralis*) caused substantial seedling mortality. Second-year seedling survival was highest on sandy soils, and third-year survival was highest at sites with higher minimum temperatures. We conclude that differential tree seedling establishment driven by low minimum temperatures in the valley bottoms is the primary factor maintaining montane grasslands of the VCNP, although this process probably operated historically in combination with frequent surface fire to set the position of the tree line ecotone. As at alpine tree lines, reversed tree lines bordering montane and subalpine grasslands can represent temperature-sensitive boundaries of the tree life form.

Key words: alpine tree line; conifer seedling; ecotone; frost damage; herbivory; high-montane meadow; inverted timberline; photoinhibition; *Pinus ponderosa*; Valles Caldera National Preserve, New Mexico.

INTRODUCTION

Montane and subalpine grasslands are prominent features of high-elevation valley bottoms in the southern Rocky Mountains, where they are also known as meadows, parks, and *valles* (Ramaley 1927, Turner and Paulsen 1976, Brown 1994, Peet 2000). Analogous graminoid-dominated communities occupy valley bottoms in many mountain ranges globally (Wardle 1971, Smith 1975, Moore and Williams 1976, Wearne and Morgan 2001). Where such grassy valley bottoms adjoin forested mountain slopes, they frequently form abrupt “reversed” or “inverted” tree line ecotones. Despite the widespread occurrence and biological importance of montane and subalpine grasslands, the factors that control woody establishment below such reversed tree lines are remarkably poorly understood. Such an understanding may be especially important, given recent, widespread forest encroachment in these com-

munities (e.g., Rochefort et al. 1994, Wearne and Morgan 2001, Moore and Huffman 2004, Coop and Givnish 2007a).

Hypotheses accounting for the tree-less condition of montane and subalpine valley bottoms have generally invoked cold temperatures, edaphic conditions, and/or interactive effects of herb competition in precluding tree seedling establishment from valleys but not adjacent slopes. Low minimum temperatures resulting from cold-air drainage and pooling in valley bottoms are thought to generate inverted tree lines of the subalpine zone of Australia and New Zealand (Wardle 1971, Moore and Williams 1976, Paton 1988). Frosts may damage seedlings, predisposing them to subsequent winter desiccation, as may occur at alpine tree lines (Tranquilini 1979). Low-temperature photoinhibition of photosynthesis is another factor that has been demonstrated to limit tree seedling growth near alpine tree line (Ball et al. 1991, Germino and Smith 1999, 2000, Johnson et al. 2004), and would also be expected to occur where cold air accumulates in valley bottoms. Wardle (1971) found that tree seedlings transplanted into a grassy subalpine basin in New Zealand survived only when grown beneath shade cloth, results that suggest both desiccation and/or photoinhibition.

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Edaphic explanations of grassy valley bottoms focus on the direct or indirect (via enhanced herb competition) effects of soil texture or moisture content. Fine-textured soils in valley bottoms have been proposed to impede conifer establishment by slowing root growth and limiting access to soil moisture (Daubenmire 1943, Patten 1963). Excessively wet soils (where drainage is impeded by high clay content and/or topography) could also maintain treeless meadows (Ives 1942, Gilfedder 1988). However, soil moisture shortages have been proposed to limit tree growth in a park in Wyoming, USA (Doering and Reider 1992).

Fine-textured, moist, and/or nutrient rich soils in valley bottoms have also been hypothesized to promote such dense growth by competing grasses that tree seedlings cannot become established (Ramaley 1927, Peet 1981, 2000, Fensham and Kirkpatrick 1992, Schauer et al. 1998). The detrimental effects of grass competition on tree seedling establishment are particularly well known for conifers of the western United States (e.g., Stewart et al. 1984), and it follows that edaphic conditions favoring grass growth could shift the balance of competition between grasses and tree seedlings. However, where tree establishment or growth is limited by environmental stress, the positive effects of facilitation by neighbors may outweigh the negative effects of competition (e.g., Callaway 1998). Facilitation by herbs may play an especially critical role in tree seedling establishment at alpine tree lines (Smith et al. 2003), but even at such sites, the relationship may not be simple: tree seedlings can show increased photosynthesis and growth *within* herbaceous cover (Maher et al. 2005), but decreased growth *above* grass cover due to greater cold-temperature photoinhibition relative to that above bare soil (Ball et al. 1997).

In addition to these factors, herbivory can affect tree establishment across tree lines. Grazing by cattle has been positively associated with tree invasion of subalpine meadows in the western United States (Dunwiddie 1977, Butler 1986). However, reindeer herbivory has been shown to decrease woody expansion across arctic tree lines in Sweden (Cairns and Moen 2004), and elk browsing in the Rockies is well known to negatively impact tree establishment, especially for aspen (*Populus tremuloides*) (Hessl and Graumlich 2002, Kaye et al. 2003). Pocket gophers (*Thomomys bottae*) can cause extensive aspen mortality in montane meadows in Arizona (USA), and may limit aspen to thin-soiled, rocky outcrops in some areas (Cantor and Whitham 1989).

The objective of this study is to use field experiments to assess the effects of thermal and edaphic factors, herbs, and ungulates on tree seedling establishment across reversed tree lines in the Valles Caldera National Preserve (VCNP), New Mexico, USA. Nearly 10 000 ha of grasslands occur on gentle slopes in valley bottoms at elevations of 2550–2750 m in the VCNP, forming abrupt tree lines with stands dominated by ponderosa pine

(*Pinus ponderosa* var. *scopulorum*) and Colorado blue spruce (*Picea pungens*) on adjacent mountain slopes. Valley bottoms of the VCNP are associated with both finer textured soils and lower minimum temperatures than adjacent slopes, but not with increased or decreased soil moisture (Coop and Givnish 2007b). Krantz (2001) also reported widespread elk and/or cattle damage to tree seedlings and saplings at forest–grassland margins in the VCNP. Accordingly, we test four hypothesized constraints on tree seedling establishment below reversed tree lines in the VCNP:

Hypothesis 1.—Cold-air drainage and accumulation on gently sloping topography in valley bottoms results in decreased tree seedling photosynthesis, growth, and/or survival than on adjacent mountain slopes.

Hypothesis 2.—Tree seedling growth and/or survival is reduced by fine-textured soils in valley bottoms.

Hypothesis 3.—Fine-textured soils of valley bottoms produce increased herb growth, which reduces tree seedling growth and/or survival.

Hypothesis 4.—Ungulate damage reduces tree seedling survival at and below reversed tree lines.

Here we present data that bear on the validity of these hypotheses, based on in situ experiments conducted on ponderosa pine seedlings from 2002 to 2004.

MATERIALS AND METHODS

Study location

All studies were conducted in the Valles Caldera National Preserve, VCNP (35°50'–36°00' N, 106°24'–106°37' W), a 24 km wide volcanic caldera in the Jemez Mountains of New Mexico, USA (see Appendix A for photo of study site). Forests at reversed tree lines are generally composed of ponderosa pine, Colorado blue spruce, and occasionally other conifers or aspen; grasslands are dominated by native bunchgrasses including species of oatgrass (*Danthonia* spp.), fescue (*Festuca* spp.), and muhly (*Muhlenbergia* spp.), native and nonnative rhizomatous grasses, including Kentucky bluegrass (*Poa pratensis*), and contain >100 other species of graminoids and forbs (Coop and Givnish 2007b). Rocks of mountain slopes are rhyolitic; valley floors are underlain by alluvium and lacustrine sediments (Smith et al. 1970). The climate is continental, semiarid, and strongly influenced by the North American Monsoon. Mean high and low temperatures at the nearest climate station (Wolf Canyon; 35°57' N, 106°45' W, 2506 m) are 3.4° and –13.5°C in January, and 24.9° and 5.7°C in July. Mean annual precipitation (1954–2004) is 576 mm. May and June are dry (6% and 5% of total annual precipitation, respectively), but are followed by frequent rains during July (15%) and August (15%). The VCNP forms the core range of a large elk herd and is used for cattle grazing. During the period of this study (2002–2004), total annual AUM's [an animal unit month is the amount of forage necessary to sustain a cow and calf for one month] on the VCNP were 879–2111 livestock and an estimated 5000–6250 elk (R. R.

Parmenter, VCNP, *unpublished data*). Range utilization of grasslands and forest margins was substantial, ranging between 19% and 45% (USDA-ARS Jornada Experimental Range, *unpublished data*).

Spatial and environmental stratification of experimental sites

To facilitate analysis of both the individual and combined effects of edaphic and thermal factors, experimental arrays were stratified by four kinds of sites. These included eight (a) silty valley bottoms (cold, low-lying areas with June–August daily minimum temperature $0.1^{\circ} \pm 0.8^{\circ}\text{C}$ [mean $\pm$ SD] and loam or silty loam soils, $52\% \pm 6\%$ silt [mean $\pm$ SD]); and eight (b) sandy back-slopes (warm mountain slopes with June–August daily minimum temperature $4.2^{\circ} \pm 1.8^{\circ}\text{C}$ and gravelly, sandy loam soils, $35\% \pm 9\%$ silt). These sites are representative of most of our study area (Coop and Givnish 2007b), where thermal and edaphic conditions are coupled to each other and to shifts from grassland to forest. We also located 16 sites where thermal and edaphic gradients were naturally decoupled, including eight sites on (c) silty backslopes (shallow benches on mountain slopes that are warmer than typical valley bottom locations, with June–August daily minimum temperature $1.9^{\circ} \pm 1.3^{\circ}\text{C}$ and loam or silt loam soils, $50\% \pm 7\%$ silt), and eight on (d) sandy valley bottoms (cold, low-lying valley bottoms with June–August daily minimum temperature $0.5^{\circ} \pm 0.7^{\circ}\text{C}$ and gravelly, sandy loam soils, $33\% \pm 9\%$ silt) such as occur on recent fluvial deposits. Although the latter two classes of sites are much less common in the VCNP, thermal and edaphic conditions at these locations fell well within the range of variation documented across the study area. Because we used only ponderosa pine in our experiments, we chose sites with south to west aspects near ponderosa pine tree lines, which represent $\sim 60\%$ of the reversed tree line ecotones in our study area (from data presented in Coop and Givnish [2007b]). Sites were spaced widely across the 35 560-ha VCNP.

To control for any confounding effects of forest canopy cover on the mountain slopes, all experimental sites were located where there was no forest canopy present. Our experiments were designed to examine the effects of underlying environmental differences between valley bottoms and mountain slopes (primary differences that could generate the shift from grassland to forest), and not the secondary effects that the forest canopy might subsequently produce. Ponderosa pine has long been known to be shade intolerant and to grow poorly beneath a forest canopy (e.g., Pearson 1940). However, shading could also promote growth by reducing photo-inhibition (e.g., Germino and Smith 1999). We thus utilized sites either in open valley bottoms or on forested slopes where canopy openings had been created by recent natural disturbance or historic logging. Because these stands were logged prior to 1962 (Balmat 2004), residual effects of logging operations (beyond tree

removal and increased understory growth) should be negligible. Where necessary, we enlarged openings by cutting regenerating trees so that none were within 20 m or a distance equal to the height of the adjacent forested stands.

Thermal conditions at each site were recorded hourly with single-channel data loggers (Hobo Pro Temp, Microdaq.com, Warner, New Hampshire, USA) shielded from sunlight with PVC drain pipe and placed 15 cm above ground level, beginning in June 2003. Soil texture, macronutrient concentrations (available P; exchangeable K, Ca, and Mg; total N), pH, and percentage of organic matter were assayed by the University of Wisconsin Soils and Plant Tissue Analysis Laboratory, Madison, Wisconsin, USA) for composite samples pooled for each site from cores (2.5 cm diameter $\times$ 15 cm depth) collected at three of the four corners of each experimental array. During summer 2004, we measured gravimetric soil moisture content from composite samples of soil cores collected at three random locations at the edge of each site. Soil moisture was sampled seven times at each plot during 2004 on the same days that we measured tree seedling growth and fluorescence ratios. To quantify the competitive environment, aboveground herbaceous biomass (clipped in three randomly placed, 1-m² samples at the edge of each site) and belowground root biomass (from a soil sample 100 cm² $\times$ 15 cm deep at the center of each 1-m² clip plot) were collected, dried and weighed in fall 2004 (see Appendix B for location, thermal and edaphic conditions, and herb biomass at each site).

Plot design

Experimental plots measured 4.4 m by at least 8.0 m (see Appendix C for a diagram of experimental layout). Within each plot, ponderosa pine seeds and seedlings were planted under four ungulate $\times$ herb treatments. To test for an effect of elk or livestock, each plot consisted of a 4.4 $\times$ 3.4 m portion enclosed with 2 m tall barbed-wire fencing, and an unfenced 4.4 m $\times$ 1.6 m portion. Each fenced enclosure contained a two-row grid of 10 cells (0.4 $\times$ 0.4 m) for seedlings separated by a 0.4-m mown buffer zone; a similar array was located outside the fence. Enclosures also contained two strips (0.3 $\times$ 3.6 m) for seeds; however, germination was low (1.5%) across all sites, and this sub-experiment will not be discussed at length in this paper. To test for an effect of herbs, we removed all aboveground plant matter and roots from the upper 15 cm of soil from 10 seedling cells (five fenced, five unfenced) randomly selected at each site, and sliced the edges of each cell with a spade to cut roots originating outside the treatment. Herbs were left intact in the remaining 10 seedling cells.

Planting and monitoring

Twenty of the 32 experimental plots were established and planted in June 2002 (six plots in sites of type a, six in b, three in c, and five in d). In June 2003, we enlarged

these plots to accommodate an additional set of two seeded strips and 10 seedling cells within the enclosure, and 10 seedling cells outside the enclosure. We also established and planted 12 additional plots in 2003, for a total of 32 plots, eight in each thermal $\times$ edaphic regimen (a, b, c, d).

One-year-old containerized ponderosa pine seedlings acquired from the New Mexico Forestry Division were transplanted into cells in late June 2002 and 2003. Seedlings were grown from seed collected nearby (~ 2400 – 2700 m elevation, Santa Fe National Forest, Jemez Mountains, New Mexico). Seedlings were grown at the Colorado State Forestry Service nursery (Fort Collins, Colorado, USA) from seed sown in June, raised in the greenhouse at 22°C for six months followed by a two-month, 4°C hardening cycle, and then transferred outdoors until summer planting. At the time of planting, seedlings consisted of a single stem (~ 10 – 15 cm in height) that bore fascicled needles and terminated in a single apical node. In 2002, we planted 15 seedlings (five each under the –ungulates/–herbs, –ungulates/+herbs, and +ungulates/–herbs treatments) at each of 20 sites, for a total of 300 seedlings. In 2003, we planted 20 seedlings under a fully crossed set of treatments (including a +ungulates/+herbs treatment) at each of 32 sites, for a total of 640 additional seedlings. Transplanted seedlings that died within two weeks of planting were replaced. During 2003, summer rainfall arrived later than expected, and we hand-watered recently planted seedlings for the first two weeks of July. For each seedling, survival and height were measured at two- to three-week intervals during each summer. The length of needles produced in 2003 and 2004 was measured in late September each year. To account for within-seedling variance in needle length, but avoid an inefficient measurement of every single needle, we selected three needles from separate fascicles at random from each seedling for measurement.

Photoinhibition

Photosynthetic efficiency was assessed using chlorophyll fluorescence of photosystem II (PSII). Chlorophyll molecules in the antennae complex of PSII are excited by light energy; some of this excitation energy is transmitted to the reaction center and converted to chemical energy, catalyzing the electron transport chain that generates ATP in the chloroplast. However, radiation absorbed by PSII that is not converted to chemical energy can also be lost as heat or fluoresced at a slightly longer wavelength. Environmental stressors that impede photochemistry can often be detected by changes in fluorescence, especially in the relation of minimum to maximum fluorescence yields. Minimum fluorescence is represented as F_o in the dark-adapted state and as F_t in the light-adapted or steady state. Maximum fluorescence, represented as F_m in the dark-adapted state and F'_m in the light-adapted state, occurs when all PSII reaction centers can no longer accept chlorophyll

excitation energy, and can be induced by a saturating pulse of light. The difference between the dark-adapted maximum and minimum ($F_m - F_o$, also represented as variable fluorescence, F_v), when expressed as a fraction of maximum fluorescence, i.e., F_v/F_m , is a measure of the maximum quantum yield of PSII and achieves optima near 0.83 for many plant species (Björkman and Demmig 1987). The difference between the light-adapted maximum and minimum, expressed as a fraction of the maximum, $(F'_m - F_t)/F'_m$, is a measure of the achieved quantum yield of PSII, and is represented as Φ_{PSII} . We sampled both F_v/F_m and Φ_{PSII} eight times at two- to three-week intervals between 31 May and 25 September 2004 using a portable chlorophyll fluorometer (Mini-PAM, Walz, Germany). Dark-adapted F_v/F_m was measured on needles that had been held in darkness for 15 min; light-adapted Φ_{PSII} was sampled under ambient sunlight. Both measurements were made on randomly selected needles that had elongated the previous year, for three randomly chosen seedlings of each cohort at each site. Sites were sampled within three-day periods between 09:00 and 16:00 hours.

Analyses

We used linear mixed-effects models to assess the effects of treatment factors on normally distributed response variables (seedling growth, photosynthetic stress) and generalized linear mixed-effects models for binomial response variables (seedling survival). Mixed-effects models are less sensitive to missing observations (in our case, due to seedling mortality) than ANOVA because variance is modeled using maximum likelihood instead of sum-of-squares (Pinheiro and Bates 2000). We also utilized mixed-effects models because our experimental design included clustering of observations (individual seedlings grouped at experimental sites, repeated fluorescence measurements on individuals); in mixed-effects models, the covariance imposed by such clustering can be parameterized as a random effect. We included site location as a random effect in all models, and seedling identity as a random effect in models of fluorescence ratios. Ungulate exposure and herb cover (two-level categorical variables) and edaphic and thermal factors (continuous variables) were modeled as fixed effects.

Treatment effects on each cohort of seeds and seedlings were tested separately. Variables measured as a proportion were arcsine square-root transformed to increase normality. To facilitate comparisons among their effects, all predictor variables were rescaled between 0 and 1. To avoid model overparameterization, we limited the number of explanatory variables in each model, using measurements of two edaphic factors (sand and mean June–August soil moisture), one thermal factor (mean June–August daily minimum temperature), herbs, ungulates, and first-order interaction terms. Model selection was conducted using S-PLUS 6.0 (Insightful Corporation 2001) as follows. First, we used

TABLE 1. Generalized linear mixed-effects model for survival of transplanted seedlings after year 1 (cohort 1, 2002–2003; cohort 2, 2003–2004) and year 2 (cohort 1, 2003–2004).

Parameter	Cohort 1, 2002–2003		Cohort 1, 2003–2004		Cohort 2, 2003–2004	
	Coefficient	P	Coefficient	P	Coefficient	P
Intercept	−4.56	0.0122	0.59	NS	−3.89	0.0021
Herbs	−2.36	0.0138				
Sand	8.51	0.0355			5.93	0.0272
Mean minimum temperature			0.58	0.0345		
Herbs × sand	4.62	0.0181				

Notes: For all tables showing results of mixed-effects models, herb cover was included as a binomial variable, 0 (absent) or 1 (present), while other experimental factors were rescaled as continuous variables between 0 and 1; coefficients (partial regression slopes) are thus directly comparable. NS, nonsignificant ($P > 0.05$).

a stepwise procedure to determine the best linear or generalized linear model not including random effects. We next added one or both random effects and tested this model using the “lme” command for linear mixed-effects models or the “glmmPQL” function for generalized linear mixed-effects models. Nonsignificant terms were then eliminated as needed to minimize Akaike’s Information Criterion (AIC).

We also tested for relationships between edaphic and thermal factors and the cover and biomass of herbaceous competitors; between seedling growth in 2003 and growth and survival in 2004; and between mean fluorescence ratios and growth rates. Because these relationships were tested at the site level, it was not necessary to include random factors, and we used stepwise linear and logistic regressions as implemented in S-PLUS.

RESULTS

Environmental conditions at experimental sites

Across experimental sites, mean 2003 growing-season (1 June–1 September) temperatures ranged between 11.8° and 16.5°C, mean nightly minimum temperatures ranged from −0.8° to 6.3°C, and mean daily maximum temperatures ranged between 25.1° and 36.0°C. The maximum number of consecutive days between temperatures that fell below 0°C was 15 days at the coldest sites and 113 at the warmest. Sand ranged from 29% to 73% of soil composition; mean soil moisture ranged between 6% and 30%. (Summary data for these and other measured variables at each experimental site are presented in Appendix B.) Herb belowground biomass (g/m^3) was strongly negatively related to percentage of sand:

$$\text{biomass} = 10\,462 \sin^{-1} \sqrt{(\text{sand})} - 16\,433$$

($r^2 = 0.41$, $\text{df} = 30$, $P < 0.0001$) but was not significantly related to soil moisture or thermal variables. However, aboveground herb biomass was unrelated to soil texture, but increased with percentage of soil moisture and daily maximum temperature [$\text{biomass} (\text{g}/\text{m}^2) = 183.7 \ln(\% \text{ soil moisture}) + 19.8 \times \text{mean } T_{\text{max}} - 776.1$; $r^2 = 0.43$, $\text{df} = 29$, $P = 0.0003$]. Herb cover increased with percentage soil moisture [$\text{percent cover} = 35.2 \ln(\% \text{ soil moisture}) -$

19.5 ; $r^2 = 0.52$, $\text{df} = 30$, $P < 0.0001$], but was unrelated to soil texture.

Seedling survival

Survival of transplanted seedlings at the end of the experiment in September 2004 was 28.7% for seedlings planted in 2002 (cohort 1) and 30.2% for seedlings planted in 2003 (cohort 2). For both cohorts, seedling survival during the first year was higher on sandy soils and lower on fine-textured soils (Table 1), but showed no relationship to thermal factors. During the second year, cohort 1 survival was not related to soil texture or herb cover, but showed a significant decrease with lower minimum temperatures (Table 1). Cohort 1 survival in 2003 was also lower where herbs were present, although the effect was only about half as strong as that for percentage sand (Table 1). We also found a significant positive effect of herbs × sand for cohort 1 survival in 2003, as expected if competition is more intense on fine-textured soils.

We attributed seedling mortality to three causes. The first of these, physiological stress (seedlings exhibited poor growth, a gradual browning and dropping of needles, and no apparent physical damage by insects or mammal herbivores), killed 37.7% of transplanted seedlings. The second source of mortality, rodent activity, was due primarily to aboveground damage by golden-mantled ground squirrels (*Spermophilus lateralis*), although we also noted cases in which seedlings had been uprooted, tunneled beneath, or buried by pocket gophers (*Thomomys* spp.). Rodents killed 29.7% of transplanted seedlings. Lastly, 2.9% of our seedlings died from other causes, including trampling by ungulates, defoliation by grasshoppers, and factors unknown. Total mortality caused by rodents or physiological stress was roughly equal across silty and sandy soils (Fig. 1). On cold sites, however, mortality was primarily caused by stress, whereas on warm sites it was caused mainly by rodents (Fig. 1).

Seedling growth and photoinhibition

Ponderosa pine seedling growth increased strongly with minimum temperature during the growing season. Height growth increased with mean minimum temper-

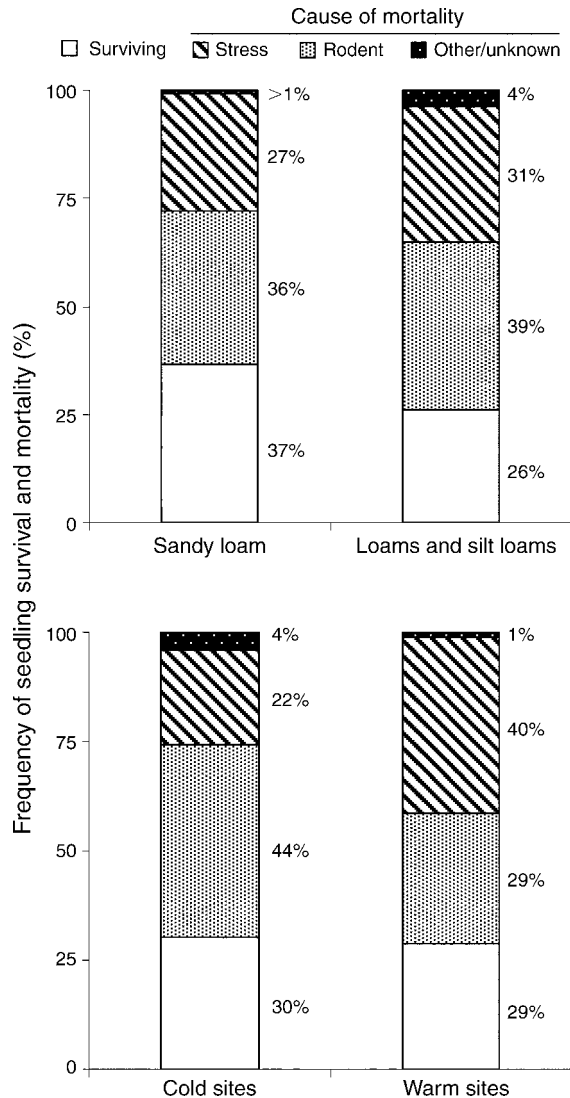


FIG. 1. Fates of transplanted ponderosa pine seedlings grouped by site-level factors. Mortality resulting from stress was distinguished by poor growth, a gradual browning out and loss of needles, and a lack of apparent animal damage. Mortality was attributed to rodents when seedlings were clearly clipped at the base, uprooted, tunneled beneath, or buried.

ature in both seedling cohorts (Table 2a, Fig. 2). The relationship of needle growth to minimum temperature was also strong (Table 2b, Fig. 2). Seedlings experiencing the lowest minimum temperatures in the valley bottoms generally exhibited stunted, reddish needles, and very little (0–5 mm) height growth even after two years (see Appendix D for photos of experimental seedlings). Needle growth in 2003 was a strong predictor of height growth in 2004 [height growth (cm) = $0.53 \times$ needle growth (cm) + 1.09; $r^2 = 0.52$, $df = 87$, $P < 0.0001$] and subsequent mortality (probability of survival = $0.04 \times$ needle growth – 0.44; $df = 116$, $P < 0.0001$), highlighting the cumulative effects of poor needle growth on later growth and survival. Herbs had no effect in most models, and only relatively minor negative effects where they were significant (Table 2a, b); no other experimental factor significantly affected growth.

Seedling growth was strongly related to the fluorescence ratios Φ_{PSII} and F_v/F_m . Both height and needle growth for cohort 1 increased with Φ_{PSII} [height growth (cm) = $13.52 \times \Phi_{PSII} - 3.80$, $r^2 = 0.23$; needle growth (cm) = $16.44 \times \Phi_{PSII} - 2.34$, $r^2 = 0.24$; $df = 89$, $P < 0.0001$ for both cases; see Fig. 3] and with F_v/F_m (height growth = $22.15 \times F_v/F_m - 13.7$, $r^2 = 0.20$, $P < 0.0001$; needle growth = $13.53 \times F_v/F_m - 7.44$, $r^2 = 0.09$, $P < 0.01$). Height and needle growth for cohort 2 also increased with Φ_{PSII} (height growth = $4.51 \times \Phi_{PSII} - 0.13$, $r^2 = 0.12$; needle growth = $11.82 \times \Phi_{PSII} - 1.31$, $r^2 = 0.25$, $df = 194$, $P < 0.0001$ in both cases; see Fig. 3) and with F_v/F_m (height growth = $2.17 \times F_v/F_m + 0.20$, $r^2 = 0.03$, $df = 188$, $P = 0.01$; needle growth = $6.05 \times F_v/F_m - 0.7$, $r^2 = 0.08$, $df = 188$, $P = 0.0001$).

These relationships indicate photoinhibition, but caused by several environmental factors (Table 3), including the minimum temperature recorded within five days preceding fluorescence measurement, percent-age sand, soil moisture, and seedling age. Neighboring herbs generally had a negative effect, or no effect, on photosynthetic performance (Table 3, Fig. 4). Fluorescence ratios showed large variation not only across sites but also across the growing season (Fig. 4). The lowest values (indicative of substantial photoinhibition) at all sites occurred when clear, cold nights combined with the

TABLE 2. Mixed-effects model for (a) height growth and (b) needle growth of transplanted seedlings after one year (cohort 1, 2003; cohort 2, 2004) and two years (cohort 2, 2004).

Parameter	Cohort 1, 2003		Cohort 1, 2004		Cohort 2, 2004	
	Coefficient	P	Coefficient	P	Coefficient	P
a) Height growth						
Intercept	10.68	<0.0001	0.57	NS	14.33	<0.0001
Herbs			–5.15	0.0156		
Mean minimum temperature	25.31	<0.0001	70.18	<0.0001	5.61	0.0365
b) Needle length						
Intercept	14.59	0.0053	25.39	0.0003	25.82	<0.0001
Herbs					–2.82	0.0176
Mean minimum temperature	72.52	<0.0001	81.80	<0.0001	25.27	0.0015

Note: NS, nonsignificant ($P > 0.05$).

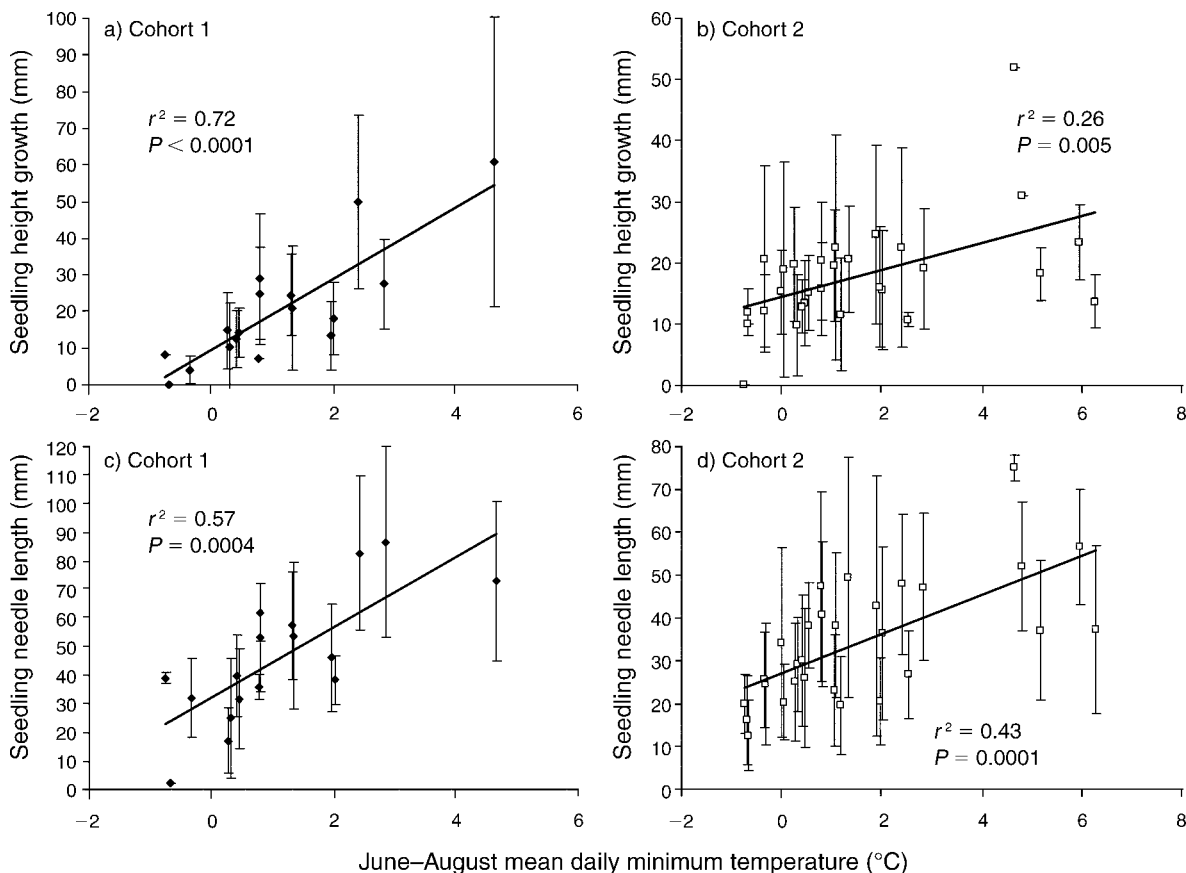


FIG. 2. (a, b) Height and (c, d) needle growth (mean $\pm$ SD) for transplanted seedlings at each site plotted against the mean daily minimum temperature, June–August 2004.

lowest soil moisture prior to the onset of the summer monsoon near the end of June; dark-adapted F_v/F_m averaged 0.62 ± 0.20 (mean $\pm$ SD) and light-adapted Φ_{PSII} averaged 0.29 ± 0.18 . The highest values occurred during the rainy season from mid-July through the end of August, also a period of warmer minimum temperatures. Across sites at the end of August, F_v/F_m averaged 0.77 ± 0.04 and Φ_{PSII} averaged 0.44 ± 0.19 .

DISCUSSION

We found the strongest support for our first hypothesis: low minimum temperature, associated with nocturnal cold-air accumulation, limits tree seedling establishment below reversed tree lines in the VCNP (Figs. 2 and 3, Table 2). At sites experiencing lower nocturnal temperatures in the valley bottoms, seedlings exhibited greatly decreased height and needle growth. Although we found evidence for low-temperature photoinhibition in the relationships of fluorescence ratios to minimum temperature (Table 3), photoinhibition does not appear to wholly account for the relationship of growth to temperature, given the strong ties of fluorescence ratios to percentage sand and soil moisture, which were themselves not strong predictors

of seedling growth. These findings suggest that growth was limited directly by freezing damage, beyond the energetic costs imposed by photoinhibition. Growing conifer needles may be particularly susceptible to frost damage; Schubert and Adams (1971) reported that temperatures lower than -3°C injured unhardened ponderosa pine needles, and temperatures during the growing season dropped below this threshold in the valley bottoms of the VCNP. Needles at experimental sites experiencing low temperatures were stunted and often appeared physically damaged (for photos, see Appendices). The compounding effects of decreased needle growth should limit subsequent energy capture and plant growth, and should increase vulnerability to other sources of mortality, and we found that needle growth was the single best predictor of both height growth and seedling survival in the following year. One likely consequence of damaged needles is increased winter desiccation; Tranquillini (1987) found that Norway spruce (*Picea abies*) needles required at least 50 consecutive days without frosts of -3°C to avoid damage during growth, but at least 90 days above -3°C to develop needle cuticles adequate to resist winter desiccation. The strong effects of frequent frosts in

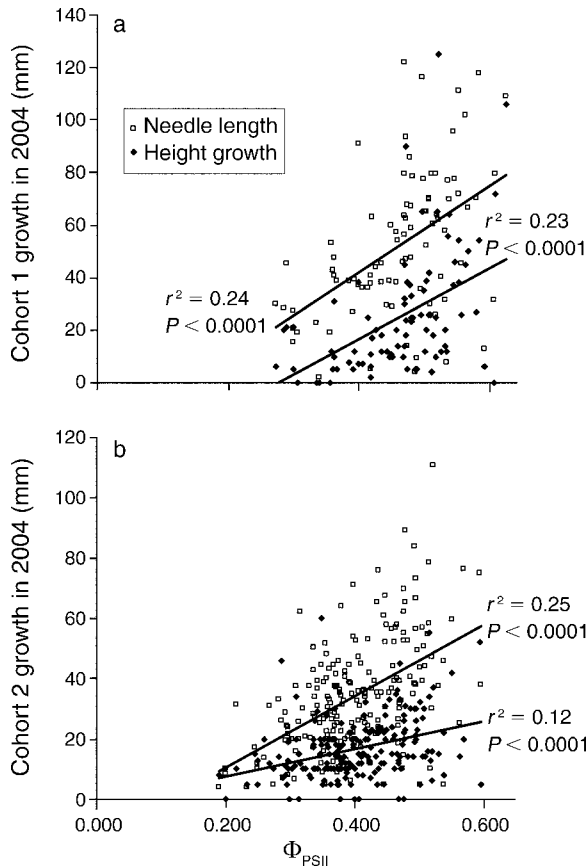


FIG. 3. Seedling growth and the light-adapted quantum yield of photosystem II [Φ_{PSII} or $(F'_m - F_o)/F'_m$] for: (a) seedling cohort 1 (3-year-old seedlings) and (b) cohort 2 (2-year-old seedlings). See Table 3 for the definitions of terms.

valley bottoms that we observed on the growth of 1–3 year-old seedlings are likely to be equally or even more detrimental to seedlings between 0 and 1 years old, and could preclude any conifer establishment at the coldest sites.

We found less support for our hypotheses that edaphic factors and herb competition impede seedling growth or survival. Percentage sand was a significant predictor of first-year transplanted seedling mortality, although the magnitude of this effect was substantially less than that of temperature on seedling growth (see Tables 1 and 2, Fig. 2). Both percentage sand and soil moisture were positively associated with photosynthetic performance (Table 3), suggesting physiological stress imposed by low soil moisture potentials; however, these factors were not related to seedling growth. Although we found support for edaphic controls on herbaceous plant cover and biomass, we found less support for any subsequent effect on ponderosa pine establishment (i.e., the herb $\times$ sand interaction term, significant in only one model), beyond a minor negative effect of competition occurring across all sites (the herb term alone, significant in several models of survival, growth, and photosynthetic performance; Tables 1–3). Taken together, these findings suggest some physiological stress imposed by low moisture potentials on fine-textured soil, possibly exacerbated by grass competition. Although these conditions exerted only minor effects on our transplanted seedlings, we cannot exclude the possibility that they decrease germination and/or seedling survival soon after germination.

The slightly negative or insignificant effects of herbs that we found contrast with positive effects of herb cover on tree seedling establishment reported at an alpine tree line in Wyoming, USA (Germino et al. 2002, Maher et al. 2005; but see Moir et al. 1999), where protective shading offered by neighboring herbs increased tree seedling photosynthetic performance (Maher et al. 2005). One explanation for these divergent findings may be the substantial differences between the two study sites in the availability of water. Maher et al. (2005) found that supplemental watering did not increase photosynthetic performance of seedlings; in our experiments, soil moisture was clearly linked to photosyn-

TABLE 3. Mixed-effects models of predictors of (a) light-adapted and (b) dark-adapted chlorophyll fluorescence ratios and their components, minimum and maximum fluorescence, for seedling ponderosa pine needles during the summer of 2004.

Parameter	a) Light-adapted chlorophyll fluorescence						b) Dark-adapted chlorophyll fluorescence ratios	
	Φ_{PSII}		F_t		F'_m		F_v/F_m	
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
Intercept	-0.002	NS	152.23	<0.0001	53.66	NS	0.510	<0.0001
Herbs	-0.018	0.0001	-4.21	0.0415	-23.14	0.0014		
Sand	0.318	<0.0001	70.71	0.0231	259.65	0.0164	0.257	0.0055
Soil moisture	0.334	<0.0001	45.01	0.0003	284.91	<0.0001	0.061	0.0318
5-day minimum temperature	0.160	<0.0001	25.66	0.0079	205.20	<0.0001	0.066	0.0018
Seedling age	0.147	<0.0001	48.38	<0.0001	155.13	<0.0001	0.052	0.0002
Seedling age $\times$ soil moisture	-0.173	0.0001	-91.27	<0.0001	-228.23	0.0010		

Notes: Terms are F_m , maximum fluorescence in the dark-adapted state; F'_m , maximum fluorescence in the light-adapted state; F_o , minimum fluorescence in the dark-adapted state; F_t , minimum fluorescence in the light-adapted or steady state; F_v , variable fluorescence (difference between the dark-adapted maximum and minimum, $F_m - F_o$); Φ_{PSII} , achieved quantum yield of photosystem II, which is the difference between the light-adapted maximum and minimum fluorescence (expressed as a fraction of the maximum, $(F'_m - F_o)/F'_m$); F_v/F_m , maximum quantum yield of PSII. NS, nonsignificant ($P > 0.05$).

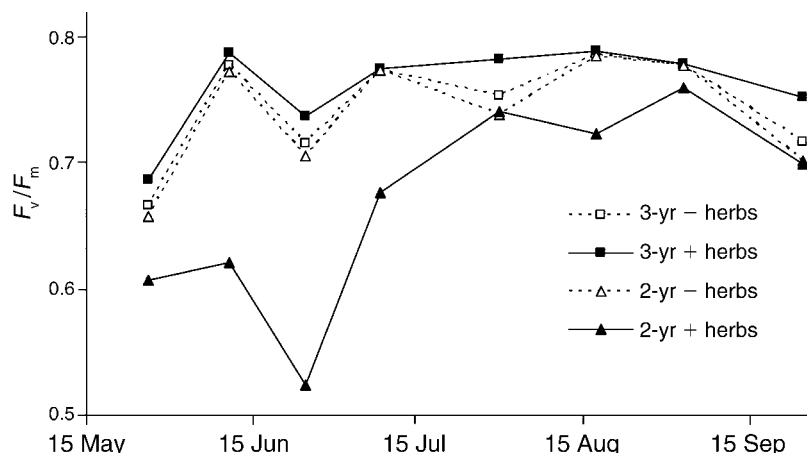


FIG. 4. Seasonal progression of mean values of the dark-adapted fluorescence ratio F_v/F_m across all experimental sites, for both cohorts of seedlings (2- and 3-year-olds) under treatments in which neighboring herbs had been left intact (“+ herbs”) or removed (“– herbs”) from the 0.16-m² cell into which seedlings were planted.

thetic performance (Table 3). The negative effects of neighboring herbs on photosynthesis were strongest for smaller, younger seedlings during the May–June dry season (Fig. 4); competition for limited soil moisture may outweigh any positive effect of shading.

We found no effect of our fencing treatment, despite clear evidence of grazing by both elk and cattle surrounding our experimental sites (J. D. Coop, *personal observation*). However, we found substantial seedling mortality resulting from rodent activity (Fig. 1). Overall, the source of mortality shifted from physiological damage at cold, low-lying sites to rodent damage at warmer sites on slopes (Fig. 1). Golden-mantled ground squirrels, responsible for much of this mortality, are most common in open ponderosa pine forests (McKeever 1964), especially in openings formed by logging (Tevis 1956), such as the clearings in which we located many of our experimental sites. Pocket gophers, which favor deeper, finer textured soils, were also responsible for seedling damage in our study, although subsequent

reductions in survival did not approach the extent reported by Cantor and Whitham (1989) for aspen shoots in a meadow in Arizona. However, rodent-imposed mortality decreased as seedlings continued to grow rapidly on slopes above the nocturnal thermal inversion in the valleys, and second-year seedling survival in our study was greater on warmer sites (Table 1).

Seedling growth in our study was more strongly related to the light-adapted fluorescence ratio Φ_{PSII} than to dark-adapted F_v/F_m , even though the latter is more commonly used as a measure of photoinhibition (Ball et al. 1995). However, both ratios and their components generally showed similar relationships to environmental variables (Table 3). Reductions in both Φ_{PSII} and F_v/F_m were generally accompanied by decreases in minimum fluorescence levels (F_t and F_o), indicating regulated, reversible photoprotective quenching mechanisms (Demmig and Björkman 1987). Similar to our findings, photoinhibition of snow gum (*Eucalyptus pauciflora*) seedlings near alpine tree line in Australia was associated with decreased F_o (Ball et al. 1991), and decreases in F_v/F_m following frosts and exposure to high light were associated with reversible, non-photochemical quenching for conifer seedlings near an alpine tree line in Wyoming (Germino and Smith 2000). In ponderosa pine, winter reductions in photosynthetic capacity are correlated with an increase in energy dissipation by xanthophyll cycle pigments (Verhoeven et al. 1999), which may be the same mechanism employed to dissipate excess light energy by seedlings experiencing environmental stress, associated with cold nighttime temperatures, low soil moisture, fine soil texture, and herbaceous vegetation (Fig. 4, Table 3) during summer months in our study.

As with alpine tree lines, the tree line ecotones that we examined appear to represent a temperature-sensitive boundary of the tree life form. In contrast with alpine tree lines, which are generally accepted to be caused by

TABLE 3. Extended.

b) Dark-adapted chlorophyll fluorescence ratios			
F_o		F_m	
Coefficient	<i>P</i>	Coefficient	<i>P</i>
405.32	<0.0001	699.96	0.0001
		931.95	0.0088
–108.37	<0.0001	675.56	<0.0001
59.39	0.0079	57.24	0.0019

lower *mean* growing-season temperatures with increasing elevation (e.g., Körner and Paulsen 2004), *minimum* growing-season temperatures constrain tree establishment in broad valley bottoms of the VCNP. Given the relationship between minimum temperatures and reversed tree lines in the VCNP, these tree lines should be highly sensitive to global climate change, and pulses of ponderosa pine invasion into grasslands during the last century appear to correspond with periods of warmer summer minimum temperatures (Coop and Givnish 2007a). However, thermal constraints on seedling growth and survival probably would have worked in combination with frequent surface fire prior to the collapse of the historic fire regime at the end of the 19th century. Reduced seedling growth in frost-prone sites would have increased the number of years that seedlings were vulnerable to low-intensity fire, possibly well beyond the 5–20 year return interval estimated from fire-scarred ponderosa pine trees bordering VCNP grasslands (Morino et al. 1998; C. D. Allen, *unpublished data*). Hence, poor seedling growth where frost was frequent, combined with a subsequently increased probability of mortality from surface fire, may have combined to maintain reversed tree lines at historic locations. Recent forest encroachment below the historic tree line may thus be attributable to both fire suppression and warmer temperatures (Allen 1984, Coop and Givnish 2007a).

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APPENDIX A

Reversed tree lines on Cerro la Jara, a small volcanic dome in the Valle Grande (*Ecological Archives* E089-066-A1).

APPENDIX B

Table characterizing each experimental site location, including the site type, year planted, soil texture, nutrient concentrations, and moisture, thermal conditions, and standing herbaceous biomass (*Ecological Archives* E089-066-A2).

APPENDIX C

Experimental site layout and a matrix of treatment combinations (*Ecological Archives* E089-066-A3).

APPENDIX D

Typical seedling height and needle growth for two-year-old seedlings at a cold site (LSAM2, mean summer minimum temperature -0.67°C) and a warm site (LSAF, mean summer minimum temperature 2.14°C) (*Ecological Archives* E089-066-A4).