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Research

Reproduction as a bottleneck to treeline advance across the circumarctic forest tundra ecotone

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The fundamental niche of many species is shifting with climate change, especially in sub-arctic ecosystems with pronounced recent warming. Ongoing warming in sub-arctic regions should lessen environmental constraints on tree growth and reproduction, leading to increased success of trees colonising tundra. Nevertheless, variable responses of treeline ecotones have been documented in association with warming temperatures. One explanation for time lags between increasingly favourable environmental conditions and treeline ecotone movement is reproductive limitations caused by low seed availability. Our objective was to assess the reproductive constraints of the dominant tree species at the treeline ecotone in the circumpolar north. We sampled reproductive structures of trees (cones and catkins) and stand attributes across circumarctic treeline ecotones. We used generalized linear mixed models to estimate the sensitivity of seed production and the availability of viable seed to regional climate, stand structure, and species-specific characteristics. Both seed production and viability of available seed were strongly driven by specific, sequential seasonal climatic conditions, but in different ways. Seed production was greatest when growing seasons with more growing degree days coincided with years with high precipitation. Two consecutive years with more growing degree days and low precipitation resulted in low seed production. Seasonal climate effects on the viability of available seed depended on the physical characteristics of the reproductive structures. Large-coned and -seeded species take more time to develop mature embryos and were therefore more sensitive to increases in growing degree days in the year of flowering and embryo development. Our findings suggest that both moisture stress and abbreviated growing seasons can have a notable negative influence on the production and viability of available seed at treeline. Our synthesis revealed that constraints on predispersal reproduction within the treeline ecotone might create a considerable time lag for range expansion of tree populations into tundra ecosystems.

Keywords: biotic interactions, climate change, range expansion, seed production, seed viability, sexual reproduction, species distribution, sub-arctic



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Introduction

Contemporary climate change has been more intense in sub-arctic ecosystems than at lower latitudes (IPCC 2014), leading to the expectation of pronounced shifts in species ranges. The sub-arctic is defined by transitions from southerly to northerly ecosystems, predominantly by the shift from forest cover to tundra (Hofgaard et al. 2012). If tree species track their fundamental climatic niche (Burrows et al. 2011), forest ecosystems should shift northwards in latitude and upwards in alpine systems. However, studies that have tested this prediction against observations have revealed that species' responses to climatic change have been variable and unpredictable (Harsch et al. 2009), suggesting that the processes controlling tree range expansion have yet to be definitively identified and may be site specific. For trees to advance beyond their current range, viable propagules must be available and disperse to microsites suitable for germination and establishment; seedlings must then overcome the ecological inertia of the ecosystem they are invading by outcompeting intact vegetation (Westman 1978) and escaping herbivory (Cairns and Moen 2004, Brown and Vellend 2014). There is a fundamental gap in our understanding of the role these ecological characteristics play in the expansion of tree species' distributions into their climatic niches under current global change.

Recent observations in the northern forest-tundra ecotone, hereafter referred to as treeline ecotone, have provided evidence of range expansion, stand infilling, or no response concomitant with warming temperatures across the circumboreal region (Harsch et al. 2009). Conversely, recession of treelines has been associated with disturbance when there are insufficient propagules available for post-disturbance colonisation (Sirois and Payette 1991, Brown and Johnstone 2012). Reproductive limitations caused by low production or poor dispersal of seeds is one of the leading explanations for delays in range shifts in response to climate warming (Svenning and Skov 2007). Whereas populations may be maintained via vegetative reproduction such as layering and resprouting (e.g. *Betula*, *Picea*, and *Larix* spp., Zasada et al. 1992), expansion of boreal tree species distributions to track current warming requires sexual reproduction and seed dispersal (Malcolm et al. 2002). Production of viable seed has high inter-annual variability and is influenced by both biotic and abiotic factors (Zasada et al. 1992). We cannot predict treeline ecotone movement via tree recruitment in tundra habitats without an understanding of factors limiting sexual reproduction in these leading edge populations.

Changes in plant distribution limits are expected to be sensitive to climate variability because of the abiotic factors that affect reproduction (Holtmeier and Broll 2005, Bykova et al. 2012). Temperature directly contributes to annual fluctuations in viable seed production of northern tree species through its influence on the initiation

and development of each step in the reproductive cycle (cone initiation, pollination, fertilization, and embryo maturation; Houle and Fillion 1993, Krebs et al. 2012). The position of the treeline ecotone has been explicitly linked to temperature limitations (Timoney et al. 1992, Sveinbjörnsson et al. 2002, Körner 2012), and we expect that climate constraints should be apparent across ecotonal boundaries in most years, regardless of annual fluctuations in productivity. A greater accumulation of growing degree days (GDD) enables an individual tree to extract more resources and accumulate more biomass than it would otherwise, increasing the likelihood that it will allocate resources to reproduction (Krebs et al. 2012). Cooler years may limit pollination and seed viability at the treeline ecotone (Hofgaard 1993, Roland et al. 2014). Seasonal weather events can also affect propagule development; e.g. through frost damage to pollen cones (Elliott 1979); likewise precipitation during pollen release can significantly reduce pollen dispersal distance (Eis 1973, Houle and Fillion 1993).

Although there is much evidence to support a link between discrete climatic variables and reproduction, we lack an understanding of the generalizability of those relationships amongst species and across space, and of how different components of weather interact to drive tree reproduction. Furthermore, biotic interactions provide a critical context that may modify the impacts of abiotic factors on reproduction and range expansion in the treeline ecotone (McIntire et al. 2016). Variations in vegetation structure such as stand density may have positive or negative effects on resource accumulation and reproductive allocation, due to competitive effects, pollination mutualisms, or alteration of the local microclimate conditions for individuals (HilleRisLambers et al. 2013, McIntire et al. 2016). Reproductive data to test the effects of these interactions are particularly scant at the latitudinal and altitudinal limit of tree species distributions, where we predict forests will advance in response to warming climatic conditions.

Here, we synthesized data from 13 sub-arctic regions to estimate constraints on the reproductive potential of dominant tree species within the treeline ecotone. To increase our understanding of how species' ranges may respond to shifting climatic envelopes, we related seed production and viability of available seed to regional climate, stand structure, and species-specific characteristics. We predicted that seed production and viability would decrease with stand density across the treeline ecotone, and that both of these would be correlated with seasonal climatic metrics. Our data are a snapshot in time of reproduction across the forest-tundra ecotone, yet they are critical data from understudied regions. Studies of functional mechanisms that link reproductive potential to environmental conditions are rare and urgently needed to help explain and predict variability in species range expansions.

Materials and methods

Study areas

We sampled reproductive structures (cones or catkins) and measured stand density in 13 regions around the terrestrial circumpolar biome (Fig. 1; Supplementary material Appendix 1 Table A1). Study regions were located in Canada (Tuktoyaktuk, Eagle Plains, Mackenzie Mountains, Churchill, Boniface River, Kangiqsualujuaq, Mealy Mountains), Norway (Porsanger, Nordreisa, Alta, and Karasjok), Sweden (Abisko), and Russia (Kola Peninsula).

Field measurements

All sampling was nested in each geographical region; plot-level data on stand density and reproduction were sampled in

stands within each geographic region (plot < stand < region). Our objective, where possible, was to capture changes in stand density across the treeline ecotone (note: stands were not measured across the ecotone in all geographical regions; Supplementary material Appendix 1 Table A2). We used fixed-area or point-centered quarter plots (Supplementary material Appendix 1 Table A1) to determine the density of trees in each stand and of the dominant species within that stand. Stands at Tuktoyaktuk and Mackenzie Mountains were composed of tree islands (isolated clusters of ramets), thus a measure of ‘stand’ density was not appropriate; these plots were assigned a density of 0.1 trees ha⁻¹ to quantify their isolation in our analyses.

Reproductive structures were sampled from a subset of individuals of the dominant species within each stand following our standard protocol (Hofgaard and Rees 2008). The sampled species were *Abies balsamea*, *A. lasiocarpa*, *Betula*



Figure 1. Study sites were located in Canada (west to east: Eagle Plains, Yukon; Mackenzie Mountains and Tuktoyaktuk, Northwest Territories; Churchill, Manitoba; Boniface River and Kangiqsualujuaq, Québec; and Mealy Mountains, Newfoundland and Labrador), Sweden (Abisko), Norway (Nordreisa, Alta, Karasjok, and Porsanger), and Russia (Kola Peninsula).

pubescens subsp. *tortuosa* (herein referred to as *B. pubescens*), *Larix laricina*, *Picea glauca*, *P. mariana*, and *Pinus sylvestris* (Supplementary material Appendix 1 Table A2). In each plot, 10 cones or catkins were collected from 10 individuals, where possible, for a total of 100 cones or catkins (except for Québec regions where five cones were collected from each of 50 individuals, see Dufour-Tremblay and Boudreau 2011, Dufour-Tremblay et al. 2012). The cones or catkins were collected from at least five branches of each individual. The number of reproductive structures per tree was visually estimated as either a single value or assigned to a density class (Supplementary material Appendix 1 Table A2). Sampling was conducted during at least one growing season in the period between 2006 and 2011 (see Supplementary material Appendix 1 for region-specific sampling periods). Collection of reproductive structures was synchronous with natural seed maturation.

Laboratory methods

Following collection, seeds were stored in cool, dry conditions to preserve their viability. Prior to germination trials, we counted the number of seeds per reproductive structure. For most species, seeds passively dehisced from the parent material or were extracted from the woody cones and catkins through agitation. However, *P. mariana* has semi-serotinous cones that require the simulation of fire for seed extraction, thus cones were treated to a regime of soaking-drying-heating prior to agitation (following Leadem et al. 1997, Sirois 2000).

Viability of seeds from each sampled tree (or pooled by stand for Norway and Sweden) were tested in a single 100 × 15 mm Petri dish, except where samples had more than 100 seeds (50 for Mealy Mountains samples) and were divided into multiple dishes. Seeds were allowed to germinate for 28 d at 18 hours of light per day at room temperature (~20°C; Eagle Plains); within growth chambers with 16 hours of light per day at 25/18°C (day/night; Churchill and Mackenzie Mountains) or 25/15°C (Québec regions and Mealy Mountains); within a greenhouse with constant light (see Walker et al. 2012 for full methods in the Tuktoyaktuk region); or at 20 hours of light per day at 20/15°C (Norway and Sweden regions). Seeds were watered with deionised water as needed. Seeds were considered germinated once their radicle grew to twice the length of the seed coat (Leadem et al. 1997). The total number of seeds, number of germinated seeds, and proportion of viable seed were calculated for each sample. We considered the number of seeds that germinated as the minimum number of viable seeds present in the sample. We assumed that seeds that did not germinate would also be unavailable for recruitment under field conditions due to dormancy or an underdeveloped or absent embryo.

Statistical analyses

Model variables

Two measures of reproductive potential were modelled: 1) number of seeds produced per cone/catkin (herein ‘seed

production’) and 2) viability of available seed, where data were available (Supplementary material Appendix 1 Table A2). We emphasize that we modelled the viability of the seed available for sampling, not the viability of all seed produced, as viable seed produced by a cone can be lost to pre-dispersal seed predation and thus not captured during our collection. Number of seeds per catkin was not counted, though we estimated number of catkins per tree. In each case, we calculated means of the measured variables by region and year.

For all regions, we calculated species-specific climate variables relevant to plant reproduction (see ‘Modelling procedure’, below, for variables). We obtained reconstructed climate data (CRU TS 3.21: Univ. of East Anglia Climatic Research Unit 2013) for study regions in Canada and Russia to explore relationships between stand density, reproductive output, and regional climate. The reconstructed climate data were not suitable for the five regions in Norway and Sweden due to topographic and maritime influences on their microclimate, thus data from the nearest climate station were used. Growing degree days (GDD) were calculated from monthly mean air temperatures as the sum of degrees per daily mean temperature over 5°C, representing the biologically active period for most sub-arctic vegetation (Sirois 2000). Climate variables were calculated for the current year of sampling (t) as well as for past climatic conditions (t_{-x}), to account for the multi-year process of seed production and maturation. Past climate calculations included one year prior to sampling (t_{-1}) for all species and two years (t_{-2}) for *Pinus sylvestris* due to its longer developmental time (Zasada et al. 1992).

Additional model covariates included conspecific stand density and cone length. We used conspecific stand density rather than total stand density for two reasons: 1) we assumed that conspecific density gives a better measure of site suitability and intraspecific competition for that particular species (Brown et al. 1995); and 2) seed viability will be partially controlled by the density of conspecifics for cross-pollination. We included average cone length of each species (Burns and Honkala 1990) as a covariate in our models as a surrogate for the potential number of embryos produced by a cone, allowing us to account for higher potential seed production in species with larger cones. In all models, all covariates were scaled to z-scores to improve model fit and allow for a comparison of parameter effect size within each model.

Modelling procedure

Our approach to modelling seed production and viability of available seed followed a process of sequential and cumulative variable selection inspired by modelling methods of Roland et al. (2014) and informed by Zasada et al. (1992) and Juday et al. (2003) as follows. We expected seasonal and inter-annual climate variables to be highly interrelated. To reduce collinearity while maintaining a high resolution of seasonal climate information, we assessed groups of seasonally related climate variables and excluded variables with VIF > 10 and correlations with other covariates of $r > 0.75$ (thresholds follow Roland et al. 2013). Seasonal variables were grouped and added to the collinearity analysis following

the order of variable addition presented in Roland et al. (2014), reflecting their occurrence throughout the cycle of bud initiation, seed development, fertilization, and embryo maturation.

We first considered climate variables related to the year of cone initiation, prior to the year of flowering (annual growing degree days (GDD) t_{-x} , May-June (herein spring) GDD t_{-x}). After determining the least related variables (i.e., lowest correlation coefficients and VIFs), we added variables for the winter and precipitation sums for the seasons between cone initiation and flowering. Next, we added annual GDD, spring GDD, minimum spring temperature (as a proxy for spring harshness), and spring and summer precipitation sums for the current (flowering and embryo development) year. In the fourth and final step, we added our stand and species characteristics: conspecific stand density and cone length. At each step, all covariates under consideration were assessed for collinearity; thus, a variable from the previous step could be eliminated if it was correlated to a new variable being added to the group.

Once we determined the final covariates (separately for each of the two models), we applied generalized linear mixed models to model count data of seed production using a Poisson distribution and success/failure data of viability of available seed from laboratory germination trials using a binomial distribution. In both models, we accounted for the uniqueness of our different regions by incorporating 'year' and 'region' as nested random effects. Due to the relative geographic separation of the five regions in Norway and Sweden, we ran our models with and without the five regions grouped. No geographic bias was detected and our final models included the five distinct study regions in Norway and Sweden. We performed model selection on the interaction terms only, using Akaike information criterion (AIC; Burnham and Anderson 2002). Bias in model fit was assessed by plotting 1) residuals versus fitted values, and 2) residuals versus each covariate in the model. All statistical analyses were conducted in R 3.3.3 (R Core Team) using the packages 'nlme' ver. 3.1-131 (Pinheiro et al. 2017) and 'glmmTMB' ver. 0.1.3 (Magnusson et al. 2017).

Additional analysis

We used our estimates of the viability of available seed, seed production per cone, cone production per tree, and stand density to calculate seed production per hectare and number of viable seeds available per hectare at a subset of our sites. Those data were only complete for Eagle Plains (*Picea mariana*), Tuktoyaktuk (*P. glauca*), Churchill (*P. glauca*, *P. mariana*, and *L. laricina*), and Mealy Mountains (*A. balsamea*, *L. laricina*, *P. glauca*, and *P. mariana*).

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.m58sp14>> (Brown et al. 2018).

Results

Seed production

Reproductive structures were collected from seven species in our 13 sub-arctic study regions (Supplementary material Appendix 1 Table A2). The number of reproductive structures per tree was highly variable during the study period, with density classes ranging from 0 to 100–1000 catkins per tree and 0 to 200 cones per tree. The number of seeds found per conifer cone was also highly variable, ranging from one (*P. glauca*, Tuktoyaktuk) to 211 (*Abies lasiocarpa*, Mackenzie Mountains; Supplementary material Appendix 1 Table A2). Among the cone-producing conifers, *Abies* spp. consistently produced more seeds per cone than the other species.

Seed production was positively associated with conspecific density and cone length, where species with longer cones produced more seeds than those with smaller cones. Overall, summer precipitation sum had the largest positive effect on seed production, and positive associations between spring growing degree days (GDD) and seed production were contingent on wet flowering (t) summers (Table 1). Seed production was negatively associated with annual GDD in both the year of cone initiation (t_{-x}) and flowering (t), with the exception that in sites with higher density of conspecific trees, seed production was positively associated with increases in annual GDD (t). Additional effects on seed production arose from interactions among annual GDD in the year of cone initiation (t_{-x}) and flowering (t), spring GDD and annual GDD in the year of flowering (t), and conspecific stand density (Table 1; Fig. 2). In general, GDD in the year of cone initiation had the greatest negative effect on seed production when it was followed by another summer with high spring or high annual GDD.

Viability of available seed

Sampled seed viability (i.e., the proportion of seed available for sampling that germinated in laboratory trials) was also highly variable between species and regions (0 to 44% viability; Supplementary material Appendix 1 Table A2). Notably, the Mackenzie Mountains region, which had the highest production of seeds per cone, had no viable seed (similar results reported for Mackenzie Mountains in 2013; S. D. Mamet, unpubl. data).

The viability of available seed was influenced by interactions among cone, stand, and seasonal climate characteristics (Table 1; Fig. 3). Unlike seed production, where longer cones produced more seeds, seed viability was negatively associated with cone length, and the length of the cone influenced a tree's response to seasonal climate variables. For example, viability of available seed was negatively associated with spring growing degree days (GDD) in the year of cone initiation (t_{-x}), except for smaller cones and for stands with higher conspecific density, both of which had higher viability of available seed when there were more GDD during the spring (t_{-x}). Viability of available seed in smaller cones also had a

Table 1. Parameter estimates with standard errors (SE) for generalized linear mixed models of seed production (number of seeds per cone; Poisson distribution; linear regression of observed versus fitted data: $R^2=0.87$, $p < 0.0001$) and viability of available seed in laboratory trials (binomial distribution; linear regression of observed versus fitted data: $R^2=0.78$, $p < 0.0001$). Covariates were scaled to z-scores, and both models included year and region as nested random effects. Empty cells for main effects indicate variables that were eliminated through the variable selection process. Significant ($\alpha=0.05$) covariate estimates are bolded. Covariates related to growing degree days are denoted with GDD. No problems with model fit were indicated via our model assessment procedure for either model.

	Seed production			Viability of available seed		
	Parameter estimate	SE	z-value	Parameter estimate	SE	z-value
Intercept	+5.03	0.35	14.5	-3.10	0.39	-7.90
Past spring GDD				-1.32	0.40	-3.33
Past annual GDD	-2.95	0.73	-4.03			
Spring GDD	+2.30	0.45	5.10			
Annual GDD	-0.48	0.14	-3.47	+1.05	0.34	3.10
Summer precipitation	+5.20	0.96	5.43	+0.67	0.32	2.11
Consppecific stand density	+0.30	0.15	2.01	+0.85	0.09	9.24
Cone length	+0.40	0.08	4.96	-2.10	0.60	-3.50
Past annual GDD × Spring GDD	+0.88	0.34	2.63			
Past annual GDD × GDD	-2.58	0.66	-3.91			
Past annual GDD × Summer precipitation	-0.11	0.30	-0.39			
Past annual GDD × Conspecific density	-1.08	0.20	-5.47			
Spring GDD × Annual GDD	+0.49	0.22	2.23			
Spring GDD × Summer precipitation	+2.59	0.47	5.56			
Spring GDD × Conspecific density	-0.33	0.16	-2.02			
Annual GDD × Conspecific density	+0.61	0.19	3.20			
Consppecific density × Cone length	-0.31	0.19	-1.67			
Past spring GDD × Cone length				-2.49	0.61	-4.11
Past spring GDD × Conspecific density				+0.58	0.10	5.95
Annual GDD × Summer precipitation				-0.44	0.24	-1.87
Annual GDD × Cone length				+1.64	0.50	3.31
Annual GDD × Conspecific density				-0.19	0.13	-1.48
Summer precipitation × Cone length				+1.01	0.35	2.89
Summer precipitation × Conspecific density				-0.55	0.09	-5.89

divergent response from larger cones to annual GDD in the year of flowering and embryo development (t), where annual GDD was positively associated with the viability of available seed in mid-sized and large cones but had little effect on species with small cones. The effects of summer precipitation also depended on cone length and conspecific density, where greater sums of summer precipitation in the year of flowering were generally positively associated with the viability of available seed, except for small cones and in sites with high conspecific density.

Estimates of viable seed per unit area

Seed production estimates were highly variable depending on species and region, and high seed production did not necessarily lead to a large number of viable seeds per unit area (Fig. 4). *Picea mariana* had the highest number of seeds produced per unit area (range: 289–1 857 273 seeds ha⁻¹; Supplementary material Appendix 1 Table A2) whereas production of viable seed was at least an order of magnitude lower (0–484 748 available viable seeds ha⁻¹). *L. laricina* produced 1636–65 682 seeds ha⁻¹, with 0–1773 of those available as viable seeds ha⁻¹. *P. glauca* produced 2–196 365 seeds ha⁻¹ and available viable seed ranged from 0.006–53 411 seeds ha⁻¹. Finally,

A. balsamea produced 3175–5054 seeds ha⁻¹, with 6–105 available viable seeds ha⁻¹.

Discussion

It is well established that the availability of niche space, i.e., suitable seedbed conditions, creates the finest filter on tree recruitment (Harper 1977). Here we address the antecedent filter: if niche space becomes available beyond the current treeline ecotone, are the individuals present in the current treeline ecotone able to produce sufficient propagules to colonise? The decrease in seed production per cone and viability of available seed with declining conspecific density from forest to tundra was widespread among our study sites. Although it is not surprising that stand density represents gradients in habitat suitability across the treeline ecotone, it may also entrain biotic limitation of reproductive processes such as pollination. The synthesis of our short-term observations of multiple species across sub-arctic treelines suggested general patterns in abiotic drivers likely to be key drivers of spatio-temporal variation in seed production and viability. Seasonal climate variables in our models, rather than simply annual or summer variables, revealed a more accurate understanding

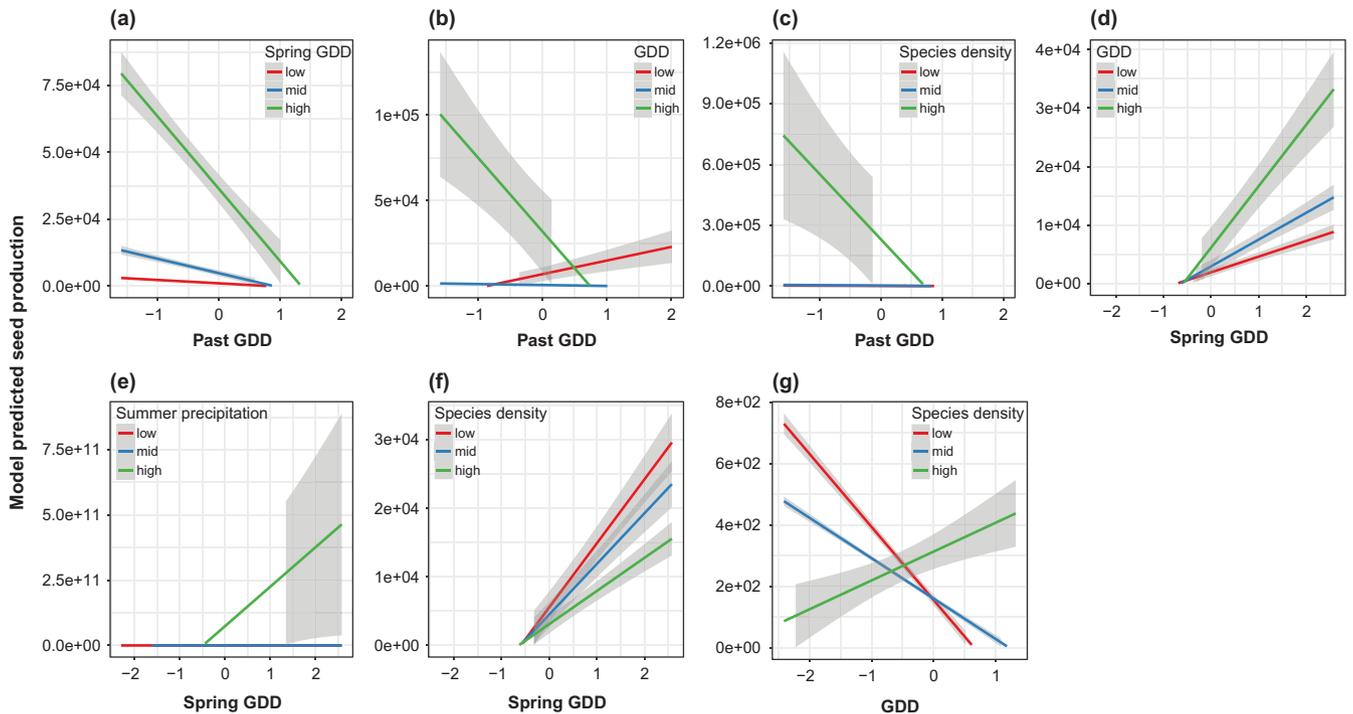


Figure 2. Generalized linear mixed model predicted values of seed production as a function of the interaction between (a) past annual growing degree days (GDD) and spring GDD divided into three categories; (b) past annual GDD and current annual GDD divided into three categories; (c) past GDD and conspecific density divided into three categories; (d) spring GDD and current annual GDD divided into three categories; (e) spring GDD and summer precipitation divided into three categories; (f) spring GDD and conspecific density divided into three categories; and (g) current annual GDD and conspecific density divided into three categories. All x-axes represent standardized z-scores of the response variables. Lines represent the linear model fit of the plotted covariates, holding all other covariates at the population means, with shading over the 95% prediction intervals. See Table 1 for parameter estimates.

of the climatic drivers of reproduction. We detected two general patterns: 1) the order of seasonal climate conditions across the years from bud initiation to flowering and embryo maturation influenced seed production and viability of available seed, but not always in the same way; and 2) high seed production in a region did not necessarily result in a large number of viable seeds available for recruitment. Here, we discuss each pattern and their implications for treeline range expansion.

Sequential climatic controls of production and viability

Our results suggest that it is not simply the number of growing degree days (GDD) that is important for the production of viable seed, but the sequence of their occurrence across the developmental period of reproductive structures. We observed divergent climate drivers of seed production quantity versus seed quality (e.g., the viability of available seed), a pattern also observed for a long-term study of alpine treeline *P. glauca* in Alaska (Roland et al. 2014). In our study, seed production was highest when a year with fewer GDDs was followed by a spring with more GDDs and a relatively wet summer (i.e., greatest precipitation sum). Those relationships appear to be particularly driven by whether or not there were two consecutive years with high GDD, with large negative

effects on seed production. That those negative effects were lessened by increased precipitation the summer of flowering suggests moisture limitations on seed production in our study populations. Further, increased GDD had positive effects on the development of viable embryos for species with large cones, but had little effect on those species with small cones. Since our study species with larger cones generally had larger seed (e.g., *Abies* spp.), this pattern leads us to hypothesize that longer periods of warmth are required for development of larger embryos.

The importance of the sequence of climatic conditions becomes even more apparent when we attempt to reconcile the divergent climate drivers of seed production quantity versus seed quality (e.g., the viability of available seed), a pattern also observed for a long-term study of alpine treeline *P. glauca* in Alaska (Roland et al. 2014). If the number of GDD in the spring of flowering is high, seed production may be reduced because of moisture stress (Eis 1973), yet if the spring is too cool, the viability of available seed may be lower due to the sensitivity of embryos to spring frost damage (Caron and Powell 1989, Meunier et al. 2007). Although our variable representing spring cold extremes (minimum May–June temperature) was eliminated during variable selection, it was highly correlated with annual GDD in the year of flowering. Frost events in late spring are difficult to predict, adding to

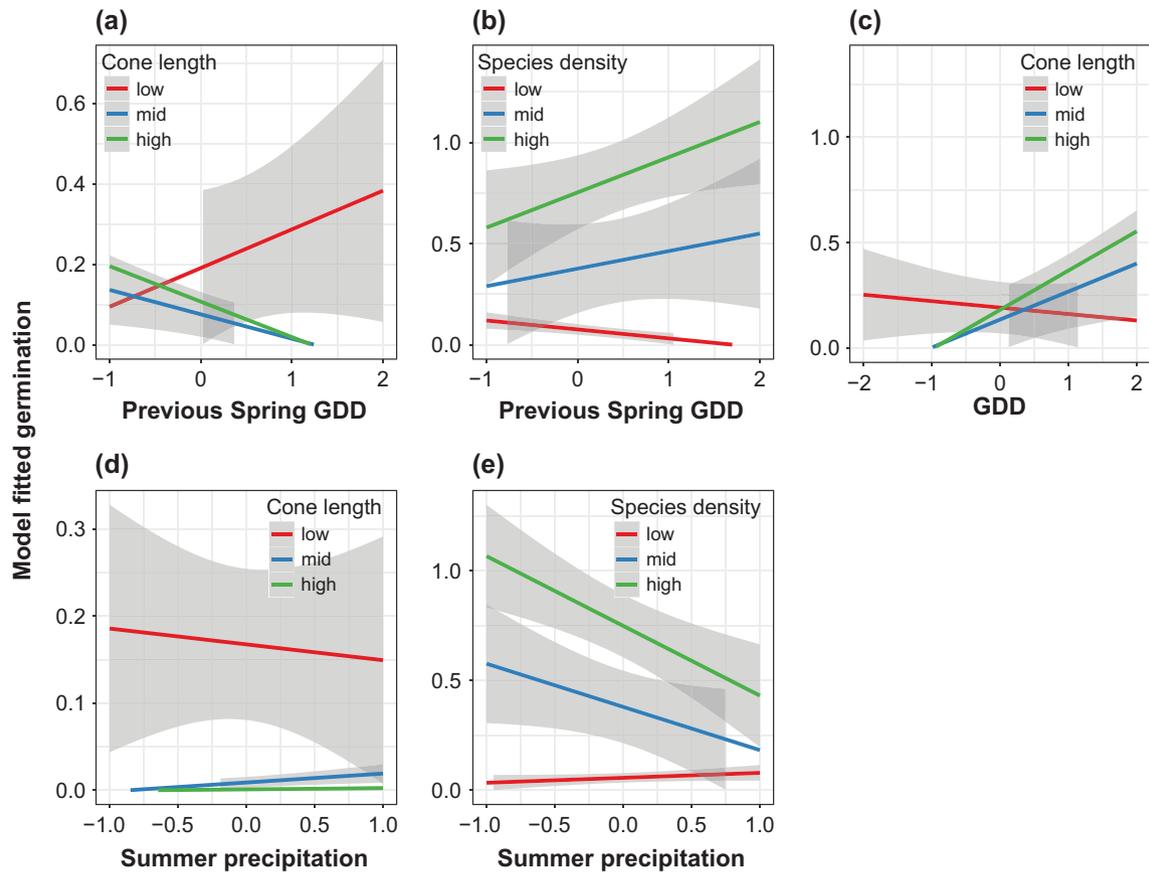


Figure 3. Generalized linear mixed model predicted values of viability of available seed as a function of the interaction between (a) previous spring growing degree days (GDD) and one length divided into three categories; (b) previous spring GDD and conspecific density divided into three categories; (c) current annual GDD and cone length divided into three categories; (d) summer precipitation and cone length divided into three categories; and (e) summer precipitation and conspecific density divided into three categories. All x-axes represent standardized z-scores of the response variables. Lines represent the model fit of the plotted covariates, holding all other covariates at the population means, with shading over the 95% prediction intervals. See Table 1 for parameter estimates.

the complexity of accurately modelling climatic controls on reproduction at the treeline ecotone.

Seed production does not beget viable seed available for dispersal

Patterns in seed production across our study regions did not consistently align with availability of viable seed, similar to findings in *P. mariana* (Sirois 2000) and *P. glauca* (Roland et al. 2014) at other treeline ecotones. In some of our study regions many seeds were produced, but little of that available seed was viable (e.g., Mackenzie and Mealy Mountains). Other studies have suggested direct climatic constraints on the maturation of viable seed (GDD threshold; Sirois 2000, Roland et al. 2014). Seed and pollen cone production are highly correlated in mature conifers (Caron and Powell 1989), suggesting that similar climate variables are important to the development of both male and female reproductive structures. In treeline ecotone populations where stand density is low, reduced proximity of conspecifics may lower the probability of pollination (Allison 1990).

The positive effect of conspecific density on the viability of available seed in our models is consistent with the hypotheses of pollen limitation previously identified in range-edge populations of *Betula* (Weis and Hermanutz 1993, Holm 1994), *Picea* (Elliott 1979, Sirois 2000), and *Larix* species (Elliott 1979).

Other weather events are also important for successful pollination. Interactions between conspecific stand density and summer precipitation (in June, July, and August; pollen dispersal occurs from May-July in sub-arctic regions; Burns and Honkala 1990) meant that higher density stands had less viable seed available when the summer had more rain, possibly due to poor pollen dispersal. Seed viability in low density stands was unaffected by summer rain, and we hypothesize that lower density stands receive very little pollen regardless of precipitation. As discussed above, our models also highlight the importance of more GDD in the spring of pollination, providing indirect support for the hypothesis that reproductive structures, particularly pollen cones (Elliott 1979), are vulnerable to early frost damage. Shorter growing seasons with late season frosts, fewer growing degree days, or

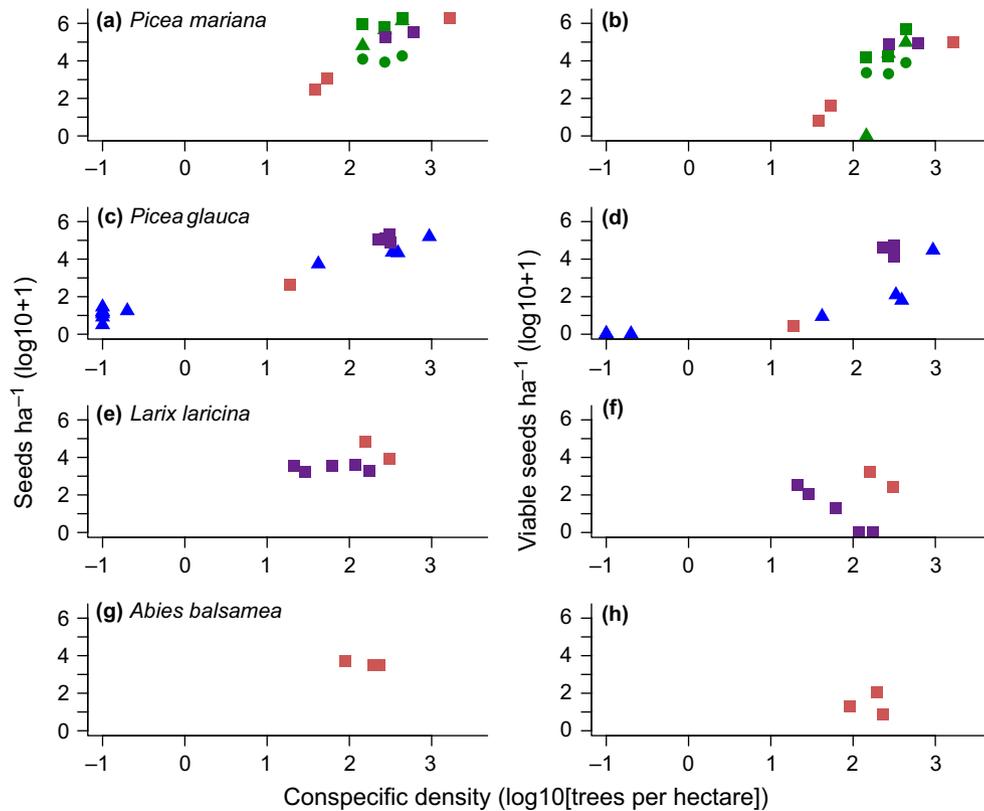


Figure 4. Relationship between number of seeds ha^{-1} (left column) and number of available viable seeds ha^{-1} (right column; both $\log_{10}+1$ scale) and conspecific stand density (trees ha^{-1} ; \log_{10} scale) for *Picea mariana* (a)–(b), *P. glauca* (c)–(d), *Larix laricina* (e)–(f) and *Abies balsamea* (g)–(h) sampled from Eagle Plains, Yukon (green), Tuktoyaktuk, Northwest Territories (blue), Churchill, Manitoba (purple), and Mealy Mountains, Newfoundland and Labrador (red) in Canada. Symbols mark the year of sampling during 2007 (circles), 2008 (squares), and 2009 (triangles).

rainy conditions during pollen dispersal may result in unfertilized or immature embryos that are unable to germinate for range expansion.

Our analyses support the concept of a sequential model of seed production, in which successful production of viable seed is influenced by a series of weather conditions occurring over multiple seasons (Juday et al. 2003, Krebs et al. 2012, Roland et al. 2014). Favourable climate conditions driven by synoptic weather patterns have been identified as triggers of regionally synchronous pulses of high seed production (e.g., ‘mast’ years; Koenig and Knops 1998). All seven species in this study exhibit masting dynamics that are hypothesized to be the result of some combination of warm, dry conditions in the growing seasons up to two years prior to cone development (see examples for our study species in Woodward et al. 1994, Houle 1999, Sirois 2000, Krebs et al. 2012, Bisi et al. 2016, Gallego Zamorano et al. 2018). However, documentation of seed production in mast years requires long time series and our study design was insufficient to assess controls over mast years. Although the data presented here represent a snapshot in time, they captured a wide range of weather conditions and species and are thus likely to have general applicability. Study sites occurred along elevational gradients within the sub-arctic region, and

our findings are relevant to other treeline ecotones with cold climates. Notably, differences in constraints on seed production compared to viability mean that insights into factors affecting the viability of available seed could readily apply to both masting and non-masting years, and may represent critical limitations on reproductive potential at treeline (Roland et al. 2014).

Reproductive potential for range expansion

Adult trees appear to require a complex series of climatic conditions to reproduce and disperse propagules that enable range expansion beyond the treeline ecotone. Viable seed availability is very low near range edges in our study regions, suggesting that many treeline ecotones are seed limited, at least some of the time. Without sufficient propagules, range expansion at these sites will be slow. The concept of shifting niches with ontogeny has been suggested for germination to post-germination (seedling) life stages (Donohue et al. 2010); here, we propose expanding that concept to adult reproduction. Even when conditions are favourable for germination, establishment, or recruitment beyond range limits, they may not be suitable to produce sufficient propagules to fill the new niche space beyond forest range limits.

The expansion of tree species ranges into tundra is a process of multiple hurdles that start with the production of viable seeds. For successful expansion, climatic conditions must sequentially favour: cone initiation with ovule and pollen production, the development of embryos to produce viable seed, post-dispersal germination, early seedling survival and growth, which may require biotic interactions with soil biota, and overwinter seedling survival. This sequence may produce a one-year old seedling beyond the species' current range; yet sustained range expansion requires new colonisers to reach reproductive maturity, which may take decades of appropriate climate conditions and potentially require the presence of some species, such as mycorrhizal fungi, and absence of others, such as herbivores and pathogenic fungi. Once we add the effect of pre- and post-dispersal seed predators (Jameson et al. 2015, Kambo and Danby 2017), availability of optimal seedbeds (Harper 1977), and seedling herbivores (Cairns and Moen 2004), it becomes clear that the requirements for range expansion are challenging and complex. The large number of potential bottlenecks makes it unsurprising that treeline ecotones are not consistently expanding in response to climate change. The abiotic constraints on sexual reproduction described here suggest that changes in climate are likely to influence the availability of viable seed at treeline. However, a complex series of sequential climate controls that interact with density-dependent processes of reproduction is likely to give rise to individualistic and episodic patterns of range expansion that are difficult to predict from simple climate-envelope models.

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Supplementary material (Appendix ECOG-03733 at <www.ecography.org/appendix/ecog-03733>). Appendix 1.