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Interactive Effects of Contact Fungicide and Cold Stratification on the Germination Rate for Five Dominant Temperate Tree Species

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Fungicide application facilitates seed and seedling survival, but these pesticides may also negatively affect germination. Moreover, it is unclear how fungicide may interact with influential environmental conditions, such as cold stratification, to affect tree seed germination. We examined how fungicide and five different cold stratification durations of increasing length influenced tree seed germination (i.e., germination fraction and germination rate) for five important species commonly found in temperate North American forests (*Abies balsamea*, *Acer saccharum*, *Picea glauca*, *Pinus resinosa*, and *Pinus strobus*). Greater cold stratification durations increased *A. saccharum* germination fraction, had positive effects on *P. strobus* and *A. balsamea* germination fractions, and decreased germination for *P. glauca*. Longer cold stratification reduced time to germination for all species, reinforcing the importance of this pregermination condition in tree seed phenology. We also found that although Captan 50W fungicide had no effect on the germination fraction for any species, it delayed the germination of *P. glauca* and *P. strobus* at specific stratification durations. We suggest that delays in tree seed germination—mediated by fungicide application, reductions in stratification duration driven by warmer winters, or both—could hinder seedling survival and performance with long-term effects on the vigor of tree seedlings used for transplanting.

Keywords: Captan 50W, germination fraction, germination rate, Pinaceae, survival analysis

Tree recruitment is a critical component of forest health, biodiversity, and production (Smith et al. 2009). Fungal pathogens, as significant agents of mortality for tree seeds and seedlings, represent a fundamental constraint on tree recruitment in nurseries, managed forests, and natural stands (e.g., Vaartaja 1956, Enebak et al. 1990, Zhong and van der Kamp 1999, O'Hanlon-Manners and Kotanen 2004, 2006). Fungicides provide a means to mitigate damage to economically and ecologically important tree species (e.g., *Pinus* spp., *Picea* spp., *Acer* spp., and *Abies* spp.) by fungal pathogens (Enebak et al. 1990, Mittal and Wang 1993, Coakley et al. 1999, Cram and Fraedrich 2010). Moreover, because changes in climatic conditions (e.g., warmer temperatures or reduced snow cover duration) may amplify fungal pathogen attack on trees in nurseries or managed forests by increasing plant stress and altering pathogen communities (Chakraborty et al. 2008, Wingfield et al. 2015) and/or alter the efficiency of fungicides to control plant pathogens (Juroszek and von Tiedemann 2011), experiments evaluating the use of fungicides to manage tree seed and seedling survival may become increasingly important to inform plant propagation and disease management strategies.

Although fungicides are commonly used to maximize and safeguard seed and seedling survival in nurseries (Landis 2008, South and Carey 2008), these pesticides may have direct deleterious effects on tree seed germination dynamics. Fungicides can reduce total germination in some tree species (Vaartaja 1956, Lock et al. 1975) or slow the rate of tree seed germination (Sato 1962). Fungicides may also interact with important environmental conditions (e.g., stratification) to alter tree seed germination (Bloomberg and Trelawny 1970). Although a reduction in germination fraction has a clear effect on the number of seeds that become seedlings, changes in germination timing may also be influential: short delays in germination can lead to important differences in plant survival (Boyer et al. 1987, Jones et al. 1997), biomass accumulation (Orrock and Christopher 2010), and competitive ability (Dyer et al. 2000). Consequently, germination speed is also likely to be an ideal indicator of tree seedling survival and performance (Barnett and McLemore

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1984, Boyer et al. 1987). It is imperative to characterize how fungicides affect germination dynamics for forest tree species within the context of differing physical pregermination conditions because fungicides often affect the number of seeds that become seedlings (e.g., Enebak et al. 1990) and the use of fungicides within plant production systems may become more common under future climate scenarios (Juroszek and von Tiedemann 2011). In particular, climate change in temperate ecosystems is expected to increase average winter temperatures, thereby reducing the duration of suitable stratification periods for many plant species (Walck et al. 2011). As cold stratification is an important component in the phenology and seedling performance of many temperate tree species (Landis 2008), considering how fungicide application influences tree germination dynamics across a gradient of shorter to longer cold stratification durations will help identify how fungicide usage may influence tree seedling survival and performance under projected future climates.

Our study objectives were to examine how the total germination fraction and the germination timing of five North America tree species are influenced by differing stratification durations and the presence of the commercial fungicide Captan 50W. Captan is a contact, phthalimide-class fungicide that can be used to limit preemergence mortality and damping-off typically caused by fungal genera, including *Fusarium*, *Pythium*, and *Rhizoctonia*, and is particularly effective for controlling fungi associated with seed decay (Neergaard 1977). Captan fungicide has a relative short persistence in soil treatments and has minimal or only transient negative effects on important plant-mycorrhizae interactions when applied at low to moderate concentrations (Vyas 1988). We focus on the effects of Captan fungicide as it has been used extensively in studies evaluating fungal pathogen attack on tree seeds in nurseries (e.g., Cayford and Waldron 1967, Enebak et al. 1990, Rai and Mamatha 2005) and natural stands (e.g., O'Hanlon-Manners and Kotanen 2004, 2006, Kotanen 2007). In addition, some work indicates that Captan may have phytotoxic effects on tree seed and seedlings (e.g., Cayford and Waldron 1967, Lock et al. 1975), suggesting that this fungicide is ideal for evaluating how contact fungicide application and differential seed stratification status could interact to alter tree germination dynamics. We hypothesize that the effect of fungicide application on germination dynamics may differ between species and that phytotoxic effects associated with fungicide application may differ based on seed stratification status. We focus on seeds of five tree species that are prevalent in northern temperate forests and whose importance is expected to be influenced by changing climate conditions: balsam fir (*Abies balsamea* [L.] Mill.), sugar maple (*Acer saccharum* [Marsh.]), white spruce (*Picea glauca* [Moench] Voss), red pine (*Pinus resinosa* [Ait.]), and eastern white pine (*Pinus strobus* [L.]) (Iverson et al. 2008).

Methods

Seed Source and Storage

We purchased seeds of five tree species (*A. balsamea*, *A. saccharum*, *P. glauca*, *P. resinosa*, and *P. strobus*) through the Wisconsin Department of Natural Resources Griffith State Nursery (Wisconsin Rapids, WI) (see Supplemental Table S1[S]) in October 2014. These species are dominant in Wisconsin hardwood and boreal forests and are ideal study species because their survival and growth as juvenile trees are important components in the sustainability of timber production and other industries (e.g., maple sugar production and tree farms). Before purchase, seeds were housed at the Hayward State Nursery (Hayward, WI) where they were stored in plastic bags within cardboard canisters at ~32° F (0° C) (Jeremiah Auer, Wisconsin Department of Natural Resources, pers. comm., May 19, 2015). Seed collection years differ by species with accessions ranging from 3–34 years old (Table S1); however, all of the tree species evaluated in this study demonstrate strong seed longevity (i.e., little to no reduction in germination and high moisture content) after prolonged cold storage (Simpson et al. 2004). For example, *P. glauca* (the oldest seed accession tested in our study) can retain >90% germination fraction after 33 years in cold storage (Simpson et al. 2004). Once in our laboratory, seeds were stored at 22–25° C with ambient relative humidity until the initiation of seed stratification.

Experimental Design and Data Collection

We used a 5 × 2 factorial study design to evaluate the effect of five different cold stratification durations and two fungicide addition levels (i.e., the presence and absence of Captan 50W fungicide) on the seed germination dynamics (i.e., germination fraction and germination rate) of five different tree species. To apply the seed stratification treatment, approximately 2,000 seeds of each species were washed with deionized water and presoaked for 48 hours. Seeds were then dried with paper towels, placed in plastic bags on top of sand, and positioned on a level surface within a refrigerator. We used an iButton (Maxim Integrated Products, San Jose, CA) to report temperature conditions within the refrigerator; seeds were stratified at 33.6° F (0.9° C). Seed stratification started on Dec. 19, 2014. Seeds were stratified into groups assigned to different stratification durations: no stratification, 14 days, 28 days, 42 days, and 56 days. Seeds assigned to each stratification treatment were stored in separate bags to minimize disturbance among stratification treatments.

At 14-day intervals, 10 groups of 10 seeds of each species were removed from stratification, and each group was placed into 1 of 10 sterile plastic Petri dishes (100 × 15 mm; Fisher brand) with a germination blotter (3.37-in. [8.57-cm] diameter; Anchor paper). To evaluate the effect of fungicide application on germination, blotters in half of the Petri dishes assigned to each species at each stratification period (replicate number, $n = 5$) were saturated with 2 ml of a fungicide solution (0.36 oz of Captan 50W/gallon [2.757 g/liter] or ~0.08% Captan solution, the manufacturer's recommended concentration for application to field soils). The replicate number was selected to maximize statistical power while simultaneously ensuring we were able to accurately record both the temporal dynamics of seed germination and estimates of seed viability. Seed density per dish was kept low (1.06 seeds/in.^{2} [0.16 seed/cm^2]) to control for any density-dependent effects on germination speed (Dyer et al. 2000, Orrock and Christopher 2010). Blotters in the remaining Petri dishes were saturated with water. After initiation of the germination trial, blotters in all dishes were kept saturated.

Seeds of four of the five species (*A. balsamea*, *P. glauca*, *P. resinosa*, and *P. strobus*) were placed in a Percival plant growth chamber (model E-41L2; Percival Scientific, Perry, IA) with a 59° F/77° F (15° C/25° C) nocturnal/diurnal temperature cycle; each temperature period lasted 12 hours. Illumination was provided for 4 hours daily (10:00 am to 2:00 pm); light conditions were chosen to meet possible pregermination condition requirements for some species

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(e.g., *Pinus* spp.; Qamaruddin and Tillberg 1989), while simultaneously matching the light limitation common within shaded nurseries or the understory of managed forests. Temperature and photoperiod conditions were informed by the US Department of Agriculture Forest Service woody plant seed manuals for *Abies* spp. (Edwards 2008), *Picea* spp. (Youngblood and Safford 2008), and *Pinus* spp. (Krugman and Jenkinson 2008). Seeds were monitored for germination once daily for 28 days, except for the 28-day stratification treatment, which was monitored for 27 days. Seeds were scored as germinated if the radicle extended ≥0.12 in. (3 mm) from the seed coat. The time and date of each germination event were recorded, and then germinated seeds were removed from dishes to prevent competitive inhibition on ungerminated seeds.

We assessed seed viability at the end of the germination study using a combination of a seed firmness testing followed by a cut test (Borza et al. 2007, Beckstead et al. 2010). In brief, at the end of the germination study, the firmness of each ungerminated seed was assessed by depressing the seed coat with a pair of blunted forceps; if the endosperm was rigid and there was little to no superficial discoloration of the seed, the seed was counted as "viable," but seeds that did not meet these criteria were subjected to a cut test. In cut testing, seed embryos were excised and examined for intact structure and superficial discoloration using a hand lens. Seeds with intact embryos that lacked superficial discoloration were considered dormant but "viable," whereas damage to or lack of an embryo was classified as a "nonviable."

Seeds of *A. saccharum* require lower temperatures (33–41° F [0.5–5.0° C]) to break dormancy (Zasada and Strong 2008). To evaluate the effects of fungicide and stratification on *A. saccharum* germination dynamics, we compared the effects of fungicide between seeds that had been stratified on slightly damp sand for 28 days and seeds that did not receive a stratification treatment. The experimental design matched that described for the other tree species except for three modifications: each factorial treatment level had 10 replicates; germination trials were conducted under identical refrigeration conditions used for stratification (see above); and the total period of evaluation was extended to 62 days with germination timing monitored every 2–5 days to accommodate the slower *A. saccharum* germination rates at colder temperatures.

Data Analysis

We used generalized linear mixed-effects models with a binomial response distribution to evaluate whether stratification and fungicide treatments influenced proportion of germination (i.e., germination fraction) for each species. Germination fraction reports the number of seeds per dish that germinated during the period of observation. Estimates of germination fraction included seeds that germinated during the 28-day period in the growth chamber for *A. balsamea*, *P. glauca*, *P. resinosa*, and *P. strobus* or during the 62-day cold period for *A. saccharum*. Stratification period, fungicide application, and the interaction of these treatments were treated as fixed effects in models addressing germination fraction independently for each species. Pairwise comparison tests of germination data using *t*-value scores were adjusted for multiplicity using the Tukey-Kramer method (Littell et al. 2006). Time to germination was the amount of time (in hours) it took for each seed, once placed in the growth chamber, to grow a radicle ≥0.12 in. (3 mm) in length. We used Cox proportional hazards analysis to evaluate the influence of stratification period, fungicide application, and the interaction between these two factors on the timing of tree seed germination; Wald χ^2 values were used to test each fixed effect (Allison 2010, McNair et al. 2012). We used hazard ratio analysis and 95% profile likelihood confidence limits to test specific hypotheses regarding the effect of fungicide application on seed germination timing at each level of stratification period and for each species. Confidence limits around hazard ratio point estimates that fell <1.0 indicated that fungicide application delayed germination at that stratification period (Allison 2010). Statistical tests returning a type I error (α) ≤0.05 were reported as statistically significant, whereas statistical tests returning a type I error (α) between 0.10 and 0.05 were interpreted as marginally significant. All analyses were conducted in SAS (version 9.3; SAS Institute, Cary, NC).

Table 1. Summary of effects of fungicide application (Captan 50W), stratification duration, and the interaction of these two fixed effects on the germination fraction and the rate of germination for five species: *Abies balsamea*, *Picea glauca*, *Pinus resinosa*, *Pinus strobus*, and *Acer saccharum*.

	Germination fraction			Germination rate		
Factor	*F*	*df*	*P*	Wald χ^2	*df*	*P*
Species: *A. balsamea*						
Fungicide (FUNG)	1.63	1, 40	0.209	1.02	1	0.312
Stratification (STRAT)	2.14	4, 40	0.094	88.10	4	<0.001
FUNG × STRAT	0.79	4, 40	0.537	5.56	4	0.235
Species: *P. glauca*						
Fungicide (FUNG)	1.68	1, 40	0.203	1.12	1	0.291
Stratification (STRAT)	5.74	4, 40	0.001	47.30	4	<0.001
FUNG × STRAT	2.07	4, 40	0.103	12.38	4	0.015
Species: *P. resinosa*						
Fungicide (FUNG)	0.00	1, 40	0.998	0.42	1	0.518
Stratification (STRAT)	0.17	4, 40	0.952	178.52	4	<0.001
FUNG × STRAT	0.06	4, 40	0.994	7.15	4	0.128
Species: *P. strobus*						
Fungicide (FUNG)	1.25	1, 40	0.270	0.47	1	0.491
Stratification (STRAT)	2.22	4, 40	0.084	111.55	4	<0.001
FUNG × STRAT	0.71	4, 40	0.593	8.623	4	0.071
Species: *A. saccharum*						
Fungicide (FUNG)	0.53	1, 36	0.471	0.82	1	0.366
Stratification (STRAT)	23.96	1, 36	<0.001	8.47	1	0.004
FUNG × STRAT	1.19	1, 36	0.283	0.80	1	0.372

Results

Proportion of Tree Seeds Germinating and Seed Viability

Captan fungicide treatment did not influence germination fraction for any of the five tree species, but stratification generated species-specific responses in tree seed germination fraction (Table 1; Figure 1). Longer stratification typically resulted in greater average germination for *A. balsamea*, *P. strobus*, and *A. saccharum*, although the trend was only statistically significant for *A. saccharum*. The greatest average number of *A. balsamea* seeds germinated after 42- and 56-day stratification lengths, but these germination proportions did not differ significantly from *A. balsamea* germinations of shorter duration (all $P > 0.155$). *P. strobus* germination fraction was marginally greater after the 56-day stratification treatment relative to treatments with no stratification ($t = 2.80$, $df = 40$, $P = 0.057$), but did not differ between any other stratification treatments (all $P \geq 0.107$). *A. saccharum* germination fraction was significantly greater after the 28-day stratification treatment relative to that of *A. saccharum* seeds from the unstratified treatment ($t = 4.89$, $df = 36$, $P < 0.001$).

The proportion of *P. glauca* seeds that germinated declined with greater stratification length (Table 1; Figure 1). Notably, *P. glauca*

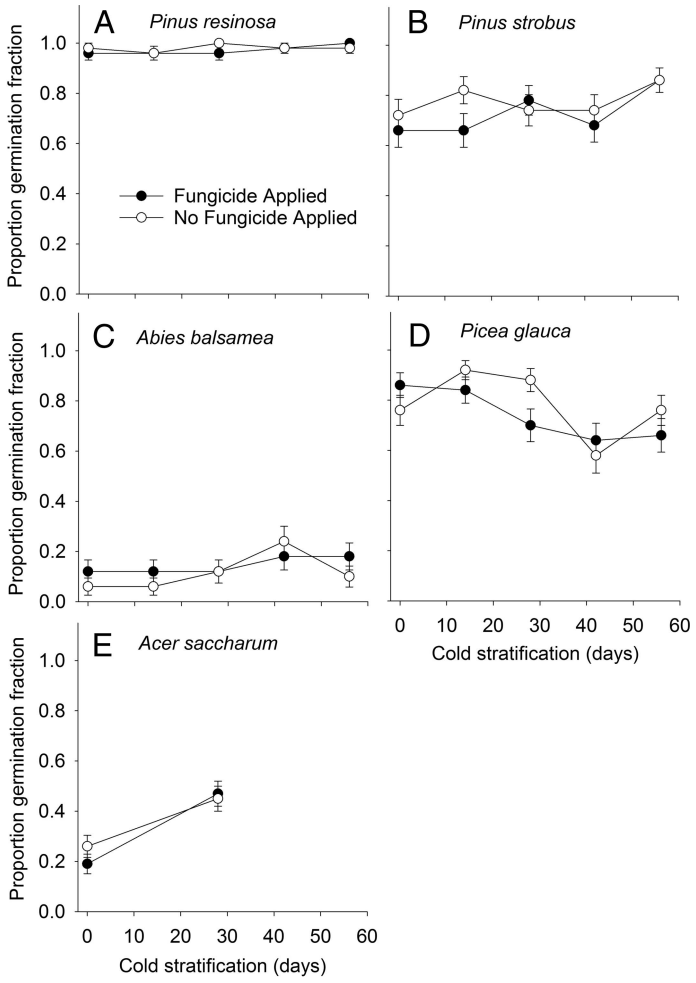

Figure 1. Germination fraction for seeds under a gradient of cold stratification (33.6° F [0.9° C]) durations and contact fungicide (Captan 50W) application. The effects of stratification duration (0, 14, 28, 42, and 56 days) and fungicide application (fungicide applied versus no fungicide applied) on proportion of germinated seeds (mean ± SE) for *Pinus strobus* (A), *Pinus resinosa* (B), *Abies balsamea* (C), *Picea glauca* (D), and *Acer saccharum* (E) are shown.

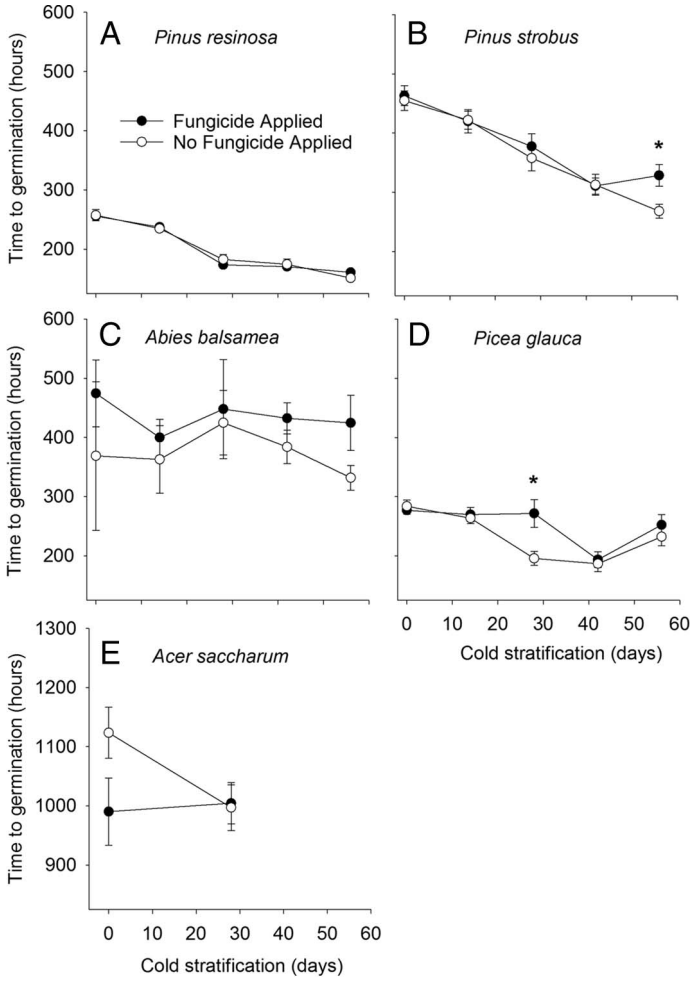

Figure 2. Rate of seed germination under a gradient of cold stratification (33.6° F [0.9° C]) durations and contact fungicide (Captan 50W) application. The effects of stratification duration (0, 14, 28, 42, and 56 days) and fungicide application (fungicide applied versus no fungicide applied) on average time to germination (mean ± SE) for *Pinus strobus* (A), *Pinus resinosa* (B), *Abies balsamea* (C), *Picea glauca* (D), and *Acer saccharum* (E) are shown. Note the difference in time range for *A. saccharum* (E) relative to all other species germination rate plots. *Levels of stratification at which 95% profile likelihood confidence limits around hazard ratio point estimates are <1.0, indicating that fungicide application significantly delayed germination at those levels.

germination fraction was lower in the 42-day stratification treatment than in any shorter stratification duration treatment (all $P < 0.047$), and fewer *P. glauca* seeds germinated in the 56-day stratification treatment than *P. glauca* seeds that were stratified for 14 days ($t = -2.90$, $df = 40$, $P = 0.045$), but there was no significant trend suggesting that fewer *P. glauca* seeds germinated after the 56-day stratification treatment relative to the seeds that were not stratified ($t = -1.67$, $df = 40$, $P = 0.462$). Neither fungicide nor stratification treatments affected *P. resinosa* germination fraction (Table 1).

Rate of Tree Seed Germination

Captan fungicide had no main effect on the germination rate of *A. balsamea*, *A. saccharum*, or *P. resinosa*, but the germination rates of all species were generally faster with longer stratification durations (Table 1; Figure 2A, C, and E). Captan fungicide did not influence *P. strobus* germination rates, but a marginally significant interaction term between stratification and fungicide main effects suggests that the influence of fungicide application on *P. strobus* germination rate may differ by stratification duration (Table 1; Figure 2B). Notably, germination rates of *P. strobus* seeds after the 56-day stratification that received fungicide were significantly slower (60-hour delay) than conspecifics that were not treated with fungicide at the same stratification duration (Figure 2B). Specifically, hazard ratios at the 56-day stratification level indicate that fungicide-treated *P. strobus* seeds germinated more slowly than untreated seeds at this stratification period (95% confidence limits, *P. strobus*: 0.334 −0.787). Similarly, *P. glauca* germination rate was influenced by an interaction between the fungicide application treatment and stratification duration (Table 1). Whereas greater stratification durations typically increased the rate of *P. glauca* germination (Figure 2D), fungicide application significantly slowed germination after 28 days of stratification (95% confidence limits, *P. glauca*: 0.270–0.661) but did not influence germination speed at any other stratification length.

Discussion

Given the widespread potential for global climate change to amplify fungal pathogen pest burdens within tree seedling nurseries and managed temperate forests (Coakley et al. 1999, Millar and Stephenson 2015, Wingfield et al. 2015) and modify physical pregermination conditions (Walck et al. 2011), it may become increasingly important to understand how fungicides will interact with plants under a variety of climate conditions to influence successful propagation (Juroszek and von Tiedemann 2011). We demonstrate that Captan 50W contact fungicide applied at moderate concentrations does not affect germination fraction for the tree species used in this study but that stratification duration plays an important role in dictating the rate of tree seed germination (McLemore 1969). Importantly, we also show that this contact fungicide can occasionally alter the rate of germination in species-specific patterns at certain stratification durations. Our results have several implications: changes in germination timing in some Pinaceae caused by Captan fungicide may affect young seedling growth; and considering the species-specific responses to varying stratification durations and pesticide application may help nursery managers maximize tree seedling vigor under future climate scenarios.

Captan Fungicide and Regeneration Dynamics in the Pinaceae

Our work adds a new facet to the forestry literature describing the phytotoxic potential associated with fungicide application on young Pinaceae (Cayford and Waldron 1967, Peterson 1970) by showing that fungicide can interact with stratification period to slow germination for some members of this family. Captan-mediated delays in germination depend strongly on stratification status for the affected species (i.e., *P. strobus* and *P. glauca*), and absolute values of change in germination timing were relatively small (Figure 2). However, as germination timing is an essential component of tree seedling vigor (Barnett and McLemore 1984, Boyer et al. 1987), even short differences in emergence timing may lead to substantial variation in juvenile tree performance. For example, a *P. glauca* seedling that emerges 82 hours before a neighboring seedling (a duration comparable to the effect of fungicide at the 28-day stratification level) (Figure 2D) has an average of 15% more aboveground biomass after 4 weeks of growth (B.M. Connolly unpub. data, Oct. 1, 2016). Emergence order is important for resource competition in plants (Jones et al. 1997, Orrock and Christopher 2010), and even short-term shifts in germination time can have persistent effects on individual survival and performance (Boyer et al. 1987, Jones et al. 1997) and may influence long-term patterns of plant dominance (Tielbörger and Prasse 2009).

Differences in early tree seedling size may interact directly with resource availability to determine patterns of survival (Tuttle et al. 1987), suggesting that by generating disparity in tree seedling size, fungicide-mediated shifts in emergence time may have the potential to influence tree seedling vigor. By incorporating recent applications of survival analysis to seed germination data (e.g., McNair et al. 2012), nursery managers now have a robust analytical tool to help predict the effects of environmental (e.g., changing abiotic conditions) and anthropogenic factors (e.g., effects of different pesticides) on tree seed germination rate and to help determine how these factors may influence tree seedling emergence, survival, and performance.

Stratification Duration and Tree Germination Dynamics

Tree species typically display unique thresholds in stratification that dictate germination fraction and rate of germination (e.g., Edwards 2008, Krugman and Jenkinson 2008, Youngblood and Safford 2008, Zasada and Strong 2008); insufficient stratification may leave seeds viable but still dormant. For example, estimates of seed viability (Supplemental Table S2) indicate that *A. balsamea* retained high viability despite relatively low percent germination during our study period, suggesting either that pregermination conditions were insufficient to completely break dormancy for this species or that the combination of seed age and long-term storage conditions may have resulted in low seed vigor (e.g., Edwards 2008). However, germination fraction and viability estimates suggest that our pregermination treatments were sufficient to break seed dormancy for the other four tree species. Interspecific differences in cold stratification requirements may alter expected patterns in tree seed emergence as cold stratification durations shift with warming winters (Walck et al. 2011).

Our results build on a large body of forestry research indicating that cold stratification is an important, often essential, pregermination requirement for many temperate tree species (Landis 2008). For the five species we studied, longer stratification durations consistently led to a faster germination rate (Figure 2), and these results are consistent with similar work conducted by others (e.g., McLemore 1969). However, as a result of global warming, the annual duration of seed stratification lengths is likely to decrease (Walck et al. 2011), generating potentially negative consequences for cumulative tree seed germination and the timing of seedling emergence. For example, optimal seed stratification lengths at 33–41° F (0.5–5° C) for stored seeds of some species evaluated in this study range from 30 to 90 days (Edwards 2008, Krugman and Jenkinson 2008, Zasada and Strong 2008) with longer stratification durations facilitating larger germination fractions and faster rates of germination. The recommended stratification requirements we evaluated align closely with natural conditions experienced by seeds of these species; soil temperatures (~0.80 in. [2 cm] depth) in two Wisconsin hardwood forest sites during the winter of 2016 remained consistently <41° F (5° C) for approximately 88–91 days and consistently <34° F (1° C) for approximately 58–62 days (B.M. Connolly unpub. data, Oct. 1, 2016). Given increased warming and greater variability in winter soil temperatures, projected outcomes of climate change (Henry 2008), our results suggest that the climate change-mediated losses of sufficient stratification conditions (e.g., less time spent at <34° F [1° C]) may decrease tree seed germination fraction and lengthen the required time for tree seeds to germinate. The likely concurrence of climate change-mediated delays in tree seedling emergence and increased likelihood of attack by fungal pathogens under new climate conditions (Chakraborty et al. 2008) highlights the importance of adequate stratification, artificial or natural (Landis 2008), and application of fungicide in actively managed systems to ensure tree seedling survival and growth.

Conclusions and Future Directions

Our work suggests Captan fungicide application may have species-specific consequences for tree seed germination rate. However, enforced stratification under controlled conditions may speed up germination to such an extent that the effects of fungicide on germination timing are minor relative to the overall positive effects of pathogen control. For example, although fungicide slowed *P. strobus* germination after 56 days of stratification (Figure 2B), the rate of germination was still faster than germination of any *P. strobus* seeds stratified for less than 42 days, suggesting that any loss of seedling vigor due to fungicide might be overcome by application of artificial

stratification. Additional studies are needed to clarify whether fungicide-mediated shifts in germination timing alter tree seedling vigor under field or nursery conditions, and further work will be necessary to determine how effective and practicable artificial stratification is as a methodology to offset the decreases in cold stratification duration projected with warmer winter climates and to determine how the application of other types of biocides/fungicide (e.g., methyl bromide, chloropicrin, dazomet, or thiram) influence tree seed germination dynamics after different cold stratification durations.

Differences in seed characteristics may also influence the response to cold stratification and fungicide application. Seed age varied among the species evaluated in this study (Supplemental Table S1) and the germination dynamics of older seeds may differ from those of more recent accessions. Seeds of the tree species used in this study, however, retain high viability after long-term cold storage (Simpson et al. 2004), suggesting that accession age may play a limited role in the germination dynamics we report. For example, the oldest and second oldest seed accessions (*P. glauca* [34 years old] and *P. resinosa* [18 years old], respectively) still demonstrated strong positive responses to cold stratification duration, the fastest germination rates (Figure 2), and the highest seed viability (both >89%) at the conclusion of our study (Supplemental Table S1). Nevertheless, seed age may alter tree seed germination dynamics and further examination of the role of cold stratification and fungicide application on conspecific seeds from a range of accession dates will inform how seed age interacts with these treatment factors to influence germination fraction and rate. Germination dynamics can also vary as a function of genetic lines within species and seed collection location (Thomson and El-Kassaby 1993) or as a result of annual variations in climate mediated via maternal effects (Rix et al. 2012), implying that systematic screening of fungicides across a range of cold stratification durations on distinct seed accessions will be necessary to predict how the tree germination will be influenced in future climates.

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