Persistent Effects of Herbaceous Species on the Infectious Lethality of Soil for Conifer Seedlings

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Abstract

Seeds of the coniferous trees *Abies balsamea*, *Picea mariana*, and *Pinus strobus* were sown in the laboratory in two soils taken from ground-layer patches differing in species composition, one of which was dominated by *Aegopodium podagraria* (goutweed). This permitted inference whether herbaceous species may affect the favorableness of the soil for establishment of these trees. Weibull distributions were fitted to the time course of aggregate seedling emergence and post-emergence mortality, enabling seedling lifespan to be inferred without monitoring of each individual. A higher percentage of *Abies* seeds developed into emerged seedlings in the goutweed soil, likely because of less pre-emergence mortality incited by pathogens. *Picea* and *Pinus* emergence percentages were similar in both soils. Most emerged seedlings died within weeks, with symptoms of diseases incited by soil- or seed-borne fungi. Although the timing of seedling emergence did not differ between soils, seedlings died more quickly on the goutweed soil, largely because of a faster development of post-emergence damping-off. Total post-emergence mortality of *Picea* and *Pinus* was greater on the goutweed soil. The

relative frequencies of the several symptoms exhibited by dying seedlings varied between the two soils, suggesting that the ground-layer species differentially affected the microbial community's composition or interactions with the seedlings. Symptom frequencies differed among tree species. Local spatial variation in herbaceous species composition appeared to produce patchy infectious lethality of soil for tree seedlings, an indirect effect that was observed after the herbaceous plants had been removed.

Introduction

The recruitment of forest tree populations from seed may be inhibited by herbaceous species or other ground-layer plants (Daniel et al. 1979, George and Bazzaz 2003, Maguire and Forman 1983). For tree species tolerant of overstory shade, this may limit advance regeneration. Suppression of tree seedling establishment by the ground layer could come about through competition for limiting resources, release of directly inhibitory allelochemicals, apparent competition - the ground layer's stimulation of other species that are the tree seedlings' natural enemies (Holt and Lawton 1994), or the ground layer's inhibition of other species that benefit the tree seedlings. Because apparent competition and inhibition of beneficial species are mediated by third parties, each represents an indirect interaction (Wooton 1994) between neighboring plant species.

An extreme degree of local dominance by the forb *Aegopodium podagraria* L. (goutweed) has been observed in one place where it was introduced into forest vegetation beyond its native Eurasian range. At the site of a former settlement in the southern boreal forest, Minnesota, USA, a 0.23-ha continuous cover of goutweed under an evergreen coniferous overstory was found to have low vascular plant richness and diversity, in comparison to that in an adjoining native forest ground layer (Garske 2000). Seedling and sapling densities of shade-tolerant coniferous trees were greatly depressed within the goutweed patch, which had existed for several decades before the data of Garske (2000) were obtained (Ahlgren and Ahlgren 1984). There were 3340 ha^{-1} of these juvenile trees amidst goutweed, vs. 18,200 ha^{-1} amidst the native ground layer (Garske 2000). All of the juvenile trees in the sample quadrats were the shade-tolerant *Abies balsamea* (L.) Miller. If we use 2000 trees ha^{-1} as a representative regional value for a fully stocked overstory of *Abies* and *Picea* with stem diameter greater than 10 cm (Bakuzis and Hansen 1965), this allows for little further mortality of juvenile trees in the goutweed patch before the future overstory may become discontinuous. Ahlgren and Ahlgren (1984) proposed that allelopathy accounted for the extreme dominance by goutweed on this site. We would add that goutweed appears to be an overbearing competitor for limiting light. We observed that goutweed produces a dense layer of closely abutting leaflets that is held higher than the leaves of conifer seedlings and most native forest herbs at the Minnesota boreal site, allowing goutweed to pre-empt photons

where light is scarce. A goutweed patch forms by lateral extension of rhizomes. Details on the structure and dynamics of goutweed clones are given by Gatsuk et al. (1980).

Our study attempted to discover whether herbaceous species, especially goutweed, differentially suppress tree recruitment through processes beyond resource competition. Because early seedling development is an especially vulnerable portion of the life cycle of many species, we focused on possible effects of the ground layer on seedling establishment through its influence on soil characteristics. The opportunity for a seedling to acquire soil nutrients generally has little influence on its early success at establishment (Harper 1977), so any ground-layer effects on nutrient availability may be assumed to have low direct importance for this phase of the seed-plant life cycle. Any observed influence on establishment by ground-layer conditioned soil could then be attributed to allelopathy or to indirect interactions. We tested the hypotheses that contrasting ground-layer composition influenced soil properties so as to affect the percentage of seedling emergence, the time course of seedling emergence, the percentage of post-emergence seedling survival, and the time course of post-emergence seedling survival.

Methods

Soil source

Because soil could not be brought promptly to the laboratory from the remote boreal site described above, soil was obtained from a human-modified northern deciduous forest site in Duluth, Minnesota, USA (46°48′ N 92°8′ W 400 m elevation), in the first week of June, 1994, after herbs had grown to nearly full size. One soil collection was made within a dense 220 m^2 patch of goutweed; the other soil was collected 1 - 2 m beyond the edge of the goutweed patch, with both collection areas on the same contour. Only goutweed grew in the ground layer where "goutweed soil" was collected. The "other soil" was collected near the non-native species *Phalaris arundinacea* L. and *Valeriana officinalis* L., where the ground-layer canopy was less continuous and extended over a much greater vertical range. Both ground-layer patches had a discontinuous overstory of *Populus balsamifera* L. about 8 m tall. Soil was removed by excavating surface blocks about 15 cm square and 7 - 10 cm deep, trimming off the outer 1 cm of soil from the cut edges of the block with a dull knife to exclude possible sap from wounded roots or rhizomes, then gently crumbling the remaining block. This was repeated until the total soil volume was about 0.05 m^3 from each type of ground layer. Evident stones, roots, rhizomes, and invertebrates were removed manually, and the remaining soil mixed by hand. Analysis of soil texture (hydrometer method) found the goutweed soil to be 70% sand, 9% silt, and 21% clay; the other soil was 65% sand, 11% silt, and 24% clay (USDA particle sizes). Soil pH (1:1 water), measured with a pH meter (SA720, Thermo Orion, Beverly, MA, USA), was 6.2 for the goutweed soil and 6.3 for the other soil.

Seed source

Seeds of the conifers *Abies balsamea*, *Picea mariana* (Miller) BSP, and *Pinus strobus* L. were obtained from the Minnesota Department of Natural Resources. Each of these species is highly dependent on seed for recruitment (Burns and Honkala 1990) and was common in the canopy at the boreal field site. The supplier reported the viabilities as 94%, 95%, and 91%, respectively. Each seed lot had been collected from a native population in Minnesota within 32′ latitude and 65 m altitude of the site from which the soil was taken. Seeds were stratified in moist paper towels at 5 °C for 60 days just before planting.

Germination experiment

Immediately after it was mixed, each soil was distributed into nine plastic flats (25 × 52 × 6 cm) having drainage holes, giving a soil depth of 3.5 cm. Both soils had a crumb structure, with aggregates up to a few mm in diameter. Seeds were sown immediately. Each flat received 48 seeds of each species in an 18 × 8 grid, with every seed being 3 cm from its nearest neighbors. We alternated the species at consecutive points in the planting grid. Seeds were covered with 3 - 6 mm of soil. We covered each flat with a transparent plastic dome that fit loosely enough to allow slight air exchange. Equal amounts of deionized water were added to each flat as a fine mist periodically throughout the experiment, which kept the soil moist but not wet enough to result in a visible sheen of liquid. Flats were placed on table tops with the two soils alternating in spatial sequence. The room was illuminated for 12 h per day by cool-white fluorescent ceiling lamps, and its temperature fluctuated within 21 - 26 °C. Counts of live and dead emerged seedlings were made daily for 45 days, except for day 44. Seedlings were not monitored individually. The covers were removed from the flats while observations were made. Each dead seedling was assigned to one category of symptoms based on Hartley et al. (1918): (1) classic damping-off (mechanical failure of the hypocotyl near the soil surface), (2) moldy shoot (visible mycelium enveloping the epicotyl and cotyledons or attached seed coat), or (3) top wilt (seedling erect and shriveled or discolored, but not visibly moldy). These symptoms are associated with infections by fungi (*sensu lato*). The date of death was the first daylight period when (1) the seedling had fallen over, (2) the mycelium had reached the shoot apex and base of the cotyledons, or (3) the cotyledons were shriveled or discolored to their base, respectively. Each dead seedling was left in place, and a 1-cm thread was laid on the soil next to it on the day of death to enable differentiation of new fatalities from old ones.

At the end of the experiment, instantaneous photosynthetic photon flux density (PPFD) was measured. A point quantum sensor (LI-190SA and LI-1000, LICOR, Lincoln, NE, USA) was held horizontally 15 cm in from each end of each flat and 4 cm above the soil surface, and the two readings per flat were averaged. The sample mean and 95% confidence interval (CI) PPFD was 6 ± 0.6 μmol m^{-2} s^{-1} for each kind of soil. For each flat the soil was then mixed, and a sample with a moist mass of about 250 g had its

water content determined gravimetrically (Θ_m) at the end of the experiment. The sample mean and 95% CI Θ_m was 0.33 ± 0.03 for the goutweed-soil flats and 0.35 ± 0.02 for the flats with the other soil.

Data analysis

The time-course of a population's germination or seedling emergence can be represented closely by a Weibull distribution (Brown and Mayer 1988). Weibull distributions can also be used to analyze disease progress in plants (Campbell 1998). We built Weibull models of seedling emergence and post-emergence mortality with SAS (SAS Institute 2004) (Appendix). The fit of the model function to the mean of the daily observations was tested with an approximation of the Komolgorov - Smirnov test that used a critical value (0.05 level) of $1.36/n^{0.5}$. From the model outputs we estimated the final mean proportion emerged, mean time to emergence of the 50th percentile of the sown seeds, mean time alive after emergence for the 25th percentile of emerged seedlings that died, and final proportion of emerged seedlings that survived. Standard errors and tests of significance were computed with 400 bootstrap runs. In accord with potential statistical dependence of plants in the same flat, bootstraps were obtained by randomly sampling flats with replacement, rather than individual plants.

Frequencies of mortality symptoms were compared between soils within species by multi-response permutation procedure (MRPP), and frequencies of symptoms were compared between species within soils by blocked MRPP, both using PC-ORD (McCune and Mefford 1999). In both types of analysis, counts were relativized per flat, and Euclidean distance and $n/\Sigma(n)$ weighting were used. For the blocked MRPP, median alignment was performed. Post-emergence days until death for each symptom was compared between soils within species with a *t*-test, using Statistix (Analytical Software 2003).

Results

Observations of cumulative seedling emergence and post-emergence mortality are summarized in Figure 1. The emergence trajectories were very similar between soils within species, being most gradual for *Abies* and most abrupt for *Picea*. The fit of the model functions (Table 1) to the means of the emergence observations was within the critical value of 0.065 for all species, soils, and days, with two exceptions: for *Pinus* on the other soil (soil 2), the difference between the model and the observed mean was 0.075 on day 12 and 0.089 on day 13. These were days when cumulative emergence was increasing especially rapidly, a situation anticipated by Brown and Mayer (1988). From model estimates of total emergence (p_e, Table 1), a greater fraction of *Abies* seeds developed into emerged seedlings on the goutweed soil than on the other soil (bootstrap $P = 0/400$). The fraction of *Picea* seeds that developed into emerged seedlings was not significantly different between the two soils, and this was also true for *Pinus*. *Picea* seedlings emerged earlier than the other two species. *Pinus* 50th-percentile

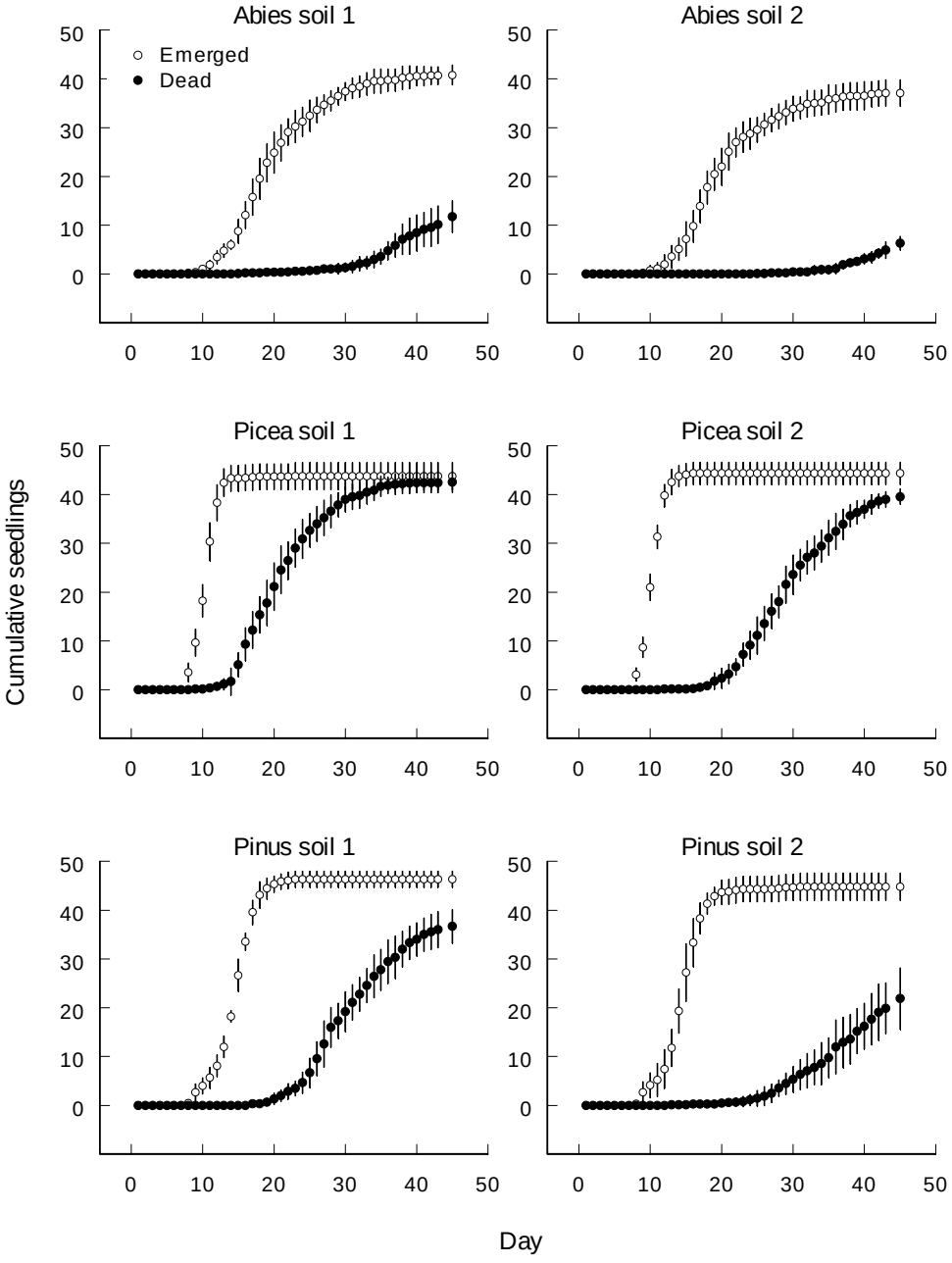

Figure 1. Cumulative seedling emergence (open circles) and cumulative mortality of emerged seedlings (solid circles) for *Abies balsamea*, *Picea mariana*, and *Pinus strobus* sown on two different forest soils in the laboratory. Soil 1 was from a patch of goutweed, soil 2 from other herbaceous species. There were 48 seeds of each tree species in each of nine flats per soil, sown on day 0. Bars represent ±1 standard deviation.

emergence occurred about 4 days later than that of *Picea*, and that of *Abies* occurred about 9 days later than that of *Picea*. Within species, the time of 50th-percentile emergence was very similar on both soils (Table 1).

The appearance of the post-emergence mortality trajectories (Figure 1) differs between soils within species, and among species within soils. Mortality began sooner on the goutweed soil. It began soonest and accumulated most rapidly for *Picea*, latest and most slowly for *Abies*. The fit of the models (Table 1) to the means of post-emergence mortality observations was within the critical value in every case. Total post-emergence survival (p_s) of *Picea* was only one-third as high on goutweed soil as on the other soil, a statistically significant difference (bootstrap $P = 0.015$) (Table 1). Total post-emergence survival of *Pinus* seedlings was four to six times as high as that of *Picea*. Total survival for *Pinus* was about one-half as high on goutweed soil as on the other soil; this seemingly large difference was not statistically significant, apparently because the delayed mortality on the other soil censored the data sufficiently to expand the standard error of the estimate. Similarly, the even later occurrence of mortality for *Abies* did not permit meaningful estimates of total post-emergence survival for that species (Table 1). Early post-emergence mortality occurred sooner on goutweed soil. The estimated time after emergence until the first 25% of total mortality was complete was shorter on goutweed soil by about 5 days (*Abies*) or 9 days (*Picea* and *Pinus*) (Table 1). Each of these between-soil differences was statistically significant (bootstrap $P = 0/400$).

All newly dead seedlings had symptoms of infections by fungi (*sensu lato*). Classic damping-off was the most frequent symptom for all three species on both soils, and was substantially more frequent on goutweed soil than on the other soil for both *Picea* and *Pinus* (Table 2). The between-soil trend for *Abies* was the same, but the effect size was smaller and the difference not statistically significant. On the other soil the lower frequencies of classic damping-off were balanced mainly by higher frequencies of moldy shoot for *Pinus* and of top wilt for *Picea*. For each of the three species-pair comparisons of frequency distributions, the effect size was less on the goutweed soil, although the difference between *Abies* and *Picea* was not statistically significant on either soil. The differences in frequency distributions were statistically significant on each soil for *Abies* vs. *Pinus* and for *Picea* vs. *Pinus*.

For each of the three species, mortality from classic damping-off tended to occur earlier on the goutweed soil than on the other soil (Table 3). The statistical significance of this is attributable in part to greater test power associated with much larger numbers of observations of this symptom, yet the between-soil absolute difference in mean days between sowing and death was also larger for classic damping-off than it was for the other two categories of symptoms for each of the species. Mean date of observed mortality from classic damping-off occurred earlier on goutweed soil by 3.3 days for *Abies*, 9.1 days for *Picea*, and 10.4 days for *Pinus*. Mean date of mortality of *Picea* from

Table 1. Estimated parameters (see Appendix) for seedling emergence and mortality. Soil 1 was from a patch of goutweed, soil 2 from other herbaceous species. Numbers in parentheses are standard error of the mean. Percentile emerged is days after sowing, percentile dead is days after emergence. For statistical significance, see text.

Parameter	Species and Soil					
	Abies soil 1	*Abies* soil 2	*Picea* soil 1	*Picea* soil 2	*Pinus* soil 1	*Pinus* soil 2
z_1	7.33	8.41	6.91	6.77	3.45	6.74
c_1	2.00	1.85	2.12	2.48	4.48	2.58
k_1	0.0708	0.0762	0.262	0.258	0.0835	0.117
z_2	0.000	2.06	1.35	3.08	6.83	0.000
c_2	2.68	4.14	1.72	2.63	1.53	3.16
k_2	0.0258	0.0269	0.0937	0.0548	0.0923	0.0366
Total emergence (p_e)	0.85 (0.014)	0.77 (0.018)	0.91 (0.018)	0.92 (0.016)	0.96 (0.011)	0.93 (0.018)
50th percentile emerged (days)	19.1 (0.35)	19.2 (1.02)	10.1 (0.10)	10.1 (0.038)	14.5 (0.10)	14.1 (0.26)
25th percentile dead (days)	24.3 (0.99)	29.5 (0.95)	6.26 (0.32)	14.9 (0.35)	12.4 (0.44)	21.7 (1.22)
Total post-emergence survival (p_s)	n.a.	n.a.	0.03 (0.012)	0.09 (0.019)	0.18 (0.034)	0.35 (0.13)

Table 2. Proportion of dead seedlings assigned to mutually exclusive categories of symptoms. Soil 1 was from a patch of goutweed, soil 2 from other herbaceous species. Means are followed by standard deviations in parentheses. *A* can be regarded as effect size in MRPP, *P* is probability of an equal or smaller delta.

Species and soil	Category		
	Classic damping-off	Moldy shoot	Top wilt
Abies soil 1	0.893 (0.141)	0.011 (0.033)	0.096 (0.117)
Abies soil 2	0.842 (0.142)	0.087 (0.112)	0.070 (0.086)
Picea soil 1	0.947 (0.038)	0.022 (0.020)	0.030 (0.024)
Picea soil 2	0.788 (0.087)	0.030 (0.024)	0.182 (0.078)
Pinus soil 1	0.886 (0.059)	0.090 (0.049)	0.026 (0.024)
Pinus soil 2	0.664 (0.072)	0.293 (0.085)	0.044 (0.050)

Abies, soil 1 vs. 2	$A = 0.009$, $P = 0.294$
Picea, soil 1 vs. 2	$A = 0.384$, $P < 0.000$
Pinus, soil 1 vs. 2	$A = 0.435$, $P < 0.000$
Abies vs. *Picea*, soil 1	$A = 0.036$, $P = 0.229$
Abies vs. *Picea*, soil 2	$A = 0.053$, $P = 0.167$
Abies vs. *Pinus*, soil 1	$A = 0.107$, $P = 0.043$
Abies vs. *Pinus*, soil 2	$A = 0.381$, $P = 0.003$
Picea vs. *Pinus*, soil 1	$A = 0.265$, $P = 0.012$
Picea vs. *Pinus*, soil 2	$A = 0.481$, $P = 0.002$

moldy shoot also occurred significantly earlier (6.6 days) on goutweed soil, despite moldy shoot having an earlier average mortality date than that of the other two symptoms on the other soil. The other symptom-species combinations had smaller differences between soils that were not statistically significant. There was only one moldy-shoot death of *Abies* on goutweed soil (day 35), with five *Abies* deaths (mean = 33.8 days) associated with that symptom on the other soil.

Discussion

Epidemiology

Post-emergence seedling disease involving mixed-species germination is a challenging system to analyze temporally. Seedling communities can be extremely dynamic, with the number of new susceptible hosts sometimes increasing rapidly and nonlinearly, while previously emerged hosts may either die or develop beyond the susceptible stage before all seedlings have emerged. These processes will often be asynchronous among species sharing the same seedbed. Although changing host

Table 3. Days from sowing until observed seedling death, by species, symptom, and soils. Soil 1 was from a patch of goutweed and soil 2 from other herbaceous species. Observations aggregated across all flats. Means followed by standard deviations in parentheses. Two-tailed Student's *t* (absolute value) followed by degrees of freedom in parentheses; decimal degrees of freedom indicate *t*-test based on unequal variances. *P* is probability of an equal or larger *t* by chance. *Abies* moldy shoot could not be tested because there was only one occurrence on soil 1.

Species and symptom	Soil			
	1 (days)	2 (days)	*t*	*P*
Abies classic damping-off	36.4 (5.6)	39.7 (4.2)	3.98 (117.0)	0.0002
Abies top wilt	32.3 (10.2)	32.8 (5.0)	0.08 (12)	0.9352
Picea classic damping-off	20.7 (5.8)	29.8 (6.2)	19.19 (639)	<0.0000
Picea moldy shoot	16.1 (3.7)	22.7 (3.6)	3.80 (17)	0.0014
Picea top wilt	21.9 (8.2)	25.0 (5.8)	1.31 (15.9)	0.2079
Pinus classic damping-off	29.9 (6.3)	40.3 (5.2)	12.58 (286.0)	<0.0000
Pinus moldy shoot	28.0 (3.7)	28.8 (4.2)	0.86 (86)	0.3909
Pinus top wilt	32.4 (4.8)	30.9 (8.4)	0.44 (15)	0.6675

abundance has been addressed in several ways in plant epidemiology generally (Campbell 1998), it appears that less has been done with seedlings and their diseases. We demonstrated one way (Appendix) in which the time course of disease may be analyzed when individual seedlings have not been monitored after time of emergence.

Because our data are not spatially explicit, we are unable to dissect the epidemics directly into primary and secondary infections, as done by Otten et al. (2003). A method (Filipe et al. 2004) that allows inference of primary vs. secondary infections from the temporal pattern of non-spatial data was validated with spatial data from a monoculture of hosts, all of which had emerged before they were challenged with a single type of pathogen. The relatively great temporal spread of emergence among our three species and the expected diversity of our pathogen communities (Cram 2003) would seem to limit the resolving power of that method for our data. Neher et al. (1987) found that less synchronous germination could either increase or decrease mortality from damping-off, depending on the host species. The earlier mortality on goutweed soil (Table 1) probably involved increased numbers of lethal primary infections, but we can draw no clear conclusions about secondary infections. Our 3-cm minimum distance between nearest-neighbor emergents is short enough to make secondary infections plausible in the laboratory (e.g., Burdon and Chilvers 1975).

The earlier mortality on goutweed soil seen for all three species in the laboratory could sometimes be more important in the field. If changing weather were to alter temperature or moisture so as to subsequently disfavor damping-off, then early

mortality may constitute a greater portion of total mortality. This could accentuate between-soil differences in total mortality. Even under the steady conditions of our laboratory, there was a positive association between earlier mortality and greater total mortality for *Picea*, and quite probably for *Pinus* as well (Table 1). Neher et al. (1992) likewise concluded that more rapid damping-off (both pre- and post-emergence) was positively associated with higher total laboratory mortality for seedlings. Pérez-Reche et al. (2012) found evidence that early mortality from soil-borne fungi was predictive of overall mortality. We suggest that this possibly general pattern merits further evaluation, using other hosts, soils, and conditions.

Interactions among pathogens and hosts

Earlier mortality in both *Picea* and *Pinus* was associated with large increases in classic damping-off's proportion of total deaths (Table 2). Through multiplying these proportions by total mortality (1 - p_s from Table 1), it can be seen that they also correspond to large increases in the absolute number of seedlings killed post-emergence on goutweed soil. Classic damping-off was also the symptom by which goutweed soil most accelerated death (Table 3). Pinaceae seedlings become more likely to escape mortality of this type as the outer tissues of their hypocotyls lose succulence during development (Cram 2003; Daniel et al. 1979). Younger seedlings are therefore more susceptible to classic damping-off. Because classic damping-off caused a large majority of the deaths (Table 2), the earlier mean time of death from it on goutweed soil (Table 3) could be used to infer that seedlings killed by classic damping-off were younger, on average, on that soil. Thus it would seem that the pathogens that incite classic damping-off were able, on the goutweed soil, to infect the hypocotyls earlier or weaken the hypocotyls to the failure point more rapidly after infection began. This succumbing to classic damping-off at a younger post-emergence age may explain the higher overall mortality that we observed for *Picea* and *Pinus* on goutweed soil, as fewer seedlings escaped the disease through further development. Although moldy shoot also tended to kill *Picea* earlier on goutweed soil (Table 3), it did not kill a higher proportion or total number of *Picea* seedlings in those flats (Table 2) and was only a minor source of mortality on either soil.

Earlier and more extensive mortality from classic damping-off on goutweed soil was associated with lower frequencies (as well as reduced absolute numbers) of deaths from moldy shoot (*Pinus*) or top wilt (*Picea*) (Table 2). These latter symptoms did not tend to have notably later mean dates of mortality than classic damping-off did (Table 3). Thus the lower frequencies of these other symptoms on goutweed soil can not be attributed to overall host pre-emption by classic damping-off, although they could result from pre-emptive mortality in any subpopulation of seedlings that is more vulnerable to these latter symptoms. Between-soil differences in the dynamics of the microbial community may explain the altered frequencies. The contrast in between-soil differences in symptom frequencies for *Pinus* vs. *Picea* (Table 2) indicates that the

interaction between the microbial community and the seedlings varied between soils in unequal ways for different host species. The most frequent occurrence of moldy shoot was, by far, on *Pinus* on the other soil (Table 2). We hypothesize that *Pinus* may be more susceptible to moldy shoot because its cotyledons may be slower to shed the seed coat and diverge. As compared to our other two species, a *Pinus strobus* seedling has 1.5 - 3 times as many cotyledons, which are 2 - 6 times as long and have serrate instead of smooth margins (Ahlgren and Ahlgren 1958).

The lower total emergence for *Abies* from the other soil (Table 1) could be ascribed to pre-emergence damping-off or to allelopathic inhibition. We would expect chemical inhibition to delay emergence, given that Lehle and Putnam (1982) found that germination was delayed even when chemical concentrations were too low to reduce total germination. Because the time for 50th-percentile emergence was the same on both soils (Table 1), it seems more likely that unequal frequencies of pre-emergence damping-off explain this modest but statistically significant difference in total emergence. An experiment that prevented pre-emergence infections would be necessary to test this hypothesis more rigorously. Interestingly, this higher pre-emergence mortality was in the soil that was associated with lower post-emergence mortality, another indication of between-soil differences in the microbial community. *Abies* was the slowest-emerging of the three species (Table 1), which lengthened its time of exposure to pre-emergence mortality from pathogens. Its total emergence from either soil was notably less than the 94% viability reported by the seed supplier, so there may have been detectable pre-emergence damping-off in both soils if the viability report was accurate. The total emergence proportions for *Picea* and *Pinus* were close to the reported viabilities, suggesting that pre-emergence damping-off or chemical inhibition were nearly nil for these species. We would expect the lengthy residence of seeds in soil in the forest from autumn/winter dispersal until spring/summer germination to raise the rates of pre-emergence damping-off in the field above the frequencies that we observed in our briefer experiment.

Processes generating ground-layer effects

Damping-off is favored by low shortwave irradiance, high humidity, and wet soil (Rotem 1978). The photosynthetic photon flux density (PPFD) of the seedling environment in our laboratory was very low (0.26 mol m^{-2} d^{-1}) and would be expected to be stressful to inadequate for seedling survival, as this number was similar to mean values in a neutrally shaded greenhouse that increased mortality in seedlings of *A. balsamea* and *P. mariana*, but not *P. strobus* (Walters and Reich 2000). Like we did, Walters and Reich (2000) observed more damping-off in *Picea* than in the other two species. Garske (2000) found June mid-day instantaneous PPFD beneath the goutweed canopy at the boreal field site to be only one-third that of our laboratory levels, with PPFD just above the boreal goutweed canopy at 4.5 times our laboratory levels. In addition, mean saturation deficit of air just above the soil surface at that field site was

3.5 g m^{-3} under goutweed during the same afternoon that it was 9.7 g m^{-3} in the native ground layer (Garske 2000), which was comparatively open like that where our other experimental soil was obtained. This deeper shade and higher humidity at the soil surface would reinforce goutweed's promotion of post-emergence damping-off via the persistent influences on soil that we observed in the laboratory.

If our results prove to be representative for goutweed patches more generally, how might a persistent effect of goutweed on soil pathogenicity develop? Packer and Clay (2004) found that the damping-off of a seedling raised the risk of primary damping-off for seedlings planted in the same soil immediately thereafter. The shade and humidity beneath a goutweed canopy that should promote damping-off may similarly leave the soil there more lethal for subsequent seedlings. As Packer and Clay (2004) point out, this increased lethality could come about through an increase in pathogen inoculum density, a decrease in activity of antagonists to the pathogens, or natural selection for greater pathogen virulence. Goutweed could also improve the soil's chemical quality for the pathogens. Fresh dead organic matter can enhance or suppress damping-off, depending on whether it preferentially promotes saprophytic growth of the damping-off pathogens or of their antagonists (Martin and Loper 1999). Wall (1984) found that addition of nutrient-rich dead plant matter promoted damping-off of *Picea mariana* and *Pinus banksiana* Lambert, but that adding nutrient-poor dead plant matter had no effect. Goutweed produces relatively high amounts of herbage (Garske 2000), and we observed its litter to decay rapidly, which suggests that it is nutrient-rich. Thus one plausible explanation is that goutweed litter favors saprophytic growth of damping-off pathogens. Martin and Loper (1999) generalized that, at least for the *Pythium* pathogen group, the incidence of damping-off in the field is more likely related to the success of saprophytic growth of the pathogen than it is to inoculum density.

A further, non-exclusive hypothesis is that goutweed soil could increase seedling susceptibility to infection. For example, Patrick and Koch (1963) and Tousson and Patrick (1963) inferred that allelochemicals in soil made plants more susceptible to root rot. Allelochemicals from goutweed could be hypothesized to directly increase susceptibility of conifer seedlings to post-emergence damping-off, perhaps by slowing the hardening of the hypocotyl or by increasing the leakage of metabolites from the seedling. The chemicals could also act indirectly on seedlings if they stimulated pathogens or inhibited soil biota that are beneficial to seedlings. Whitehead et al. (1982) analyzed a soil supporting a pure stand of goutweed for phenolic substances and found that it did not appear to have especially high concentrations or unusual combinations of specific compounds. Phenolics, of course, are not the only type of compounds that may be allelochemically operative.

Our results show some parallels to the findings of Wardle (1959). He observed that post-emergence damping-off of *Fraxinus excelsior* tree seedlings in the forest was

much more common within dense patches of *Mercurialis perennis* than it was where the herbage of this forb had been trimmed away or where other species dominated the ground layer; densities of newly emerged *Fraxinus* seedlings were not affected by this ground-layer variation. *Mercurialis* forms a dense and relatively tall canopy early in the growing season (Hutchings and Barkham 1976), similar to what we observed for goutweed. Wardle (1959) measured deep shading by the *Mercurialis* canopy, yet *Fraxinus* seedlings survived well, albeit in a suppressed condition, under even deeper shade as long as *Mercurialis* was absent. Our results indicate that the promotion of damping-off by a plant can be brought about not only by an immediate effect of the plant on physical factors near the soil surface, but also through an effect on the soil that persists for at least a few weeks beyond the presence of the plant, whereas Wardle (1959) did not report a persistent effect. No visible litter remained in the soil that we used for the experiment, distinguishing our results from those (e.g., Daniel and Schmidt 1972) that demonstrate lethality of some kinds of litter to seeds.

Spatial patterns of damping-off may also be associated with the locations of seeds and seedlings relative to mature conspecifics (e.g., Packer and Clay 2000). Our soils came from a site where the species of the seeds we used were not present in the vegetation, eliminating this potentially confounding variable. Wardle (1959) apparently did not consider overall dispersion of the host (*Fraxinus*), but his research has the virtue of including multiple examples of one ground-layer patch type (*Mercurialis*). Our work and that of Wardle (1959) can be viewed as the beginnings of evidence that herbaceous mosaics can generate sizable short-distance variation in fatal disease risk and pathogen-host dynamics for tree seeds or young seedlings. More complex studies, which would examine multiple examples of selected ground-layer patch types and at the same time account for dispersion of host species, would be of benefit. For now, it appears that spatial heterogeneity of damping-off within a plant community may result not only from dispersion of a host species, but also from the distribution of other plant species. These indirect effects illustrate that there can be apparent competition between ground-layer and tree species, in addition to resource competition.

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Appendix

To model emergence times, we defined p_e to be the probability that a seed is viable and would eventually become an emerged ("live") seedling. Let S be the random variable for emergence time. The cumulative emergence distribution function is $F(t) = P(S \leq t) = 1 - \exp(-(k_1(t - z_1))^{c1})$ for $t > z_1$ where k_1 estimates the rate of increase of

emergence for the population once emergence has begun, z_1 estimates the time lag between sowing and the emergence of the first seedling, c_1 is a shape parameter for the time-course curve, and t is time since sowing. The corresponding probability density function is $f(t) = c_1 k_1^{c1} (t - z_1)^{c1-1} \exp(-(k_1(t - z_1))^{c1})$ for $t > z_1$. For a seedling that emerges on day t, the contribution of this datum to the likelihood when estimating the parameters is the probability that the seedling will eventually emerge and the probability that the observed emergence is on day t. Let D be the day of emergence. For a seed that will eventually emerge, the probability of its emergence on day t is $P(D = t \mid \text{viable}) = F(t) - F(t - 1)$. For finding maximum likelihood estimates, the contribution of an emerged seedling to the likelihood is Lik(observed emerged) = P(viable) $P(D = t \mid \text{viable}) = p_e[F(t) - F(t - 1)]$ since seedlings are observed and recorded once daily. An advantage of using this probability of an observed day in the likelihood rather than the density function is that the density function approaches infinity for $c_1 < 1.0$ at $t = z_1$. For seedlings that have not emerged by the last day of observation, the likelihood has two components: (1) the probability that the seedling would never emerge and (2) the probability that the seedling would eventually emerge but had not done so by the last day of observation. Let T_f be the final observation time. Lik(not emerged) = $1 - p_e + p_e[1 - F(T_f)]$. The loglikelihood for all emergence information is LogLik (emerged) = $\Sigma\log$[Lik(observed emerged)] + $\Sigma\log$[Lik(nonemerged)].

For the times until death the complication is that we do not know which emergence time goes with which time of death. Hence we don't know the time from emergence until death for each individual seedling. The time of observed death is the sum of the emergence time (D) plus L, the post-emergence time alive. The post-emergence survival time can only be estimated in aggregate, based on the observed distributions of emergence time (D) and total time until death ($D + L$). We modeled L with a Weibull distribution with parameters k_2, z_2 and c_2. Only seedlings that emerged before the last observation day give us information about L. Let $G(t)$ and $g(t)$ be the cumulative distribution function and density function for L. For seedlings that emerged before the last day, the distribution is a truncated Weibull: $G(t) = P(L \leq t \mid \text{emerged}) = G(t)/G(T_f)$.

The probability that $D + L$ is less than or equal to some given t is a convolution of the distributions of D and L: $H(t) = P(D + L \leq t \mid \text{emerged}) = \int_{z_1}^{t - z_2} f(x)G(t - x)dx$. The limits on the integration are determined by where the product is nonzero. We cannot write this integral as an explicit function, so we numerically approximate the integral. We partition the interval z_1 to $t - z_2$ into $x_0 = z_1$, $x_1 = x_0 + \in x$, $x_2 = x_1 + \in x$, $\rfloor$, $x_n = t - z_2$. We chose Δx, the width of an interval, to be about one day by letting n equal round($t - z_2 - z_1 + 1$). We approximated $f(x)\Delta x$ with $F(x_{i+1}) - F(x_i)$. This again avoids the problem of an unbounded density function. $H(t)$ is estimated by $\Sigma(F(x_{i+1}) - F(x_i))G(t - (x_i + x_{i+1})/2)$.

Some individuals develop beyond the susceptible stage without succumbing to these maladies that are specific to post-emergence seedlings. Let p_s be the probability that an emerged seedling survives the post-emergence period of risk. We observed only part of the post-emergence risk period, the part until day T_f. For an observed death at day t, the probability is Lik(observed death) = $(1 - p_s)$ [H(t) - H(t - 1)]. A live emerged seedling at the end of observation occurs if either (1) the seedling would survive the whole post-emergence risk period or (2) the seedling would not survive the whole post-emergence risk period but its time of death occurred after T_f. For a surviving seedling the likelihood contribution is Lik(unobserved death) = $p_s + (1 - p_s)(1 - H(T_f))$. The loglikelihood of the death times is LogLik(death) = Σlog[Lik(observed death)] + Σlog[Lik(unobserved death)]. If we assume that the lifetimes of seedlings and the emergence times of seedlings are independent, then the loglikelihood of all data is LogLik(emergence) + LogLik(death). The goal is to maximize this loglikelihood over the parameters p_e, k_1, z_1, c_1, p_s, k_2, r_2, and c_2. This maximization was accomplished using SAS (SAS Institute 2004) procedure NLMIXED using its general likelihood capability.

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