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LOGS AS REFUGES FROM FUNGAL PATHOGENS FOR SEEDS OF EASTERN HEMLOCK (*TSUGA CANADENSIS*)

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Abstract. The importance of fallen logs as recruitment sites for seedlings of some forest trees commonly is explained by the hospitable physical conditions such nurse logs provide. We used fungicide treatments to test whether logs also provide a refuge from pathogenic soil fungi for seeds of eastern hemlock (*Tsuga canadensis*). We tested two seedlots. For one seedlot, fungicide significantly improved survival of seeds in the soil, while seeds in logs were unaffected. This suggests that logs provide a refuge from pathogenic soil fungi. For the second seedlot, fungicide did not significantly improve survival either in logs or in soil, even though survival of control seeds was significantly lower in soil. This suggests that factors other than fungi must contribute to the success of seedlings on logs. Together, these results indicate that logs provide both physical and biotic benefits to seeds of eastern hemlock. When pathogens are important, logs provide enemy-free space; when risks from pathogens are low, logs still provide an improved physical environment.

Key words: eastern hemlock; forests; fungal pathogens; nurse logs; seed mortality; Tsuga Canadensis.

INTRODUCTION

Within forested ecosystems, regeneration of some tree species is linked to the presence of ''nurse'' logs, which act as foci for the establishment of seedlings (Burns and Honkala 1990*a*, *b*). For instance, the highly shade-tolerant conifer, eastern hemlock (*Tsuga canadensis* (L.) Carrière), commonly germinates and establishes on decaying logs or tree stumps (Ward and McCormick 1982, Godman and Lancaster 1990, Anderson and Gordon 1994). Several mechanisms have been proposed to explain this pattern. Increased moisture and decreased moisture fluctuations in decaying logs may reduce desiccation of the sensitive seeds and seedlings of this species, in comparison with the drier forest floor (Tubbs 1996). Alternatively, the seedlings of this small-seeded species may be less likely to be smothered by leaf litter on the bare surfaces of decaying logs and stumps than on the ground (Corinth 1996). Other advantages of logs may include a warmer microhabitat (Godman and Lancaster 1990) and freedom from competition from faster growing sugar maple (*Acer saccharum*) seedlings (Tubbs 1996). Here, we provide experimental evidence of another proposed mechanism (Zhong and van der Kamp 1999): logs may provide a refuge from pathogenic soil fungi.

Losses of seeds and seedlings to soil-borne pathogens are better documented in agricultural systems (e.g., Burdon and Shattock 1980, Alexander 1992, Agrios 1997) than in natural soils. Still, the existing evidence suggests that many species suffer considerable losses of seeds and seedlings to pathogens in natural soils as well. For example, work in tropical forests has documented significant losses of seedlings and buried seeds to fungal pathogens (Augspurger 1983, Augspurger and Kelly 1984, Dalling et al. 1998). Similarly, Packer and Clay (2000, 2003) found substantial impacts of soil pathogens on seedlings of the temperate forest tree, *Prunus serotina*. Other studies have demonstrated losses of seeds to soil fungi in such disparate habitats as northern Australian shrublands (Lonsdale 1993), English grasslands (Leishman et al. 2000), and Ontario old fields (Blaney and Kotanen 2001, 2002). The presence in seeds of physical and chemical mechanisms that inhibit fungal attack (Howe and Vande Kerckhove 1981, Halloin 1983, Hendry et al. 1994) also suggests that fungal pathogens are an important source of seed mortality in many natural systems.

The pathogens primarily responsible for these losses probably include both parasitic ''damping-off'' organisms (e.g., Packer and Clay 2000) and opportunistic

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soil fungi (e.g., Crist and Friese 1993, M. Schafer and P. M. Kotanen, *unpublished manuscript*). Neither of these groups is likely to be prominent in decaying wood. Only a limited number of specialized fungi are able to break down wood, which is both nutrient-poor and largely composed of chemically recalcitrant cellulose and lignin; the ability to break down lignin is especially unusual (Zabel and Morrell 1992). Wood chemicals also may have a fungitoxic or fungistatic effect (Boddy 1992). These constraints lead to significant differences between fungal communities in soil and those in wood (Boddy 1992).

In this study, we investigated the extent to which escape from seed-attacking soil fungi may contribute to the success of eastern hemlock seeds on logs. We accomplished this by monitoring the survival of known numbers of seeds inserted into both logs and the forest floor. These seeds were either treated with fungicide or with water as a control. We predicted that if logs serve as a refuge from soil fungal pathogens, then fungicide should improve survival of seeds on the forest floor more than it improves survival on logs. If logs do serve as a refuge from pathogens, this would support an unconventional explanation for the dependence of species such as eastern hemlock on logs for recruitment. Ultimately, the lack of suitable nurse logs could result in declines in pathogen-sensitive species.

METHODS

Study site

This experiment was conducted in mature forest located within the 350-ha University of Toronto Koffler Scientific Reserve at Jokers Hill, Regional Municipality of York, Ontario (44°02′ N, 79°31′ W). This forest is dominated by sugar maple, although eastern hemlock, American beech (*Fagus grandifolia*), and eastern white pine (*Pinus strobus*) are also common. Within the forest, two substrate types were used: fallen logs and the forest floor. Sixteen replicates (sites), each with one log and one adjacent forest floor plot, were used. Forest floor plots were situated within 5 m of each log. Logs were chosen only if they were sufficiently decomposed to allow insertion of seeds. Each log was identified to species. Soil and log samples for measuring moisture content were collected from each site following at least 24 h of dry weather. The soil and wood were weighed, dried for 3 d at 50°C, and then reweighed.

Susceptibility to fungal pathogens

Two different seedlots of eastern hemlock were used in this experiment. Both seedlots were bulk-collected from multiple trees. One seedlot was gathered by the authors at Jokers Hill in the fall of 2000 and subsequently stored frozen. However, since the effects of this storage method on viability were unknown, a second, highly viable seedlot donated by The National Tree Seed Centre (Canadian Forest Service, Fredericton, New Brunswick, Canada) also was used. These seeds were collected near Petawawa, Ontario (46°00′ N, 77°27′ W), in 1989, and subsequently were stored frozen. Henceforth, these two seedlots are referred to as the Jokers Hill seedlot and the Petawawa seedlot. These source populations are separated by ∼270 km.

Twenty seeds of each seedlot were suspended between two pieces of nylon stocking stretched within a plastic slide mount (Polaroid 35 mm slide mounts, Polaroid Corporation, Cambridge, Massachusetts, USA), for a total of 64 slide mounts/seedlot. This technique allows good seed recovery, while also enabling seeds to be in close contact with the soil and soil organisms. Slide mounts were subjected to one of two treatments: (1) control, in which the slide mount was saturated in water; and (2) fungicide, in which the slide mount was saturated in a fungicide solution. The fungicide used was Maestro 75DF (active ingredient Captan 75% by weight; Zeneca Corporation, Stoney Creek, Ontario, Canada). The fungicide was diluted in water to a concentration of 1:100, as recommended by the manufacturer for use as a dip for bulbs and tubers. Captan is a nonsystemic heterocyclic nitrogen fungicide that was chosen because of its effectiveness in controlling a large number of fungi in the Oomycota, Ascomycota, and Basidiomycota (Sharvelle 1961, Torgeson 1969, Neergaard 1977), and in particular, for its control of seed-rotting fungi (Neergaard 1977). It has minimal effects on endomycorrhizal fungi, while its effects on ectomycorrhizae are mixed (Trappe et al. 1984) and species dependent (Vyas 1988). In addition, we performed laboratory trials indicating that Captan did not affect germination of eastern hemlock seeds in sterilized soil ($P > 0.5$).

Slide mounts were placed in the field in October of 2001; early in the annual cycle of seed dispersal, as dispersal occurs throughout the fall and winter for this species (Godman and Lancaster 1990). Each log or adjacent forest floor location received four slides (two per seedlot, each of which included one fungicide-treated slide mount and one control slide mount). Slits large enough to accommodate a single slide mount were made in each log using a knife, in a line 0.5 m apart and at least 0.5 m from the end of each log. Slide mounts were inserted so that the top edge was just visible. Slides were inserted into the forest floor in a similar manner. During the burial period, fungicide and water were applied monthly, unless snow cover prohibited application, for a total of four applications. Either 10 mL of water or fungicide solution, as required, was applied to each slide mount using a needleless syringe.

Treatment of seeds following retrieval from the field

In May of 2002, the slide mounts were recovered from each site and brought to the lab for processing. Although seed germination may occur until the end of July in the northern part of eastern hemlock's range (Fowells 1965), at Jokers Hill abundant germination was observed to occur prior to the end of May. In the laboratory, the slide mounts were opened, and germinated seeds were counted and removed. The remaining seeds were spread over potting soil in plastic cell packs (40 × 47 × 55 mm deep), one slide mount per cell, and placed in the greenhouse. Cells were kept moist and the top 1 cm of soil stirred monthly to prevent moss buildup and to bring any seeds that had become buried to the surface. Cells were rotated randomly on a weekly basis. Seeds were checked weekly for 3 mo, and germinated seeds were counted and removed. At the end of the germination period, ungerminated seeds were recovered by placing the pot contents into a sieve and rinsing with water to remove soil.

Viability testing of ungerminated seeds

To determine viability of ungerminated seeds at the end of the study period, seeds were treated with tetrazolium chloride, which stains living tissue red (Hendry and Grime 1993). Up to 5 ungerminated seeds per slide mount were randomly selected for staining, as available. Since no specific protocol for eastern hemlock could be found, the methods determined by the International Seed Testing Association (1997) for treating eastern white pine seeds were used.

Statistical analysis

The principal variables analyzed were the proportion of seeds that germinated in the field, the proportion of ungerminated seeds that germinated in the greenhouse, and the proportion of residual ungerminated seeds deemed viable by tetrazolium staining. Prior to analysis, these proportions were normalized by arcsin square-root transformation (Kirk 1982). A standard randomized block factorial ANOVA design (Kirk 1982) was used to investigate substrate and treatment effects with field sites used as the blocking factor, and Type III sums of squares were used throughout. Substrate moisture and the effect of log species on germination were analyzed using simple, one-way ANOVAs. The Tukey-Kramer HSD test was used for all a posteriori comparisons ($P < 0.05$). Degrees of freedom vary because a few replicates were not recovered, and because seed germination occasionally left too few seeds for further testing. Means are reported (±1 SE).

RESULTS

The decaying logs used comprised four tree species: six eastern hemlock logs, five sugar maple logs, four white birch (*Betula papyrifera*) logs, and one eastern white pine log. Log type had no effect on field germination for either the Jokers Hill seedlot ($F_{3,7} = 0.81$, $P = 0.53$) or the Petawawa seedlot ($F_{3,7} = 1.22$, $P = 0.37$). Moisture differed between substrates ($F_{1,30} = 318.87$, $P < 0.0001$): logs were significantly wetter (63.0% ± 0.5%) than forest soil (15.5% ± 0.5%).

Susceptibility to fungal pathogens

A preliminary ANOVA including both seedlots indicated a significant three-way (seedlot × treatment × substrate) interaction ($F_{1,100} = 5.35$, $P < 0.05$). This result means that the seedlots responded differently to the same combinations of fungicide and substrate. Therefore, all subsequent analyses treated each seedlot separately.

Field germination.—The proportion of Jokers Hill seeds germinating in the field differed between substrates: significantly more seeds germinated on logs than in the forest soil (48.6% and 27.8%, respectively; Table 1a, Fig. 1a). Fungicide addition did not significantly affect field germination, although there was a tendency towards higher germination in fungicide treatments for both log and forest floor substrates ($P = 0.22$; Table 1a, Fig. 1a). There was no significant interaction between substrate and treatment (Table 1a). In contrast, the proportion of Petawawa seeds germinating in the field did not differ on average between substrates or between treatments (Table 1b, Fig. 1b), though a significant interaction between substrate and treatment was detected (Table 1b, Fig. 1b). A posteriori analyses indicated that the germination of seeds on logs did not differ between treatments: germination was similar for seeds in the control treatment and the fungicide treatment (70.0% and 65.5%, respectively). However, there was a difference in germination between treatments on the forest floor: germination was significantly greater for seeds in the fungicide treatment than in the controls (79.4% and 49.3%, respectively; Table 1b, Fig. 1b).

Greenhouse germination and viability testing.—Following field germination, the mean number of seeds per replicate available for placement in the greenhouse was 10.89 ± 0.16 and 8.66 ± 0.14 from forest floor and log substrates, respectively. These figures were only slightly reduced following greenhouse germination: 10.83 ± 0.16 and 8.58 ± 0.14 seeds subsequently remained for tetrazolium staining from the forest floor and log substrates, respectively.

For both seedlots, neither fungicide addition nor substrate had a significant effect on germination in the

TABLE 1. Results of randomized-block factorial ANOVAs of viability of *Tsuga canadensis* seeds.

	Germination				Viability of ungerminated seeds	
	Field		Laboratory			
Factor	df	*F*	df	*F*	df	*F*
a) Jokes Hill						
Substrate	1	13.351***	1	1.360	1	1.108
Treatment	1	1.553	1	3.195	1	2.992
Substrate × treatment	1	0.001	1	0.151	1	0.026
Error	42		41		38	
b) Petawawa						
Substrate	1	0.119	1	0.047	1	0.116
Treatment	1	3.241	1	0.471	1	1.974
Substrate × treatment	1	8.671**	1	1.412	1	0.006
Error	43		39		32	

Notes: ''Substrate'' refers to the habitat in which seeds were placed (log or forest floor); ''treatment'' refers to application of fungicide or water (control). Site was treated as the blocking factor. All factors were tested over the residual error term, using error df as the denominator df.

** $P < 0.01$; *** $P < 0.001$.

greenhouse (Table 1a, b). Similarly, tetrazolium testing indicated that the viability of seeds recovered at the end of the germination period did not vary between substrates or treatments for either seedlot (Table 1a, b), though there was a tendency for greater viability of fungicide-treated seeds ($P = 0.09$ and $P = 0.17$, for Jokers Hill and Petawawa seedlots, respectively).

Laboratory germination and tetrazolium results indicate that the vast majority of viable seeds germinated in the field. For both seedlots, greenhouse germination was extremely low (<1.0% of seeds that did not germinate in the field) in contrast to high field germination (66.0% for Petawawa seeds and 37.7% for Jokers Hill seeds). Similarly, tetrazolium staining of ungerminated seeds indicated that their viability was low (<10.0% for the Jokers Hill seeds and <15.0% for the Petawawa seeds). Therefore, patterns of germination in the field provide a far more complete and accurate assessment of patterns of fungal attack than greenhouse or tetrazolium tests.

DISCUSSION

Fungicide improved the survival of Petawawa seeds on the forest floor, but not on logs, indicating that fungal pathogens caused significant mortality only to seeds buried in the ground. As well, survival of untreated seeds on logs was similar to survival of fungicide-treated seeds in soil. These results suggest that for this seedlot, logs are indeed providing a refuge from fungal pathogens. In contrast, Jokers Hill seeds were not significantly affected by fungicide in either substrate, though there was a trend toward higher germination of treated seeds. The increased germination of this seedlot on logs is therefore likely to be the result of factors other than protection from fungal pathogens, possibly the higher moisture content of logs. It also may be that the higher absolute germination of Petawawa seeds compared to Jokers Hill seeds led to a greater power to detect treatment effects. Conservatively, our results indicate that for those seedlots at risk of attack by fungi, logs provide a refuge.

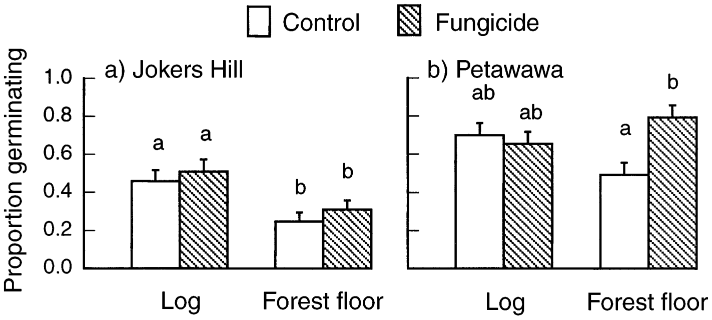

FIG. 1. Germination of eastern hemlock seeds subjected to two treatments (control [water] and fungicide) in two different substrates (decaying logs and the forest floor). Data indicate mean proportion of seeds germinating in the field + 1 SE. Bars sharing the same letters are not significantly different ($P > 0.05$).

For seedlings of some small-seeded trees, including eastern hemlock and yellow birch (*Betula alleghaniensis*), logs are believed to provide a refuge from such hazards of the forest floor as competition, smothering by litter, and desiccation (Erdmann 1990, Anderson and Gordon 1994, Corinth 1996). Such factors generally are used to explain the importance of nurse logs for the regeneration of these species; for example, within one stand in the Upper Peninsula of Michigan, coarse woody debris accounted for 57% of eastern hemlock regeneration, while only covering 10% of the forest floor (Corinth 1996). Our results indicate logs also can provide seeds of eastern hemlock with a refuge from pathogenic soil fungi, a result that may apply to other tree species as well. For instance, Zhong and van der Kamp (1999) found that seeds of Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) buried in undisturbed forest soil were less viable than those buried in either logs or mineral soil, and further demonstrated that this loss of viability was correlated with the incidence of fungal seed pathogens. Unlike our study, however, they did not use fungicides to directly demonstrate that these pathogens were responsible for the low viability of seeds in the forest floor.

Seeds of eastern hemlock are attacked by at least seven species of fungi, including both internally borne and soil fungi (Godman and Lancaster 1990). Some, such as *Botrytis* spp. (LeMadeleine 1980) or certain damping-off pathogens (Goerlich and Nyland 2000) can kill seeds or delay germination, whereas others, such as *Rhizoctonia*, attack soon after germination (Ruth 1974). Logs are unlikely to protect against internally borne pathogens, but may offer protection against both pre- and post-germination pathogens present in the external environment. Initial colonization of wood in contact with the ground is by common soil and litter moulds such as *Fusarium* and *Penicillium* (Dix and Webster 1995), but specialist saprophytic wood decay fungi soon replace these soil fungi (Rayner and Boddy 1988, Zabel and Morrell 1992). Since eastern hemlock seedlings establish on wood in the latter stages of decay, such as the logs used in our experiment, soil pathogens are unlikely still to be present.

There are a number of possible explanations for the different responses of the two seedlots to fungicide. For example, the two parent hemlock populations may have differed in their resistance to pathogens. Alternatively, differences in age, collection procedure, screening, and storage may have led to variation in seed chemistry, dormancy, and viability between the two seedlots. Such variation might have led to differences in susceptibility to pathogens. As an example, the greater germinability of Petawawa seeds may have increased their susceptibility to fungal attack, since exudates released throughout germination can stimulate attack by fungi (Garrett 1970, Burdon 1987). Regardless of their explanation, differences between these two populations do not alter the conclusion that, for susceptible seeds, logs act as refuges from fungal pathogens.

Our work provides further evidence that logs play an important role in the ecology of eastern hemlock, as well as other forest trees. This interaction may have consequences beyond those already recognized for recruitment. For example, the lack of coarse woody debris in young or managed forests effectively may increase the susceptibility of hemlock populations to diseases that are insignificant in more structurally diverse settings. As a result, the absence of suitable nurse logs may not just reduce recruitment, but may also enhance the importance of natural enemies of this species, altering population dynamics and favoring more disease-resistant genotypes.

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