

Losses of seeds of temperate trees to soil fungi: effects of habitat and host ecology

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Received: 17 June 2005 / Accepted: 22 February 2006 / Published online: 28 March 2006
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Abstract Evidence suggests that impacts of fungal pathogens on tree recruitment tend to be greater in the forest understory than in openings, and that shade-tolerant trees are less vulnerable than shade-intolerant species. To investigate the role that harmful soil fungi may have in reducing regeneration of temperate trees, we applied fungicide to buried seeds of matched pairs of species differing in their relative shade tolerance and/or successional status (*Acer negundo* versus *Acer saccharum*, *Prunus virginiana* versus *Prunus serotina*, and *Pinus strobus* versus *Tsuga canadensis*), in three habitats that differed in their degree of openness (old field, forest gap, and forest understory). Our results indicated that soil fungi reduced germination of *A. negundo*, *P. virginiana*, *P. serotina*, and *T. canadensis*, and reduced viability of ungerminated seeds of *P. strobus*; no significant effects of fungi on seeds of *A. saccharum* were detected. However, we found seeds were not less likely to survive following burial in forest understory than in gaps. As well, results for only one species pair (*A. negundo* versus *A. saccharum*) were consistent with the prediction that shade-intolerant or successional species should be more susceptible to fungal attack than mature forest species. These results contrast with other studies of temperate and especially tropical forest trees.

Keywords Forest gaps · Forest understory · Old fields · Pathogenic fungi · Seed banks

Introduction

The ecology of seed pathogens has been little studied in comparison with such other sources of seed mortality as predation, drought, and senescence (Kremer 1993; Chambers and MacMahon 1994; Baskin and Baskin 1998; Thompson 2000; Gilbert 2002). Despite this, studies of both seeds (Forget 1997; Dalling et al. 1998; Kitajima and Augspurger 1989; Tomita et al. 2002; O'Hanlon-Manners and Kotanen 2004a, b) and seedlings (Augspurger 1983, 1984; Augspurger and Kelly 1984; Kitajima and Augspurger 1989; Stanosz 1994; Forget 1997; Wenny and Levey 1998; Masaki and Nakashizuka 2002; Packer and Clay 2000, 2003; Reinhart et al. 2003; Reinhart and Callaway 2004) indicate that pathogens may have substantial impacts on patterns of regeneration in natural systems. For instance, the tendency of many forest trees to recruit primarily in gaps may in part reflect differences in the impacts of pathogens between openings and the surrounding forest (Roberts and Gilliam 1995). One explanation for these differences may be that soil moisture, temperature, light, proximity to sources of infection, and other factors are less favorable for pathogens in gaps than in the forest understory (Augspurger 1983, 1984; Augspurger and Kelly 1984). Improved growing conditions in gaps and

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other open areas also may allow host plants to escape pathogens through better defenses or more rapid growth to invulnerable size (e.g., Shure and Wilson 1993; Seiwa 1998). Finally, sufficiently large gaps might be refuges from pathogens accumulating near conspecific plants (e.g., Augspurger 1983, 1984; Augspurger and Kelly 1984; Dalling et al. 1998; Packer and Clay 2000, 2003). Consequently, dispersal into gaps can provide a refuge from seed and seedling pathogens associated with moist, shady understory habitats (Augspurger 1983, 1984; Augspurger and Kelly 1984; Kitajima and Augspurger 1989; Forget 1997; Wenny and Levey 1998; O'Hanlon-Manners and Kotanen 2004a). Conversely, shade-intolerant trees may be excluded from nongap sites in part because of greater vulnerability to fungi than shade-tolerant species: Augspurger and Kelly (1984) found that seedlings of pioneer trees were more susceptible to attack by fungal pathogens than those of shade-tolerant species.

Although much of the evidence of pathogen impacts on forest recruitment comes from tropical systems (Augspurger 1983, 1984; Augspurger and Kelly 1984; Kitajima and Augspurger 1989; Forget 1997; Wenny and Levey 1998; Dalling et al. 1998), recent work has indicated that pathogens also may reduce the recruitment of temperate forest species (Stanosz 1994; Packer and Clay 2000, 2003; Masaki and Nakashizuka 2002; Tomita et al. 2002; Reinhart et al. 2003; O'Hanlon-Manners and Kotanen 2004a, b; Reinhart and Callaway 2004). Our objective in this study was to determine whether impacts of soil fungal "pathogens" (including true pathogens, decay organisms, and any other fungal antagonists) on seeds of a range of temperate trees vary either among habitat types, or among trees associated with those habitat types. We accomplished this by monitoring the germination of known numbers of seeds of paired species differing in relative shade tolerance and/or successional status, and exposed to three different habitats: old field, forest gap and forest understory. Old field and forest understory habitats represent either extreme of a continuum of light conditions: gaps are intermediate between these two extremes. Seeds were treated either with fungicide or with water as a control, in order to address the following questions:

- (1) Are impacts of soil fungi greatest in understory, gap, or open habitats?

- (2) Do fungal impacts differ between colonists of open sites versus mature forest species?

Methods

Study site

This experiment was conducted in old field and mature forest habitats 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). Ten replicates (sites) of each of three habitat types were used, for a total of 30 sites: mesic old field, forest gap, and forest understory. The old field sites selected were at least 50 years old (as indicated by aerial photographs), and were dominated by a mixture of grasses (principally *Poa compressa* L. and *Poa pratensis* L.) and forbs (notably *Solidago canadensis* L. and *Aster* spp.). While *Acer saccharum* Marsh. and *Tsuga canadensis* (L.) Carr. dominated the forest sites, including both gap and understory plots, numerous other tree species such as *Acer rubrum* L., *Fagus grandifolia* Ehrh., *Ostrya virginiana* (Miller) K. Koch, *Pinus strobus* L., and *Tilia americana* L. also were common. Multiple treefall sites were chosen to represent forest gaps, and understory habitats were selected from areas with full, or almost full, canopy cover. Gap dimensions, estimated by measuring the smallest and largest lengths, ranged from 493 to 2980 m², with a mean size of 986 m². Ring analysis of *A. saccharum* saplings determined that all gaps were in the range of 2–5 years old, with a mean of 4.0 years. Local soils typically are sand or sandy loams beneath a <50 cm organic-rich surface layer. Nomenclature follows Morton and Venn (1990).

Experimental species

Seeds of six tree species were used in this experiment. All of these species commonly occur at Jokers Hill. These species consisted of three matched pairs, which differed in their relative shade tolerance and/or successional status. Consequently, for each pair, one species is more reliant on open habitats for recruitment, and one is more typical of forest sites. Two

congeneric pairs of deciduous species, *Acer negundo* L. (Manitoba Maple) versus *A. saccharum* Marsh. (Sugar Maple), and *Prunus virginiana* L. (Choke Cherry) versus *P. serotina* Ehrh. (Black Cherry), and one pair of noncongeneric coniferous species, *Pinus strobus* L. (Eastern White Pine) versus *Tsuga canadensis* (L.) Carr. (Eastern Hemlock), were used; in each of these pairs, the second species listed is more strongly associated with mature forest habitats. *A. saccharum* is the dominant deciduous forest tree throughout much of southeastern Canada (Godman et al. 1990; Farrar 1995). This species is considered very shade-tolerant, and in understory habitats, seedlings can persist for many years as a “sapling bank” until released by a gap in the canopy (Godman et al. 1990; Farrar 1995). In contrast, *A. negundo* is a fast-growing, short-lived weedy shrub or small to medium tree (Overton 1990; Farrar 1995). It is shade-intolerant (Farrar 1995), and typically occurs in open or semi-open habitats rather than in mature forest. *Prunus serotina* is a medium-sized mid-successional forest tree, though it is considered shade-intolerant, and likely often recruits in gaps (Baker 1950; Auclair and Cottam 1971; Marquis 1990; Farrar 1995; Packer and Clay 2003). In contrast, *P. virginiana* is a short-lived shade-intolerant shrub or small tree that is typically found in open or shrubby sites (Farrar 1995). Finally, *T. canadensis* is a highly shade-tolerant, late-successional forest species (Anderson and Gordon 1994). This slow-growing species is extremely long-lived and may survive for 800 years or more (Godman and Lancaster 1990). In contrast, though *P. strobus* frequently is a component of mature forests, it is only moderately shade-tolerant (Farrar 1995), and commonly functions as a successional species or even as a pioneer species in old fields (Wendel and Smith 1990).

Exposure of seeds to pathogens

All seedlots were collected from multiple trees and subsequently stored frozen. *Acer negundo* seeds were collected from within and around the University of Toronto at Mississauga. All other seeds were donated by The National Tree Seed Centre (Natural Resources Canada, Canadian Forest Service, Fredericton, New Brunswick), and were all collected near Petawawa, Ontario (46°00' N, 77°27' W), or in the case of

P. strobus, near Lac Lynch, Quebec (46°21' N, 77°10' W).

Seed bags were constructed from nylon stockings. Each bag was filled with 100 ml of sieved soil collected in the previous week, mixed with 20 seeds of a single tree species (7200 seeds in total). The soil used to fill each bag originated at the site where that bag was subsequently buried; thus, each bag contained local soil. Seed bags were subjected to one of two treatments prior to burial: (1) control, in which the seed bag was saturated with water, and (2) fungicide, in which the seed bag was saturated with a fungicide solution. The fungicide used was Maestro 75DF (75% Captan by weight; Zeneca Corp., Stoney Creek, ON, Canada) diluted to a concentration of 1:100, which is the concentration recommended by the manufacturer for use as a dip for bulbs and tubers. Captan (*N*-trichloromethylthio-4-cyclahexene-1,2-dicarboximide), a nonsystemic heterocyclic nitrogen fungicide, was selected for its effectiveness against a broad range of Oomycota, Ascomycota and Basidiomycota (Sharville 1961; Torgeson 1969; Neergaard 1977), including seed rotting fungi (Neergaard 1977). Captan often is used agriculturally to protect seeds, and has been successfully used in similar ecological field studies (e.g., Dalling et al. 1998).

For each species at each site, two seed bags (one per treatment) were buried 0.5 m apart to a depth of 5 cm. Bags were placed in the field in October 2001 and recovered in May 2002; this period approximates conditions experienced by a seed between dispersal and germination. During the burial period, fungicide and water were re-applied monthly, unless snow cover prohibited application, for a total of four applications. On each occasion, either 20 ml of water or fungicide solution, as required, was applied to each bag using a needleless syringe. These treatments were intended to counteract loss of Captan by leaching or degradation.

Germination techniques

Following recovery, seed bags were opened and the germinated seeds were counted and removed. Ungerminated seeds were then transferred to the lab for germination trials. The experimental species have very different germination requirements (Burns and Honkala 1990a, b); therefore ungerminated seeds were subjected to treatments which differed accord-

ing to species. The germination techniques used were those shown to give the highest germination for each species in earlier lab trials, and were adapted from information provided by the National Tree Seed Centre, New Brunswick (Table 1). We chose this approach instead of a single standardized germination treatment because our main interest was in the effects of fungicide on the potential germination of each species, rather than in estimating germination rates under standard conditions. *Acer negundo* seeds and associated soil were spread over potting soil in 10 cm plastic pots then placed in a greenhouse under light banks. *Tsuga canadensis* and *P. strobus* seeds and associated soil were spread over potting soil in 10 cm plastic pots and placed in a seed germinator (12 h graded 5C–25C light/dark cycle at 90% humidity). *Prunus serotina* and *P. virginiana* seeds were cold stratified for 2 months prior to spreading over potting soil in 10 cm plastic pots which were then placed in the seed germinator (12 h 5C/25C light/dark cycle, 90% humidity). For *A. saccharum*, seed bag contents were mixed with an equivalent quantity of sand in polybags, moistened and placed in a dark refrigerator. The bags were opened weekly and the contents shaken to ensure adequate aeration; water was added as necessary. Each set of seeds was checked weekly for 3 months, and germinated seeds were counted and removed.

At the end of the study period ungerminated seeds were treated with tetrazolium chloride, which stains living tissue red (Hendry and Grime 1993), to determine viability. Where available, up to five seeds per site were randomly selected for staining. Staining protocol varied according to species. Each species, with the exception of *T. canadensis*, was treated according to the protocols determined by the International Seed Testing Association (1997). Since no specific protocol for *T. canadensis* could be found, the methods for treating *P. strobus* were used.

Table 1 Seed germination treatments used

Species	Cold-stratified?	Germination substrate	Germination location
<i>Acer negundo</i>	No	Potting soil	Greenhouse
<i>Acer saccharum</i>	No	Sand in polybag	Refrigerator
<i>Prunus serotina</i>	Yes	Potting soil	Seed germinator
<i>Prunus virginiana</i>	Yes	Potting soil	Seed germinator
<i>Pinus strobus</i>	No	Potting soil	Seed germinator
<i>Tsuga canadensis</i>	No	Potting soil	Seed germinator

See text for details

Statistical analyses

The proportion of seeds that germinated in the field, the proportion of intact seeds that germinated in the lab, and the proportion of ungerminated seeds that were found to be viable according to tetrazolium tests, were analyzed using a standard split-plot factorial ANOVA design (Kirk 1982) with field sites used as the blocking factor. Data from the three tests for each species were not combined since they considered different response variables, and since each test used only a subset of the seeds available for the previous test. Proportions were arcsin-transformed prior to analysis, and Type III sums of squares were used throughout; degrees of freedom vary because germination occasionally left too few seeds for further testing. The Tukey–Kramer HSD test was used for all a posteriori comparisons ($P < 0.05$). Means are reported \pm one standard error. Germination tests likely underestimate viability, even when combined with tetrazolium testing. However, this should not bias comparisons between experimental treatments.

Species were analyzed separately because each responded differently to the same treatments. As well, each species required its own germination and staining treatments, meaning that absolute viability estimates for different species are not directly comparable. This limitation does not apply to within-species tests of treatment effects; therefore, we have focussed primarily on the manner in which each species responded to habitat and fungicide.

Results

Acer negundo and *Acer saccharum*

Fungicide improved the germination of *A. negundo* seeds in the field (Table 2, Fig. 1a): more seeds

Table 2 Results of split-plot factorial ANOVAs

Species	Factor	Germinated (field)		Germinated (lab)		Viability of ungerminated seeds	
		df	F	df	F	df	F
<i>Acer negundo</i>	Habitat	2,27	0.648	2,27	3.308	2,20	0.041
	Treatment	1,27	4.759*	1,27	0.533	1,20	0.573
	Habitat×treatment	2,27	1.376	2,27	0.214	2,20	1.224
<i>Acer saccharum</i>	Habitat	2,27	0.442	2,27	3.640*	2,26	0.908
	Treatment	1,27	2.985	1,27	0.225	1,26	3.250
	Habitat×treatment	2,27	3.172	2,27	0.225	2,26	0.908
<i>Prunus virginiana</i>	Habitat	2,27	2.552	2,27	1.084	2,22	1.208
	Treatment	1,27	1.106	1,27	10.984**	1,22	0.113
	Habitat×treatment	2,27	0.410	2,27	0.316	2,22	0.649
<i>Prunus serotina</i>	Habitat	2,27	7.168**	2,27	0.394	2,26	6.701**
	Treatment	1,27	3.046	1,27	15.070***	1,26	3.246
	Habitat×treatment	2,27	2.127	2,27	1.135	2,26	2.923
<i>Pinus strobus</i>	Habitat	2,27	5.241*	2,27	5.192*	2,26	4.712*
	Treatment	1,27	3.244	1,27	1.367	1,26	8.818**
	Habitat×treatment	2,27	0.188	2,27	0.859	2,26	2.517
<i>Tsuga canadensis</i>	Habitat	2,27	0.971	2,27	0.341	2,25	1.491
	Treatment	1,27	6.795*	1,27	2.158	1,25	0.072
	Habitat×treatment	2,27	1.412	2,27	0.407	2,25	3.180

“Habitat” refers to the habitat to which seeds were exposed (forest understorey, forest gap, or old field); “treatment” refers to the application of fungicide or water (as a control)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

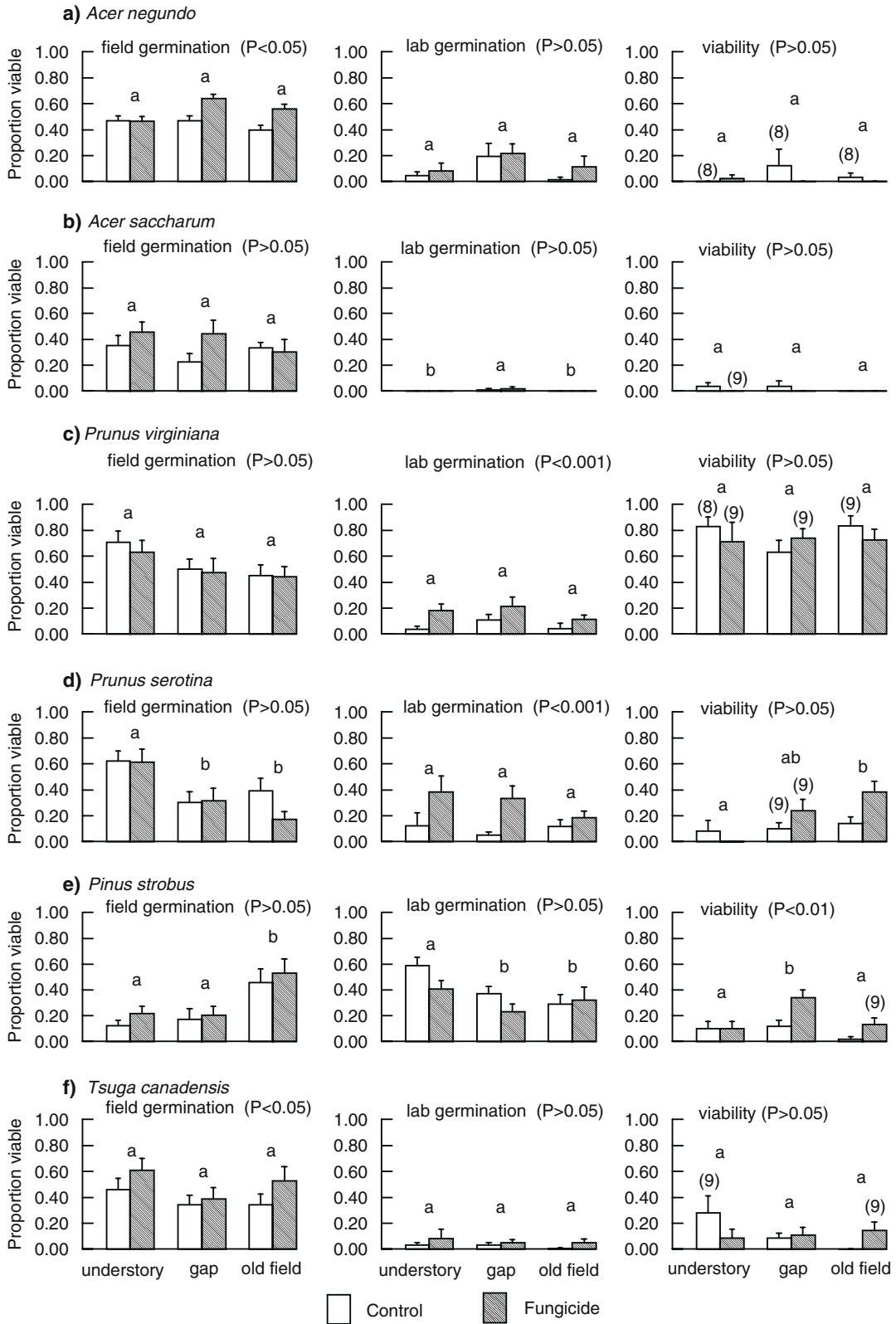
germinated in the fungicide treatment (55.5%) than in the controls (44.7%) (Table 2, Fig. 1a). There were no significant between-habitat differences, though the positive effects of fungicide tended to be greater in gaps and old fields than in understorey sites (Fig. 1a). Germination in the lab was not influenced by habitat or fungicide (Table 2, Fig. 1a). Tetrazolium staining indicated viability of the residual, ungerminated seeds was low (<2.5%) and did not depend on habitat or fungicide treatment ($P > 0.05$).

All tests for treatment or habitat effects on *A. saccharum* field germination were nonsignificant, though fungicide tended to improve viability (Table 2, Fig. 1b). In contrast, lab germination differed among habitats (Table 2, Fig. 1b), though means were very small and must be treated with caution: more seeds from forest gaps (1.5%) germinated than did seeds from forest understoreys (0%) or old fields (0%). Tetrazolium staining indicated viability of ungerminated seeds was low (<3.0%) and did not depend on habitat or fungicide treatment ($P > 0.05$).

Prunus virginiana and *Prunus serotina*

Field germination of *P. virginiana* did not differ among habitats or fungicide treatments (Table 2, Fig. 1c). In contrast, more seeds germinated in the lab in the fungicide treatment (16.8%) than in the control treatment (6.3%) (Table 2, Fig. 1c), though there still were no significant between-habitat effects. Tetrazolium staining suggested many ungerminated seeds were dormant rather than dead: viability of ungerminated seeds was high (>65%) but did not depend on habitat or fungicide application ($P > 0.05$).

Field germination of *P. serotina* seeds differed among habitats (Table 2, Fig. 1d): more seeds germinated in forest understoreys (62.0%) than in forest gaps (31.0%) or old fields (28.5%). Fungicide addition did not have a significant effect on field germination, though it did increase lab germination: more seeds germinated in the fungicide treatment (30.1%) than in the control treatment (9.8%) (Table 2, Fig. 1d). There were no significant between-habitat effects for seeds germinating in the lab. Tetrazolium staining indicated that the viability of ungerminated seeds was not af-



◀ **Fig. 1** Viability of tree seeds subjected to two treatments, fungicide and water (as a control), in three different habitats, forest understory, forest gap, and old field. Data indicate mean proportion of seeds estimated as viable+SEM ($n=10$ unless otherwise indicated in brackets). P -values indicate the results of the tests on the effect of fungicide addition. Differences among habitats, as detected by a posteriori tests, are indicated by lower case letters; habitats sharing the same letter do not differ significantly ($P>0.05$)

ected by fungicide, but did vary among habitats ($F_{2,26} = 6.70$, $P < 0.01$): more ungerminated seeds were found to be viable from old fields (26.3%) than from forest understories (4.2%), while viability of seeds from gaps was intermediate (17.0%).

Pinus strobus and *Tsuga canadensis*

The addition of fungicide did not have a significant effect on the germination of *P. strobus* seeds in the field, although more seeds tended to germinate in the fungicide treatment (31.9%) than in the control treatment (25.3%) (Table 2, Fig. 1e). In contrast, field germination did differ among habitats (Table 2, Fig. 1e): more seeds germinated in old fields (49.6%) than in either forest gaps (19.0%) or forest understory habitats (17.3%). Lab germination also varied among seeds from different habitats (Table 2, Fig. 1e): more seeds from forest understory habitats (50.1%) germinated in comparison with seeds from forest gaps (30.2%) or old fields (30.5%). Fungicide addition did not affect lab germination. Tetrazolium staining indicated that the viability of ungerminated seeds was affected by fungicide addition ($F_{1,26} = 8.82$, $P < 0.01$): more seeds were found to be viable in the fungicide treatment (19.3%) than in the control treatment (8.0%). Between-habitat differences were also found ($F_{2,26} = 4.71$, $P < 0.05$): more ungerminated seeds from forest gaps (23.0%) were found to be viable in comparison with seeds from either old field (7.4%) or forest understory (10.0%) habitats.

The germination of *T. canadensis* seeds in the field did not differ among habitats (Table 2, Fig. 1f). However, the addition of fungicide did alter the number of seeds germinating in the field: more seeds germinated in the fungicide treatment (51.0%) than in the control treatment (38.3%) (Table 2, Fig. 1f). Lab germination was not affected by habitat or fungicide addition. Tetrazolium staining indicated viability of ungerminated

seeds was moderate (7–18%, depending on treatment) but did not depend on habitat or fungicide ($P > 0.05$).

Discussion

Are impacts of soil fungi greatest in understory, gap, or open habitats?

Most of our experimental species in the present study showed some evidence of seed loss to soil fungi. These fungi might kill dormant seeds, might attack germinating seeds in the field, or might colonize seed coats and subsequently kill young seedlings in the lab. It is not possible to separate these categories of enemies in our experiment, but they likely overlap, and in any case produce the same result: a seed that dies before or shortly after germination.

Evidence from several systems suggests that moist, shady habitats such as those typical of the forest understory increase the risk of fungal attack by reducing plant growth and/or promoting fungal abundance and growth (Augspurger 1983, 1984; Augspurger and Kelly 1984; Kitajima and Augspurger 1989; Forget 1997; Wenny and Levey 1998; Schafer and Kotanen 2003; O'Hanlon-Manners and Kotanen 2004a). For example, in forests near Brisbane, Australia, only eucalypts resistant to the root rot pathogen *Phytophthora cinnamomi* are able to establish and grow in the moist soils found in depressions and swales, limiting vulnerable species to the drier ridges (Burdon and Shattock 1980; Weste 1986). Similarly, we previously have found that seeds of the shade-intolerant species White Birch (*Betula papyrifera*) are more vulnerable to fungi in understory sites than in openings (O'Hanlon-Manners and Kotanen 2004a). In contrast, the present study did not find seeds to be more susceptible to attack in the forest understory relative to gaps and old fields, as indicated by the complete absence of habitat×treatment interactions. In part, this may be because winter conditions reduce physical differences between gaps and the forest understory, and consequently differences in fungal activity. Greater survival in one habitat is instead likely to be explained by factors other than escape from fungal pathogens. For example, the higher field germination of *P. serotina* seeds in the forest understory compared with the other habitats might be explained by this species' require-

ment for a stable moisture supply for germination (Marquis 1990), which may be better met in the forest understory than in more exposed habitats. In contrast, the greater field germination of *P. strobus* in old fields might be expected for a species that can be an aggressive invader of open habitats (Wendel and Smith 1990), while greater lab germination for *P. strobus* seeds recovered from the forest understory may have been a consequence of low field germination due to poor light conditions: most viable seeds from the old fields already may have germinated.

Do fungal impacts differ between colonists of open sites versus mature forest species?

For one species pair, there was weak evidence that the less shade-tolerant member was more damaged by seed-attacking fungi. While field results indicated that *A. negundo* seeds treated with fungicide exhibited higher germination than those in the control treatment, *A. saccharum* showed no significant response to fungicide application, though fungicide did tend to improve field germination. Reinhart and Callaway (2004) also documented negative effects of soil biota on growth of seedlings of *A. negundo*. *Acer saccharum* may enjoy lower fungal attack thanks to pathogen avoidance. The seeds of this species germinate best at 1C, and therefore germinate earlier than any other North American forest tree species, often while snow is still on the ground (Godman et al. 1990). In consequence, seedlings will be older, larger, and therefore less vulnerable, by the time warmer temperatures encourage the growth of many pathogens. This has been shown for *Acer mono*, in which early germinating seedlings in some sites are less likely to be killed by predators and fungi than late-germinating seedlings (Seiwa 1998). Surviving *A. saccharum* seedlings can then persist in a juvenile state for many years (Godman et al. 1990); this may be a safer option than risking exposure to seed pathogens belowground.

The two other species pairs tested did not clearly follow this trend, however: there was no evidence the more shade-tolerant/late successional species enjoyed lower losses to fungi. In part, this may be because these pairs are less strongly divided into very shade-intolerant versus very shade-tolerant species. For the cherries, lab germination results indicated that both species suffered similar losses of seeds to fungi, perhaps reflecting the fact that both are relatively light-loving (Farrar 1995).

Other studies also have demonstrated pathogen impacts on seedlings of the mature forest species of the pair, *P. serotina* (Packer and Clay 2000, 2003; Reinhart et al. 2003). Both conifers also provided evidence of pathogen impacts, though at different stages. Field germination of the very shade-tolerant *T. canadensis* was reduced by fungi. Other studies have identified a variety of fungal pathogens that attack seeds (LeMadeleine 1980; Godman and Lancaster 1990; Goerlich and Nyland 2000) and seedlings (Ruth 1974) of this species, and recent evidence suggests that fungal soil pathogens help to explain its tendency to establish on logs rather than the forest floor (O'Hanlon-Manners and Kotanen 2004b). In contrast, *P. strobus* showed no significant effects of fungicide on germination, but tetrazolium tests indicated that fungi instead substantially reduce the viability of ungerminated seed. Numerous fungi have been isolated from seeds of this species; some apparently occur on seeds in the field, while others colonize following collection (Mittal and Wang 1987).

Conclusions

Our data provide evidence that seeds of most of our experimental species are subject to attack by fungal pathogens: with the exception of *A. saccharum*, the addition of fungicide always had some measurably positive effect on seed survival. Significant results were sparse, their effect sizes were small, and their ecological importance may be eclipsed by other controls on recruitment such as shade tolerance, growth rate, and competitive ability. Still, our results demonstrate that the potential for soil fungi to affect forest regeneration does exist, and our estimates probably are conservative: it is unlikely that our fungicide treatments achieved more than partial control. Nonetheless, we did not find that seeds of these species were less likely to be attacked in gaps than in the forest understory; this is not an artifact of using insufficiently large gaps, since very large openings (old fields) gave the same result, and since another similar study *did* demonstrate such an effect for *Betula papyrifera* (O'Hanlon-Manners and Kotanen 2004a). As well, the different tree species we studied behaved idiosyncratically, and mature forest species were not consistently less vulnerable to fungi. Why our results differ from studies of tropical forests (e.g., Augspurger 1983, 1984; Augspurger and Kelly

1984; Kitajima and Augspurger 1989) is unclear. To some extent, this may simply reflect the choice of species, since other temperate trees do seem to exhibit the expected patterns of mortality (O'Hanlon-Manners and Kotanen 2004a). Still, perhaps higher fungal activity in tropical sites makes such patterns more pronounced. Fall seed dispersal followed by spring germination also may mean that temperate seeds are protected from fungi for much of their post-dispersal period by low winter temperatures and frozen soils, particularly if they germinate early, as does *A. saccharum*.

Acknowledgements This research was supported by an NSERC PGS to DO'H-M and NSERC Research and Equipment Grants to PMK, with additional funding from Human Resources Development Canada. Stephanie deLorenzo, Pamela O, Michelle Schafer and Lesley Cresswell provided field and lab assistance. The National Tree Seed Centre kindly donated some of the seeds used in this experiment. Special thanks to Murray Koffler for his donation to the University of Toronto of the property that has become the Koffler Scientific Reserve at Jokers Hill. This is a publication of the Koffler Scientific Reserve at Jokers Hill.

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