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Evidence that fungal pathogens inhibit recruitment of a shade-intolerant tree, White Birch (*Betula papyrifera*), in understory habitats

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Abstract Evidence from tropical forests suggests understory habitats are associated with a high risk of disease, which may prevent the establishment of vulnerable tree species; in contrast, canopy gaps can act as refuges from these pathogens. However, few studies have investigated the impacts of pathogens on regeneration in temperate forests. To determine whether losses to fungi of seeds of *Betula papyrifera*, a light-loving species, varied between habitats that differed in their degree of openness, we applied fungicide to seeds buried in old fields, treefall gaps, and forest understory sites. We found that the application of fungicide significantly reduced losses in all habitats, relative to control values. This effect was habitat-dependent: the benefit of fungicide was greater in forest understory than in openings. This suggests that *B. papyrifera* is prevented from establishing in understory environments in part by its susceptibility to pathogen attack, and not solely because of a high light requirement.

Keywords Disease, Forest gaps, Old Fields, Recruitment, Seed banks

Introduction

Gaps in forest ecosystems play an important role in the regeneration of both tropical and temperate trees (Platt and Strong 1989; Brokaw and Busing 2000). Treefall gaps provide saplings of mature forest species with opportunities for recruitment into the canopy, while larger openings are important for the establishment of shade-intolerant trees (but see Hubbell et al. 1999; Brokaw and Busing 2000; Dalling et al. 2002). These benefits usually are explained as a release from competition, primarily for light. However, patterns of gap creation and occupancy may be amplified or altered by pathogens (Roberts and Gilliam 1995). In particular, pathogens that attack seeds and seedlings have the potential to restrict recruitment of vulnerable tree species to a subset of otherwise suitable habitats (Augspurger 1983; Augspurger and Kelly 1984; Dalling et al. 1998; Packer and Clay 2003; O'Hanlon-Manners and Kotanen 2003).

Impacts of pathogens depend on factors which can differ significantly between openings and the forest understory, including soil moisture, temperature, light, and the density and location of seedlings (Augspurger 1983; Augspurger and Kelly 1984; Kitajima and Augspurger 1989; Dalling et al. 1998; Schafer and Kotanen 2003). The occurrence and severity of disease in a particular habitat will depend upon whether these factors favor pathogen infection, growth, and development over plant growth and development (Schoeneweiss 1981; Shure and Wilson 1993). For instance, pathogen activity is influenced by temperature and moisture conditions, which can affect the survival of inoculum, spore germination, infection, and sporulation. As a result, fungal activity in tropical forests is greater in moist, shady understory microenvironments than in gaps (Augspurger 1983; Augspurger and Kelly 1984; Kitajima and Augspurger 1989). In addition, both individual plants and plant populations may be more vulnerable to attack in

suboptimal habitats: poor conditions may weaken plants and predispose them to attack (Schoeneweiss 1981), whereas favorable conditions may allow plants to escape enemies through better defenses or more rapid growth to invulnerable size (Shure and Wilson 1993). For example, Australian *Eucalyptus* susceptible to the root rot pathogen *Phytophthora cinnamomi* exhibit only limited damage when attacked in favorable habitats, but are killed when also exposed to conditions of waterlogging, low nutrient content and poor soil structure (Burdon and Shattock 1980; Weste 1986).

Although seed and seedling pathogens may influence patterns of tree recruitment, few studies have examined this interaction, and most of these studies focus on tropical forests. Our objective in this study was to determine the extent to which soil fungi are responsible for habitat-dependent mortality of seeds of a temperate, deciduous, shade-intolerant tree, *Betula papyrifera* Marsh. (White Birch). Our goal was accomplished by monitoring the survival of known numbers of seeds in experimental plots. These seeds were either treated with fungicide or water (as a control), in order to address the question:

Are fungal impacts on seeds of B. papyrifera greatest in understory or more open habitats?

Our results indicate that *B. papyrifera* suffers greater losses of seeds to fungal pathogens in the forest understory than in open habitats, and suggest that pathogens may contribute to the shade-intolerant nature of this 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 (mesic old field, forest gap, and forest understory) were used, for a total of 30 sites. 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. dominated the forest sites, including both gap and understory plots, other species such as *Tsuga canadensis* (L.) Carr., *Fagus grandifolia* Ehrh., and *Pinus strobus* L. also were common. Multiple treefalls were chosen to represent forest gaps, and understory habitats were selected from areas with full, or almost full, canopy cover. The treefall gaps used ranged from 493m² to 2980 m² in area, with a mean size ($\pm$ SEM) of 986 ± 236 m². Tree ring analysis determined that all gaps were in the range of 2-5 years old, with a mean ($\pm$ SEM) of 4.0 ± 0.3 years.

The study species

Betula papyrifera is a very shade-intolerant tree that is typically found at forest edges, lakeshores and roadsides (Farrar 1995), and large disturbed sites such as clearcuts and recent burns (Safford et al. 1990). Although fast growing, this tree is short-lived, rarely surviving longer than 140 to 200 years (Safford et al. 1990). Pollen and female catkins are produced by this monoecious tree from mid-April to early July (Brinkman 1974), and seed ripening occurs from early August until mid-September, with dispersal occurring from September through November (Safford et al. 1990). The extremely light, winged seeds, averaging 3 million per kilogram, are easily dispersed by the wind and a few may travel long distances (Safford et al. 1990). Germination usually occurs in the spring following dispersal (Safford et al. 1990). This species is common in successional habitats at Jokers Hill.

Effects of pathogens on germination

The National Tree Seed Centre (Canadian Forest Service, Fredericton, New Brunswick) donated the seeds used in this study. These seeds were collected near Petawawa, Ontario (46°00' N, 77°27' W) in 1989, and subsequently were stored frozen.

Seed bags were constructed from nylon stockings, and each was filled with 20 seeds mixed with 100 mL of sieved soil collected from the field in the previous week and subsequently air-dried. Soil from different sites was not mixed, so that seed bags at each site contained only soil collected at the same location. Seed bags were subjected to one of two treatments: 1) "control", in which the seed bag was saturated with water prior to burial, and 2) "fungicide", in which the seed bag instead 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, a nonsystemic heterocyclic nitrogen fungicide, is effective against a broad range of Oomycota, Ascomycota and Basidiomycota, including seed rotting fungi (Neergaard 1977).

In June 2001, two seed bags, one per treatment, were buried 0.5 m apart at each site to a depth of 5 cm. While buried, fungicide and water were re-applied monthly for a total of five treatment applications: either 20 mL of water or fungicide solution, as required, was applied to each bag using a needleless syringe. Bags were recovered in October 2001, and their contents were spread over potting soil in 10 cm plastic pots, which then were placed in a greenhouse under light banks. Pots were kept moist and the top 1 cm was carefully stirred monthly to prevent moss buildup and to bring any buried

seeds to the top; earlier trials found that this technique gave the highest germination. Pots were checked weekly for three months and germinated seeds were counted, recorded and removed.

Viability testing of ungerminated seeds

Tetrazolium chloride, which stains living tissue red, was used to determine the viability of ungerminated seeds recovered at the end of the study period. Up to five seeds per treatment per site were randomly selected for staining, as available. The staining protocol used was that employed by Houle and Payette (1990). Seeds were transversely cut and the embryos excised. Embryos were then soaked in the stain for approximately 36 hours, after which they were rinsed and evaluated. Only those embryos staining completely red were considered viable.

Statistical analysis

The proportion of experimental seeds that germinated in the greenhouse, and the proportion of ungerminated seeds that were found to be viable according to tetrazolium tests, were analyzed by a standard split-plot factorial ANOVA design with field sites used as the blocking factor. Proportions were arcsin-transformed prior to analysis, and Type III sums of squares were used throughout.

Results

No germination occurred in the field. In the greenhouse, the proportion of seeds germinating was affected by fungicide addition: significantly more seeds germinated from the fungicide treatment (25%) than from the control treatment (11%) (Table 1, Fig. 1a). There were no significant between-habitat effects, but there was a significant habitat x treatment interaction (Table 1, Fig. 1a): fungicide always increased germination, but the

magnitude of this effect varied among habitats. The biggest difference between treatments occurred in the forest understory: 8% of seeds germinated in the control treatment compared to 32% in the fungicide treatment. The smallest difference between treatments occurred in the old fields: 12% of seeds germinated in the control treatment compared to 21% in the fungicide treatment. Tetrazolium staining indicated that the viability of ungerminated seeds was low (4.6%), and did not vary among habitats or between treatments (Table 1, Fig. 1b).

Discussion

Diseases of seeds (e.g., Kirkpatrick and Bazzaz 1979; Lonsdale 1993; Blaney and Kotanen 2001, 2002; O'Hanlon-Manners and Kotanen 2003; Tomita et al. 2002) and seedlings (e.g., Augspurger 1984; Augspurger and Kelly 1984; Packer and Clay 2000, Masaki and Nakashizuka 2002, Packer and Clay 2003) have significant impacts on regenerating plants in a wide range of natural ecosystems. Our results similarly indicated that losses of seeds of *B. papyrifera* to fungi are an important source of mortality: fungicide addition more than doubled the number of seeds that survived to germination, and improved survival in all habitats. Seeds were more susceptible to attack in the shady forest understory than in openings, as also has been reported for tropical trees (Augspurger 1984; Augspurger and Kelly 1984; Kitajima and Augspurger 1989). This effect was so strong that the pattern of greater survival in openings observed for the control seeds was the reverse of that observed when fungal pathogens were suppressed: fungicide-treated seeds tended to survive better in the understory than in open habitats, possibly because of a reduced risk of desiccation. Since no seeds germinated in the field, reduced losses to fungi in openings must reflect reduced fungal activity rather than improved growth or defence of this shade-intolerant tree.

It may be that shade-intolerant species such as *B. papyrifera* are especially vulnerable to pathogen attack, either because they allocate fewer resources to defence as a result of a growth-defence tradeoff (Coley et al. 1985; but see Almeida-Cortez et al. 1999; Messina et al. 2002), or as an evolutionary consequence of their typical occurrence in low-pathogen habitats where strong defences are not required. Augspurger (1984) and Augspurger and Kelly (1984) did in fact find that seedlings of pioneer trees were more susceptible to attack by fungal pathogens than those of shade-tolerant species. On the other hand, a study involving seeds of six temperate forests trees (O'Hanlon-Manners and Kotanen, in review) found that although most of these species were significantly affected by fungal pathogens, the risk was not greater for less shade-tolerant species, and gaps did not appear to provide an escape.

In summary, our results indicate that the shade-intolerant *B. papyrifera* is more vulnerable to fungal seed pathogens in the forest understory than in its usual, open habitats. Fungi are not the only factor excluding *B. papyrifera* from the forest understory; in particular, low light levels mean that seedlings that germinate in this habitat have a low probability of survival. Nonetheless, pathogens may reinforce or even help to create the "shade-intolerant" behaviour of this species by reducing its success in low-light environments.

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Table 1 Results of split-plot factorial ANOVAs performed for the proportion of seeds germinating in the greenhouse and the proportion of ungerminated seeds that were found to be viable according to tetrazolium staining. "Habitat" refers to the habitat to which seeds were exposed (forest understory, treefall gap, or old field); "treatment" refers to the application of fungicide or water (as a control).

Factor	Germinated (greenhouse)		Tetrazolium staining	
	<i>df</i>	<i>F</i>	<i>df</i>	<i>F</i>
habitat	2	0.345	2	0.570
treatment	1	28.742***	1	3.387
habitat x treatment	2	4.003*	2	0.585
error	27		27	

* = $P < 0.05$; *** = $P < 0.001$

Figure legends

Fig. 1

Viability of *B. papyrifera* seeds subjected to two treatments, fungicide and control (water), in three different habitats, forest understory, forest gap, and old field. Data indicate mean ($\pm SE$, $n=10$) proportion of seeds germinating in the lab or viable according to tetrazolium tests. *P*-values indicate the results of tests of the effect of fungicide addition; see Table 1 for complete ANOVA results.

Fig. 1