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Fungi-mediated mortality of seeds of two old-field plant species[1]

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ORROCK, J. L. (Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011) AND E. I. DAMSCHEN (Department of Zoology, North Carolina State University, Raleigh, NC 27695). Fungi-mediated mortality of seeds of two oldfield plant species. J. Torrey Bot. Soc. 132: 613–617. 2005.—Fungi are a significant source of seed mortality. Although the seeds of pokeweed, *Phytolacca americana*, are known to contain antifungal compounds, it is unknown if these compounds confer the presumed benefit of increased persistence of *P. americana* seeds in soil compared to seeds of potential competitors. We buried seeds of *P. americana* with seeds of a sympatric species, *Chamaecrista fasciculata*, that is not known to exhibit antifungal properties. Half of the buried seeds were treated with a fungicide. After three months, seeds were exhumed. Treatment of buried seeds with fungicide significantly increased the number of intact *C. fasciculata* seeds recovered (82% recovered with fungicide addition, 39% recovered from controls). Recovery of intact *P. americana* seeds was 100% for both control and fungicide treatments. Results from a complementary experiment using non-buried seeds in a growth chamber experiment confirmed that *P. americana* was much less susceptible to fungal attack compared to *C. fasciculata*. Our work demonstrates that the antifungal properties of *P. americana* significantly improved survival of *P. americana* seeds in the seedbank. Moreover, this increased survival may promote *P. americana* persistence in communities where species without antifungal properties, such as *C. fasciculata*, also exist.

Key words: fungi, Savannah River Site, seed, seedbank, survival.

The presence of antifungal compounds in the seeds of many plant species (e.g., Broekaert et al. 1995, Minami et al. 1998, Liu et al. 2000) is often interpreted as an adaptive response to fungal seed pathogens (Broekaert et al. 1995), which can have a substantial influence on seed mortality (Crist and Friese 1993, Leishman et al. 2000, Blaney and Kotanen 2001, 2002; Schafer and Kotanen 2003, 2004). However, rarely do studies that examine antifungal compounds in seeds document whether or not the antifungal properties conferred by such compounds increase seed persistence in the seedbank, and studies that document mortality of seeds due to fungi rarely employ seeds known to exhibit antifungal properties. Hence, although antifungal compounds have been documented in the lab (e.g., Liu et al. 2000), and susceptibility of seeds to fungi is known to vary widely among plant species and habitat type (e.g., Leishman et al. 2000, Blaney and Kotanen 2001, Schafer and Kotanen 2004), the ecological significance of seeds known to exhibit antifungal properties is rarely demonstrated. In addition to the direct benefit of a presumed increase in seedbank persistence, antifungal properties may have indirect benefits. Because many soil fungi are generalists that attack a variety of seeds (Dix and Webster 1995, Blaney and Kotanen 2001, 2002; Schafer and Kotanen 2004), those species with antifungal properties may indirectly benefit via increased mortality of potential competitor species with fungi-susceptible seeds.

We examined whether fungi affected the survival of seeds of pokeweed, *Phytolacca americana*, and partridge pea, *Chamaecrista fasciculata*. We selected these two species because *P. americana* is known to possess antifungal compounds effective against several different soil

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fungi (Minami et al. 1998, Liu et al. 2000) whereas assays of the seeds of *C. fasciculata* have found no antifungal compounds (Raskin and Poulev 2002). Importantly, seed mortality has been shown to affect the recruitment of adult plants of *P. americana* (Orrock et al. 2003) and *C. fasciculata* (3 times more *C. fasciculata* plants when exclosures prohibit access by seed predators, unpublished data), suggesting that fungi-mediated seed mortality may have direct consequences for plant recruitment. Both *P. americana* and *C. fasciculata* are common inhabitants of recently-disturbed, early-successional habitats in the southeastern United States (Radford et al. 1968). As such, these species are also putative competitors and the possession of antifungal properties by *P. americana* may reduce competition with *C. fasciculata* if antifungal properties promote seedbank persistence.

Previous work has documented that susceptibility of seeds to fungal attack is variable among plants within the same community (e.g., Leishman et al. 2000, Blaney and Kotanen 2001, 2002; Schafer and Kotanen 2003, 2004), and that seeds possess antifungal compounds (e.g., Minami et al. 1998; Liu et al. 2000). We combine these approaches to evaluate if two species that differ in antifungal properties have differential susceptibility to fungi in the seedbank. The goal of our study was to evaluate the importance of antifungal compounds in *P. americana* by determining if soil fungi differentially affect the persistence of *P. americana* and *C. fasciculata* in the seedbank under natural conditions. We complement this study of seedbank survival with a growth chamber experiment using non-buried seeds to further evaluate the susceptibility of *P. americana* and *C. fasciculata* to destruction by fungal attack.

Methods. This study was conducted at the Savannah River Site, a National Environmental Research Park (NERP) near Aiken, South Carolina, USA. The study site was located in a mature forest dominated by planted longleaf pine, *Pinus palustris*. As is typical of mature *P. palustris* forests, the soil of the study area was covered by a layer of pine litter. The study site represents a common forest type in the region, where seeds of *P. americana* and *C. fasciculata* exist as components of the seedbank (unpublished data). In the study area, both species generally set seed from July–November, with initiation of germination in early March (Radford et al. 1968).

Mature *P. americana* fruits were collected in the study area on July 28, 2003. Seeds were removed from fruits by sieving and were then thoroughly washed and allowed to dry; seeds are approximately 2.5–3 mm in size and oval in shape (Radford et al. 1968), mean seed weight is 0.007 g ± 0.002 SE ($N = 10$). This treatment ensured that fruit-free seeds were used, because naturally disseminated *P. americana* are cleaned of fruit by passage through the digestive tract of vertebrate frugivores (McDonnell et al. 1984). Seeds of *C. fasciculata* were obtained from a local commercial supplier (Adams-Briscoe Seed Company, Jackson, Georgia) from plants harvested in July 2003. Seeds of *C. fasciculata* are 2.5–4.0 mm in size and also oval in shape (Radford et al. 1968); mean seed weight is 0.01 g ± 0.008 SE ($N = 10$). On December 20, 2003, seeds were buried in envelopes constructed of fiberglass window screening (approximately 1 mm mesh). Burial of seeds in December may make our estimate of fungi-mediated mortality slightly conservative because seeds of *P. americana* and *C. fasciculata* are naturally dispersed from July through November (Radford et al. 1968).

Envelopes with seeds were approximately 4 × 6 × 0.5 cm. This method ensured complete recovery of intact seeds and also allowed seeds and soil to freely intermingle. Each envelope contained either 20 seeds of *P. americana* or 20 seeds of *C. fasciculata*. A pair of envelopes, one envelope of *P. americana* and one of *C. fasciculata*, was buried at each location. Each pair was randomly assigned to one of two treatments: fungicide application or control (no fungicide application), resulting in 10 pairs of untreated seed envelopes and 9 pairs that were treated with fungicide. Fungicide treatment consisted of thoroughly dusting the seed envelopes with fungicide powder (active ingredient: Captan, 75% by weight). Captan is active against a wide variety of soil fungi in the Ascomycota, Oomycota, and Basidiomycota (Torgeson 1969, Neergaard 1977), is particularly effective against seed-rotting fungi (Neergaard 1977), and has been successfully used in studies of the effect of fungal pathogens on seed survival (Blaney and Kotanen 2001, 2002; Schafer and Kotanen 2003).

Each pair of seed envelopes was buried approximately 8 cm deep. Pairs were haphazardly spaced with the constraint that pairs be 3–4 meters apart. Burial was performed with care to minimize disturbance to the soil and litter and any litter removed during burial was replaced.

In total, we buried 19 pairs of envelopes, for a total of 380 seeds of each species (19 envelopes per species x 20 seeds per envelope). Any differences in the proportion of intact seeds among species and treatments are ascribed to differences in antifungal properties, and thus susceptibility to fungi, of the two species.

Seeds were exhumed on March 28, 2004; this duration is similar to other studies of fungi-mediated seed losses (e.g. Blaney and Kotanen 2001) and coincides with spring rains that lead to pulses of germination. Exhumed seeds were thoroughly rinsed to remove soil and fungicide. Seeds were removed from envelopes and examined. Seeds were considered 'intact' if they were recovered with hard, unbroken seed coats and no visible decay. Seeds that germinated during burial were also considered 'intact' (the outcome of our results did not differ if germinants were not included in the count of intact seeds). Intact seeds were subjected to a germination trial to assess viability. Each of the 38 total seed samples was placed on wetted filter paper in a Petri dish on May 1, 2004. Dishes were randomly arranged along a single level within a Percival PGC 15.5 growth chamber (approximate light output 1000 μmol m^2 s^{-1} from cool white florescent and incandescent bulbs). The growth chamber was set to 14 h day / 10 h night photoperiod, with temperatures of 34 and 27 °C, respectively. This regime was chosen as a compromise between the conditions used in other germination studies of *P. americana* germination (Farmer and Hall 1970, Edwards et al. 1988). Seeds were checked daily; distilled water was added if necessary and germinated seeds were removed. Seeds were considered to have germinated when the radicle was $>$1mm in length (Farmer and Hall 1970).

Visible fungal growth became evident during the germination trials and eliminated a large proportion of *C. fasciculata* seeds (0.58 ± 0.06; 95% confidence interval) before they could germinate and a negligible proportion of *P. americana* seeds (0.02 ± 0.01). Because this contamination may have occurred after seeds were exhumed and washed (and fungicide was removed), germination trial data for buried seeds were unsuitable for testing the effects of fungicide on the viability of buried seeds.

The presence of pathogenic fungi in the growth chamber provided an additional opportunity to test the susceptibility of *P. americana* and *C. fasciculata* to fungal attack. Using non-buried *P. americana* and *C. fasciculata* seeds, we conducted germination trials in the growth chamber with and without fungicide addition. In this experiment, 20 seeds of *C. fasciculata* or *P. americana* were placed in a Petri dish on wet filter paper (as for the buried seeds described above). Each dish received either no fungicide or fungicide (Captan) mixed at the recommended strength of 2 ml Captan per 500 ml water. For each species and treatment, ten dishes were created, resulting in 40 total dishes (ten replicates × two species × two treatments). Seeds were monitored daily from April 29, 2004 to June 6, 2004. At the conclusion of the experiment, the number of intact seeds was counted. As with buried seeds, non-buried seeds in the growth chamber experiment were considered intact if they had germinated during the course of the experiment or had hard, intact seed coats with no visible signs of decay.

The proportion of intact seeds in each experiment (survival of buried seeds and non-buried seeds) was the dependent variable in our statistical analyses. Because the dependent variables were proportions, we performed our analyses using a generalized linear model with a logit link function and bivariate response distribution (Littell et al. 2002). For both analyses, strong interactions among fungicide treatment (control or fungicide) and species (*P. americana* or *C. fasciculata*) were found. As a result, we tested the importance of simple effects using linear contrasts (Littell et al. 2002). All analyses were performed using SAS v. 8.1; means are presented ± 95% confidence intervals.

Results. Fungicide treatment did not affect the proportion of intact buried *P. americana* seeds: all exhumed *P. americana* seeds were intact regardless of treatment (Fig. 1A; $\chi^2 < 0.001$, $P = 1.00$, df $= 1$). However, fungicide treatment increased the proportion of intact *C. fasciculata* seeds (Fig. 1A; $\chi^2 = 76.5$, $P < 0.01$, df $= 1$). The proportion of intact control *C. fasciculata* seeds was significantly lower than control *P. americana* seeds ($\chi^2 = 227.1$, $P < 0.01$, df $=$ 1) and fungicide-treated *P. americana* seeds (χ^2 $= 211.9$, $P < 0.01$, df $= 1$). Although *C. fasciculata* survival was increased by the addition of fungicide, survival of fungicide-treated *C. fasciculata* was still less than fungicide-treated *P. americana* ($\chi^2 = 49.1$, $P < 0.01$, df $=1$) or control *P. americana* seeds (Fig. 1A; $\chi^2 = 52.8$, $P < 0.01$, df $= 1$). Although fungal contamination made germination trial results inappropriate for analysis of fungicide effects on seed

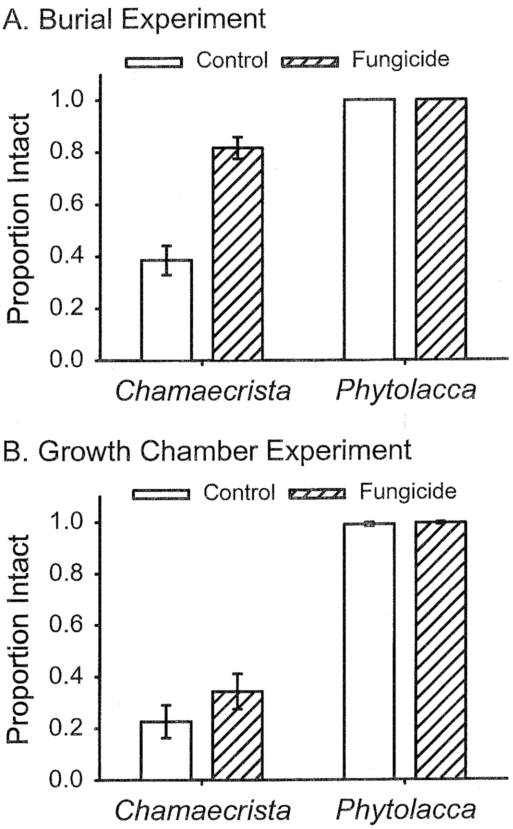

FIG. 1. The effect of fungicide on the proportion of intact seeds of *Phytolacca americana*, a species known to contain antifungal compounds, compared to *Chamaecrista fasciculata*, a species not known to possess antifungal compounds. **A.** The proportion of intact *C. fasciculata* seeds recovered after burial from December 12, 2003 to March 28, 2004 was significantly improved by the addition of fungicide prior to burial. Fungicide did not affect the proportion of intact *P. americana* seeds recovered. Seeds were considered intact if they had hard, undamaged seed coats after burial or if they successfully germinated while buried. **B.** The proportion of non-buried seeds remaining intact after germination trials in a growth chamber where fungal contamination resulted in seed losses. Trials were conducted from April 29, 2004 to June 6, 2004. Fungicide treatment significantly increased survival of *C. fasciculata* but not *P. americana*. Regardless of treatment, significantly more *P. americana* were intact than *C. fasciculata*. In both panels, error bars represent ± 95% confidence intervals.

persistence, the data nonetheless provided additional support for the importance of antifungal compounds for increasing *P. americana* seed survival relative to *C. fasciculata*. Averaged across treatment, the proportion of buried *P. americana* seeds that subsequently survived fungal attack in the growth chamber was 0.98 ± 0.01, whereas the proportion of buried *C. fasciculata* that subsequently survived was 0.42 ± 0.06.

Results of the non-buried seed trials were qualitatively identical to the results with buried seeds (Fig. 1B). Fungicide treatment significantly increased the proportion of intact *C. fasciculata* ($\chi^2 = 6.60$, $P = 0.01$, df = 1) but did not increase the proportion of intact *P. americana* ($\chi^2 = 0.34$, $P = 0.56$, df = 1). The proportion of intact control *P. americana* seeds was greater than control *C. fasciculata* ($\chi^2 = 298.9$, $P < 0.01$, df = 1) and fungicide-treated *C. fasciculata* ($\chi^2 = 229.7$, $P < 0.01$, df = 1). Similarly, the proportion of intact fungicide-treated *P. americana* was greater than control (Fig. 1B; $\chi^2 = 306.8$, $P < 0.01$, df = 1) and fungicide-treated *C. fasciculata* ($\chi^2 = 237.6$, $P < 0.01$, df = 1).

Discussion. Although other studies have documented that fungal pathogens have important effects and that seeds vary in susceptibility to fungi (Kirkpatrick and Bazzaz 1979, Crist and Friese 1993, Leishman et al. 2001, Blaney and Kotanen 2001, 2002; Schafer and Kotanen 2003, 2004), this study is among the first to show that seeds with known antifungal properties exhibit greater persistence in the seedbank, even over relatively short periods of exposure (Fig. 1A). Over longer periods, antifungal properties may explain why *P. americana* seeds can remain viable in soil for at least 40 years (Toole and Brown 1946). Fungi led to substantial losses of *C. fasciculata*, as has been found for other old-field plant species (Blaney and Kotanen 2001, Schafer and Kotanen 2004).

The antifungal properties of *P. americana* seeds may take the form of physical characteristics (e.g., seed coat thickness) or antifungal compounds. Because we did not isolate antifungal compounds from the seeds and experimentally manipulate their presence for logistical reasons, we ultimately cannot distinguish between these two possibilities. However, the substantial evidence regarding the presence of antifungal compounds in *P. americana* (Minami et al. 1998, Liu et al. 2000) and their absence in *C. fasciculata* (Raskin and Poulev 2002) suggests that any differences in fungal susceptibility are due, at least in part, to the presence of antifungal compounds. Regardless of the mechanisms underlying the antifungal properties of *P. americana* seeds, we have documented that these properties lead to substantial increases in *P. americana* short-term survival in the seedbank, while the susceptibility of *C. fasciculata* to fungi can lead to substantial losses (Fig. 1); further experiments conducted over longer time periods using seeds where antifungal compounds have been experimentally manipulated would be a fruitful area of research.

As adaptations, antifungal compounds may serve two important, interrelated functions: antifungal compounds directly reduce losses to pathogenic fungi and may also indirectly increase the success of *P. americana* against potential competitors without antifungal properties (e.g., *C. fasciculata*) that experience higher mortality due to fungi. These two advantages of antifungal compounds may play an important role in structuring plant communities, given the variability in fungal susceptibility found among different plant species (e.g., Blaney and Kotanen 2001, Schafer and Kotanen 2004). Because susceptibility to soil fungi may vary among habitats depending upon physical conditions of the seedbank (Blaney and Kotanen 2001), the advantage of antifungal compounds is also likely to vary among habitats. For example, antifungal compounds may be most advantageous in lowland habitats with moist soils and potentially greater risk of fungal attack (Blaney and Kotanen 2001). Future work focusing on the prevalence of antifungal compounds in plant species with different competitive abilities and the large-scale variation in the prevalence of pathogenic fungi will help provide more insight into the importance of antifungal compounds in the ecology and evolution of plants.

Literature Cited

BLANEY, C. S. AND P. M. KOTANEN. 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. J. Appl. Ecol. 38: 1104–1113.

BLANEY, C. S. AND P. M. KOTANEN. 2002. Persistence in the seed bank: the effects of fungi and invertebrates on seeds of native and exotic plants. Ecoscience 9: 509–517.

BROEKAERT, W. F., F. R. G. TERRAS, B. P A. CAMMUE, AND R. W. OSBORN. 1995. Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Phys. 108: 1353–1358.

CRIST, T. O. AND C. F. FRIESE. 1993. The impact of fungi on soil seeds: implications for plants and granivores in a semiarid shrub-steppe. Ecology 74: 2231–2239.

DIX, N. AND J. WEBSTER. 1995. Fungal ecology. Chapman and Hall, London, UK. 549 p.

EDWARDS, M. E., E. M. HARRIS, F. H. WAGNER, M. C. CROSS, AND G. S. MILLER. 1988. Seed germination of American pokeweed (*Phytolacca americana*). I. Laboratory techniques and toxicity. Am. J. Bot. 75: 1794–1802.

FARMER, R. E. JR. AND G. C. HALL. 1970. Pokeweed seed germination: effects of environment, stratification, and chemical growth regulators. Ecology 51: 894–898.

KIRKPATRICK, B. L. AND F. A. BAZZAZ. 1979. Influence of certain fungi on seed germination and seedling survival of four colonizing annuals. J. Appl. Ecol. 16: 515–527.

LEISHMAN, M. R., G. J. MASTERS, I. P. CLARKE, AND V. K. BROWN. 2000. Seed bank dynamics: the role of fungal pathogens and climate change. Funct. Ecol. 14: 293–299.

LITTELL, R. C., W. W. STROUP, AND R. F. FREUND. 2002. SAS for linear models. SAS Institute, Cary, NC. 496 p.

LIU Y., J. LUO, C. XU, R. REN, C. PENG, G. WU, AND J. ZHAO. 2000. Purification, characterization, and molecular cloning of a seed-specific antimicrobial protein from Pokeweed. Plant Phys. 122: 1015–1024.

MCDONNELL, M. J., E. W. STILES, G. P. CHEPLICK, AND J. J. ARMESTO. 1984. Bird-dispersal of *Phytolacca americana* L. and the influence of fruit removal on subsequent development. Am. J. Bot. 71: 895–901.

MINAMI, Y., S. HIGUCHI, F. YAGI, AND K. TADERA. 1998. Isolation and some properties of the antimicrobial peptide (Pa-AMP) from the seeds of pokeweed (*Phytolacca americana*). Biosci. Biotech. Biochem. 62: 2076–2078.

NEERGAARD, P. 1977. Seed Pathology, Volume 1. Halsted Press, New York, NY. 1187 p.

ORROCK, J. L., B. J. DANIELSON, M. J. BURNS, AND D. J. LEVEY. 2003. Spatial ecology of predator-prey interactions: corridors and patch shape influence seed predation. Ecology 84: 2589–2599.

RADFORD, A. E., H. E. AHLES, AND C. R. BELL. 1968. Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill, NC. 1245 p.

RASKIN, I. AND A. POULEV. 2002. Elicited Plant Products. United States Patent Application no. 20020132021.

SCHAFER, M. AND P. M. KOTANEN. 2003. The influence of soil moisture on losses of buried seeds to fungi. Acta Oecol. 24: 255–263.

SCHAFER, M. AND P. M. KOTANEN. 2004. Impacts of naturally-occurring soil fungi on seeds of meadow plants. Plant Ecol. 175: 19–35.

TOOLE, E. H. AND E. BROWN. 1946. Final results of the Duvel buried seed experiment. J. Agron. Res. 72: 201–210.

TORGESON, D. C. 1969. Fungicides: an advanced treatise. Volume II. Chemistry and Physiology. Academic Press, New York, NY. 540 p.