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Heat-Treatment Effects on Seed Bank Species of an Old-growth Douglas-fir Forest

Abstract

Although many studies have investigated the density and species composition of soil seed banks, few studies have investigated the effect of disturbance on seed banks. An earlier field study (Clark 1991) showed that the density of buried seeds of an old-growth Douglas-fir forest after logging and slash-burning was significantly less than the seed bank density of an adjacent, undisturbed old-growth Douglas-fir forest. The purpose of the present study was to investigate the mechanisms for this seed bank reduction. Six species found in the old-growth seed bank received experimental heat treatments that included six combinations of temperature, duration and soil moisture. Generally, germination percentages were not significantly affected when seeds were heated at 50C for one hour in either the wet or dry soil, but were significantly reduced at 100C for 15 min in both the wet and dry soils. When the seeds were heated at 75C for 15 min, germination was significantly reduced in the wet soil only. Because of the seeds' sensitivity to heat, few of these seed bank species are likely to be available for establishment after fire, even one of low intensity. This experimental study supports the hypothesis that seed mortality from elevated temperature during fire reduced seed bank densities of the old-growth forest and does not support the hypothesis that the reduction was due to stimulation of germination by heat. Although this study tested species with different growth forms and longevities, germination responses were generally the same for all species.

Introduction

Substantial numbers of dormant seeds have been found in the soil of most habitats (Leck *et al.* 1989). This soil seed bank provides an immediate source of propagules for revegetation after disturbance (Roberts 1981, Pickett *et al.* 1987). The persistent seed bank also maintains a reservoir of genotypes that can provide substantial flexibility to environmental changes, buffering the population from extinction and preserving the species within a community (Templeton and Levin 1979). Although numerous studies have investigated the seed density and species composition of soil seed banks (Leck *et al.* 1989), relatively few studies have investigated the effect of disturbance on seed bank density and composition (Roberts and Dawkins 1967, Uhl *et al.* 1981, Mallik and Gimingham 1985, Smith and Kadlec 1985, Hassan and West 1986, Kauffman 1986). High temperatures from fire generally lower the seed bank density either by seed mortality or by heat-stimulated seed germination, thus favoring the survival of species with heat-resistant seeds or with seeds that are stimulated to germinate by heat.

Both natural fires and slash-burning after clearcut harvesting are important disturbances in the coniferous forests of the Pacific Northwest. We recently compared the seed bank density and composition of a recently disturbed old-growth Douglas-fir forest with the seed bank of an adjacent undisturbed old-growth Douglas-fir forest (Clark 1991). The disturbed site was logged in late fall 1985 and the slash burned with a low-intensity fire in July, 1986. Temperatures of the mineral soil during the fire were between <52C and 177C at the surfaces, between <39C and 139C at 2 cm, between 52C and 66C at 4 cm, and <52C at 6 cm (M. V. Wilson, unpublished data). (The duration of the temperature exposure was not measured.) Seed bank samples collected the following spring showed that the disturbed seed bank density (18.4/m^2) was significantly less than the old-growth seed bank (88.6 seeds/m^2) (Clark 1991).

The purpose of the present study was to investigate the mechanisms for this seed bank reduction using an experimental approach in a controlled laboratory setting. The specific objective was to determine the response of seeds to various temperatures, durations of heating, and soil conditions (wet or dry).

Study Species

All herb and shrub species found in the soil seed bank of an old-growth Douglas-fir forest (Clark 1991) were selected for experimental heat treatment except for Watson's willow-herb (*Epilobium watsonii*), soft rush (*Juncus effusus*), and Pacific blackberry (*Rubus ursinus*), which were not included because of difficulty in germinating the seeds of these species. The remaining six study species included an early successional annual, wood groundsel (*Senecio sylvaticus*), an early successional biennial, common thistle (*Cirsium vulgare*), two herbaceous perennials, Dewey's sedge (*Carex deweyana*) and fireweed (*Epilobium angustifolium*), and two old-growth woody understory species, western rhododendron (*Rhododendron macrophyllum*) and salal (*Gaultheria shallon*). Four of these species, *Gaultheria shallon*, *Rhododendron macrophyllum*, *Epilobium angustifolium* and *Senecio sylvaticus*, are also important components of the initial vegetation following disturbance by logging (Dyrness 1973, Halpern 1989, Clark 1991). Little information is known about seed densities at different soil depths and the seed longevity of these species in old-growth Douglas-fir forest seed banks (Kellman 1970, 1974).

Evidence from an earlier study (Clark 1991) showed that relatively few individuals established from seed the first year after disturbance at the study site. Kellman (1974) suggests that only logged sites contiguous to an established early successional community would receive appreciable migrant seed of early successional species due to the inefficient movement of these species' seeds through forest stands. Since our site was not adjacent to such seed sources, a large portion of the few individuals establishing from seed the first year probably came from seed bank propagules. The second year after disturbance brought a tremendous increase in individuals establishing from seed (Clark 1991). The source of these seeds was probably on-site seed dispersal from the few annuals establishing from seed in the first year, particularly *Senecio sylvaticus*. Thus, although seed bank densities were low compared to other forests (Strickler and Edgerton 1976, Moore and Wein 1977, Kramer and Johnson 1987, Morgan and Neuenschwander 1988, and Fyles 1989), plants establishing from the seed bank might strongly influence revegetation starting in the second year after disturbance.

Methods

Seeds were collected at maturity from plant populations growing at the study site located in the H.J. Andrews Experimental Forest, a National Science Foundation Long-Term Ecological Research Site in the western Cascade Mountains of Oregon. The collection site, about 800 m elevation, was dominated by old-growth western hemlock (*Tsuga heterophylla*) and Douglas-fir (*Pseudotsuga menziesii*) with some western redcedar (*Thuja plicata*). The understory included dwarf Oregon grape (*Berberis nervosa*), twinflower (*Linnaea borealis*), red huckleberry (*Vaccinium parvifolium*), *Rhododendron macrophyllum* and *Gaultheria shallon*. The habitat-type is classified as western hemlock/rhododendron/dwarf Oregon grape (Dyrness *et al.* 1974). After seed collection in the summer of 1988, the seeds were stored dry at 4C until experimental treatments were conducted in early 1990. Preliminary germination tests necessitated this schedule.

Experimental treatments included six combinations of temperature, duration and soil moisture: seeds heated in dry soil at 50C for one hour, 75C for 15 min and 100C for 15 min, and seeds heated in moist soil at the same three temperatures and durations. These conditions were selected to match soil temperature data collected during the 1986 slash burn following logging near the site (M. V. Wilson, unpublished data) and to match moisture extremes found between the wetter soils of spring and late fall and the drier soils of summer.

For each heat treatment, two test tubes (2.5 cm diameter x 15 cm deep) per species were placed in a water bath. Each tube was filled with 20 seeds mixed with approximately 15 cm^3 dry soil; 10 ml of water was added to the second tube 3 minutes before being placed in the water bath. (For *Cirsium vulgare*, 10 seeds were used in each test tube). This procedure was replicated five times per experimental treatment. These methods are modifications of methods commonly used by other researchers investigating heat effects on seed germination (Gratkowski 1962, Keeley *et al.* 1985, Mallik and Gimingham 1985, Kauffman 1986, Bell *et al.* 1987, Keeley 1987).

In preliminary tests, thermistors were placed at four locations in a test tube filled with 15 cm^3 soil: at the surface, in the center midway down the tube, along the side next to the glass midway down, and at the bottom. Temperatures were recorded during each of the six experimental conditions. These tests showed that (a) it took approximately 7-10 min for the soil temperature in the test tubes to equilibrate, (b) the temperature of the soil at equilibrium was the same as the temperature of the water bath, with the exception of the dry soil, which was 98C at the 100C treatment, and (c) the soil temperature after equilibration was uniform throughout the test tube during each treatment.

After each heat treatment the contents of each test tube were emptied into a Petri dish. In addition, to make sure no seeds were left behind, the residues in the test tubes with wet soil were rinsed with distilled water, filtered, air dried and added to the appropriate Petri dish. Five controls (no heat treatment) for each species were treated identically as the experimental treatments; that is, the same procedures for transferring soil and seeds were followed for the controls as were used for the experimental treatments.

The Petri dishes were placed in a controlled environment with 14 hours of light and moistened with distilled water as needed. Preliminary tests showed that *Gaultheria shallon*, *Senecio sylvaticus* and *Rhododendron macrophyllum* germinated best with alternating temperatures of 20C for 14 hours and 15C for 10 hours. The other species germinated best at alternating temperatures of 30C for 14 hours and 20C for 10 hours. Therefore, these temperatures were used to promote germination. The number of germinating seeds was counted until no new germination occurred for at least 14 days. Seeds were considered germinated when the shoot could be seen.

Differences in final germination rates between treatments and controls were tested for statistical significance with a Mann-Whitney test (Conover 1980).

Results and Discussion

For five of the six species, germination percentages were not significantly affected compared to unheated controls when seeds were heated at 50C for one hour in either the wet or dry soil (Table 1). The single exception was *Epilobium angustifolium* for which germination was significantly reduced in the wet soil at 50C. Germination percentages of all six species were significantly reduced at 100C for 15 min in both the wet and dry soils (Table 1). When the seeds were heated at 75C for 15 min, germination was significantly reduced in the wet soil only. No treatment produced significantly higher germination than the control. Thus, seeds of these seed bank species appear to be killed at the relatively low temperatures of 75C and 100C, with the effect stronger in wet soil.

Several factors may explain why the wet and dry soil treatments differed at 75C. The increased seed mortality with wet soils might have been due to increased heat conductivity through the moist seed coats, causing death of interior seed tissue. The seeds in the wet soil could also have imbibed more water than those in the dry soil. Seeds, particularly those of herbaceous species, that absorb moisture beyond 20% of their dry weight can be quite sensitive to heat (Parker and Kelly 1989), possibly because enzymes are more susceptible to heat denaturation when hydrated than when dry (Salisbury and Ross 1985). Although preliminary tests at 75C showed that dry and wet soils differed little in temperature, the wet soil at 75C took an average of about 9 min to cool to 50C after removal from the water bath compared to the average time of 5 min for the dry soil; thus, more seeds could

ABLE 1. Mean seed germination percentages following experimental heat treatments of six species found in the seed bank of an old-growth Douglas-fir forest. The treatments were: W50, 50C for 60 min in wet soil; W75, 75C for 15 min in wet soil; W100, 100C for 15 min in wet soil; D50, 50C for 60 min in dry soil; D75, 75C for 15 min in dry soil; D100, 100C for 15 min in dry soil; and CON, control. n=5. Differences in germination percentages between treatment and control were tested using the Mann-Whitney test.

SPECIES	TREATMENTS						
	W50	W75	W100	D50	D75	D100	CON
Carex deweyana	85	0**	0**	89	89	0**	89
Cirsium vulgare	34	0**	0**	44	32	6*	62
Epilobium angustifolium	0**	0**	0**	26	30	0**	20
Gaultheria shallon	21	0**	0**	20	10	1**	17
Rhododendron macrophyllum	37	0**	0**	33	48	2**	41
Senecio sylvaticus	72	0**	0**	72	76	29**	87

* $P < 0.05$
** $P < 0.01$

have been killed because of the longer temperature durations.

Germination percentages of all species were significantly reduced after heating to 100C in both wet and dry soils. This experimental study thus supports the hypothesis that the general reduction in the mean seed bank density after logging and slash-burning of the old-growth Douglas-fir forest (Clark 1991) was caused by seed mortality from elevated temperature during fire. This general reduction contrasts with survival of temperatures greater than 100C by seeds of several species of mixed coniferous forests (Strickler and Edgerton 1976) and of chaparral (Keeley *et al.* 1985). The results presented here do not support the hypothesis that reduction was due to stimulation of germination by heat, as sometimes occurs in Douglas-fir forests (Gratkowski 1962), heathlands (Mallik and Gimingham 1985), chaparral (Keeley *et al.* 1985), and mixed coniferous forests of Sierra Nevada (Kauffman 1986).

Although this study tested several species with different growth forms and longevities, germination responses were similar (Table 1): all species were killed at relatively low temperatures and none were stimulated by heat to germinate (Table 1). Other studies report a diversity of germination responses to heat treatments among species within a single ecosystem (Floyd 1966, Keeley *et al.* 1985, Mallik and Gimingham 1985, Bell *et al.* 1987). Species whose seeds are able to survive elevated temperatures or whose seeds are physically stimulated by heating are sometimes more prominent early in succession after disturbance (Floyd 1966, Keeley *et al.* 1985, Morgan and Neuenschwander 1988). Similarity of responses to elevated temperatures seen in this study, however, argues against fire as an agent favoring the seeds of one of the six study species over another.

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