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Brief Report

The Response of *Botrytis cinerea* to Fire in a Coast Redwood Forest

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Abstract: Coast redwoods (*Sequoia sempervirens*) are long-lived trees that create deep shade and litter layers, and have limited recruitment from seedlings. *Botrytis cinerea* is an airborne fungal pathogen that attacks redwood seedlings. *B. cinerea* lives as a saprotroph in dead plant matter or as a necrotroph in live tissue. In the coast redwood forest, accumulated leaf litter may provide inoculum for subsequent infections, limiting redwood seedling recruitment. Here, we examine the response of *B. cinerea* to fire in the coast redwood forest. We measured the abundance of airborne *B. cinerea* spores in paired burned and unburned plots using a selective and diagnostic medium. In a greenhouse experiment, we grew seedlings in four different treatments: (1) burned soil with no leaf litter, (2) unburned soil with no leaf litter, (3) burned soil with leaf litter collected from the burn plot, (4) unburned soil with leaf litter collected from the unburned plot. Spore trapping showed no difference in the abundance of airborne spores in the paired plots. The seedling experiment showed that disease was greatest and survival lowest when grown in burned soil; leaf litter collected from burned plots reduced survival while leaf litter from not-burned plots increased survival. These results indicate that fire did not affect airborne *B. cinerea* and post-fire conditions did not provide favorable growth conditions for coast redwood seedlings.

Keywords: *Botrytis cinerea*; gray mold; wildfire-disease interaction; coast redwood forest; *Sequoia sempervirens*

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1. Introduction

Botrytis cinerea, commonly known as gray mold, is an airborne fungal plant pathogen that infects over 200 crop species and 100 woody and herbaceous plant species [1]. *B. cinerea* is an economically important pathogen, causing over $10 billion dollars in post-harvest crop damage each year and an estimated 15–50% of fruit loss in post-harvest production [2,3]. Infection occurs when spores land on host plants and secrete cell-wall-degrading enzymes that allow the penetration of host cells and the subsequent use of host cells as food [4]. *B. cinerea* has a flexible life cycle as it is able to kill live plant tissue as a necrotrophic pathogen, as well as survive in dead plant matter as a saprotroph [5].

B. cinerea threatens coast redwood seedling recruitment (*Sequoia sempervirens*; Cupressaceae), as their tender tissues allow pathogens to easily invade [6,7]. When young seedlings are infected, mycelium grows from the germinating seed into the stem or roots of the seedling, first decaying the seed coat then the whole seedling [6,8]. Disease symptoms include chlorosis on leaves, cankers on stems, drooping leaves, and eventual seedling mortality [9]. Older seedlings that are infected at 1–2 years of age have stunted growth [1].

Fungicides are used in agriculture and tree nurseries to manage gray mold. However, controlling *B. cinerea* is difficult due to the wide range of host species, the ability of the pathogen to survive harsh environmental conditions, and its ability to produce conidia and sclerotia in both live and dead plant matter [10]. In the coastal redwood forest, fire-suppressive land management has led to an accumulation of dead plant material, which may serve as inoculum for infection [11].

Fire is a natural process that removes dead plant material and supports nutrient cycling, leaving exposed mineral soil that provides supportive growing conditions for coast redwood seedlings [7,12]. Wildfire can also shape the impact of plant pathogens in wild systems. Exposure to smoke reduces the spore germination of the western gall rust fungus [13]. Fire has been used to control dwarf mistletoe infection in lodgepole pine trees by killing parts of the host plant that are infected [13]. In contrast, fire-related damage on trees can increase disease as wounds allow pathogens to invade more easily [11]. Here, we explore whether fire may be useful to reduce the presence of *B. cinerea* in the redwood forest.

In this study, we first quantified the difference in the abundance of airborne *B. cinerea* in the redwood forest in burned and unburned plots through the use of a selective and diagnostic medium. In a greenhouse experiment, we then measured disease development and survival of redwood seedlings grown in four different treatments: (1) burned soil with no leaf litter, (2) unburned soil with no leaf litter, (3) burned soil with leaf litter collected from the burned plot, (4) unburned soil with leaf litter collected from the unburned plot. We expected that the abundance of airborne *B. cinerea* will be lowest in burned plots due to the elimination of inoculum sources in accumulated leaf litter, and that disease severity would be lowest in redwood seedlings grown in burned soil without leaf litter, favored by exposed mineral content and lack of inoculum.

2. Materials and Methods

2.1. Study Site

The Campus Natural Reserve (CNR) is located on the campus of the University of California, Santa Cruz (U.S.A.) upon the unceded territory of the Awaswas-speaking Uypi Tribe. On 17 August 2022, 0.25 ha of forest burned following a lightning-ignited fire (Figure 1; 37.004778, −122.065694). The fire was quickly extinguished, but a week later reignited from smoldering logs, resulting in a total of 0.53 ha of the CNR being burned (A. Jones, personal communication, 2 March 2023). The fire effectively cleared leaf litter and understory shrubs from the forest, but did not kill mature trees.

Figure 1. Location of burn site on the University of California Santa Cruz campus. Blue circle shows approximate extent of burn site.

2.2. Media

Botrytis spore trapping medium (BSTM) followed Edward and Seddon [14] glucose, 2 g; $NaNO_3$, 0.1 g; K_2HPO_2, 0.1 g; $MgSO_4{\bullet}7H_2O$, 0.2 g; pentachloronitrobenzene, 0.02 g; Maneb 80 (80% manganese ethylenebisdithiocarbamate), 0.02 g; fenarimol, 0.012 g; tannic acid, 5 g; agar no. 3 20 g. The solution was stirred while heating to a boil and poured into Petri plates while it was still hot. Botrytis-selective medium (BSM) followed Edward and Seddon [14]: glucose, 2 g; $NaNO_3$, 0.1 g; K_2HPO_2, 0.1 g; $MgSO_4{\bullet}7H_2O$, 0.2 g; pentachloronitrobenzene, 0.02 g; Maneb 80 (80% manganese ethylenebisdithio-carbamate), 0.02 g; rose bengal, 0.05 g; tannic acid, 5 g; agar no. 3, 20 g.

2.3. Spore Trapping and Counting

Sampling took place in 10 sets of paired plots located at the site of the 2022 lightning burn on UCSC Campus Natural Reserve in December 2022 (Trial 1) and February 2023 (Trial 2). Transects started at the divide between the burned and unburned land types and continued 15 m into both land types, with sampling at the 5 m and 15 m marks (Figure 2). We sampled at the 5 and 15 m marks to capture the differences in spore abundance at different places within each land type to see if the pathogen may be dispersing among the plots. At the 5 and 15 m marks, 3 Petri plates with BSTM were placed at soil level in each plot for 16 h. At the end of 16 h, plates were collected and incubated in a dry, cool room. Eight days later, we identified colonies by counting brown spots characteristic of developing *Botrytis* colonies on the BSTM under a Darkfield Quebec Colony Counter. A total of 106 plates were set out across the two trial dates.

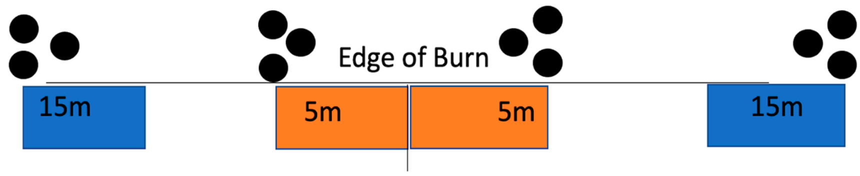

Figure 2. Spore trapping design. Each color block represents one paired plot. Circles represent Petri plates with BSTM medium.

2.4. Seed Germination and Seedling Growth

Coast redwood seeds were purchased from Sheffield's Seed Company (Syracuse, NY, USA; https://www.sheffields.com/; accessed on 25 January 2023). Seeds were stratified in cold water for 24 h before they were sown into seed flats that contained Sunshine Mix soil (Sungro Horiculture (Agawam, MA, USA); https://www.sungro.com/; accessed on 25 January 2023). Seeds were then placed into an incubator with 12 h cycles of 18 °C and 20° until germination, 1 to 3 weeks later.

Once seedlings germinated, they were placed into 25.4 cm long conetainers (Ray Leach cone-tainers, Stuwe and Sons, Inc., Tangent, OR, USA). Sunshine Mix soil (Sun Gro Horticulture, Agawam, MA, USA) filled the bottom half of the conetainer and the rest was filled with soil collected from either burned or unburned soil collected from the field site. The soil on half of the seedlings were covered with leaf litter that was collected from either the burned or unburned field sites. The litter from the burned site was all material that fell after the fire, whereas that from the unburned site included both old and new litter. Seedlings were place in two contiguous conetainer racks (arranged as a single long rack) with treatments in a completely randomized design. Seedlings were kept in a greenhouse that was on a 12 h cycle of 18 °C and 20 °C. Seedlings were watered by hand every other day, and to ensure there was minimal air flow to reduce risk of pathogen spread on air currents, a plastic cover was placed above the conetainer racks. A total of 390 seedlings were grown.

Measurements of height, number of leaves, and disease severity (Table 1) were collected weekly. Seedlings that survived each week had measurements taken and dead seedlings were not measured. Measurement data available in Supplementary Materials.

Table 1. Disease Severity Ranking system for *Botrytis* infection on coast redwood seedlings.

Rank	% Plant Affected	Associated Symptoms
0.00	0%	None
0.25	1–10%	Leaf yellowing appears on tips of leaves
0.50	10–20%	Leaf yellowing appears on the whole leaf
1.00	20–50%	Leaf yellowing on one or more whole leaves, leaves start to drop
1.25	50–70%	One or more leaves has turned white, top half of plant fallen over
1.50	70–90%	One or more leaves white and yellow, top half of the plant fallen over
2.00	90–100%	Plant has no color, plant completely fallen down, and is dead

2.5. Statistical Analysis

Statistical analysis was performed in R Studio version 4.1.3. We tested for differences between burned and unburned plots at the different meter marks using two-factor (burn status and distance from edge) Analysis of Variance (ANOVA) with blocking. Plates that were lost in the field (n = 14) were excluded from this analysis. Cox proportional log-rank tests were performed for each treatment to compare survival rates among the seedlings.

3. Results

3.1. Spore Trapping

There was no significant difference in the abundance of airborne spores of *B. cinerea* in burned and unburned plots (Figure 3; Trial 1: $F_{3,10} = 0.08$, $p = 0.970$; Trial 2: $F_{3,12} = 0.950$, $p = 0.447$). There was no significant difference in the abundance of airborne *B. cinerea* spores between the 5 and 15 m sampling marks (Figure 3; Trial 1: $F_{4,10} = 5.66$, $p = 0.693$; Trial 2: $F_{4,12} = 1.693$, $p = 0.216$).

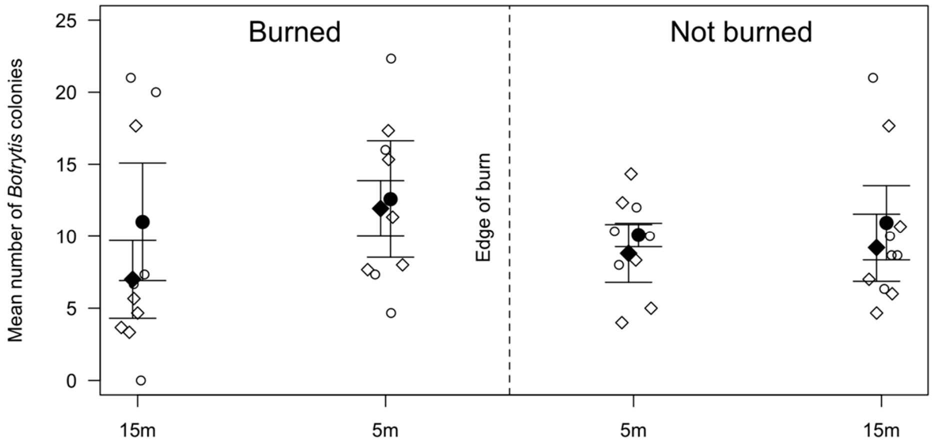

Figure 3. *Botrytis* colonies captured on selective medium on paired burned and unburned plots. Trial 1 results are shown as circles and Trial 2 results are shown as diamonds. Filled shapes are the means for each trial.

3.2. Seedling Measurements

Seedlings grown with leaf litter were consistently larger than their counterparts grown without leaf litter (Figure 4). This effect of litter did not depend on whether soil had been burned or not; burning had no effect on plant height (Figure 4).

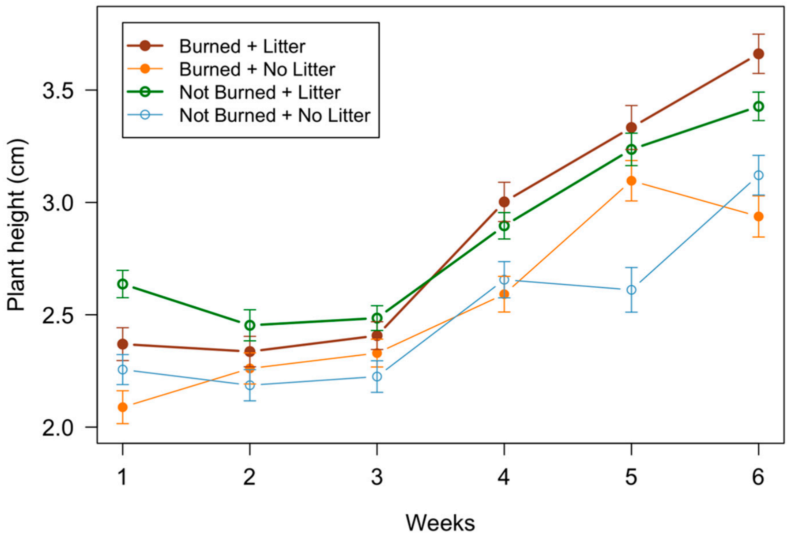

Figure 4. Mean plant height (± standard error) of redwood seedlings grown in a factorial greenhouse experiment with burned/not-burned soil and with or without leaf litter (starting n = 96 for each treatment).

In contrast, the presence of litter did not have a consistent impact on the number of leaves produced by seedlings (Figure 5). However, in the absence of leaf litter, seedlings grown in burned soil produced up to 50% more leaves than did those grown in not-burned soil (Figure 5).

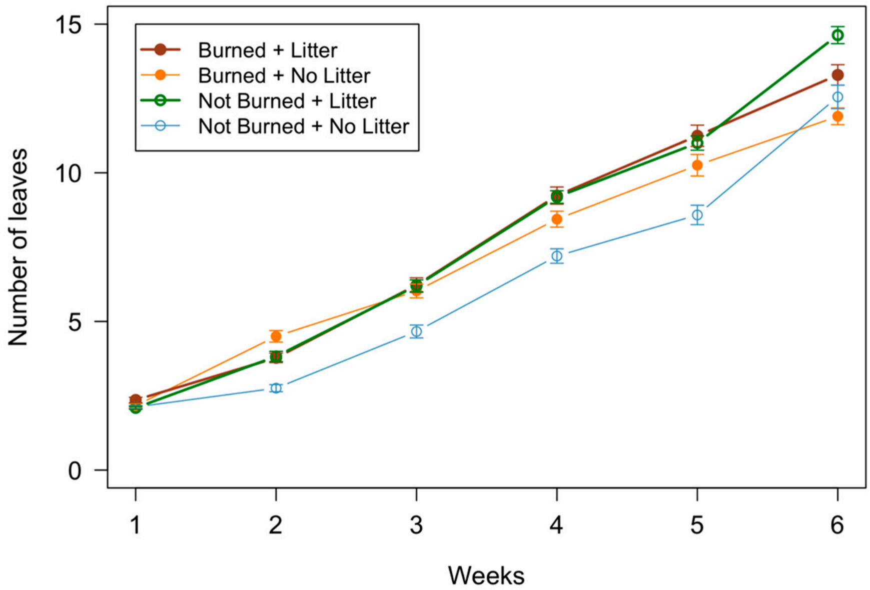

Figure 5. Mean number of leaves (± standard error) of redwood seedlings grown in a factorial greenhouse experiment with burned/not-burned soil and with or without leaf litter (starting n = 96 for each treatment).

3.3. Disease Development

The majority (65%) of the seedlings in the experiment developed diseased symptoms consistent with gray mold infection. *Botrytis* infection was confirmed by placing symptomatic plants onto BSM. At nearly all time points, disease severity was greater in burned soil than in not-burned soil, with no consistent effect of litter (Figure 6).

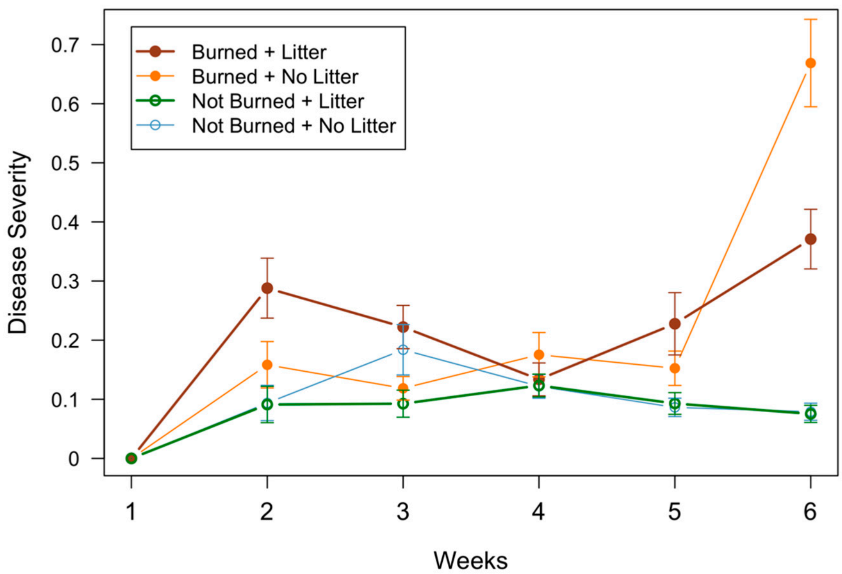

Figure 6. Mean disease severity (± standard error) in redwood seedlings grown in a factorial greenhouse experiment with burned/not-burned soil and with or without leaf litter (starting n = 96 for each treatment).

3.4. Survival Analysis

Seedlings had significantly lower survival in burned than in not-burned soil when litter was present (likelihood ratio test = 37.85, $p < 0.00001$), but there was no significant effect of burning in the absence of litter (LRT = 0.66, $p = 0.4$) (Figure 7). Leaf litter significantly increased the survival of seedlings in not-burned soil (LRT = 32.01, $p < 0.00001$), but survival was marginally significantly lower when litter was added to burned soil (LRT = 3.55, $p = 0.06$) (Figure 7).

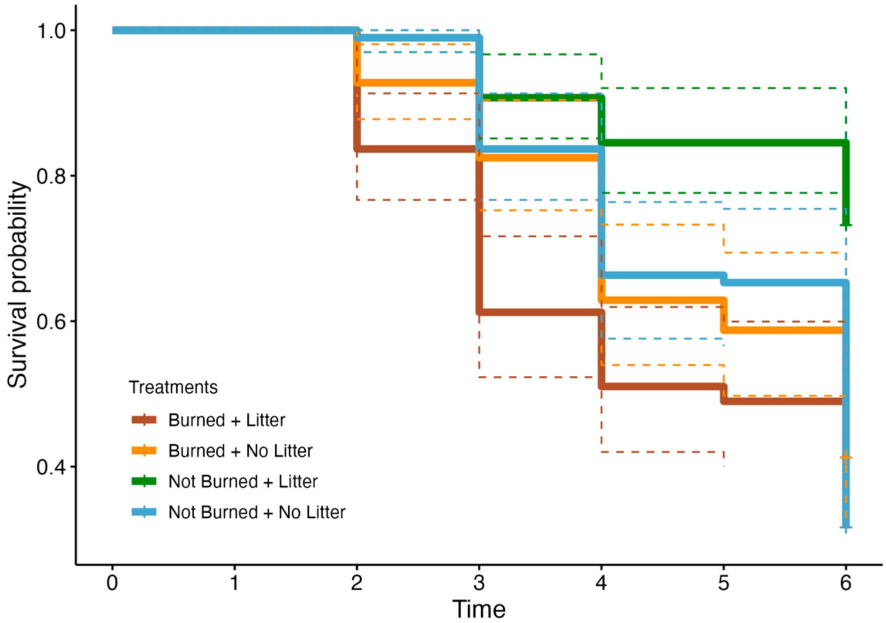

Figure 7. Time to death for coast redwood seeds that were grown (n = 96 per treatment). Dashed lines indicate 95% confidence intervals for solid lines of same color.

4. Discussion

We expected that the abundance of airborne *B. cinerea* would be lower in burned plots than unburned plots, since fire can eliminate the dead plant material that serves as a source of inoculum for infection [11]. The results of the spore trapping showed no difference in the number of airborne *B. cinerea* colonies formed in the burned and unburned plots, or between plates placed 5 to 15 m from the burn edge. Some research has indicated that wildland fires can aerosolize and transport microbes, leading to an increase in the concentration of airborne microbes [15–17], although this effect is not universal [18]. Camacho et al. [19] found that airborne fungal spores of all kinds of fungi roughly doubled in concentration during a window of 9 to 13 days after forest fires on Madeira Island, Portugal, but that fungal spore density decreased to normal ranges quickly thereafter. Our sampling happened several months after the fire; it is possible that there was a transient change in spore abundance before we sampled. While there is significant work on the effects of wildfires on fungi in forest soils [20–23], we are aware of no other literature that specifically addresses airborne fungal spore abundance, and none focused on *Botrytis*.

The lack of difference in airborne spores of *B. cinerea* suggests that sources other than forest floor litter dominate airborne inoculum. Because the fire did not scorch the tree canopy itself (some 20–40 m above the ground), infected foliage may contribute to the presence of gray mold in the area through dropping infected leaves or shedding fungal spores. At the time of sampling, a small amount of fresh leaf litter was present in the burned plots, dead leaves having fallen from the canopy above. Previous work has documented abundant and diverse fungal communities in the redwood forest canopy [24] including *Botrytis* [25].

We expected that seedlings grown in unburned soil with leaf litter would have the lowest growth rate and number of leaves formed as the leaf litter may contain inoculum for *B. cinerea* infection that would reduce their growth. Instead, we found that disease severity was greatest in burned soil with no consistent impact of litter (Figure 6), seedlings grown with litter were consistently taller (Figure 4), and there were no consistent effects of litter or burning on leaf number (Figure 5). This suggests that fire does not create soil conditions suitable for *S. sempervirens* seedlings. Redwood resprouts commonly appear after fires; resprouts may be more tolerant than seedlings of the soil conditions following fire [26]. Additionally, fire can diminish the fungal and bacterial communities present in soil, and a loss of beneficial microbes might contribute to the low survival rate for seedlings grown in burned soil [27].

Leaf litter decreased survival of seedlings if they were grown in burned soil, but had no effect on seedling survival in unburned soil (Figure 7). Disease severity was lowest in seedlings grown in unburned soil with leaf litter and highest in seedlings grown in burned soil with leaf litter. This suggests that fresh leaf litter may contain more inoculum than older leaf litter, possibly contributing to more disease and lower survival rates in seedlings.

5. Conclusions

Our results indicate that fire has no effect on the presence of airborne *B. cinerea* spores, at least for small-scale, low-intensity wildfires. We were surprised to find that seedlings grown in soil from burned areas had greater disease severity and lower survival than those grown in soil from non-burned areas. Surviving seedlings were consistently taller when grown in the presence of litter, and had greater survival in litter from non-burned areas than in fresh litter from burned areas. Future research should focus on looking at how various degrees of burning impacts airborne spore presence and how leaf litter age impacts disease severity on seedlings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijpb15010008/s1, Data file: SeedlingMeasurements_Rojas_Gilbert.csv.

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