# FROM THE LABORATORY TO THE FIELD: INVESTIGATING THE EFFECT OF BIOCHAR ON FOREST INSECTS

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Stacey Lee Rice

Approved by:
Major Professor: Stephen P. Cook, Ph.D.
Committee Members: Edwin Lewis, Ph.D.;
Luc Leblanc, Ph.D.; Deborah Page-Dumroese, Ph.D.
Department Administrator: Edwin Lewis, Ph.D.

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## ABSTRACT

Insects provide numerous ecosystem services in a forest environment, such as pollination, nutrient cycling, as well as providing disturbance. Defoliating lepidoptera such as Douglas-fir tussock moth (*Orgyia pseudotsugata* Lepidoptera: Erebidae) attack healthy trees, predominantly true firs (*Abies sp.*), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and spruce (*Picea sp.*), and convert tree foliar biomass into frass, contributing soil organic matter within the forest ecosystem that benefit soil organisms. In outbreak years, high densities of *O. pseudotsugata* may consume all available foliage. Defoliation of large forest stands allows for light to penetrate to the undergrowth and accelerate the regenerative succession of the forest stand. Wood boring insects including bark beetles help to cycle nutrients by increasing the rate of breakdown of woody material which also contributes to accumulation of soil organic matter.

Biochar is a carbon-rich material made via thermochemical decomposition of organic matter in a high temperature, low oxygen environment. Biochar can be created from many types of organic material such as discarded slash material during logging of overstocked stands or beetle killed trees, and used as a soil amendment to restore degraded soils. Biochar has been used to sequester carbon, and to increase soil water holding capacity and plant available water. Forest insects may be exposed to biochar when the material is applied to surface organic horizons and downed trees. The results of recent laboratory studies show a potential negative effect on insects exposed to biochar material, although field experiments are necessary to establish how insects are affected by the application of biochar in a forest system.

In the first experiment, direct exposure of *O. pseudotsugata* to biochar either on the surface of or incorporated within synthetic diet negatively affected survival and weight gain of the insects. Although the physiological effects of biochar are unknown, the low 10% volume/volume biochar treatment potentially may lead to compensatory feeding by the insects. Two field experiments were performed using sections (bolts) of ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson), to determine if biochar applied to the bark surface (1) interfered with attack or emergence of the pine engraver beetle *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae) when bolts were baited with a pheromone lure, or (2) altered species richness or abundance of insect assemblages on non-baited bolts. Similar mean density of nuptial chambers and emergence indicated both control and biochar treated bolts were suitable habitat for *I. pini*. Species richness was greater in the non-treated control bolts compared to the bolts treated with biochar for emerged insects in the test that compared bolts that did not receive a pheromone treatment. Red turpentine beetles, *Dendroctonous valens (LeConte)* (Coleoptera: Curculionidae: Scolytinae) were more abundant in non-treated control bolts as compared with the biochar treated bolts. Colonization by other insect taxa were not found to be significantly different between non-treated control bolts and bolts treated with biochar, although the insects that emerged from each bolt varied.

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## SIMPLE SUMMARY

The novel use of carbon-rich biochar as a soil amendment in forest systems may be beneficial in the restoration of disturbed sites due to its ability to increase soil water holding capacity, potentially reduce drought stress in surrounding vegetation and aid in long-term carbon sequestration. As biochar is utilized in forest management, it is necessary to establish the potential effects that it may have on insects and other invertebrate assemblages. The results of recent laboratory studies demonstrate a potential for negative impacts on insects. Examining direct exposure of insects to biochar in a laboratory experiment may help us understand what effects biochar may have on insects that come into direct contact with the material. Along with direct exposure, biochar applications in the field would result in the surface and possible contamination of insect nutrient sources. To determine the impacts of ingesting biochar, we reared Douglas-fir tussock moth, *Orgyia pseudotsugata,* on synthetic diet to examine the insect's survival and longevity.

## ABSTRACT

The use of biochar as a soil amendment in forest ecosystems can be beneficial in the restoration of degraded soils. Forest insects such as the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough) (Lepidoptera: Erebidae), may be exposed to biochar when the material is applied. Two experiments were conducted using biochar either (1) applied to the surface of the diet at three rates (0, 5, and 10 mg) or (2) incorporated into synthetic diet at four rates (0, 10, 20, and 40% volume/volume). The objective of both experiments was to determine if biochar on the surface or incorporated into a synthetic diet affected development and survival of *O. pseudotsugata* larvae. In both experiments, there was a significant decrease in estimated time to larval mortality in all biochar treatments compared to untreated controls. In the surface-applied biochar experiment, there was a significant difference in larval weight gain at day 12 between the control and 10 mg biochar treatments. In the experiment with biochar incorporated into the diet, mean larval weight at day 12 was highest in the low (10%) biochar treatment compared to all other treatments, although weight gain was only significantly different between the low- and high-concentration (40%) biochar treatments. Our results suggest that larvae, feeding on a low amount of biochar in the synthetic diet, may respond by engaging in compensatory feeding behavior. Fewer surviving larvae in the biochar treatment groups may contribute to the lack of significance found in the comparison of weight gain at day 24 in each experiment.

**Keywords:** forest defoliator; soil amendment; compensatory feeding

## INTRODUCTION

Douglas-fir tussock moth (*Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Erebidae)) is a native forest insect in the western United States and Canada that primarily feeds on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), true firs (*Abies* sp.) and spruce (*Picea* sp.) trees [1]. Each female moth may lay up to 200 eggs, all in a single egg mass, with the number of eggs per mass depending upon the phase of the infestation, as well as other environmental conditions [2]. Young larvae feed exclusively on new needles and, as the larvae mature, they switch to older, tougher needles. Past land management practices such as extensive timber harvesting and fire suppression have led to dense, overstocked stands that are dominated by shade-tolerant host species [3]. These stands are more susceptible to defoliator outbreaks than were presettlement forests [4]. Periodic outbreaks of *O. pseudotsugata* occur every 8 to 12 years when natural controls are unable to keep the population in check and can last 2 to 5 years, with defoliation contributing to growth loss, weakened trees, top-kill and tree mortality over large areas [5–7]. During an outbreak, suppression of *O. pseudotsugata* can be achieved with applications of chemical or microbial insecticides [5,8], bole-injected systemic insecticides [9], insect growth regulators [10], or mating disruption pheromones [7,11].

There is a complex of natural enemies associated with *O. pseudotsugata* including a naturally occurring nuclear polyhedrosis virus [12] as well as multiple parasitoids and predators [11,13,14]. In addition to these, the toxicity of monoterpenes and diterpene acids, which are present in host plant foliage, may also contribute to maintaining low population densities of insect herbivores [15]. Lockner et al. [16] reported that the direct exposure of five individual monoterpenes present in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) foliage increased larval mortality of *O. pseudotsugata* larvae reared on synthetic diet. In addition to the existing natural enemies and occasional suppression techniques, a long-term management strategy to improve tree health may help protect susceptible forest stands against *O. pseudotsugata* and other defoliating insects. The application of biochar may improve overall tree resistance to defoliating insects and benefit tree growth at the same time.

Biochar is a carbon-rich co-product of biomass pyrolysis [17], that is created in a high-temperature, low-oxygen environment [18]. Biochar can be made from any organic feedstock material such as woody residues, and could then be returned to the surface organic horizons [19–21]. Biochar has been used on forest [22,23] range [24], mine reclamation [25], and agricultural [20,26–29] soils as a method of improving greenhouse gas emissions [23,30], sequestering carbon [21] and improving soil properties [27-29,31,32]. Biochar may contribute to reducing plant herbivory by insect pests, either through physical contact with the material, or by improving overall plant resistance to herbivory. It may also be useful in priming the expression of plant defense-related genes [33], where improving overall plant resistance to herbivory may affect developmental and reproductive performances of the insect feeding on the plant [34].

*Orgyia pseudotsugata* larvae may come into contact with biochar on the foliage of the host tree, the surrounding understory vegetation and seedings, as well as the surrounding soil as larvae disperse between trees. Field observations indicate that larvae disperse with silk ballooning, or drop to the ground and crawl up to nearby trees and understory vegetation to find new foliage [1,2]. The effects of biochar in forest sites on herbivorous insects such as *O. pseudotsugata* has not been thoroughly examined. Cook and Rodrigues de Andrade Neto [35] noted a significant reduction in adult survival for three of the four insect species examined when they were in direct contact with dry biochar in confined arenas. *Formica obscuripes* (Forel) (Hymenoptera: Formicidae), *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae) and *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogossitidae) had significantly reduced survival while *Enoclerus sphegeus* (Fabricius) (Coleoptera: Curculionidae: Scolytinae) did not. In addition, decreased survival and fecundity and increased duration of development has been reported for the brown rice planthopper, *Nilaparvata lugens* (Homopera: Delphacidae) reared in arenas with high concentrations of dry biochar [36].

Based on the potential to enhance soil carbon storage and to alter soil properties and processes with the addition of biochar on forest sites, we hypothesized that biochar would impact forest insects by altering their development and survival after ingestion. Therefore, we conducted two feeding trial experiments with the objective of determining if biochar either added to the surface, or incorporated into a synthetic diet altered the development and survival of *O. pseudotsugata* larvae in a controlled environment.

## MATERIALS AND METHODS

### *Insects and Synthetic Diet*

A total of 374 *O. pseudotsugata* larvae from five egg masses were used in two feeding trial experiments. The egg masses used were collected in October 2017 from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.) from two infestations in Packer John State Forest (44.18199, −116.04811 and 44.18450, −116.07502), approximately 93 km north of Boise, ID. Egg masses were maintained from October until April 2018 in a protected, outdoor enclosure in Moscow, ID (USA) where temperatures ranged from –6 °C to 14 °C. In May 2018, individual egg masses were affixed to the lids of approximately 475 mL clear plastic rearing containers with an approximate surface area of 56.75 $cm^2$, with nylon mesh material glued over an opening in the lid for ventilation and filled approximately 25% with synthetic diet (Spruce Budworm Diet, Frontier Scientific Services, Newark, DE, USA). A 3 cm strip of Fluon® Insect-a-slip insect barrier (BioQuip Products, Rancho Dominguez, CA, USA) was painted on the inside rim of the rearing container. Rearing containers were maintained at 22 °C in a 12:12 h (light:dark) regimen in environmental growth chambers (Percival Scientific, Perry, IA, USA). Only egg masses and larvae that appeared to be non-parasitized and disease-free were used. No parasitoids emerged from the selected egg masses, and no evidence of virus was observed. An open dish filled with deionized water provided humidity within each chamber.

Following egg hatch, larvae were reared under the same conditions as described above. Once larvae molted to the second instar, verified with discarded exuvia and observable true tufts (located at the prothorax and the first, second, and eighth abdominal segments) [2], they were removed from rearing containers, weighed, and randomly assigned to experiment and treatment (described below) with larvae from each egg mass evenly distributed among all treatments. One larva was placed in the appropriate container (approximately 60 mL plastic cups with cardstock lids) and maintained under the conditions described above for the duration of the experiment. In each experiment and biochar-diet combination, the diet (with appropriate treatment) was replaced as necessary due to desiccation, appearance of mold, or larval consumption.

In each experiment, larvae that appeared to be unhealthy at the start of the trial were eliminated from the experiment, leading to uneven sample sizes. For the duration of each experiment, larval survival was monitored daily, with mortality assigned to larvae that did not move or respond to stimulation with a paintbrush. Surviving larvae were weighed on days 12 and 24. Assays were conducted from June to July 2018.

### *Biochar*

Biochar used in this experiment was produced by pyrolysis of mixed conifer sawmill residues (primarily Douglas-fir and lodgepole pine (*Pinus contorta* Douglas ex Loudon)) in a gasification system (Tucker Engineering Associates, Locust, NC, USA). After gasification, the biochar was characterized by Anderson et al. as: pH = 10.2, moisture content = 2.94%, bulk density (dry) = 0.165 Mg $m^{-3}$, carbon = 91.5%, nitrogen = 0.89%, C:N = 103.0, BET surface area = 15.0 $m^2g^{-1}$, energy = 33.98 MJ $kg^{-1}$ and a particle size distribution of <44 μm to 6.35 mm, centered around 0.84 mm [37]. Biochar was dried for 48 h at 35 °C to remove residual moisture before being ground and sifted into ≤105 μm sized particles using metal mesh particle sieves.

### *Experiment 1: Surface-Applied Biochar Treatments*

*Orgyia pseudotsugata* larvae were reared on synthetic diet (Spruce Budworm Diet, Frontier Scientific Services, Newark, DE, USA) with dry biochar placed onto the surface (approximate surface area of 12.57 $cm^2$). After initial weights were measured at the second instar stage, individual larvae were randomly assigned to diet-biochar treatment containers with 53, 57 and 51 larvae examined in the 0, 5 and 10 mg treatments, respectively (total sample size = 161).

### *Experiment 2: Incorporated Biochar Treatments*

*Orgyia pseudotsugata* larvae were reared on synthetic diet (Spruce Budworm Diet, Frontier Scientific Services, Newark, DE, USA) with biochar uniformly mixed throughout the diet. Biochar at 0, 10, 20 and 40% (volume/volume) of the diet mixture was incorporated into the diet prior to being poured into containers. After initial weights were measured at the second instar stage, individual larvae were randomly selected and placed on the various diet-biochar mixtures, with 54, 53, 55 and 51 larvae examined in the 0, 10, 20 and 40% biochar treatments, respectively (total sample size = 213).

### *Statistical Analysis*

All statistical analysis was conducted using SAS 9.4 analytical software [38]. Probit analysis was conducted for each experiment (biochar either on surface of, or incorporated into diet) and each treatment level to obtain lethal time (LT) estimates for 50% ($LT_{50}$) and 95% ($LT_{95}$) larval mortality of each treatment level with 95% confidence intervals. Non-overlapping confidence intervals were used to determine significant differences ($p < 0.05$). Kaplan–Meier curve analyses with Log-Rank tests and Tukey–Kramer adjustment for multiple comparisons, were used to analyze differences ($p < 0.05$) in mean larval survivorship for each experiment. Comparisons of initial larval weight (at day 0) were made among individual egg masses. Larval weight gain for two time intervals was calculated by subtracting initial larval weight (at day 0) from the weight of the same individual at day 12, and subtracting larval weight at day 12 from the weight at day 24. Comparisons of initial larval weight among egg masses, as well as larval weight gain among treatments within both food and biochar experiments (surface or incorporated biochar with synthetic diet), were made using generalized mixed models procedures (PROC GLMMIX) with lognormal distribution and $p < 0.05$ used to determine significance.

## RESULTS

### *Experiment 1. Surface-Applied Biochar Treatments*

Of the larvae that were reared on synthetic diet with dry biochar applied to the surface, 62.3% were alive at day 12 in the control (0 mg biochar) and 49.1% were alive at day 24. In the 5 mg biochar treatment, larval survival to day 12 was 47.4% and 19.3% were alive at day 24. In the highest treatment concentration (10 mg biochar), 35.3% of the larvae were alive at day 12 and 21.6% were alive at day 24.

There was a significant decrease in time to mortality ($p < 0.05$) for larvae exposed to either the 5 or 10 mg treatments of surface-applied biochar compared with the 0 mg control (Table 1.1). However, time to mortality between the individuals exposed to the 5 mg and 10 mg treatments was not significantly different.

**Table 1.1** Lethal time (LT) estimates to 50% ($LT_{50}$) and 95% ($LT_{95}$) mortality (maximum likelihood) with 95% confidence intervals (CI) for *O. pseudotsugata* larvae reared on synthetic diet with dry biochar material applied to the surface. Within a column, estimates of LT followed by the same letter are not significantly different.

| **mg Biochar** | **$LT_{50}$ (days)** | **95% CI** | **$LT_{95}$ (days)** | **95% CI** |
|---|---|---|---|---|
| 0 | 18.5 a | 17.1–19.7 | 54.7 a | 50.6–59.8 |
| 5 | 12.7 b | 11.6–13.8 | 38.9 b | 35.8–42.8 |
| 10 | 10.9 b | 10.0–12.0 | 35.5 b | 32.3–39.5 |

Analysis of Kaplan–Meier survival curves with multiple comparisons indicated differences in the survival of larvae exposed to 0 mg compared to 10 mg surface-applied biochar treatments ($p = 0.0383$). These comparisons showed control individuals lived longer on average than those feeding on biochar treated (both 5 mg and 10 mg) diet, although the difference in survival was not significant comparing the control and 5 mg biochar treatment ($p = 0.2591$) or the two surface-applied biochar treatments ($p = 0.6866$) (Figure 1.1).

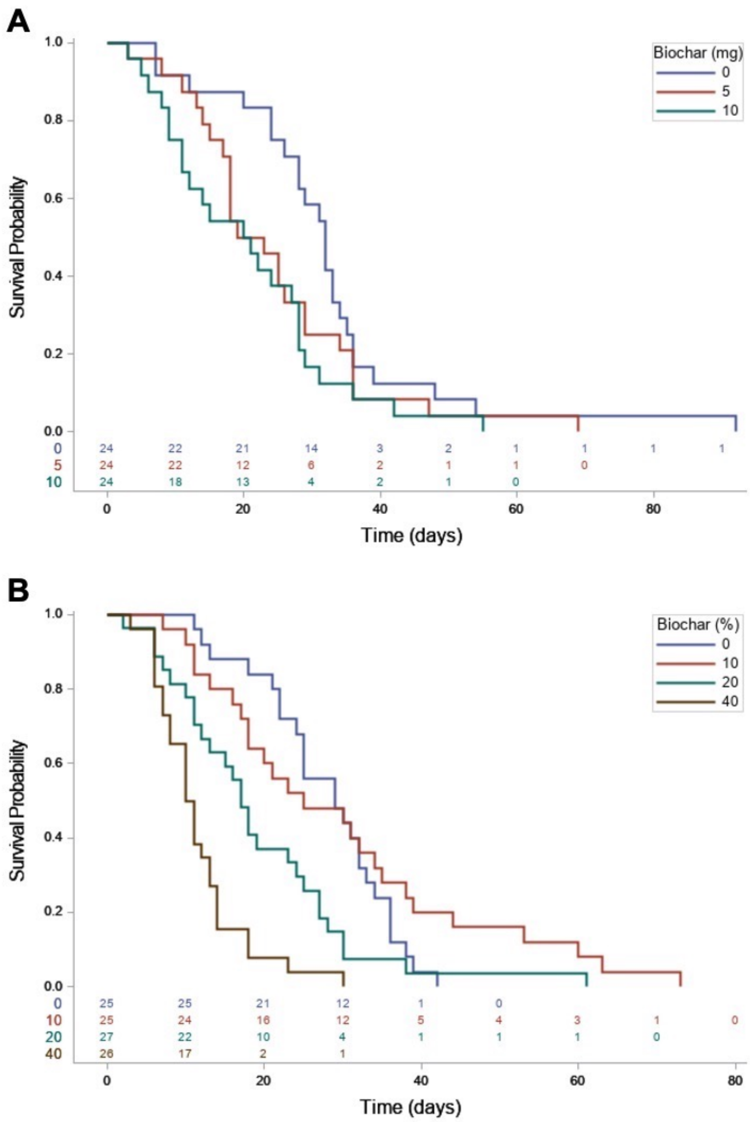


**Figure 1.1** Kaplan–Meier survival curves for *O. pseudotsugata* larvae reared on (**A**) synthetic diet with dry biochar applied to the surface at three concentrations (0, 5 and 10 mg) and (**B**) biochar incorporated into synthetic diet at four rates (0, 10, 20 and 40% (by volume)) of the diet mixture. For each time interval, estimated survival percentage (survival probability), is calculated as the number of larvae surviving divided by the number of total larvae at the start of experiment for each treatment group. Counts of surviving larvae at each time point for each treatment are included beneath survival curves.

Initial mean larval weights at day 0 were similar for all second instar larvae, independent of egg mass ($p = 0.2013$), prior to being randomly assigned to diet-surface biochar treatments (Figure 1.2A). Although mean larval weights appear to be similar among the treatments for each time point (Figure 1.2B,C), there was a significant difference ($p = 0.0462$) in larval weight gain from day 0 to day 12 (F= 2.32; $p > F = 0.1069$; df = 2, 60) between the control (0 mg biochar) and the treatment with the greatest amount of surface-applied biochar (10 mg biochar) (Figure 1.3A). However, there were no significant differences found when comparing larval weight gain between the control and low (5 mg biochar) treatments ($p = 0.1762$) or comparing the low- and high-concentration surface-applied biochar treatments ($p = 0.4336$) (Figure 1.3A). No significant difference was found in mean larval weight gain from day 12 to day 24 (F = 0.75; $p > F = 0.4831$; df = 2, 21) among all surface-applied biochar treatments ($p > 0.25$) (Figure 1.3B).

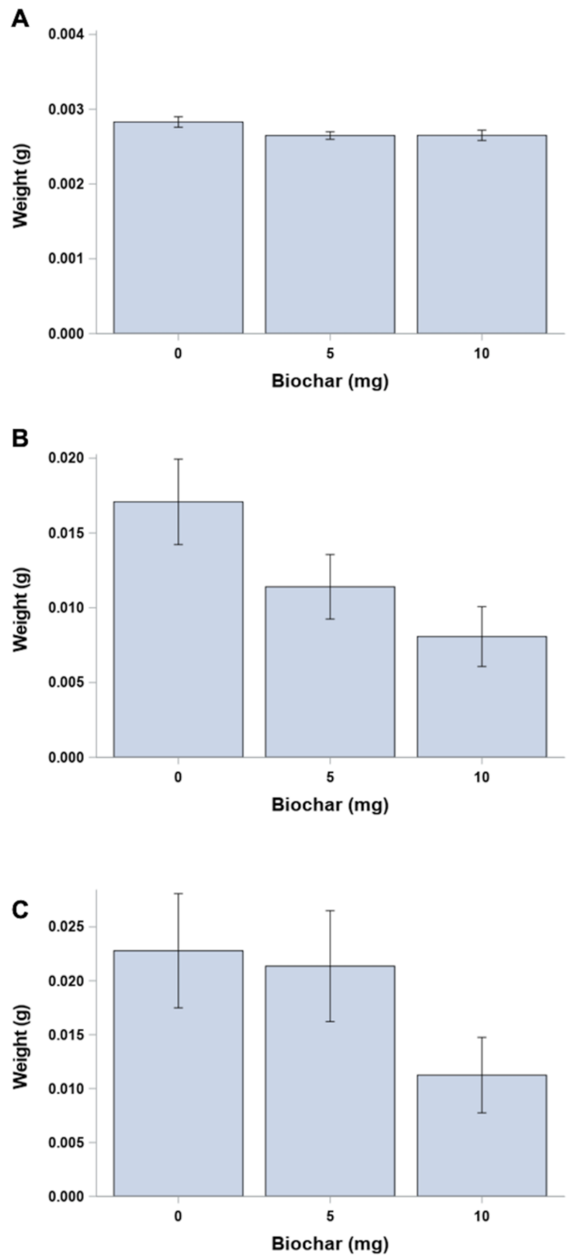


**Figure 1.2** Mean larval weights (g ± SEM) of *O. pseudotsugata* larvae reared on synthetic diet with dry biochar applied to the surface at three concentrations (0, 5 and 10 mg). Initial weights were taken at (**A**) day 0 for all second instar larvae prior to randomly assignment to each treatment rate, then again at (**B**) day 12 and (**C**) day 24 for surviving larvae.

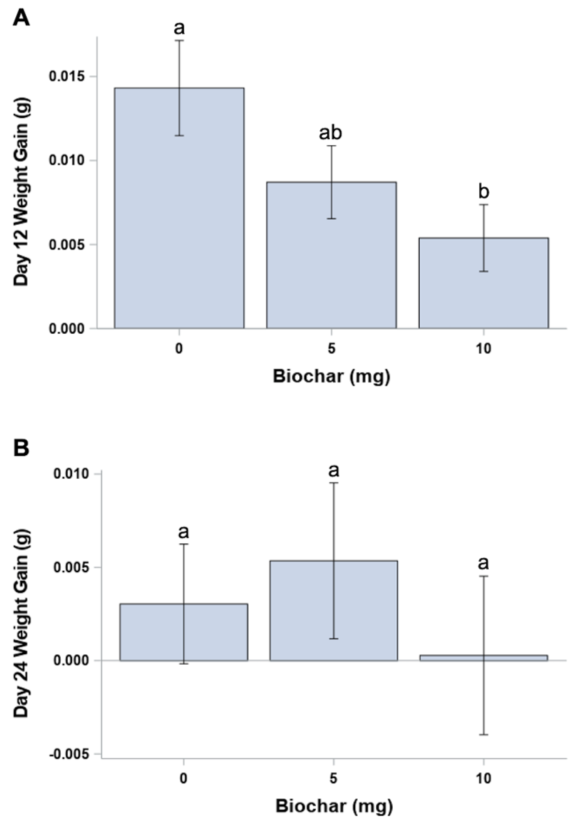


**Figure 1.3.** Mean (± SEM) weight gain at 12 day intervals for individual larvae measured at (**A**) day 12 and (**B**) day 24 for *O. pseudotsugata* reared on synthetic diet with dry biochar applied to the diet surface at three concentrations (0, 5 and 10 mg). Means with the same letter are not significantly different ($p < 0.05$) based upon analysis of variance results.

***Experiment 2. Incorporated Biochar Treatments***

Of the *O. pseudotsugata* larvae that were reared on biochar-incorporated diet, 66.7% were alive at day 12 in the control (0% biochar) and 46.3% were alive at day 24. Larval survival in the low concentration (10% biochar) treatment was 56.6% at day 12 and 32.1% at day 24. In the 20% biochar treatment, 50.9% of the larvae were alive at day 12 and 20.0% were alive at day 24. Furthermore, in the 40% incorporated biochar treatment, 25.5% were alive at day 12 and only 1.96% (one individual) was alive at day 24.

There was a significant decrease in time to mortality ($p < 0.05$) for larvae in each of the incorporated biochar treatments (10, 20 and 40%) compared to the untreated control (Table 1.2). Ingestion of the biochar material in the larval diet significantly decreased the lethal time estimation to 50% and 95% mortality as compared to the control, as each increase in the volume of biochar in the diet corresponded to a decreased time to mortality (Table 1.2).

**Table 1.2.** Lethal time (LT) estimates to 50% and 95% mortality (maximum likelihood) with 95% confidence intervals (CI) for *O. pseudotsugata* larvae reared on synthetic diet mixed with multiple concentrations of dry biochar material by volume. Within a column, estimates of probability of mortality followed by the same letter are not significantly different.

| (% Biochar) | $LT_{50}$ (days) | 95% CI | $LT_{95}$ (days) | 95% CI |
|---|---|---|---|---|
| 0 | 18.7 a | 17.6–19.8 | 42.0 a | 39.2–45.5 |
| 10 | 16.2 b | 14.9–17.4 | 51.9 b | 47.8–57.0 |
| 20 | 12.4 c | 11.3–13.4 | 36.6 c | 31.8–38.1 |
| 40 | 8.9 d | 8.1–9.6 | 19.0 d | 17.3–21.4 |

Analysis of Kaplan–Meier survival curves with multiple comparisons showed a significantly greater probability of survival of control individuals compared to those feeding on 40% incorporated biochar treatment ($p < 0.0001$), as well as for larvae in 10% compared to 40% incorporated biochar treatments ($p < 0.0001$). Larval survival was also significant comparing the 10% and 20% incorporated biochar treatments ($p = 0.0320$). Overall, larvae in the 40% treatment showed the lowest probability of survival throughout the experiment. These comparisons showed control individuals lived longer on average than those feeding on biochar treated food, up until 24 days. Larvae in the 10% biochar treatment showed the greatest probability of survival after 24 days, even compared to the control individuals. The difference in larval survival was not significant comparing the control and the 10% biochar treatment ($p = 0.9348$), the control and 20% biochar treatment ($p = 0.1571$) or the two highest concentrations (20% and 40%) incorporated biochar treatments ($p = 0.1299$) (Figure 1.1).

The average larval weight, independent of egg mass, at day 0 was similar, with no significant difference in weight among egg mass ($p = 0.2800$) prior to their random assignment to individual diet-biochar treatments (Figure 1.4). Overall, larval weight gain from day 0 to day 12 was highest in the low (10%) biochar treatment compared to all other treatments, although it was only significantly different ($F = 1.61$; $p > F = 0.1932$; df = 3, 82) between the low- (10%) and high-concentration (40%) biochar treatments ($p = 0.0373$) (Figure 1.5A). *p*-values are greater than 0.10 for all other comparisons of incorporated biochar treatments (Figure 1.5A). No significant difference in larval weight gain was found among the biochar treatments from day 12 to day 24 (*p* values greater than 0.39 for all comparisons) (Figure 1.5B). Fewer larvae survived to days 12 and 24 in each biochar-incorporated treatment compared to the control (0% biochar), with only a single larva surviving to day 24 in the highest (40%) biochar treatment.

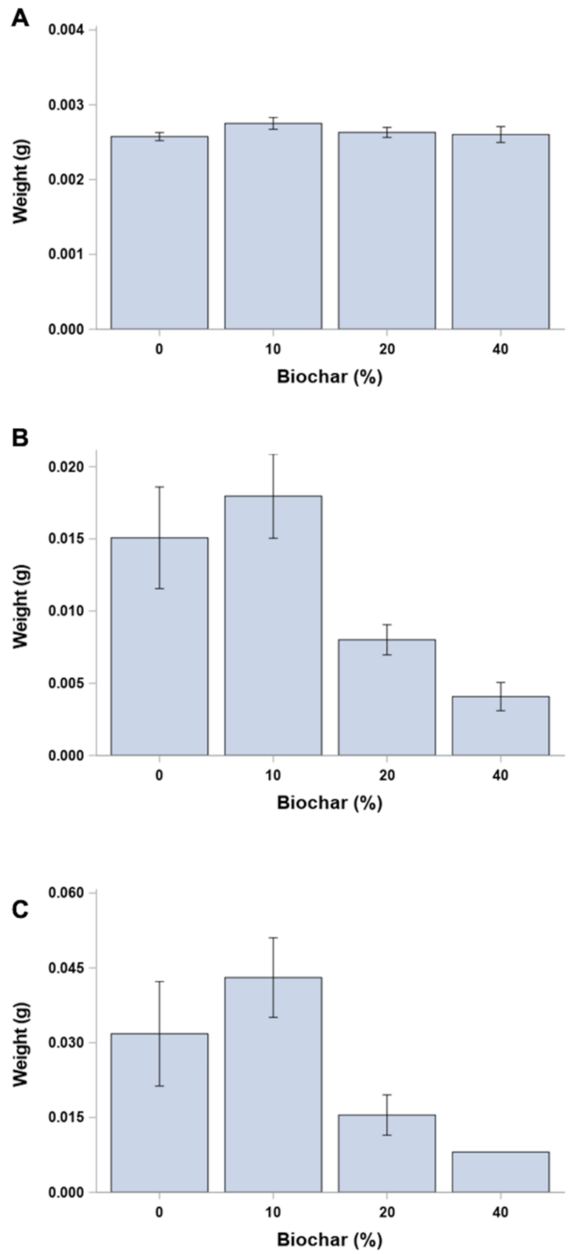


**Figure 1.4.** Mean larval weights (g ± SEM) of *O. pseudotsugata* larvae reared on synthetic diet with biochar incorporated into diet at four rates (0, 10, 20 and 40% (by volume)) of the diet mixture. Initial weights were taken at (**A**) day 0 for all second instar larvae prior to random assignment to each diet-biochar treatment, then again at (**B**) day 12 and (**C**) day 24 for surviving larvae. Only 1 larva survived to day 24.

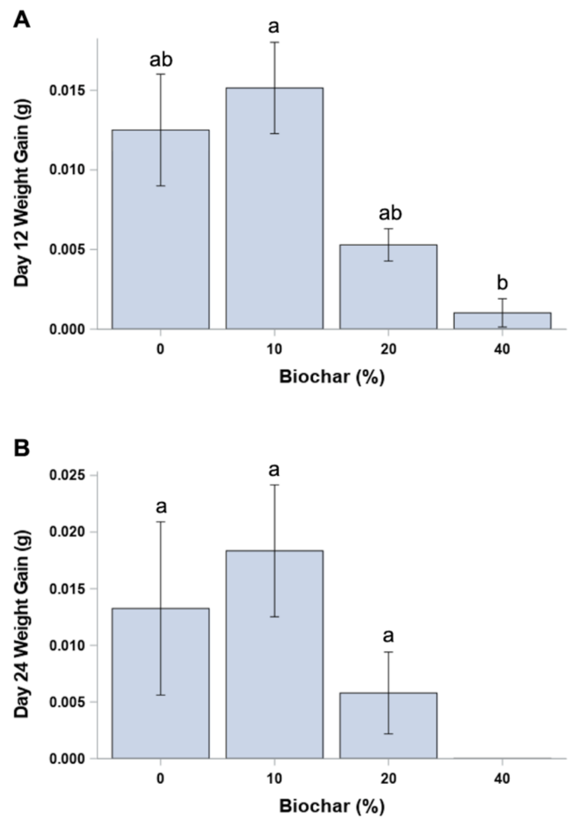


**Figure 1.5.** Mean (± SEM) weight gain at 12 day intervals for individual larvae measured at (**A**) day 12 and (**B**) day 24 for *O. pseudotsugata* reared on synthetic diet with biochar incorporated into diet at four diet:biochar (volume/volume) rates (0, 10, 20 and 40% biochar). Only 1 larva survived to day 24 and was not included in this analysis.

The average larval weight gain from day 12 to day 24 was not significantly different ($F = 1.61$; $p > F = 0.1932$; df = 3, 82) among the control (0%), 10%, and 20% treatments ($p > 0.39$) (Figure 1.5B). The weight gain data for the single surviving larva in the highest concentration (40% biochar) treatment were not included in the analysis.

## DISCUSSION

Biochar material made from woody residues and applied as a soil amendment has been shown to improve soil health indices and is becoming a tool to sequester carbon on forest [22,23], range [24], mine reclamation [25], and agricultural sites [20,26–29]. In the laboratory, biochar can have a deleterious effect on survival [35] as well as development and fecundity [36] of insects that are directly exposed to the material in enclosed arenas. Further, studies on cereal grain pests have shown that in the laboratory biochar can decrease fertility and population growth [33], and these results in combination with our lab study show a potential for management of insect populations. However, field applications of biochar may impact insect populations and have not been thoroughly studied for forest insects that may be exposed to applied biochar. For example, freshly hatched *O. pseudotsugata* larvae descend from egg masses on silk strands and disperse with the wind to surrounding trees and understory vegetation where they begin to feed. Dispersing larvae may land on the ground and crawl to nearby vegetation [2,5–7]. When biochar is applied, a portion of the material becomes airborne and settles on foliage of trees and understory plants. Larvae would potentially be exposed to and ingest biochar if it were present on the foliage of trees and understory plants or on the soil surface.

The biochar used in our study consisted of ≤105 μm sized particles, a small portion sifted from the manufactured biochar material. Efficient field application of biochar will likely consist of a mixture of particles ranging in size from large chunk to nano-particles, depending on the method of manufacture and application. Cook and Rodrigues de Andrade Neto [35] demonstrated a potential for negative impacts of insect species exposed to dry biochar material, with both fine (<150 mm) and coarse (>1.0 mm) particle sizes showing a decrease in survival. Moisture content as well as physical size of the biochar may affect how insects exposed to and potentially ingesting biochar may respond.

In our first experiment, larvae were in direct contact with, and ingested biochar applied to the surface of the diet. Although we found statistically significant differences in the estimated time to mortality between the control treatment and each biochar treated group, by the end of this study, there was a reduced sample size which may have contributed to a lack of significance in time to mortality between the low- (5 mg) and high-concentration (10 mg) biochar treatments (Table 1.1). Fewer surviving larvae in the biochar treatment groups may also contribute to the lack of significance among all three treatments in experiment 1 when comparing larval weight gain from day 12 to day 24 (Figure 1.3). Further, several larvae were observed feeding restricted to a small area of the treatment cup, which may have been an attempt to avoid ingesting the biochar. However, larvae were exposed to biochar on the surface of the diet and therefore biochar was at least initially ingested prior to the larvae continuing to feed on diet underneath the surface layer of biochar.

In the second experiment, larvae consumed the biochar which was uniformly incorporated into the diet. Our results suggest that the amount of biochar in the low (10%) treatment may result in larvae engaging in compensatory feeding behavior [39]. This behavior results in the consumption of more food of a lower quality in response to a decrease in dietary nutrients. The increased consumption may result in there being no decrease in mean weight compared with larvae reared on the control (0% biochar) diet. Several laboratory studies have shown that compensatory feeding occurs with insects fed with synthetic diets that are low in nitrogen or protein [39,40]. The high 103.0 C:N ratio for the biochar used in this study may possibly contribute to an overall lowered available N concentration in the diet, especially in those diets with biochar directly incorporated into the food. Addition of carbon in the form of biochar would alter or dilute the nutrients of the diet in a way that the larvae would need to consume more of it to reach similar stages of development compared to the diet with no biochar added (the control). A lower available N in the diet would therefore be consistent with a compensatory feeding theory. Additionally, the reduced sample size for the biochar-treated diets may have contributed to lack of significance among all four treatments comparing the weight gain of the larvae at day 24 (Figure 1.5B).

Larvae were able to ingest and presumably pass biochar through their digestive tracts (Figure 1.6), although the physiological effects of the biochar, and specifically the mode of action, are unknown and should be addressed in future research. The alkaline pH and potential water holding capacity [21,37] of the porous biochar may affect the conditions of the larval digestive tracts and interrupt their ability to digest nutrients or absorb nutrients. Based upon the lower larval survival to 24 days and lower weights at each time point, it is possible that larvae reared on the biochar treated synthetic diet failed to develop normally.

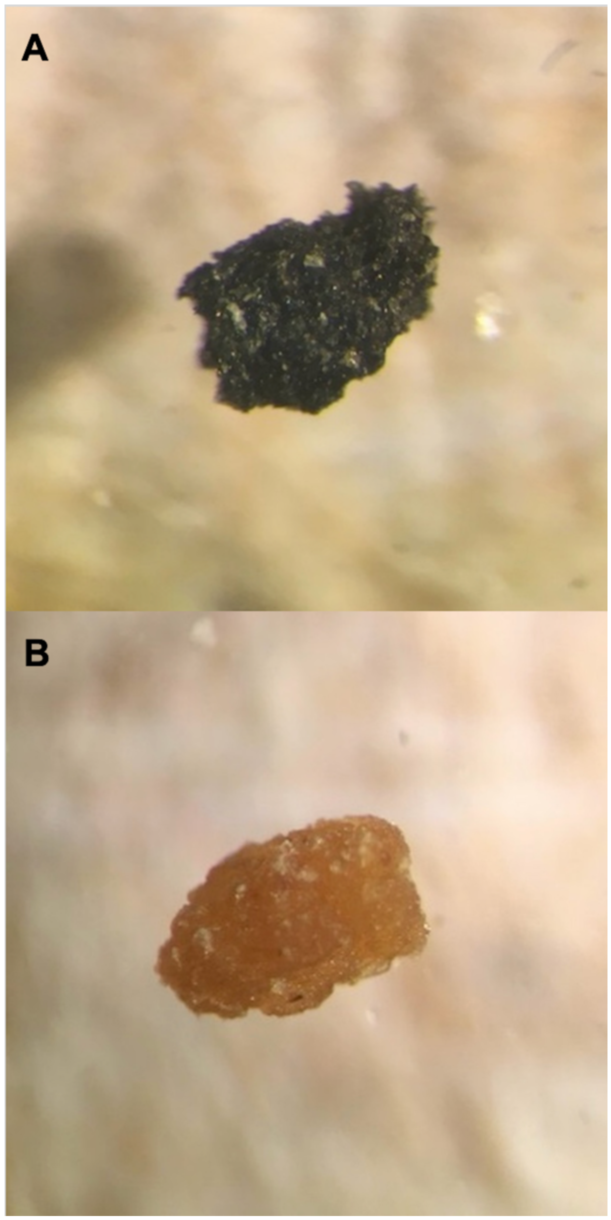


**Figure 1.6.** *Orgyia pseudotsugata* frass viewed at 40× magnification, collected from larvae reared on synthetic diet in (**A**) the 10% biochar treatment and (**B**) the control 0% biochar treatment.

## CONCLUSIONS

Our laboratory studies showed that the addition of biochar on herbivorous insect food sources had deleterious consequences on survival and weight gain of *O. pseudotsugata.* Biochar has the potential to impact tree resistance mechanisms, and the potential use of biochar applied to tree foliage and surrounding soil for suppression of herbivorous insects is undetermined. This work should be extended to long-term, large-scale field plots with known insect populations to assess the potentially suppressive effects of biochar. It may be possible that biochar will have direct impacts on insects ingesting the material, as well as improving overall tree health and ability to defend against insect herbivory.

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# CHAPTER 2: DOES SURFACE-APPLIED BIOCHAR ALTER INSECT UTILIZATION OF DOWNED PONDEROSA PINE (*PINUS PONDEROSA* LAWSON & C. LAWSON) BOLTS?


## ABSTRACT

Biochar can be created as part of harvest operations in overstocked stands, after wildfire, or from trees killed by insects or disease and used as a soil amendment to restore degraded soils, sequester carbon, and increase soil water holding capacity and plant available water. Forest insects may be exposed to biochar when the material is applied to surface organic horizons and downed trees. How biochar affects insects' ability to locate and utilize downed woody material in the forest is undetermined. Two field experiments, with freshly downed sections (bolts) of ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson), were conducted to determine if applied biochar (1) at a rate equivalent to 3.2 Mg $ha^{-1}$ (1.30 tons $acre^{-1}$) interfered with attack or emergence of the pine engraver beetle *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae) when bolts were baited with a pheromone lure, or (2) at a rate equivalent to 6.2 Mg $ha^{-1}$ (2.50 tons $acre^{-1}$) altered species richness or abundance of insect assemblages on non-baited bolts. In the first experiment, similar mean density of nuptial chambers and emergence indicated both control and biochar-treated bolts were suitable habitat for *I. pini*. In the second experiment, non-treated control bolts had higher species richness compared to the bolts treated with biochar. Red turpentine beetles, *Dendroctonous valens* (LeConte) (Coleoptera: Curculionidae: Scolytinae) were found more in non-treated control bolts compared with biochar-treated bolts. Utilization of bolts by other insect taxa such as longhorn beetles (Coleoptera: Cerambycidae) was similar in non-treated control bolts and biochar-treated bolts, although multiple insect taxa were found in such low numbers that no differences were found.

**KEYWORDS**

Downed woody residue, forest management, soil amendment, Scolytinae, Cerambycidae

## INTRODUCTION

Insects provide numerous ecosystem services contributing to the balance and health of forest systems including toward decomposition and nutrient cycling (Furniss and Carolin 1977). Many taxa including bark beetles (Coleoptera: Curculionidae: Scolytinae), round-headed wood borers (Coleoptera: Cerambycidae), flat-headed wood borers (Coleoptera: Buprestidae) and woodwasps (Hymenoptera: Siricidae), help to cycle nutrients by increasing the rate of breakdown of woody material which also contributes to accumulation of soil organic matter (Furniss and Carolin 1977). Some of these insects also introduce microbial associates that can assist in metabolism, breaking down lignin and beginning the decomposition process (Adams et al. 2013, Hofstetter et al. 2015, Paine et al. 1997).

Biochar is a carbon-rich product created by the breakdown of organic biomass (Biederman and Harpole 2013) in a high temperature, low oxygen environment (Bridgewater, 2004) for land application. The application of biochar to surface organic horizons in forest stands can sequester carbon while increasing soil nutrient retention (Borchard et al., 2019), water holding capacity (Abit et al. 2012, Lehmann et al. 2006, Lehmann and Joseph 2009), plant available water (Edeh et al. 2020; Razzaghi et al. 2020) and provides other ecosystem services (Blanco-Canqui 2021). In a forest system, biochar is applied to the soil surface and surrounding vegetation without tilling activities. Biochar becomes vertically incorporated into the soil structure over time as precipitation and freeze/thaw activities naturally disperse the material and gradually allow it to penetrate soil horizons. The amount of time it takes for biochar to be incorporated into soil organic horizons may vary by soil structure, climate and site characteristics (Blackwell et al. 2009). The impact of biochar on the ability of insects to locate and utilize host material, however, has not been thoroughly examined and there is little information on how it may affect them. Therefore, it is necessary to establish the potential effects of biochar applications on insect assemblages.

The pine engraver, *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae) typically colonizes weakened, stressed, and recently killed trees such as fallen trees or logging residue (Cognato 2015, Hoffstetter et al. 2015). *Ips pini* males that successfully attack a host tree create a single nuptial chamber where they mate with two to six female beetles (Cognato 2015). After mating, each female constructs an egg gallery radiating out from the nuptial chamber and oviposits in niches cut into the sides of the gallery (Furniss et al. 1977). In some *Ips* species, multiple males may use a single nuptial chamber and multiple females may create egg galleries coming from each single chamber (Cook et al. 1985). Therefore, the density of nuptial chambers provides an estimate of attack density but not of the number of individuals attacking a host. *Ips pini* use aggregation pheromones released by colonizing male beetles in combination with host tree-emitted compounds to attract conspecifics (Furniss et al. 1978, Wood 1982, Wegensteiner et al. 2015). Ipsenol, ipsdienol, and *cis*-verbenol are the main semiochemicals produced in the beetle's gut when the male beetle feeds on host phloem (Wood 1982) or are oxidation by-products of host tree terpene compounds (Cognato 2015).

When applied to forest soils, biochar will land on most exposed surfaces, including surface organic matter, downed coarse and fine woody residues, seedlings, and understory plants. The application rate and method will determine the extent of the soil surface that is covered which influences the level of exposure to the material of larval and adult insects. It is unknown whether biochar affects insects' ability to locate and utilize host material in the forest, but recent laboratory studies demonstrate a potential negative impact of biochar on insects and the infectivity of entomopathogenic nematodes (Yaman et al. 2021). A second study reported that contact with dry biochar decreased survival in three of the four species examined including *Formica obscuripes* (Forel) (Hymenoptera: Formicidae), *I. pini* and *Temnochila chlorodia* (Mannerheim), but survival of *Enoclerus sphegus*

(Fabricius) was not affected (Cook and Rodriguies de Andrade Neto 2018). Another study reported decreased fecundity and survival with an increased time of development for the brown rice planthopper, *Nilaparvata lugens* (Stal) (Homopera: Delphacidae) reared in arenas with high concentrations of dry biochar (Hou et al. 2015). In addition, Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Erebidae) reared on a synthetic diet showed a decrease in survival corresponding with an increase in biochar concentration as well as evidence of potential compensatory feeding when ingesting diet containing a low (10% volume/volume) concentration of biochar (Rice-Marshall et al. 2021). Yaman et al. (2021) concluded that biochar application, depending on feedstock, may have detrimental or indifferent impacts on some beneficial nematode species such as *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae).

The effect of biochar on insect utilization of downed woody material has not been investigated. Our experiments were designed to examine the potential impacts of surface-applied biochar on insect utilization of sections (hereafter, bolts) of ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson). The specific objectives were to (1) determine if applied biochar interfered with attack or emergence of *I. pini* when bolts were baited with a pheromone lure and (2) determine if biochar alters either species richness or abundance of insects utilizing treated bolts.

## MATERIALS AND METHODS

### *Field Sites*

Field sites were located at the University of Idaho's Experimental Forest, West Hatter Unit, 46°50'12.3"N, 116°51'48.9"W, 954.3 m elevation, approximately 12.0 km south of Potlatch, ID in Latah County. The field site is characterized as a mixed conifer stand, primarily ponderosa pine, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and grand fir (*Abies grandis* (Douglas ex D. Don) Lindl). The soil is a complex of predominantly Vassar in a Vassar-Jacot-Aldermand soil series (generally, ashy over loamy, amorphic over isotic, frigid Typic Udivitrands; Soil Survey Staff 1999) with 30 to 65 percent slopes. The surface organic horizon (inclusive of the $O_a$, $O_e$, and $O_i$) was 2.5 cm of slightly decomposed plant material. The mineral soil consisted of approximately 53 cm of volcanic ashy silt loam on top of 28cm of coarse sandy loam which is underlain with 46 cm of gravelly loamy coarse sand (Soil Survey Staff 2022). The understory consisted of a mixture of plants such as Columbia brome (*Bromus vulgaris* (Hook.) Shear), Oregon boxleaf (*Paxistima myrsinites* (Pursh) Raf. (Celastraceae)) northern twinflower (*Linnaea borealis* L.), Idaho goldthread (*Coptis occidentalis* (Nutt.) Torr. & A. Gray), fragrant bedstraw (*Galium triflorum* Michx.) hookspurred violet (*Viola adunca* Sm.), Nootka rose (*Rosa nutkana* C. Presl) and bride's bonnet (*Clintonia uniflora* (Menzies ex Schult. & Schult. f.) Kunth). Temperature and precipitation were measured at a nearby climatic monitoring station, Potlatch 3 NNE USC00107301, 46°57'37.1" N, 116°51'18" W. Overall in 2015, mean annual air temperature was 9.9 °C, with 55.4 cm of precipitation, and in 2018 mean annual air temperature was 8.6 °C and average annual precipitation was 65.0 cm.

### ***Biochar***

Biochar was made in a gasification system (Tucker Engineering Associates, Locust, NC) by pyrolysis of mixed conifer sawmill residues (including Douglas-fir and lodgepole pine (*Pinus contorta* Douglas ex Loudon). Biochar used in these two experiments is from the same manufacturer and lot as was used in previous experiments (Anderson et al. 2013, Rice-Marshall et al. 2021), and has pH = 10.2, moisture content = 2.94%, bulk density (dry) = 0.17 Mg $m^{-3}$, carbon = 91.5%, nitrogen = 0.89%, C:N = 103.0, BET surface area = 15.0 $m^2g^{-1}$, energy = 33.98 MJ $kg^{-1}$ and a particle size distribution of <44 $\mu$m to 6.35 mm, centered around 0.84 mm (Anderson et al. 2013).

### ***Experimental Procedures***

Field exposure for the first experiment occurred in July 2015 and lasted 8 days during the field-testing period. Mean temperatures in July 2015 were 20.6 °C, with 0.3 cm precipitation. To create the bolts, five ponderosa pine trees were felled and two, adjacent 75 cm long bolts were cut from the base of each tree. The adjacent bolts from individual trees were considered a single, paired replicate. Bolts within a pair were placed on the ground a minimum of 2.5 m apart, with similar canopy cover and exposure conditions. Each pair was separated by a minimum distance of 50.0 m. Bolt diameters were measured at the midpoint (ranging from 9.2 to 15.3 cm) and used to estimate the total surface area of the individual bolts (Table 2.1). Biochar treatments were randomly assigned to bolts in each replicate pair which consisted of a non-treated, control bolt and a bolt that was surface-treated in situ with approximately 215 g of biochar, applied manually at a rate of approximately 3.2 Mg $ha^{-1}$ (1.3 tons $acre^{-1}$). To assure that *I. pini* were attracted to the bolts, each bolt was baited near its midpoint with a pheromone pouch (ipsdienol; Lot No. 3075; Synergy Semiochemical Corp, Burnaby, BC). After 8 days in the field, bolts were removed and cut into three 25cm sections. They were placed into individual emergence containers (BugDorm-1, MegaView Science Education Services Co., LTD.) and maintained in the laboratory under ambient conditions with a range of approximately 20-24 °C. Within-bolt moisture conditions were not monitored. *Ips pini* were collected daily as they emerged for a period of six weeks. When no emergence occurred from any bolt for a period of 7 days, the bark was removed to limit further foraging by larvae of wood-boring insects and to preserve evidence of *I. pini* nuptial chambers. Total nuptial chambers and emerged *I. pini* beetles were counted.

Table 2.1. Bolt surface area ($cm^2$) measurements in each experiment (mean surface area ± SEM) where individual bolts within a pair were randomly assigned to treatments. A paired comparison using Student's t-test was used to compare mean surface areas. Within a column, the same letters after each mean surface area ± SEM indicate no significant difference.

| Treatment | Experiment 1 | Experiment 2 |
|---|---|---|
| Surface-applied biochar | 7301.1 ± 602.47 a | 4587.7 ± 224.04 a |
| Control (no biochar) | 7464.4 ± 508.28 a | 4585.2 ± 205.92 a |

The second experiment was deployed in June 2018 when the mean air temperature was 14.2 °C and when 3.2 cm of precipitation occurred at the site. Ten ponderosa pine trees were felled and two, adjacent 1.0 m bolts removed. The adjacent bolts cut from each tree were considered a paired replicate. Both end diameters of each bolt were measured, and the average of the two (ranging from 13.3 cm to 17.2 cm) was used to estimate surface area (Table 2.1). Bolt pairs were kept in the field, 5.0 m apart, under similar canopy cover and exposure conditions, with each pair separated by a minimum distance of 30.0 m. Bolts were not baited with any lure. One randomly selected bolt from each pair was treated with approximately 425 g of biochar manually dusted on the upper surface, approximately 2.0 cm thick which approximates an overall application rate of 6.2 Mg $ha^{-1}$ (2.5 tons $acre^{-1}$). After 35 days in the field the bolts were cut into two 50 cm sections, transferred to the lab, and placed in individual plastic emergence containers (Rubbermaid, United Solutions Inc. Leominister, MA), with both sections from a single bolt placed in the same container. Nylon mesh material was glued over openings cut on each of the four sides for ventilation. Bolts were maintained at ambient laboratory conditions with a range of approximately 20-24 °C. Within-bolt moisture conditions were not monitored. Insects were collected daily as they emerged. After allowing the insects to emerge for one year, the bolts were peeled of bark and split to collect non-emerged larvae and adult insects. Adult insects were identified to species where possible, and larvae obtained from the split bolts were identified to the family level.

***Statistical analysis***

All statistical analyses were conducted using SAS 9.4 analytical software (2016 SAS Institute Inc., Cary, NC, USA). Bolt surface area was compared between treated and control sections using a paired Student's t test for each experiment.

In the first experiment, *I. pini* attacks were quantified by counting individual nuptial chambers and dividing by the total surface area ($cm^2$) for each bolt. Emergence was calculated by dividing the total number of emerged *I. pini* adults by the surface area ($cm^2$) for each bolt. Paired t tests were used to compare attack and emergence densities of *I. pini* between biochar treated and non-treated (control) bolts.

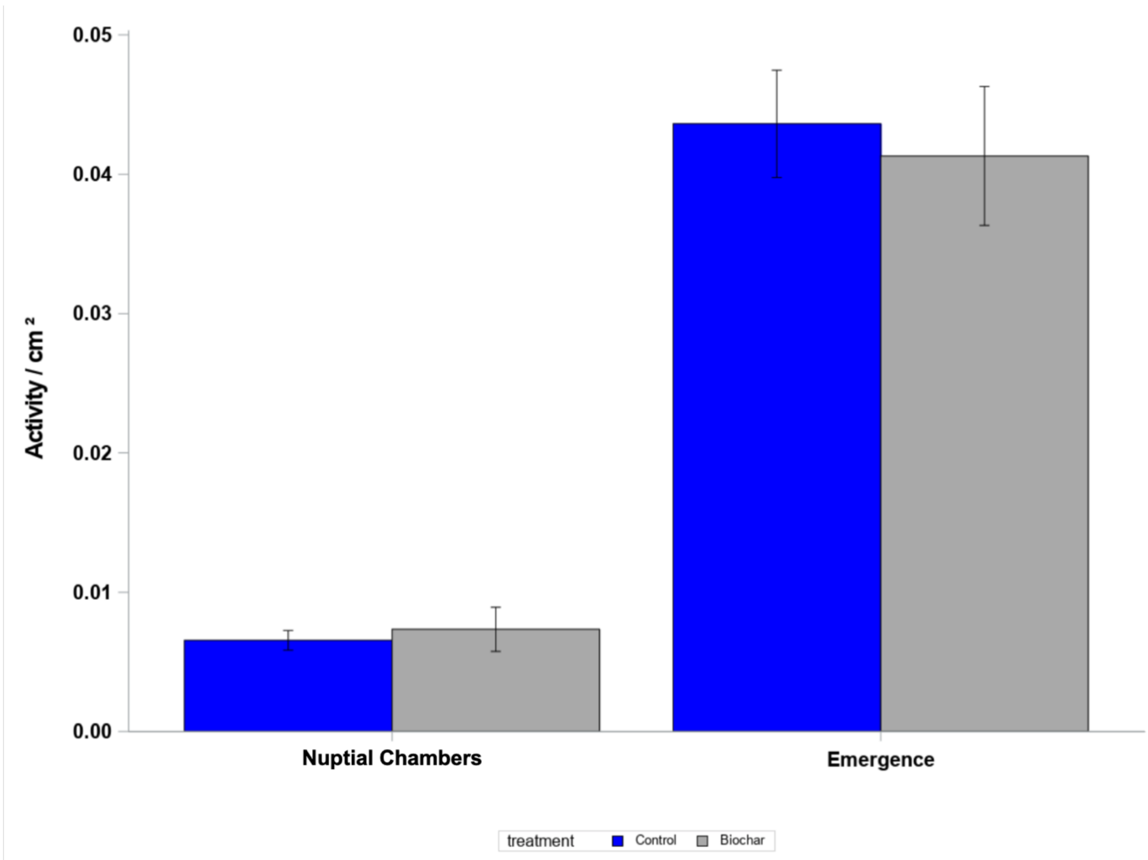
In the second experiment, the total number of individual insects (abundance) as well as the total number of species (richness) in a bolt were measured. Using all ten pairs of bolts, a paired t test was used to compare the total abundance of emerging insects between treated and control bolts. Paired t tests were also used to compare species richness between the ten treated and control bolts pairs. Further paired Student's t tests were used to compare insect emergence within a taxon for pairs of bolts from which that taxa emerged.

## RESULTS

***Experiment 1***

A total of 259 nuptial chambers and 1494 emerged *Ips pini* adults were found in the five biochar-treated bolts, while the five control bolts had a total of 250 nuptial chambers and 1640 emerged *I. pini* beetles. The surface areas of the biochar treated bolts were not significantly different than the non-treated control bolts (P = 0.8400; Table 2.1). Density of nuptial chambers ($P$ = 0.5056; Figure 2.1) and emergence (($P$ = 0.7322; Figure 2.1) were similar in control and biochar-treated bolt which indicates no difference in habitat utilization for *I. pini* based on treatment.

Figure 2.1. Mean density ( ± SEM) of nuptial chambers and emerged *Ips pini* adults per $cm^2$ of bark surface on biochar treated and non-treated control bolts.




***Experiment 2***

**Richness:**

The surface areas of the biochar treated bolts were not significantly different than the non-treated control bolts ($P$ = 0.9959; Table 2.1). On average, a greater number of species, (2.90 ± 0.46 (SEM)) emerged from the non-treated control bolts compared to the bolts treated with biochar (1.80 ± 0.29) ($P$ = 0.0318).

Very few taxa were still in the bolts after a year and these included adults and several larval *Dendroctonus valens* (*LeConte*) (Coleoptera: Curculionidae: Scolytinae), a single adult *Anthaxia aeneogaster* Laporte and Gory (Coleoptera: Buprestidae) as well as several larval Buprestidae, adult *Monochamus clamator*

LeConte and *Monochamus obtusus* Casey (Coleoptera: Cerambycidae) and larval Cerambycidae, as well as Siricidae larvae. When comparing the number of species that were only found in the post-emergence split bolts, no difference was found between the average number of species in the control bolts (0.10 species ± 0.10) and treated bolts (0.30 species ± 0.21) ($P$ = 0.1679), with several of the bolts having no insects present in them when they were examined.

Comparing both the number of individual taxa that either emerged or were found in the split bolts after the emergence period (with no species that were counted in the emerged category being double-counted in the split bolt category), the difference between the average number of taxa in the control bolts (3.00 ± 0.49) remained higher than in the treated bolts (2.10 ± 0.38) but the P value was slightly higher ($P$ = 0.0676).

**Abundance:**

Overall, a total of 77 insects were found in the biochar treated bolts, while a total of 998 insects were found in the non-treated control bolts. *Dendroctonus valens* were significantly more abundant in non-treated control bolts compared to those bolts that were treated with biochar ($P$ = 0.0278; Table 2.2). Larval beetles found within the bolts after the emergence period were also significantly more abundant in the control bolts, the majority of which were *D. valens* ($P$ = 0.0068). For all other emerged insects, no differences in abundance were found between biochar treated and non-treated controls. However, the braconid wasp, *Coeloides sympitys* Mason (Hymenoptera: Braconidae), fungus beetle *Silvanoprus* sp. (Coleoptera: Silvanidae), minute pirate bug, *Anthocoris* sp. (Hemiptera: Anthocoridae) and flat bug *Aradus* sp. (Hemiptera: Aradidae) were only associated with non-treated control bolts. Larval wood wasps in the family Siricidae, and two species of flat-headed wood borers (Coleoptera: Buprestidae), *Anthaxia aeneogaster* Laporte and Gory and *Melanophila acuminata* (DeGeer) only emerged from biochar-treated bolts, although larval Buprestidae were found in equal numbers in both treated and non-treated bolts.

Table 2.2. Mean number (± SEM where applicable) of insects by taxa that emerged from bolts that received a surface treatment of biochar versus non-treated control bolts, and the proportion of bolts within each treatment that had taxa emerge from them. Insects listed to lowest possible identification. *P*-value for comparison of abundance using Student's paired t-test.

| | **Biochar Treated** | | **Control** | | |
|---|---|---|---|---|---|
| **Taxa** | **Number of Individuals (mean ± SEM)** | **Number of bolts** | **Number of Individuals (mean ± SEM)** | **Number of bolts** | ***P*-value** |
| Curculionidae | | | | | |
| *Dendroctonus valens* LeConte | 3.00 | 1 | 60.83 ± 17.79 | 6 | 0.0278 |
| *Hylastes nigrinus* (Mannerheim) | 1.00 | 1 | 0 | 0 | NA |
| *Ips integer* (Eichhoff) | 2.00 | 1 | 0 | 0 | NA |
| *Ips pini* (Say) | 9.00 | 1 | 7.00 | 1 | 0.9185 |
| *Mecinus* sp. | 1.00 | 1 | 0 | 0 | NA |
| *Pissodes* sp. | 2.00 | 1 | 1.00 | 1 | NA |
| Larval* | 0 | 0 | 1.40 ± 0.24 | 5 | 0.0068 |
| Cerambycidae | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Megasemum asperum* (LeConte) | 0 | 0 | 1.00 | 1 | NA |
| *Monochamus clamator* LeConte | 2.60 ± 0.67 | 5 | 3.25 ± 1.03 | 4 | 0.9161 |
| *Monochamus obtusus* Casey | 11.00 | 1 | 7.50 ± 6.50 | 2 | 0.3753 |
| Larval* | 2.75 ± 1.18 | 4 | 2.67 ± 1.67 | 3 | 0.7355 |
| Braconidae | | | | | |
| *Coeloides sympitys* Mason | 0 | 0 | 3.00 ± 1.00 | 3 | 0.1966 |
| Buprestidae | | | | | |
| *Anthaxia aeneogaster* Laporte and Gory | 3.00 ± 1.00 | 2 | 0 | 0 | 0.1291 |
| *Melanophila acuminata* (DeGeer) | 1.00 | 1 | 0 | 0 | NA |
| Larval* | 1.75 ± 0.75 | 4 | 2.00 ± 0.71 | 4 | 0.9020 |
| Trogossitidae | | | | | |
| *Temnochila chlorodia* (Mannerheim) | 1.50 ± 0.50 | 2 | 2.33 ± 0.88 | 3 | 0.3900 |
| Cleridae | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Enoclerus lecontei* (Wolcott) | 2.00 | 1 | 1.00 | 1 | NA |
| Silvanidae | | | | | |
| *Silvanoprus* sp. | 0 | 0 | 125.00 | 1 | NA |
| Siricidae | | | | | |
| Larval* | 2.00 ± 1.00 | 2 | 0 | 0 | 0.2863 |
| Diprionidae | 0 | 0 | 1.00 | 1 | NA |
| Anthocoridae | | | | | |
| *Anthocoris* sp. | 0 | 0 | 3.67 ± 1.45 | 3 | 0.1238 |
| Aradidae | | | | | |
| *Aradus* sp. | 0 | 0 | 1.50± 0.50 | 2 | NA |
| Tortricidae | 1.00 | 1 | 1.00 | 1 | NA |
| Formicidae | 0 | 0 | 1.00 | 1 | NA |

* Only found in bolts split after emergence period was over

## DISCUSSION

The use of biochar as a soil amendment may be beneficial in forest systems, especially in the restoration of sites disturbed with drought, wildfire, or post-harvest if soils are degraded. Carbon-rich biochar material can contribute to long-term carbon sequestration and potentially reduce drought stress in surrounding vegetation because it can increase soil water holding capacity (Page-Dumroese et al. 2017; Sarauer et al. 2018) and plant available water (Blanco-Canqui, 2017), depending on soil texture and organic matter content. As the use of biochar is incorporated in forest management, it is necessary to determine the potential effects that it may have on insects and other invertebrate assemblages. Although direct exposure of insects to biochar in a controlled laboratory setting may reduce weight gain, survival and fecundity (Cook and Neto, 2018, Hou et al. 2015; Rice-

Marshall et al. 2021), direct exposure to biochar in the field may yield different results.

In our first experiment, biochar on the bark surface did not impede the ability for *I. pini* to locate, attack, or emerge from host material that had been baited with the pheromone ipdienol to ensure attack. The density of *I. pini* nuptial chambers and emerged beetles was not significantly different between biochar treated and non-treated bolts indicating that attack and within bolt survival was similar between treatments.

In our second experiment, *D. valens* more frequently emerged from bolts that were not treated with biochar. Only three individuals emerged from a single biochar-treated bolt. This may indicate that *D. valens* actively avoided bolts treated with biochar or that biochar may possibly inhibit the ability for *D. valens* to locate host material. Although *D. valens* pheromone was not used in the second experiment, *D. valens* produces their own aggregation pheromone, attracting increasing numbers of conspecifics, and there may be a pheromone-mediated behavior that we cannot directly account for. Colonization by other insect taxa were not found to be different between non-treated control bolts and bolts treated with biochar, although the insects that emerged from each bolt varied. Some insects collected from the bolt material were only associated with non-treated control bolts, such as the parasitoid wasps (*Coeloides sympitys*), minute pirate bugs (*Anthocoris* sp.) and fungus beetles (*Silvanoprus* sp.) and fungus-feeding flat bugs (*Aradus* sp.), although the low number of bolts that each species was found on probably affected the statistical results. The fungus beetles, *Silvanoprus* sp. only emerged from a single control bolt. Other insect taxa such as the Buprestid beetles *Anthaxia aeneogaster* and *Melanophila acuminata* and larval Siricid wasps, were only found in the biochar treated bolts. Siricid wasps may be attracted to the biochar material, as these wasps often oviposit in trees affected by fire (Costello et al. 2011). *Ips pini* were not prevalent in this study, as they were not common in our bolts. Response by natural enemies change with the colonization by bark beetles. It is possible the parasitoid Braconid wasps found only in the control bolts were not responding to the biochar, but more the lack of hosts in the biochar-treated bolts.

As these bolts were undisturbed and monitored for emergence for a significant period of time (12 months), predacious insects such as *Temnochila chlorodia* (Mannerheim) and *Enoclerus lecontei* (Wolcott), which were found in both the control and biochar treated bolts, may have reduced the number of insects that would have otherwise survived to emerge from the bolts. Both *Temnochila* and *Enocleus* beetles are abundant generalist predators and can have a substantial impact on bark beetle populations as their larvae and adults both feed on bark beetle eggs, larvae and adults (Person 1940, Wegensteiner et al. 2015).

Studies have shown that depending on the soil type and management system, biochar impacts on soil health and crop productivity respond at different rates; with plant growth responses ranging from -29% to 324% with biochar application rates from 0.5 to 135 t $ha^{-1}$ (Glaser et al. 2002; Bista et al. 2019). Responses of soil properties and wheat shoot and root growth to four rates of wood biochar (0, 11.2, 22.4, and 44.8 Mg $ha^{-1}$) and two fertilizer rates (no fertilizer and fertilizer) with Walla Walla, Washington silt loam soil led to a recommended biochar application rate to be 22.4 Mg $ha^{-1}$ and less (without fertilizer) for increasing soil organic matter, soil pH, phosphorus, potassium, sulfur contents, and the shoot and root biomass of wheat (Bista et al. 2019). In this study, the Walla Walla silt loam soil was mixed with biochar, with and without fertilizer and placed in individual pots, approximating the tilling of biochar into soil in agricultural systems. In forest systems, tilling is not feasible, although biochar can be applied to the soil surface or mixed into the mineral soil during restoration activities (Dumroese et al. 2017). In a recent field study of tree diameter response to biochar application, out of the three rates studied (0 Mg ha-1, 3 Mg ha-1 and 25 Mg ha-$^{1}$) an application rate of 25 Mg $ha^{-1}$ of biochar was recommended for maximum carbon sequestration and potential to benefit soil properties. Rates of 2.0 Mg $ha^{-1}$ to 25.0 Mg $ha^{-1}$ (1-10 tons $acre^{-1}$) are currently being recommended for forest sites (Page-Dumroese et al. 2017).

## CONCLUSIONS

Our first field study showed that *I. pini* utilization of ipsdienol-baited ponderosa pine was not affected by the application of biochar at 3.2 Mg $ha^{-1}$ (1.30 tons $acre^{-1}$). However, in non-baited bolts, utilization by *D. valens* was affected by the application of 6.2 Mg $ha^{-1}$ (2.50 tons $acre^{-1}$) of biochar. The colonization and tunneling behaviors of bark beetles such as *I. pini* and *D. valens*, and wood borers including Cerambycidae and Buprestidae contribute to decomposition of woody material and create infection routes for wood rotting fungi (Furniss and Carolin 1977). If biochar is applied to the surface of a downed tree this may result in possible interference in the ability of insects to locate or to initiate colonization of the host material, it may then further interfere with, accelerate, or prolong woody residue decomposition rates. The application of biochar may mimic the natural disturbance process of deposition of charcoal material following a wildfire (Harvey et al., 1979; DeLuca and Aplet, 2008; Matovic, 2011; Dumroese et al. 2017), but this rate of conversion during a wildfire is 1-10% of the biomass burned (DeLuca and Aplet 2008) which is likely less than the targeted application rates of biochar. Although the application of biochar may temporarily impede some insect activity on downed woody material, precipitation events should move at least some of the biochar from the bark surface to the soil organic horizons and ultimately the mineral soil. Therefore, application of biochar during the autumn, prior to rain and freeze/thaw events is recommended to limit the exposure of numerous forest insects.

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## CONCLUSIONS

The research in this thesis examined the impacts of biochar on insects using multiple experiments. In the first experiment, Douglas-fir tussock moth (*Orgyia pseudotsugata*) larvae were exposed to biochar either on the surface of or incorporated within synthetic diet. Biochar both on or within synthetic diet negatively affected survival and weight gain of *O. pseudotsugata*, and the low 10% volume to volume biochar to diet treatment led to potential compensatory feeding. *Orgyia pseudotsugata* are defoliating insects, consuming large quantities of foliar biomass predominantly on true firs (*Abies* sp.), Douglas-fir (*Pseudotsuga menziesii*), and spruce (*Picea* sp.) hosts (Furniss et al. 1977; Wickman, 1981; Pederson et al. 2020). *Orgyia pseudotsugata* were used as model organisms for studying the impact biochar on insects when the material is ingested throughout development. Further research is necessary to establish the specific physiological effects of biochar on insects and other invertebrates.

In the next series of experiments, field studies were conducted to first assess whether biochar affected the attack or emergence of the fir engraver beetle (*Ips pini*) on downed ponderosa pine (*Pinus ponderosae*) segments (bolts) baited with aggregation pheromone, and then to assess whether biochar altered species richness or abundance of insect assemblages on non-baited bolts. We found that biochar on the bark surface did not impede the ability for *I. pini* to locate, attack, or emerge from host material that had been baited with the pheromone ipdienol to encourage attack. In the second experiment, greater species richness was associated with non-treated control bolts compared to biochar treated bolts. Also, more *D. valens* emerged from control bolts that were not treated with biochar, and only three individuals emerged from a single biochar-treated bolt. This suggests that *D. valens* avoided bolts treated with biochar or that biochar may inhibit the ability of *D. valens* to identify or locate host material. The research suggests the application of biochar on downed woody material may interfere with the ability of some insects to locate or initiate colonization of host material, while other insects remain unaffected. Biochar may thus accelerate or prolong rates of woody residue decomposition.

Given that biochar amendments may improve soil properties (Marris, 2006; Waqas et al. 2014; Wang et al. 2016; Lahori et al. 2017; Zhang et al. 2017), increase soil water holding capacity (Page-Dumroese et al. 2017, Sarauer et al. 2018) plant available water (Blanco-Canqui, 2017), and sequester carbon (Lehmann and Joseph, 2009), it is useful for soil restoration in mine reclamation (Rodriguez-Franco et al. 2021), and a soil amendment within agricultural (Glaser et al. 2002; Lehmann et al. 2006; Marris, 2006; Waqas et al. 2014; Zhang et al. 2017), rangeland (Gao and DeLuca, 2020) and forest systems (Page-Dumroese et al. 2017; Sarauer et al. 2018). As biochar is utilized as a soil restoration tool, it is necessary to continue examining the potential effects that it may have on insects and other invertebrate assemblages. Biochar application may approximate the natural disturbance associated with charcoal deposition following a wildfire (Harvey et al., 1979; DeLuca and Aplet, 2008; Matovic, 2011; Dumroese et al. 2017). With precipitation events, biochar material moves into soil organic horizons and ultimately into the mineral soil. To limit the exposure of numerous forest insects, it is recommended that biochar be applied in the autumn prior to rain and freeze/thaw events.

As biochar has the potential to impact tree resistance mechanisms, there may be potential for biochar applied to tree foliage and surrounding soil to aid in the suppression of herbivorous insects. Large-scale field plots with known insect populations could be used to assess the potentially suppressive effects of biochar over the long term. It is possible that ingestion of biochar impacts insect survival and development and may at the same time improve tree health and resistance to insect herbivory. Further long-term field studies should improve the understanding of how biochar affects insect activity associated with downed woody material, the rate of insect-mediated decomposition, as well as insect species richness and abundance.

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